OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Public Health Goals

SECOND PUBLIC REVIEW DRAFT

Trihalomethanes in Drinking Water:

Chloroform
Bromoform
Bromodichloromethane
Dibromochloromethane

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PREFACE

The Public Health Goal (PHG) technical support documents provide information on health effects from contaminants in California drinking water. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals. These documents and the analyses contained therein 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.

Under the California Safe Drinking Water Act of 1996 (Health and Safety Code section 116365), the Office of Environmental Health Hazard Assessment (OEHHA) develops PHGs for drinking water contaminants in California based exclusively on public health considerations. OEHHA periodically reviews PHGs and revises them as necessary based on the availability of new scientific data. This document presents PHGs for four trihalomethanes that are created through the disinfection of water.

This release constitutes the second public review draft for the PHGs for four individual trihalomethanes – chloroform, bromoform, bromodichloromethane and dibromochloromethane. The first public review draft was released in October 2018. The documjent was subsequently peer reviewed. The draft is different from the draft PHG document for total trihalomethanes released for public review in 2010. Unlike the 2010 draft, the current document develops PHGs for the individual compounds; these PHGs were derived using up to date methodology including benchmark dose and pharmacokinetic modeling, updated drinking water ingestion rates, and dermal/inhalation exposure estimates from household tap water use.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are based solely on scientific and public health considerations without regard to economic considerations, MCLs adopted by SWRCB consider economic factors and technological feasibility. State law requires that MCLs be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory and represent only non-mandatory goals. Under federal law, MCLs established by SWRCB must be at least as stringent as the corresponding federal MCL if one exists.

CONTENTS

List of Commonly Used Abbreviations

ADD – acceptable daily dose

ASF – age sensitivity factor

BDCM – bromodichloromethane

BMD – Benchmark Dose

BMDL – 95% lower confidence limit on the benchmark dose

BMR – Benchmark Response

CAS # – Chemical Abstract Service number

$CHCl_3$ – chloroform

$CHBr_3$ – bromoform

CNS – central nervous system

CSF – cancer slope factor

CYP – cytochrome p450 enzyme

DBCM – dibromochloromethane

DBP – disinfection byproduct

DWI – daily water intake

GD – gestation day

hrs/day – hours per day

IARC - International Agency for Research on Cancer

ip – intraperitoneal

Kp – permeability coefficient

log K_{ow} – log of the octanol-water partition coefficient

LD_{50} – lethal dose to 50% of test animals

L/kg-day – liters per kg body weight per day

LI – labeling index

LOAEL – Lowest Observed Adverse Effect Level

MCL – Maximum Contaminant Level

µg/L – micrograms per liter

$\mu g/m^3$ – micrograms per cubic meter

µg/g – micrograms per gram

mg/kg – milligrams per kilogram

mg/kg-day – milligrams per kilogram body weight per day

mM – millimolar

mmol/kg – millimoles per kilogram

nmol/mg – nanomoles per milligram

NCI – National Cancer Institute

ng/g – nanograms per gram

NOAEL – No Observed Adverse Effect Level

NTP – National Toxicology Program

OEHHA – Office of Environmental Health Hazard Assessment

PHG – Public Health Goal

PND – post-natal day

POD – point of departure

ppb – parts per billion

ppm – parts per million

RSC – relative source contribution

SWRCB – State Water Resources Control Board

THM – trihalomethane

UF – uncertainty factor

UF_A – interspecies uncertainty factor

UF_H – intraspecies uncertainty factor

US EPA – United States Environmental Protection Agency

SUMMARY

Trihalomethane Public Health Goals

This document presents proposed public health goals (PHGs) for the four major regulated trihalomethanes (THMs) found in drinking water as a result of disinfection methods: chloroform ($CHCl_3$), bromoform ($CHBr_3$), bromodichloromethane (BDCM; $CHBrCl_2$), and dibromochloromethane (DBCM; $CHBr_2Cl$). A PHG is the concentration of a contaminant in drinking water that is estimated to pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. The four proposed PHGs represent concentrations of the THMs in drinking water that do not pose significant health risks, including risks of cancer. This document also identifies health protective concentrations for adverse health effects other than cancer. The table below provides the PHGs (based on cancer effects) and health-protective concentrations for the four THMs. The concentrations in the table are given in units of parts per billion (ppb), which for water on a weight/weight basis is the same as micrograms per liter (µg/L).

Table S.1. PHGs and Non-Cancer Health-Protective Drinking Water Concentrations for Trihalomethanes

Chemical Name	Public Health Goal (PHG) (ppb): Cancer effects	Health Protective Concentration (ppb): Non-cancer health effects
Chloroform	0.4	170
Bromoform	0.5	430
Bromodichloromethane	0.06	13
Dibromochloromethane	0.1	110

PHGs are not regulatory requirements, and are based solely on protection of public health without regard to cost impacts or other factors. PHGs form the basis of California's Maximum Contaminant Levels (MCLs) for drinking water, which are established by the State Water Resources Control Board, and each MCL must be set as close to the corresponding PHG as is economically and technically feasible. California MCLs may be set at the same or a more stringent level than the federal MCLs established by the US Environmental Protection Agency (US EPA). Both the current California and federal MCLs of 80 ppb for total trihalomethanes represent the highest allowable annual average sum of the concentrations of chloroform, bromoform, BDCM, and DBCM.

PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public

health professionals. The PHGs are based on comprehensive analyses of information on the toxicology of each compound. For volatile chemicals like the THMs, they also take into account multiple routes of exposure – dermal, inhalation and oral - from drinking the water, showering, taking a bath, washing dishes, and other household uses of tap water.

Necessity of Disinfection

Disinfection of drinking water is a necessity to avoid infectious diseases in the general public from microbial contamination of drinking water supplies. Disinfection by chlorination or chloramination results in the formation of disinfection byproducts (DBPs), in drinking water. These disinfection byproducts include the THMs.

Of the more than 600 DBPs that have been identified to date, THMs are among the most prevalent chemicals resulting from chlorination and chloramination. In California drinking water, the four THMs covered in this document are commonly detected. Average levels of individual THMs measured in 2014-2015 in California ranged from 4.9 to 8.8 ppb, with maximum measurements ranging from 55 to 119 ppb, depending on the individual THM.

In considering exposures to the THMs, it is important to keep in mind the hazards of microbial pathogens in drinking water. The World Health Organization in its 2011 report *Guidelines for Drinking-Water Quality* discusses the issue as follows:

"Disinfection is of unquestionable importance in the supply of safe drinking-water. The destruction of pathogenic microorganisms is essential and very commonly involves the use of reactive chemical agents such as chlorine…
The use of chemical disinfectants in water treatment usually results in the formation of chemical by-products. However, the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection efficacy not be compromised in attempting to control such by-products."

Further, as noted by the International Agency for Research on Cancer (IARC) (2004):

"There are substantial and irrefutable benefits of disinfection of water supplies by chemical methods, including chlorination. Any major change to these programmes would need to be evaluated fully as to its costs and benefits with regard not only to the need to maintain microbiological safety but also to the possible long-term adverse effects of alternatives to chlorination."

Derivation of the PHGs and Health-Protective Concentrations

A number of adverse health effects have been observed in studies in experimental animals exposed to the trihalomethanes, including kidney and liver toxicity, reproductive toxicity, and carcinogenicity. Human drinking water studies suggest an association between exposure to DBPs (including THMs) and bladder cancer.

The proposed PHGs for the THMs are based on the cancer endpoint because it is the most sensitive effect. The PHG for each THM is set at a level where the cancer risk is estimated to be one per one-million persons exposed over a 70-year lifetime. The PHGs as well as the non-cancer health-protective concentrations include considerations of sensitive subpopulations,

such as infants and children. They account for the greater drinking water intake rates adjusted for body weight for infants, and the potential greater effect of exposures early in life on cancer risk compared to adult exposures. These considerations are included in estimating cancer risk across the lifetime. An overview of the toxicity and the calculation of PHGs and the non-cancer health-protective concentrations for the four THMs follows.

Chloroform

Cancer effects: Chloroform produced kidney and liver tumors following oral exposure in rats and mice (NCI, 1976; Jorgenson *et al.*, 1985; Roe *et al.*, 1979), kidney tumors in mice following inhalation exposure (Nagano *et al.*, 1998) and kidney tumors in rats following combined inhalation and oral exposures (Nagano *et al.*, 2006). Since 1987, chloroform has been listed under California's Proposition 65 as a carcinogen, and in 1988, US EPA classified it as a Group B2 probable human carcinogen (2018c). In 1999, the International Agency for Research on Cancer (IARC) concluded that chloroform was possibly carcinogenic to humans (Group 2B). In 2016, the National Toxicology Program (NTP) concluded chloroform is reasonably anticipated to be a human carcinogen. Extensive genotoxicity tests have been conducted on chloroform, with mixed results. The positive results were typically mild and occurred at high or cytotoxic concentrations. Nevertheless, available evidence indicates chloroform is capable of inducing genetic toxicity under various experimental conditions.

To determine the health-protective concentration for cancer, that is, the concentration of chloroform in drinking water that is associated with an estimated one-in-one-million risk of cancer for people exposed over a lifetime, OEHHA first derived a cancer potency for chloroform of 0.014 milligrams per kilogram of bodyweight per day $(mg/kg\text{-}day)^{-1}$. This number is the geometric mean of potency estimates derived from several datasets on liver and kidney tumors in rodents. The cancer potency was then used to derive the proposed PHG for chloroform of 0.4 ppb.

Non-cancer effects: In animal experiments, exposure to chloroform by inhalation or the oral route induced adverse non-cancer effects in the kidney, liver, nasal epithelium, and developmental toxicity. Chloroform has been on the Proposition 65 list on the basis of reproductive toxicity (developmental endpoint) since 2009.

To determine a health protective concentration for non-cancer health effects, OEHHA performed a dose-response analysis of the rodent kidney toxicity results reported in the studies by Yamamoto *et al.* (2002) and Nagano *et al.* (2006). Dose inputs to the analysis took into account dose- and route- dependent pharmacokinetics using the physiologically-based pharmacokinetic (PBPK) model published by Sasso *et al.* (2013). OEHHA derived a benchmark dose of 3.04 mg/kg-day, and after applying uncertainty factors, calculated the acceptable daily dose as 0.017 mg/kg-day. The resulting health-protective concentration for non-cancer health effects is 170 ppb.

Bromoform

Cancer effects: NTP (1989a) concluded there was some evidence for carcinogenic activity of bromoform in male rats and clear evidence for carcinogenic activity in female rats, based on

observed increased incidences of uncommon neoplasms of the large intestine in the NTP bioassay. Since 1991, bromoform has been listed as a carcinogen under Proposition 65. US EPA has classified bromoform as a probable human carcinogen, Group B2, based on sufficient evidence of carcinogenicity in animals, namely an increased incidence of tumors following oral administration of bromoform in rats and intraperitoneal administration in mice (US EPA, 2018a). Although the overall data are mixed, positive results have been reported for a number of mutagenicity and genotoxicity assays in bacterial, mammalian, and insect test systems. Thus, the weight of the available evidence indicates bromoform is mutagenic and genotoxic.

To determine the PHG, OEHHA first derived a cancer potency of 0.0109 $(mg/kg\text{-}day)^{-1}$ from the data for the combined incidence of adenomatous polyps and adenocarcinoma in the large intestine of female F344/N rats reported in the 1989 NTP bioassay. The cancer potency was then used to derive the proposed PHG for bromoform of 0.5 ppb.

Non-cancer effects: Results of experimental exposure of laboratory animals to bromoform indicate that the liver, kidney, and colon are key targets for toxicity. As well, there is evidence for reproductive toxicity of bromoform.

OEHHA used the increased relative liver weight data from Tobe et al (1982) to determine the health protective concentration for non-cancer health effects. OEHHA derived a benchmark dose of 12.9 mg/kg-day and, after application of uncertainty factors, an acceptable daily dose of 0.043 mg/kg-day. The resulting health-protective concentration for non-cancer health effects is 430 ppb.

Bromodichloromethane

Cancer effects: Exposure of laboratory animals to BDCM given in corn oil induced tumors in the liver, kidney and large intestine. In 1987, NTP reported statistically significant increases following exposure to BDCM in incidences of adenomatous polyps and adenocarcinoma of the large intestine and renal tubular cell adenoma and carcinoma in both sexes of rats, significant positive trends for increased incidence of adenoma and adenocarcinoma of the kidney in male mice, and adenoma and carcinoma of the liver in female mice. NTP considered these large intestine and kidney tumors in rats and the kidney and liver tumors in mice to be clear evidence of carcinogenic activity under the conditions of this study. BDCM has been listed since 1990 as a carcinogen under Proposition 65. IARC (1991, 1999a) concluded there was sufficient evidence in experimental animals for BDCM carcinogenicity, thereby classifying BDCM as possibly carcinogenic to humans (Group 2B). US EPA (2018b) classified BDCM as a probable human carcinogen, Group B2, on the same basis as IARC. NTP (2016) classified BDCM as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. Although the overall data are mixed, a number of positive results were obtained for mutagenicity and genotoxicity assays in bacterial and mammalian systems. Thus, the weight of evidence suggests that BDCM is mutagenic and genotoxic.

To determine the PHG, OEHHA first derived a cancer potency of 0.087 $(mg/kg\text{-}day)^{-1}$ using the incidence of hepatocellular adenoma and carcinoma in female $B6C3F_1$ mice given BDCM in

corn oil in the NTP (1987) study. The cancer potency was then used to derive the proposed PHG for BDCM of 0.06 ppb.

Non-cancer effects: BDCM produced liver, kidney, and reproductive toxicity in laboratory animals.

To determine the health-protective concentration for BDCM for non-cancer health effects, OEHHA applied the benchmark dose approach to data on liver toxicity from the NTP (1987). Using the derived benchmark dose of 3.4 mg/kg-day for liver histopathological changes in male rats and applying uncertainty factors, the acceptable daily dose is 0.013 mg/kg-day. The resulting health-protective concentration for non-cancer health effects is 13 ppb.

Dibromochloromethane

Cancer effects: IARC (1999a) classified DBCM as a Group 3 carcinogen, not classifiable as to its carcinogenicity to humans, based on limited animal carcinogenicity data and inadequate human data on DBCM carcinogenicity. In 1992, US EPA classified DBCM as a possible human carcinogen, Group C, based on 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) exposed mice and rats to DBCM by corn oil gavage and found statistically significant increases in incidence of hepatocellular adenomas and combined adenomas and carcinomas in high-dose female mice. In male mice, a statistically significant increased incidence of hepatocellular carcinomas and combined adenomas and carcinomas was observed in the high-dose group. NTP concluded that there was equivocal evidence for carcinogenicity in males and some evidence of carcinogenicity in females. Although the overall data on DBCM's carcinogenicity are mixed, positive results have been obtained for mutagenicity and genotoxicity in mammalian and bacterial test systems. The overall weight of evidence suggests that DBCM is mutagenic and genotoxic.

Given the evidence that DBCM is genotoxic, the positive results from the NTP study in mice, and the structural similarity to other carcinogenic THMs, OEHHA is treating DBCM as a carcinogen. To determine the PHG, OEHHA first derived an estimated cancer potency of 0.0445 $(mg/kg\text{-}day)^{-1}$ based on the combined incidence of liver adenoma and carcinoma in female $B6C3F_1$ mice in the NTP (1985) study. The cancer potency factor was then used to derive the proposed PHG for DBCM of 0.1 ppb.

Non-cancer effects: Results from animal toxicology studies show that the liver and kidney are key targets for toxicity of DBCM. Reproductive toxicity has also been observed in animal studies. To determine the health-protective concentration for DBCM for non-cancer health effects, OEHHA estimated an acceptable daily dose for DBCM from data on liver toxicity in rats from the chronic NTP (1985) study. Using the benchmark dose of 3.4 mg/kg-day for liver histopathological changes in male rats and applying uncertainty factors, the acceptable daily dose is calculated as 0.013 mg/kg-day. The resulting health-protective concentration for non-cancer health effects is 110 ppb.

1. INTRODUCTION

Purpose

The purpose of this document is to estimate health-protective concentrations for the four major regulated trihalomethanes (THMs) found in drinking water as a result of disinfection methods – chloroform ($CHCl_3$), bromoform ($CHBr_3$), bromodichloromethane (BDCM; $CHBrCl_2$), and dibromochloromethane (DBCM; $CHBr_2Cl$) – and to develop public health goals (PHGs) for each individual THM. These assessments are based on comprehensive analyses of information on the toxicology of each compound. 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}) for exposures over a 70-year lifetime. In these assessments, when estimating lifetime cancer risks, OEHHA accounts for the early-life sensitivity to carcinogens and enhanced water intake relative to bodyweight of the young.

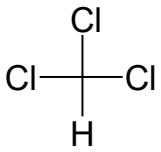
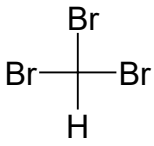
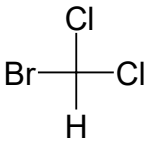
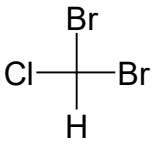
Disinfection is a critically important process for control of microbial contamination of drinking water, for it protects against cholera, typhoid fever, amoebic dysentery, giardiasis, and other enteric diseases, some of which can be life-threatening (WHO, 2011). Other waterborne diseases may result in diarrhea, and are likely to have serious consequences in infants and the elderly. However, disinfection by chlorination or chloramination leaves residual toxic byproducts in the drinking water such as THMs, and alternative disinfection methods produce other groups of disinfection byproducts (DBPs) that also carry risks of adverse health outcomes. Of the more than 600 DBPs that have been identified (Richardson et al, 2007), THMs are among the most prevalent chemicals resulting from chlorination and chloramination. In California waters, the four THMs covered in this document are commonly detected (see Chapter 2).

The US government and the State of California have adopted drinking water standards in the form of maximum contaminants levels (MCLs) for chemical contaminants that are created during drinking water disinfection. Both the current state and federal MCLs are set at 80 micrograms per liter (80 μg/L) for the total concentration of THMs in drinking water. The determination of the MCL by the State Water Resources Control Board balances the important benefits of water disinfection against the risks of exposure to residual toxic byproducts in the drinking water, as well as cost and technical feasibility.

Chemical Identity

The chemical formulas, structures, synonyms, and Chemical Abstract Service (CAS) numbers for chloroform, bromoform, BDCM, and DBCM are listed in Table 1.1.

Table 1.1. Chemical Identity of the four THMs

Characteristic	Chloroform	Bromoform	BDCM	DBCM
Synonyms	trichloromethane	tribromo-methane	bromodichloro-methane, dichlorobromo-methane	dibromochloro-methane, chlorodibromo-methane
Formula	$CHCl_3$	$CHBr_3$	$CHBrCl_2$	$CHBr_2Cl$
Molecular weight (g/mol)	119.38	252.73	163.83	208.28
Structure				
CAS number	67-66-3	75-25-2	75-27-4	124-48-1

Organization of the Document

This PHG document is organized into 11 chapters with supporting appendices. Chapter 1 provides a description of the chemical properties of the four THMs covered in this document. Chapter 2 describes THM production, use and occurrence in the environment. In Chapter 3, THM exposures via tap water are reviewed. The information on exposure in that chapter is used in the calculation of PHGs for each individual THM. Chapter 4 describes the pharmacokinetics of the individual THMs. Chapters 5 through 8 provide comprehensive toxicological profiles for each THM compound.

In Chapter 9, the mechanisms of action of carcinogenicity are reviewed for each THM. Chapter 10 provides the dose-response assessment for both the non-carcinogenic and carcinogenic effects of each THM for calculation of the PHGs, which is described in Chapter 11. Chapter 11 also provides risk characterization of the THMs, and discusses the uncertainties in estimating PHGs for the individual THMs. The benefits of water disinfection versus health risks associated with THM exposures are also discussed.

2. PRODUCTION, USE AND ENVIRONMENTAL OCCURRENCE

Some of the important physical and chemical properties of the THMs that affect environmental occurrence and exposure are provided in Table 2.1. THMs occur in drinking water primarily as a result of water disinfection. There have been historical uses of the THMs, most of which have been discontinued. These are discussed in the next section. The chapter ends with a discussion of environmental occurrence in water, air, soil, foods and beverages, and marine biota.

Table 2.1. Physical and Chemical Properties of the four THMs

Property	Chloroform	Bromoform	BDCM	DBCM
Color	colorless	colorless to yellow	colorless	colorless to pale yellow
Physical state (at ambient pressure and temperature)	liquid	liquid	liquid	liquid
Odor	pleasant, ether-like	similar to chloroform	no data	no data
Odor threshold in water (mg/L)	2.4	0.51	(1.68 in air)	no data
Taste threshold in water (mg/L)	12	no data	no data	no data
Melting point (°C)	−63.6	8	−57	−20
Boiling point (°C)	61.7	149	90	120
Flammability limits	not flammable	not flammable	not flammable	not flammable
Solubility in water at 20 °C (g/L) in organic solvents	8.1 miscible with alcohol, benzene, and lipophilic solvents	3.0 miscible with alcohol, benzene, and lipophilic solvents	4.5 very soluble in ethanol, ether, and acetone	4.4 (at 22 °C) soluble in ethanol, diethyl ether, acetone, benzene
Specific gravity at 20 °C	1.484	2.899	1.980	2.451
Partition coefficients Log K_{ow} Log K_{oc}	 1.97 1.44-2.79	 2.4 2.06	 2.0 no data	 2.24 1.92
Vapor pressure at 20 °C (mm Hg)	160	5	50	76
Henry's law constant (atm-m^3/mol)	3.67×10^{-3}	5.35×10^{-4}	2.12×10^{-3}	7.83×10^{-4}

References: ATSDR, 1997, 2005; HSDB, 2006, 2009a-c; Lide, 2005; Mabey *et al.*, 1982; Mackay and Shiu, 1981; Merck, 2006; Munz and Roberts, 1987; IPCS, 2004

The log of the octanol-water partition coefficient (log K_{ow}) of approximately 2 for these THMs indicates that they partition more by a factor of approximately 100 into lipids than into water.

Production and Use

Information on the production and current or former uses of the four THMs is provided below.

Chloroform

The most common commercial manufacturing process for chloroform is the chlorination of methyl chloride (ATSDR, 1997). In 2007, the U.S. production capacity for chloroform was 765 million pounds/year (HSDB, 2009a). Chloroform is primarily ($\geq$ 90 percent) used to manufacture HCFC-22, a hydrochlorofluorocarbon widely used as a refrigerant and feedstock for fluoropolymers. Chloroform is otherwise used in the manufacture of pharmaceuticals and agricultural products and as laboratory reagents.

Chloroform was formerly used as a sweetener, an anesthetic, and a medicinal component of cough syrups, toothpastes, liniments, and toothache compounds (ATSDR, 1997). These uses have been discontinued due to the toxicity of chloroform.

Bromoform

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, 2009b). Bromoform has been used as an intermediate in organic synthesis, an ingredient in fire-resistant chemicals, a solvent for waxes, greases and oils, a sedative and a cough suppressant (IARC, 1991; HSDB, 2009b; ATSDR, 2005). Currently it is mainly used as a laboratory reagent, in geological assays for mineral ore separations and in the electronics industry in quality assurance programs (ATSDR, 2005).

Bromodichloromethane

BDCM is no longer produced or used commercially in the US, with no import expected (HSDB, 2009c; ATSDR, 2018). 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, 2018; HSDB, 2009c). BDCM has been used as an intermediate in organic synthesis, a fire extinguisher fluid ingredient, a solvent for fats, waxes and resins, and a heavy liquid for mineral and salt separations (ATSDR, 2018; IARC, 1991; Merck, 2006).

Dibromochloromethane

DBCM is no longer produced commercially in the U.S. (HSDB, 2006). DBCM has been used as a chemical intermediate in the manufacture of fire extinguishing agents, aerosol propellants, refrigerants and pesticides (IARC, 1991; ATSDR, 2005). DBCM is used as a laboratory chemical reagent and intermediate in organic synthesis (HSDB, 2006).

Environmental Occurrence

Drinking Water

General

The four regulated THMs are byproducts produced during disinfection of water by chlorination or chloramination. The amount of each THM present in various drinking water supplies is dependent on factors such as organic content, temperature, salinity, pH of the water, and type of chlorinating agent (Sadiq and Rodriguez, 2004; Rodriguez *et al.*, 2004).

When chlorine is added to water as a disinfectant, it forms hypochlorous acid, which can then react with organic materials in the water to form multiple halocarbon compounds (NRC, 1980; Wallace, 1997). The THMs are also formed when ozone, chlorine dioxide or chloramine is used as the disinfectant, although usually at lower levels (NRC, 1986).

The proportions of individual THMs can be significantly affected by the amount of bromide in the source waters (McGuire and Meadow, 1988; Krasner *et al.*, 1989; Chen and Weisel, 1998; Keegan, 1998; Nieuwenhuijsen *et al.*, 2000). Sources of bromide include saltwater intrusion, connate water (ancient geologically trapped seawater), oil-field brines, and industrial and agricultural chemicals.

Occurrence in California Drinking Water

OEHHA calculated population-weighted statewide average concentrations of THMs for water systems with positive measurements post water treatment. The calculation used results from samples collected and tabulated by the State Water Resources Control Board (SWRCB)[1] in 2014 and 2015 from 3,498 locations in drinking water utility distribution systems in California. The mean concentrations for each THM are shown in Table 2.2, and are also plotted in Figure 2.1 below. The Table and Figure both indicate moderate variations in the levels of individual THMs across water systems in California.

[1]Division of Drinking Water - Water Quality Analyses Database Files. State Water Resources Control Board. California Environmental Protection Agency. Accessed on December 13, 2017 at https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/EDTlibrary.shtml.

Table 2.2. Statewide Population-Weighted Mean Concentrations and Maximum Concentrations of THMs in Community Drinking Water Systems in California in 2014-2015

THM	Population-weighted mean concentration [a] (µg/L)	Maximum concentration measured (µg/L)
Chloroform	8.8	119
BDCM	6.2	79
DBCM	7.5	71
Bromoform	4.9	55
Total THMs	27	229

[a] For water systems with at least one positive THM sample.

The calculations proceeded as follows. Sampling location identification information and corresponding water quality monitoring data were extracted from the Water Quality Monitoring Database maintained by the SWRCB. Time-weighted averages were determined for each sample location by year. This means that within a year, a sample was weighted by the number of days between the day the sample was taken and the day the next sample was taken. This resulted in a yearly average concentration for each sampling location within each system. The concentrations by year were averaged together to calculate one concentration for each sampling location. Then, all sampling locations within a system were averaged together to achieve one concentration for each system. Next, system averages were adjusted based on any contributing wholesale system, if applicable. If a water system purchases water from a wholesale system, the wholesale system's data were incorporated based on the fraction of water that is supplied to the retail system. Lastly, the system concentrations were weighted by the populations they serve to calculate a population-weighted statewide average for all water systems with data with at least one contaminant greater than zero. If a system had no detection (zero concentration average) for all four chemicals, it was excluded from the calculation.

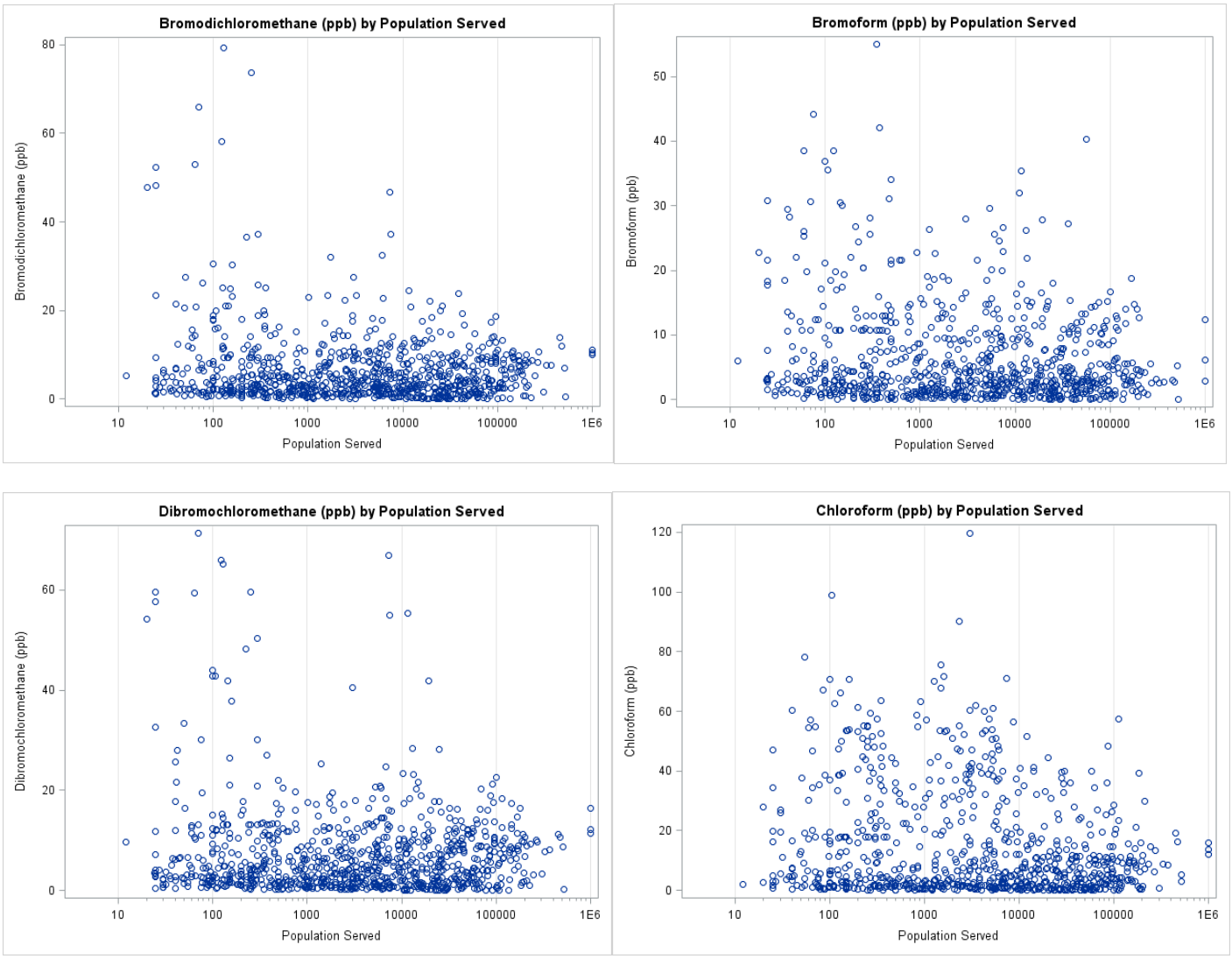


Figure 2.1. Average level of THMs in water systems in California (2014-2015) testing positive for at least one THM

Swimming Pools

Swimming pools that have been disinfected through chlorination or bromination are an additional source of exposure to THMs (Silva *et al.*, 2012; Lourencetti *et al.*, 2012; Lee *et al.*, 2010). In a study of indoor swimming pools disinfected using chlorine-based techniques in Lisbon, Portugal, water THM concentrations ranged from 10.1 to 155 micrograms per liter (µg/L), with 6.5 percent of samples representing values over 100 µg/L (Silva *et al.*, 2012). Chloroform was detected at the highest concentration in all pools, and was followed by BDCM, DBCM, and bromoform, which were detected in 99, 34, and 6 percent of the samples, respectively. Bromoform was the dominant THM in a study of swimming pools using bromine agents for water disinfection (Lourencetti *et al.*, 2012).

Swimmers and staff who work in and around chlorinated swimming pools constitute a subpopulation that is potentially at higher risk of increased exposure to chloroform and other THMs. Following up on their earlier study, Aggazzotti *et al.* (1998) found that chloroform was the predominant THM in water and ambient air in chlorinated indoor pools. BDCM and DBCM were always present but at lower levels than chloroform, and bromoform was rarely present.

A British study of 300,000 pregnant women found that those who swam regularly received greater chloroform doses than did non-swimmers (Whitaker *et al.*, 2003). The study simulated 90 days (the last trimester) of chloroform uptake via tap water ingestion, bathing, showering and swimming for each pregnant woman to determine the average daily uptake for all pathways. A median chloroform concentration of 52 µg/L (range, 13-365 µg/L) was found in the study for indoor swimming pools, and concentrations of chloroform supplied to households via tap water were categorized into low (< 20 µg/L), medium (≥ 20 to < 40 µg/L) and high (≥ 40 µg/L). Chloroform uptake during swimming ranged from 0 µg/day for the two-thirds of pregnant women who did not swim to 22 µg/day for the most frequent swimmers. While swimming had the largest impact on uptake, chloroform uptake via bathing and showering was more significant than chloroform uptake via tap water ingestion.

Air

Chloroform exists solely as a vapor in the atmosphere (HSDB, 2009a). Low concentrations are found in the air in both urban and rural areas, emitted from anthropogenic and natural sources (US EPA, 1985a). Natural sources include production by tropical red algae and red seaweed. Marine and terrestrial environments were estimated to be equal contributors of global biogenic chloroform emission.

In the US, 387,700 pounds of chloroform were disposed of or released into the environment by industrial facilities in 2015 (US EPA, 2017). Gentner *et al.* (2010) observed strong seasonal differences in anthropogenic emissions of chloroform; emissions were 2.5 times greater in summer than in fall. The authors identified landfills and water chlorination as possible seasonal sources, and rule out biogenic contributions due to reported insignificant biogenic emissions of chloroform in southern California ecosystems (Rhew *et al.*, 2008). Although early estimates suggested that 10 percent of global chloroform emissions was anthropogenic, newer studies propose that the anthropogenic contribution may actually be closer to 50 percent (Worton *et al.*, 2006; Trudinger *et al.*, 2004).

According to the US Environmental Protection Agency (US EPA) Toxics Release Inventory, 136,000 pounds of bromoform were disposed of or released into the environment by industrial facilities in the United States in 2015 (US EPA, 2017). Atmospheric concentrations of BDCM and DBCM are expected to be small because they are not produced commercially or are used in small quantities, as discussed in the Production and Use section, and natural production is relatively low (ATSDR, 2005, 2018). Similar to chloroform, the three brominated THMs are expected to exist solely in the vapor form in the atmosphere (HSDB, 2006, 2009b-c).

The air surrounding swimming pools and hot tubs also can contain significant levels of THMs (McCulloch, 2003; Wallace, 1997). A study of Lisbon swimming pools reported air chloroform

concentrations ranging from 45 to 373 µg/m^3; 24 percent of the samples have values above 136 µg/m^3, which is considered a high-exposure value (Silva *et al.*, 2012).

Another source of THMs in indoor air is evaporation from chlorinated water during activities such as use of chlorine bleach, hypochlorite-containing detergents, showering, bathing, clothes washing, and dish washing (Wallace, 1997, Howard and Corsi, 1998; Olson and Corsi, 2004). Exposure to these indoor sources can exceed exposure from outdoor ambient air.

Soil

Based on their physical properties, the THMs are expected to be mobile in soil and be easily volatilized from moist soil surfaces (HSDB, 2009a). These properties together with moderate water solubility mean that the THMs will partition to water and air more than to soil (ATSDR, 1997). Chloroform was detected in eight percent of 425 sediment samples from US EPA's Storage and Retrieval (STORET) database, with median concentrations less than 5 micrograms per kilogram (µg/kg) (Staples *et al.*, 1985). In 581 sediment samples, the mean BDCM concentration was 10.8 µg/kg (range, non-detected to 55 µg/kg), while DBCM and bromoform were not detected (US EPA, 1985b). BDCM was detected in nearly all the soil air samples, at concentrations from 0.03 to 0.31 nanograms per liter (ng/L), with higher levels at deeper depths.

Chloroform has been found at 1 to 2 ppm in a peat bog in New Brunswick, Canada (Silk *et al.*, 1997). 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 study suggested that chloroform is not formed in the top layer of soil, but in deeper soil layers.

Soils may also become contaminated with the THMs through spills, waste disposal in landfills, or the discharge of chlorinated water (ATSDR, 2005).

Food and Beverages

Prior to 2006, the US Food and Drug Administration routinely tested foods and beverages for THMs (except DBCM) in the Total Diet Study program. Market baskets from the most recent published report of the Total Diet Study testing positive for THMs were collected between September 1991 and October 2003. In a subset of 358 food items listed in the report, 137 items contained chloroform (US FDA, 2006). Chloroform was detected at levels below 100 parts per billion (ppb) in eggs, dairy products, meats, fish, fruits, vegetables, deli/fast food, sweets, baked goods, cereal, salted snacks, oil, salad dressings, beverages and baby food. Food items with levels of chloroform above 100 ppb included: sour cream (4-176 ppb), various cheeses (American, cheddar, Swiss and cream cheese; 3-230 ppb), roast beef (2-184 ppb), bacon (2-115 ppb), white bread (2-115 ppb), muffins (3-157 ppb), ice cream (11-118 ppb), tortilla chips (2-136 ppb), oil-popped popcorn (2-184 ppb), and fast food items such as French fries and cheeseburger (2-146 ppb), pepperoni pizza (2-164 ppb), chicken nuggets (2-104 ppb), and fried chicken leg (2-113 ppb) and breast (2-143 ppb). The highest levels of chloroform found were in sunflower seeds (15-467 ppb) followed by teething biscuits for babies (334 ppb).

BDCM was found in 46 of the food items tested, including foods in the following categories: dairy and cheese products, cooked or processed fish and meats, eggs, fresh and cooked

produce, deli items, bakery goods, peanut butter, and various juices, sodas and candies. These items contained BDCM at levels below 20 ppb. The highest levels of BDCM were in fast food hamburger (37 ppb), followed by apple juice (33 ppb) and tap water (10-30 ppb). Bromoform was not detected in any of the items.

A study in the US testing 11 foods and 17 beverages prepared using chlorinated drinking water found THMs in every sample, especially chloroform, although low or trace levels of brominated THMs were also detected (Huang and Batterman, 2009). Tea had the highest chloroform levels (from 3 to 121 μg/L), followed by coffee (from 3 to 13 μg/L), rice (9 μg/L), soups (from 0.4 to 3.0 μg/L), vegetables (< 1 μg/L), and baby food (< 0.7 μg/L).

A study conducted in northeastern Spain showed that bromoform and chloroform were among the most abundant contaminants found in agricultural irrigation waters, with concentrations ranging between 1.5 and 5.1 μg/L (Calderón-Preciado *et al.*, 2011).

Montesinos and Gallego (2014) analyzed 40 beverages (teas, isotonics, fruit beverages, tonics, and sodas) purchased at local markets in Spain for THMs. Chloroform, BDCM, and DBCM were present in all samples, whereas bromoform was found in a much lower fraction of samples and at much lower concentrations. The observed ranges were: 1.0 to 43 μg/L for chloroform; 0.09 to 7.8 μg/L for BDCM; less than 0.05 to 1.3 μg/L for DBCM; and non-detectable to 0.17 μg/L for bromoform. The researchers also analyzed 60 fruit juices (100% natural juices, reconstituted juices, and nectars) and found varying concentrations of THMs depending on the volume and quality of the treated water used in producing the beverages. Overall, the 100% natural juices contained the lowest total THM (TTHM) concentrations followed by reconstituted juices and nectars, with average values of TTHM at 0.47, 3.5, and 8.3 μg/L, respectively.

A study testing chloroform levels in milk from 43 farms supplied to three processing companies in Ireland found all samples had chloroform concentrations above 2 μg/kg (Ryan *et al.*, 2013). Average chloroform values for the three milk processors ranged from 3.4 to 6 μg/kg..

Marine Biota

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 tons per year (Dewulf and Van Langenhove, 1997).

Bromoform, BDCM, and DBCM are also produced naturally by marine algae (IARC, 1991). Bromoform production has been measured in giant kelp (*Macrocystis pyrifera*) collected from the southern California coastal region (HSDB, 2009b). Palmer and Reason (2009) estimated sea surface contributions of bromoform to the global sea-air flux and found that tropical oceans represented approximately 75 percent of the flux.

3. EXPOSURE TO THMS VIA TAP WATER

The principal source of exposure of the general population to THMs is expected to be tap water from drinking water systems disinfected by chlorination (ATSDR, 1997; NTP, 2016a; IARC, 1999b), with the exception of exposures via disinfected swimming pool water (see Chapter 2).

Ingestion of THMs in Tap Water

For oral ingestion rates used to calculate PHGs, OEHHA uses age-specific water ingestion estimates derived from a nationwide survey of food and beverage intake from approximately 20,000 individuals (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset; see OEHHA, 2012). These age-specific intake rates are normalized to body weight and expressed as liters of water ingested per kilogram of body weight per day (L/kg-day). The amount of drinking water ingested per unit body weight is higher in infants than in adults (Table 3.1). For non-cancer endpoints, the time-weighted average (TWA) daily water ingestion rate for a 70-year lifetime for the general population is generally used. However, if there is a particularly sensitive age group, the high-end (i.e., the 95th percentile parameter) estimates of the age-specific water ingestion rate for the subgroup will be used in the PHG calculations, instead of the time-weighted average (OEHHA, 2012). In deriving PHGs, OEHHA is mandated[2] to consider sensitive subgroups, such as infants and children, who may be at greater risk of adverse health effects due to exposure to drinking water contaminants than the general population.

Table 3.1. OEHHA-derived oral ingestion rates for the various life stages used for exposure modeling of contaminants in drinking water (OEHHA, 2012)

Life stage	Age range (years)	Drinking rate (L/kg-day)
Fetus	NA[a]	0.047[b]
Infant	0 to < 2	0.196
Child	2 to < 16	0.061
Adult	16 to 70	0.045

[a]Not applicable.

[b]The fetus is assumed to be exposed to the same mg/kg dose as the pregnant mother (whose ingestion rate is slightly higher than that denoted for "adult").

Multi-Route Exposure Considerations from Tap Water Use

In addition to oral ingestion, exposure to THMs in tap water can result from inhalation or dermal absorption during bathing or showering. This is because THMs are volatile organic compounds that can be released into the air from tap water (McKone, 1987).

[2]Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 et seq.)

Studies on inhalation and dermal exposures: Chloroform

Studies have established inhalation and dermal absorption as important routes of exposure to chloroform from tap water, and are highlighted below.

Jo *et al.* (1990a,b) demonstrated that chloroform levels in exhaled air were significantly higher in individuals exposed via inhalation and dermal absorption during showering compared to an exposure restricted to the inhalation route alone. They examined inhalation and dermal exposures by measuring chloroform concentration in the exhaled breath of volunteers, with and without rubber wetsuits, following showers using normal tap water at approximately 40 °C containing 5 to 35 ppb chloroform. The dermal and inhalation routes were estimated to contribute an equivalent amount of chloroform to body burden during showering.

Weisel and Jo (1996) showed elevated chloroform concentrations in exhaled breath in subjects after both inhalation and dermal exposures during showering. Breath concentrations were also elevated after dermal exposure via bathing. 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 route.

Blancato and Chiu (1993), using a physiologically-based pharmacokinetic model (PBPK) model for chloroform exposures from tap water, showed that levels of chloroform in expired breath varied depending on the route of exposure. In contrast to ingestion exposure, exposures via inhalation or dermal absorption produced exhaled breath containing elevated chloroform levels for extended periods of a few hours, indicating extensive distribution throughout the body prior to metabolism.

Corley *et al.* (1999, 2000) demonstrated temperature-dependent changes in physiology and exhalation kinetics using a PBPK model for dermal absorption of chloroform from bath water at concentrations below 100 ppb. A significant decrease was observed in the amount of chloroform exhaled as exposure temperatures decreased from 40 °C to 30 °C.

Studies on inhalation and dermal exposures: THMs

Kerger *et al.* (2000, 2005) evaluated airborne concentrations of THMs in three homes in an arid urban area, each approximately 1,000 square feet. Two homes had standard refrigeration-type central air conditioning and the third had a central evaporative cooling system supplied with chlorinated tap water containing more than 85 ppb TTHM. THMs were concurrently measured on four test days in tap water and air both outside and in selected rooms. No bromoform was detected in any sample. Air samples were collected prior to, during, and after water use for 16 shower and 7 bath events (Kerger *et al.*, 2000). The increase in average airborne concentration during showers, expressed as micrograms per cubic meter ($\mu g/m^3$) in the shower enclosure or bathroom air per µg/L in water, was 3.3 for chloroform, 1.8 for BDCM, and 0.5 for DBCM (N = 12), and during baths was 1.2 for chloroform, 0.59 for BDCM, and 0.15 for DBCM (N = 4). The relative contribution of each chemical to the air 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 being 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 US 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 THM concentrations (similar to outdoors) were found in the living room and bedroom air for the home with evaporative cooling, while the refrigeration-cooled homes showed significantly higher THM levels (by three-fold to four-fold). 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) measured levels of THMs in the blood of 31 adults exposed to THMs via 3 different scenarios (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 scenarios of 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)** documented elevated levels of individual THMs in human blood based on a field study conducted in Texas and Georgia on mothers who had given birth to healthy infants from June 1998 through May 1999. Two 10-milliliter (mL) whole blood samples were collected from each participant before and immediately following showering. Blood levels of THM species varied substantially across populations depending on both water quality characteristics and water use activities (Lynberg *et al.*, 2001). 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. 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 shower events. Results indicated that THMs in the blood rose significantly following 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 specific chemical and concentration differences in their respective tap waters (Miles *et al.*, 2002).

Egorov *et al.* (2003) reported that TTHM 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). Concentrations of chloroform in breathing zone air in bathrooms during showering were 330 ± 260 µg/m^3, and 2 ± 2 µg/m^3 in bedrooms of local residents. The mean concentration of chloroform was 3.2 µg/m^3 in exhaled air samples collected before showering and 110 µg/m^3 after showering.

Jo *et al.* (2005) used measurements of THM concentrations in tap water and indoor and outdoor air in Korean households using municipal tap water treated with ozone-chlorine or chlorine 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 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. In this study, the THM exposure estimates from ingestion were similar to those from showering.

Lévesque *et al.* (2002), in a study of chloroform exposure during showering, found mean chloroform levels in shower stall air and water, respectively, of 147 ± 56.2 μg/m^3 and 20.1 ± 9.0 μg/L. The mean increase in exhaled air chloroform levels at the end of a 10-minute shower was 33 ± 14.7 μg/m^3. The increase in exhaled air chloroform level was only associated with chloroform in the shower stall air.

Xu *et al.* (2002) estimated the steady state Kp of THMs in aqueous solution across human skin with *in vitro* diffusion chambers. 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 centimeters per hour (cm/hour) (Xu and Weisel, 2005).

Prah *et al.* (2002) developed a dermal exposure system to study the pharmacokinetics of DBPs following a 60 minute exposure of the hand and forearm of human subjects to tap water. The system was constructed of inert and impervious materials, and was able to exclude all other routes of exposure. Measurable DBPs were absorbed as demonstrated by the blood concentrations taken from 14 human subjects before, during, and after the one-hour exposure. The DBPs measured in the tap water were chloroform (56.5 ng/ml), bromodichloromethane (18.2 ng/ml), and dibromochloromethane (1.4 ng/ml). Peak concentrations in the blood of volunteers were 110 picograms per milliliter (pg/ml), 10.2 pg/ml, and 1.7 pg/ml for chloroform, bromodichloromethane, and dibromochloromethane, respectively.

Multi-Route Exposure Estimates from Tap Water Use

As the studies described in the previous section demonstrate, the principal routes by which the general population may be exposed to THMs in tap water are via oral ingestion, inhalation of indoor air containing volatilized THMs, and dermal absorption following direct skin contact (e.g., from washing and bathing). Exposure assessment models have been developed using experimental data to evaluate the contribution of inhalation and dermal exposure routes to overall exposure from tap water (McKone, 1987, 1989; Jo *et al.*, 1990a,b; 2006b).

US EPA (2006b) found that, at low environmental levels, no saturation mechanisms or interactions among the THMs relevant to uptake is likely. Thus, the actual levels are not critical to the illustration of relative uptakes from the different routes. OEHHA uses the CalTOX multimedia exposure model, which was developed by Lawrence Berkeley National Laboratory for the California Department of Toxic Substances Control (DTSC, 1994), to determine the dermal and inhalation exposures to THMs resulting from their presence in tap water. 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, except for DBCM, for which the available physicochemical parameters were manually entered and those of chloroform were used in place of missing values. Human breathing rates, drinking water consumption and body surface area values were set according to the OEHHA (2012) recommended values for the various life stages. The values used are shown below in Table 3.2.

Table 3.2. OEHHA-derived exposure parameters for various life stages used for CalTOX modeling to estimate exposures to THMs in tap water via different routes (from OEHHA, 2012)

Life Stage	Age Range (years)	Drinking Rate (L/kg-day)	Inhalation rate (m^3/kg-hr)	Body Surface Area (m^2/kg)
Fetus[b]	N/A[a]	0.047	0.015	0.029[b]
Infant	0-2	0.196	0[c]	0.059
Child	2-16	0.061	0.031	0.045
Adult	16-70	0.045	0.012	0.029

[a]Not applicable
[b]The adult body surface area parameter is used for pregnant women. Fetuses are assumed to be exposed to the same mg/kg-day dose as the pregnant mother.
[c]Infants are expected to be exposed to negligible levels of chemicals in tap water via inhalation (compared to other pathways) because they typically do not shower or flush toilets. These are the dominant inhalation exposure scenarios; therefore the inhalation pathway is excluded for infants.

The CalTOX model was run to determine the proportion of total exposures to THMs in tap water attributed to the ingestion, inhalation and dermal routes. 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. Specific values for input parameters and some model outputs are provided in Appendix A.

CalTOX predicted that inhalation exposures to the various THMs are somewhat similar, and that contributions from dermal exposure to THMs in tap water are negligible at all life stages (approximately 2 to 8 percent for the various THMs and life stages). Although chloroform and BDCM have higher Henry's law constants than bromoform and DBCM, which would produce much higher air concentrations in an equilibrium state, CalTOX also considers diffusion in water and air in the water-to-air mass transfer modeling. In the CalTOX exposure model, water-to-air transfer for the THMs is limited by their diffusion in water, resulting in relatively comparable indoor and bathroom air concentrations and exposures via the inhalation route.

As discussed above, exposure can occur via inhalation when a THM volatilizes out of water, as well as via dermal absorption of a THM across the skin. The daily water intake equivalent (DWI) is expressed in units of liter equivalents (L_{eq}) per kilogram of body weight per day (L_{eq}/kg-day). Liter equivalents represent the amount of tap water one would have to drink to account for the daily exposure to a chemical in tap water through oral, inhalation, and dermal routes. The daily

water consumption rate values for the three routes are shown in Table 3.3. These values are used in the derivation of the PHGs for the THMs in Chapter 11.

Table 3.3. Consumption of THMs in liter equivalents per kilogram of bodyweight per day via the ingestion, inhalation and dermal routes

Life Stage	THM	Ingestion (L_{eq}/kg-day)	Inhalation (L_{eq}/kg-day)	Dermal (L_{eq}/kg-day)	Total (L_{eq}/kg-day)
Fetus[a]	Chloroform	0.047	0.018	0.007	0.072
	Bromoform		0.015	0.010	0.073
	BDCM		0.018	0.008	0.073
	DBCM		0.018	0.009	0.074
Infant (0 to < 2 years)	Chloroform	0.196	0	0.014	0.210
	Bromoform			0.021	0.217
	BDCM			0.016	0.212
	DBCM			0.019	0.215
Child (2 to < 16 years)	Chloroform	0.061	0.039	0.011	0.110
	Bromoform		0.032	0.016	0.109
	BDCM		0.037	0.012	0.110
	DBCM		0.038	0.015	0.113
Adult (16 to 70 years)	Chloroform	0.045	0.015	0.007	0.067
	Bromoform		0.012	0.010	0.068
	BDCM		0.014	0.008	0.067
	DBCM		0.014	0.009	0.069
Lifetime (0 to 70 years)	Chloroform	0.053	0.019	0.008	0.080
	Bromoform		0.016	0.012	0.081
	BDCM		0.019	0.009	0.081
	DBCM		0.019	0.011	0.083

[a]Exposure occurs via the mother

4. PHARMACOKINETICS

Absorption

THMs are readily absorbed through the lungs and gastrointestinal tract. Dermal absorption can also be significant, especially following swimming and bathing in chlorinated water.

Oral Absorption

Much of the available data on oral absorption of THMs is for chloroform. Toxicological findings in both laboratory animals and humans indicate that chloroform is rapidly absorbed from the gastrointestinal tract following oral exposure, as evidenced by observations of rapid onset of toxicity in animals within minutes of oral gavage, as well as in people after accidental ingestion (US EPA, 1985a).

Oral absorption efficiency is estimated to be over 90 percent. 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 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) indicating that the majority of ingested chloroform is absorbed. This has also been demonstrated for bromoform, BDCM and DBCM, as inferred from the vast majority of the radiolabeled compounds being recovered in blood, tissue and expired air (Mathews *et al.*, 1990; Mink *et al.*, 1986).

Several studies have shown that the vehicle can have pronounced effects on the rate and extent of gastrointestinal absorption of chloroform; specifically, administration in oil may slow down this rate (Fry *et al.*, 1972; Brown *et al.*, 1974; Taylor *et al.*, 1974). For example, in a study of fasted male Wistar rats given a single 75-mg/kg dose of chloroform by gavage in either aqueous solution or corn oil, Withey *et al.* (1983) reported that when chloroform was given in water an almost instantaneous peak was detected in blood chloroform concentration, which was 6.5 times higher than when the corn oil vehicle was administered. Similar observations have been made of more rapid absorption occurring following exposure in aqueous solution than in oil for bromoform, BDCM and DBCM in mice and rats (Parra *et al.*, 1986; Lily *et al.*, 1994, 1998).

Inhalation Absorption

Uptake of inhaled vapors is driven by equilibration of the partial pressures of a chemical in tissues with the partial pressure of the chemical in air (US EPA, 1994a). Partition coefficient describes 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 chloroform readily passes from air to blood in alveoli and that pulmonary absorption is substantial.

Extensive data are available for pulmonary absorption of chloroform (versus the other THMs) because of its earlier use as a gaseous anesthetic. The rate of pulmonary uptake of chloroform in humans is initially rapid, decreasing as the concentration of chloroform in tissues approaches saturation (Raabe, 1988), after which the rate of exhalation equals the rate of absorption. US 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).

Yoshida *et al.* (1999) studied absorption and metabolism of chloroform, BDCM, and DBCM in low-level inhalation exposures in Sprague-Dawley rats. At 1 ppb exposures, the rats absorbed more chloroform (0.33 nmol/hr/kg) than BDCM (0.072 nmol/hr/kg) or DBCM (0.11 nmol/hr/kg).

Dermal Absorption

Several studies have demonstrated that THMs can be absorbed through intact skin. Percutaneous absorption of the pure liquid forms of the THMs occurs slowly, and is limited by the thickness of the epidermis, by the moderate lipophilicity of these chemicals, and by defatting of the stratum corneum (US EPA, 1985a). THMs can also be dermally absorbed from water and water vapor (Brown *et al.*, 1984; Bogen *et al.*, 1992a; Xu and Weisel, 2005; WHO, 2006; Tan *et al.*, 2007).

Dick *et al.* (1995), in a study measuring absorption of chloroform through ventral skin of the forearm of volunteers, reported greater absorption with water than ethanol as the vehicle (8.2 percent vs. 1.7 percent) based on detection in expired air and urine. Tsuruta (1975) measured the *in vivo* rate of absorption of liquid chloroform (0.5 mL) across the shaved skin of mice over a 15-minute period and reported that 0.2 percent of the applied dose was absorbed (Tsuruta, 1975). Bogen *et al.* (1992a), who measured the rate of percutaneous absorption of chloroform in a dilute aqueous solution in female hairless guinea pigs, pointed out that the human dermal absorption estimate of Jo *et al.* (1990a,b) (see Chapter 1), who estimated an equivalent contribution of chloroform to body burden from dermal and inhalation routes while showering, was not significantly different from the value they obtained for hairless guinea pigs. However, Dick *et al.* (1995) found that, based on *in vitro* percutaneous absorption of chloroform in excised human skin in a dilute aqueous solution, the permeability coefficient across human skin was 10 fold less than across the hairless guinea pig model described by Bogen *et al.* (1992a) (Dick *et al.*, 1995).

Xu *et al.* (2002) measured dermal absorption of chloroform, bromoform, BDCM, and DBCM through experiments *in vitro* using human skin. The permeability coefficients of THMs ranged from 0.16 to 0.21 cm/hour with the donor solution at 25° C. Bromoform had the highest permeability coefficient value, whereas chloroform was the least permeable through the skin, demonstrating a direct relationship between permeability and octanol/water partition coefficients. Using US EPA methodology Xu *et al.* (2002) estimated that the daily dose from bathing was 40-70 percent of the daily ingestion dose.

Leavens *et al.* (2007) measured both oral and dermal absorption in 110 human volunteers following exposure to water containing 36 µg/L ^{13}C-labeled BDCM to evaluate the pharmacokinetics of BDCM. Oral and dermal exposures were separated by at least a week, and precautions were taken to avoid inhalation exposure. Sequential whole blood samples were taken during and for up to 24 hours after exposure. Oral exposures were from one bolus of drinking water and dermal exposures were estimated for 1 hour of submersion of an arm. These authors reported a significant contribution of dermal exposure to the total exposure to BDCM in tap water. Although the oral doses were greater than the dermal doses, the maximal blood concentrations attained after dermal exposure were 25 to 100 times greater than after oral administration. As well, the clearance was considerably longer after dermal exposure, indicating significant first pass hepatic metabolism via the oral route.

Distribution

The lipophilicity of the THMs, as expressed by log octanol/water partition coefficients of 1.97 to 2.40 (93 to 250 times more soluble in octanol than in water), means that body weight and fat content will influence distribution and retention of the chemicals after absorption. Uptake and storage of the chemicals in adipose tissue can be substantial, especially in individuals with more body fat (US EPA, 1985a).

Chloroform readily distributes throughout the body (Lavigne and Marchand, 1974; Brown *et al.*, 1974; Taylor *et al.*, 1974; Löfberg and Tjälve, 1986). In humans and experimental animals, it concentrates 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 in the liver and kidney. Within the latter two organs, radioactivity from ^{14}C-chloroform is typically concentrated in the tissue immediately surrounding the hepatic centrilobular vein and in the renal cortex. McConnell *et al.* (1975), analyzing postmortem tissues from eight United Kingdom residents who were not occupationally exposed to chloroform, reported detectable levels of chloroform in liver (1 to 10 ppm), kidney (2 to 5 ppm), brain (2 to 4 ppm), and adipose tissues (19 to 68 ppm). These data, together with the partition coefficients for human tissue published by Steward *et al.* (1973), indicate that the concentrations of chloroform are highest in adipose tissue and organs with a higher fat content.

In mice, Taylor *et al.* (1974) observed distinct differences in the distribution of radioactivity between males and females of three strains (LP, CBA, C57BL) after a single gavage dose of ^{14}C-chloroform. Females exhibited the same general distribution of chloroform as males, with radiolabel detected in the stomach, intestine, bladder, liver, and kidney, except that they had significantly less radioactivity in their kidneys and significantly ($0.01 < p < 0.02$) more radioactivity in their livers compared to males. The ratio of renal radioactivity in males to that in females varied from 2.6 (in the C57BL mice) to 3.8 (in the CBA mice).

Similar to chloroform, bromoform is rapidly absorbed and distributed to liver, brain, kidney, blood, and fat tissues, with the highest concentrations found in fat (Parra *et al.*, 1986). Mink *et al.* (1986) studied distribution of radiolabeled bromoform, BDCM and DBCM in fasted male SD rats and male B6C3F$_1$ mice following gavage in corn oil of 100 mg/kg for rats and 150 mg/kg for

mice. Tissue radioactivity levels were measured eight hours after dosing. In the rat, the total organ content of radioactivity was 2.1 percent of the dose for bromoform, 3.3 percent for BDCM, and 1.4 percent for DBCM. For all three THMs, the stomach (contents removed), liver, and kidneys contained higher levels than bladder, brain, lung, muscle, pancreas, and thymus. In mice, the radioactivity recovered in organs was 4.6 percent of the dose for bromoform, 3.2 percent for BDCM, and 5.02 percent for DBCM. An additional 10 percent of the dose of bromoform was recovered in blood of mice, which the authors thought might be due to formation of carboxyhemoglobin, based on an earlier report by Anders *et al.* (1978) of elevated levels of carboxyhemoglobin in SD rats following intraperitoneal administration of bromoform.

Some of the available data on the distribution of BDCM come from studies of repeated exposures in rats. Rats given 0.5 mg or 5 mg of BDCM in corn oil gavage once daily for 25 days exhibited average BDCM concentrations of 1 or 23 μg/L in serum and 51 or 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, respectively, for both dose levels. Mathews *et al.* (1990) also conducted repeated-exposure experiments, as well as single-dose exposures, to study BDCM distribution. Male F344/N rats were 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. Consistent with observations of Mink *et al.* (1986), the liver, kidney, and stomach had the highest tissue-to-blood ratios of radiolabeled BDCM. In another experiment, 10 or 100 mg/kg of BDCM was administered daily to male F344/N 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, which is similar to the results after a single dose. Thus, repeated-dose administration did not result in altered disposition or bioaccumulation of BDCM.

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; Murray *et al.*, 1979). In humans, chloroform has been detected in placental cord blood in concentrations approximating that of maternal blood (Dowty *et al.*, 1975). Danielsson *et al.* (1986) observed fetal age-dependent differences in the distribution of chloroform, with accumulation detectable in the brain during early gestation, distribution occurring evenly throughout tissues during mid-gestation, and accumulation evident in the liver, blood, respiratory tract, oral mucosa, and esophagus late in gestation. In single exposure inhalation experiments (duration of 5 hours) using pregnant rats, Withey and Karpinski (1985) reported a linear correlation between concentrations of chloroform in air and in maternal and fetal blood. The investigators observed lower concentrations in fetal blood than in maternal blood, with a fetal to maternal blood concentration ratio of 0.32 over the exposure range tested (from 111 ppm to 1984 ppm), which might be attributable to the generally low fat content of fetal tissues.

As part of a reproductive and developmental study, Christian *et al.* (2001b) analyzed BDCM in parental tissues and fluids and F_1 generation tissues in both SD rats and New Zealand White rabbits. BDCM was administered in drinking water at concentrations of 0, 50, 150, 450, or

1,350 ppm. In rats, BDCM was only detectable in the milk from one female in the 1,350-ppm group, at a level of 0.38 µg/g. BDCM was not detected in placentas, amniotic fluid, or fetal tissue collected on gestational day (GD) 21 or in plasma from weanling pups on postpartum day 29. The lack of detectable BDCM in the majority of samples contrasts with the findings of other studies in rats (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). In rabbits, BDCM was found at concentrations of 0.15 and 0.17 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.

Pellizzari *et al.* (1982) detected DBCM in one of 42 samples of breast milk from women in urban areas (level not reported), indicating that DBCM can be distributed to breast milk.

Metabolism

The next section provides an overview of the metabolism of THMs, with an introduction to the major metabolic pathways and some of the key enzymes involved. Subsequent sections describe in greater detail the metabolism of each individual THM compound.

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 (US EPA, 1997; 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 the oxidative and reductive pathways, similar to chloroform (US 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 4.1.

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 (CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (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 ($\bullet CHX_2$).

These highly reactive radicals may also form covalent adducts with a variety of cellular macromolecules. Evidence supporting this metabolic scheme is presented below.

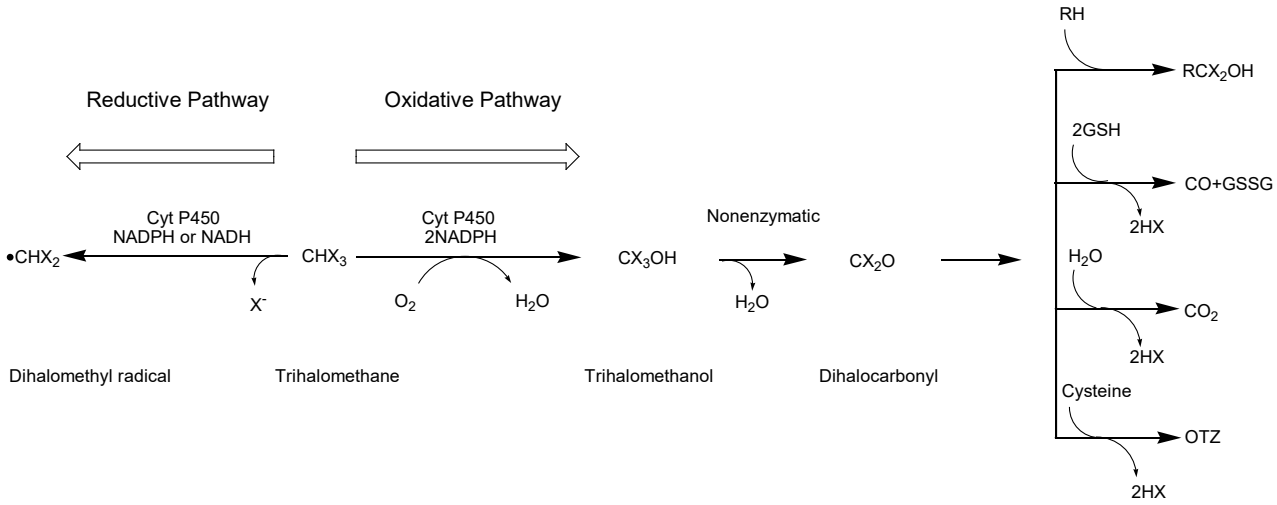


Figure 4.1. Proposed Metabolic Pathways for the Brominated Trihalomethanes
R: cellular nucleophile (protein, nucleic acid)
GSH: reduced glutathione
GSSG: oxidized glutathione
OTZ: oxothiazolidine carboxylic acid
Cyt P450: cytochrome P450
Adapted from Stevens and Anders (1981) and US EPA (2005a)

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 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 believed 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, whereas 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. 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. 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 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^-).

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) exposed the standard mutagenicity tester strain TA1535 and a strain transfected with the rat *Gstt1-1* gene to BDCM. They found evidence that mutagenicity of BDCM is enhanced by a GSTT-mediated conjugation with glutathione; they also noted that the comparatively low affinity of the GSTT-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 GSTT in bioactivation of BDCM and other brominated THMs in experiments with transfected *S. typhimurium*. They found mutagenicity of bromoform and DBCM was markedly enhanced in the strain expressing *Gstt1-1*; the observed mutagenic potency (number of revertants per ppm) was 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, including the target organs for carcinogenicity.

Proposed routes for GSTT-mediated metabolic activation of the brominated THMs are illustrated Figure 4.2.

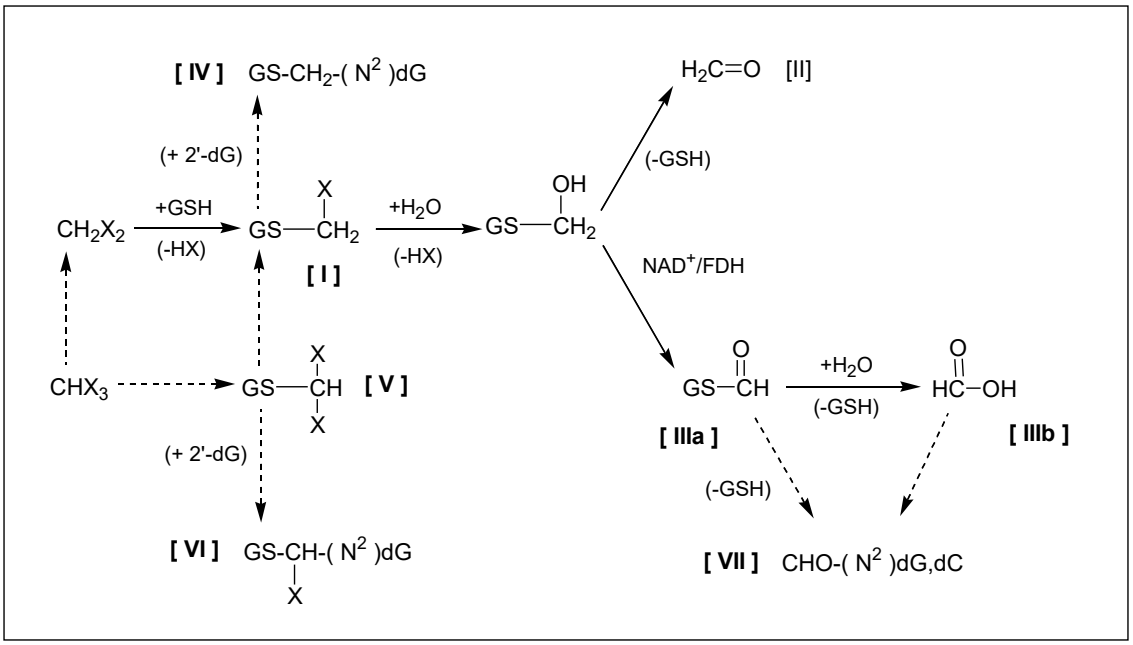


Figure 4.2. Proposed Routes for Glutathione *S*-Transferase Theta-Mediated Metabolic Activation of the Brominated Trihalomethanes
Solid arrows represent known pathways (modified from Gargas *et al.*, 1986a); dashed arrows represent proposed pathways lacking direct experimental evidence
GSH: glutathione
(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
Taken from DeMarini *et al.* (1997)

Chloroform Metabolism

As discussed previously, chloroform is rapidly and extensively metabolized by an oxidative pathway, which produces phosgene, and a reductive pathway, which produces 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 nasal turbinates (Ahmed *et al.*, 1980; US EPA, 1997; ILSI, 1997).

Löfberg and Tjälve (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.

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, the extremely short half-life of trichloromethanol has made it difficult to isolate (Ilett *et al.*, 1973; Mansuy *et al.*, 1977; 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; Docks and Krishna, 1976). Oxidative reaction is believed to involve multiple cytochrome P450 enzymes. The high-affinity process was catalyzed by CYP2E1, as indicated by kinetic studies, correlation with chlorzoxazone 6-hydroxylation, and inhibition by monoclonal antihuman CYP2E1 antibody and 4-methylpyrazole. The low-affinity phase was essentially catalyzed by CYP2A6, as indicated by correlation with coumarin 7-hydroxylase, inhibition by coumarin and anti-CYP2A6 antibody.

In vitro and *in vivo* chloroform bioactivation in the liver and kidney was reported to be similar in F344/N and Osborne-Mendel rats in both metabolism and toxicokinetics (Gemma *et al.*, 2004). Chloroform metabolism was highly 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.* (1980) 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.

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 2-oxothiazolidine-4-carboxylic acid (Branchflower *et al.*, 1984). Kluwe and Hook (1981) showed that chloroform caused depletion of renal glutathione in mice *in vivo*, 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 and 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, and both hydrogen and chloride ions. It has also been proposed that carbon monoxide is formed by the reductive dechlorination of chloroform via a carbene ($C1_2C$:) intermediate (Wolf *et al.*, 1977).

Constan *et al.* (1996, 1999) demonstrated that inhibition of CYP2E1, which would essentially eliminate metabolism of chloroform to phosgene, prevents the toxicity of chloroform in mice, providing evidence that oxidative metabolism of chloroform is critical to its toxicity. 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 about 75 percent of covalent binding following treatment with chloroform is to phospholipids; this is consistent with phosgene activity, and suggests that the major pathway for chloroform metabolism is the oxidative pathway (ILSI, 1997; US EPA, 1998c). Furthermore, addition of glutathione to the test system completely negated binding to liver microsomes, as would be expected for the oxidative metabolic pathway. Reductive metabolism has only been documented *in vivo* following induction of cytochrome P450 enzymes with phenobarbital, with negligible activity seen in non-induced 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.

There is some evidence that the dichloromethyl radical, $\bullet CHC1_2$, is formed by reductive dehalogenation of chloroform (Tomasi *et al.*, 1985). 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.

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; 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-treated rats. However, substantially fewer adducts were formed anaerobically than in aerobic incubations. These observations indicate that both oxygen-dependent and oxygen-independent metabolism may contribute to chloroform toxicity.

Lipscomb *et al.* (2004) demonstrated that differences in CYP2E1 content of microsomal protein among individuals and between rats and humans are largely responsible for observed differences in metabolism of chloroform and other low-molecular-weight halogenated compounds *in vitro*. Apparent maximum rate of metabolism (V_{max}) 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 micro molar (µM) were demonstrated *in vitro* for rats and human organ donors, respectively. The specific activity of CYP2E1 toward chloroform in rats and humans was 5.29 and 5.24 pmol/min/pmol CYP2E1, respectively.

Species differences exist in the extent of chloroform metabolism to CO_2 (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 mice metabolized chloroform to carbon dioxide to the greatest extent (about 85 percent) and rats 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).

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, whereas the cytochrome P450 inhibitor SKF525-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, 1981) found that formation of carbon monoxide from bromoform *in vitro* is markedly stimulated by 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, and 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.

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 as measured by electron spin resonance (ESR) spectroscopy was greater 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 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 GSTT-mediated conjugation pathways (DeMarini *et al.*, 1997; Pegram *et al.*, 1997). Proposed routes for GSTT-mediated metabolic activation of bromoform are illustrated in Figure 4.2.

Bromodichloromethane Metabolism

BDCM is largely metabolized to carbon dioxide as noted previously, and is also metabolized to carbon monoxide *in vitro* (Ahmed *et al.*, 1977) and *in vivo* (Anders *et al.*, 1978). 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 believed to share common oxidative and reductive pathways for metabolism.

Tomasi *et al.* (1985), who studied anaerobic activation of BDCM using hepatocytes from phenobarbital-induced male Wistar rats, showed that, as with bromoform, production of a free radical intermediate was greater for BDCM than for chloroform, as well as anaerobically compared to under aerobic conditions; less production was observed when incubated with cytochrome P450 inhibitors. These data suggest that free radical formation depends on cytochrome P450-mediated reductive metabolism.

The isoform-specific substrate results suggest that BDCM is a suicide inhibitor of CYP2B1/2 but not of CYP2E1. Lilly *et al.* (1997a) obtained evidence in rats using a CYP2E1 inhibitor (*trans*-dichloroethylene) that CYP2E1 is the major isoform involved in metabolism of BDCM at inhalation exposures of 100 to 3,200 ppm. 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 but not that of other cytochrome P450 isoforms in human liver microsomes. CYP1A2 and CYP3A4 also metabolized BDCM in human liver microsomes, as well as in a recombinant cytochrome 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 both human liver microsomes and a recombinant system (Allis and Zhao, 2002), estimating that 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 this enzyme is induced (demonstrated in rats administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) (Allis *et al.*, 2002).

As discussed for bromoform, studies in *S. typhimurium* strains engineered to express the rat *Gstt1-1* provide evidence for a third mechanism of BDCM bioactivation and mutagenicity via one or more *Gstt*-mediated conjugation pathways (DeMarini *et al.*, 1997; Pegram *et al.*, 1997). Proposed routes for Gstt1-1-mediated metabolic activation of BDCM are Figure 4.2.

Dibromochloromethane Metabolism

Metabolism of DBCM is believed to share common oxidative and reductive pathways with the other brominated THMs. As noted above, the initial, rate-limiting reaction of oxidative metabolism is insertion of oxygen at the C–H bond of THMs to produce a trihalomethanol (CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (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. DBCM is largely metabolized to carbon dioxide as noted previously, and also 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, respectively, at 180 minutes. DBCM concentrations in blood and adipose 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. Oxidative metabolism of DBCM was influenced by glutathione concentration in the liver. The rate of formation of carboxyhemoglobin and bromide was decreased after glutathione depletion due to pretreatment with buthionine sulfoximine, and was increased after pretreatment with butylated hydroxyanisole. 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 to phenobarbital-pretreated rats indicated that CYP2B1 and CYP2B2 also play a role in DBCM metabolism. Oxidation of DBCM to carbonyl halogenides, which are electrophilic and very unstable intermediates that readily react with nucleophiles in tissues, may be involved in its toxic action.

DeMarini *et al.* (1997) demonstrated the role of GSTT1-1 in bioactivation of DBCM and other brominated THMs as discussed earlier.

Cytochrome P450 Isoforms Involved in THM Metabolism

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 the concentration of chloroform 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 cytochrome P450 (CYP) isoforms and subsequently administered a single dose (0.1 ml/kg, 0.2 ml/kg, or 0.5 ml/kg) of chloroform by gavage in corn oil. The inducers used were phenobarbital (for CYP2B1/2), n-hexane (for CYP2E1), and 2-hexanone (for CYP2B1/2 and CYP2E1). Liver damage, as determined by serum enzyme activity and histopathology, was greatest at the mid-dose 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).

Inter-individual Variability in Metabolism

Metabolism of DBPs is mediated by enzymes from both the cytochrome P450 (CYP) and glutathionine *S*-transferase (GST) families. Moreover, toxicity of the THMs has been shown to be at least partly related to bioactivation by the CYP2E1 isoform (US EPA, 1994a; Van Vleet and Schnellmann, 2003). Thus, sensitivity to the toxic effects of THMs among humans is influenced by inter-individual variation in these enzymes, particularly CYP2E1 and the GST family. This section defines subpopulations of concern for exposure to THMs by identifying some of the known factors associated with altered sensitivity to THM toxicity.

CYP2E1 Expression within the Human Population

Research on CYP2E1 variation in humans has focused primarily on the liver, which is the major CYP2E1-expressing tissue, although other tissues also express the enzyme (Neafsey *et al.*, 2009). CYP2E1 protein content and catalytic activity among individuals have been examined with the use of microsomal preparations from donor liver tissue. To determine CYP2E1 activity *in vitro*, oxidation rates are measured for test compounds specific toward CYP2E1 activity. The muscle relaxant, chlorzoxazone, has been widely used for this purpose based on immunoinhibition studies and strong correlations observed between enzyme levels and catalytic activity of CYP2E1 and chlorzoxazone hydroxylation.

Overall, variability of CYP2E1 expression and enzymatic activity in human liver is generally reported to be within the range of 4- to 34-fold (Neafsey *et al.*, 2009; Lipscomb *et al.*, 2003; Carriere *et al.*, 1996). Further, Guengerich and Turvy (1991) observed variation of two orders of magnitude in enzyme levels of CYP2E1 in microsomal samples from 100 donors, including 36 normal, healthy subjects without liver disease (Figure 4.3).

Benzene metabolism by CYP enzymes is largely via CYP2E1. A study of benzene metabolism by human liver microsomal preparations from 12 donors (Nedelcheva *et al.*, 1999) found that at a non-saturating concentration of 0.2 mM benzene, rates of formation of products covalently bound to microsomal proteins varied 23-fold among individual samples.

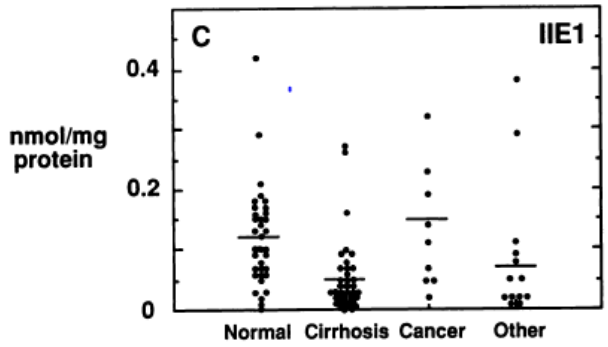


Figure 4.3. Levels of CYP2E1 enzyme in liver microsomal samples from normal, cirrhotic, metastatic cancer and other diseased subjects
Each circle represents one donor and mean values are indicated with horizontal lines
Source: Guengerich and Turvy, 1991

In vivo evidence of variable CYP2E1 metabolism comes from a study investigating the disposition of chlorzoxazone following oral administration in young healthy Caucasian men (n = 20) and Japanese men (n = 20) (Kim *et al.*, 1996). Results indicated lower CYP2E1-mediated metabolism by Japanese subjects, as evidenced by significantly elevated plasma concentrations of chlorzoxazone and reduced elimination rates in the Japanese men compared to Caucasian men. Additional studies with microsomes prepared from liver tissue from donors provides additional evidence of variation in CYP2E1 activity among humans. Table 4..1 summarizes results from individual studies on CYP2E1 variation.

One consideration to be made when relying on chlorzoxazone to probe for CYP2E1 activity is that other CYPs, namely CYP1A2 and CYP3A4, are implicated in the metabolism of chlorzoxazone (Lipscomb *et al.*, 1997; Shimada *et al.*, 1999). This is worth mentioning because hepatic CYP1A2 content may be as high as, if not higher than, that of CYP2E1 (Guengerich and Turvy, 1991; Ono *et al.*, 1995; Shimada *et al.*, 1994). Data of Ono *et al.* (1995) suggest that CYP1A2 and CYP2E1 contributions to chlorzoxazone metabolism are about the same at the physiological concentrations (30-60 µM) typically used in probe studies. Using recombinant CYP expression systems to study the relative activity of CYP1A2 and CYP2E1 toward chlorzoxazone metabolism, Shimada *et al.* (1999) found that CYP2E1 had a 3-fold lower V_{max}/K_m ratio relative to CYP1A2 due to a much lower K_m (higher substrate affinity) for CYP1A2.

Human liver microsomes with high CYP2E1 content owed the majority of their chlorzoxazone metabolism to CYP2E1, whereas a mixture of the two CYPs was implicated when CYP2E1 expression was lower, and CYP1A2 involvement was favored over 2E1 at lower substrate concentrations (Shimada *et al.*, 1999). This study also showed CYP3A4 involvement in chlorzoxazone oxidation, in addition to CYP2E1 and CYP1A2 (Shimada *et al.*, 1999). The individual contributions of these CYPs to the metabolism of chlorzoxazone were shown to be largely dependent upon their relative abundance in liver microsomes.

Table 4.1. Observed Variations in Human Hepatic CYP2E1 activities and protein content

Reference	Observed variation[a]	Methodology	Other observations
Wrighton *et al.*, 1986	7-fold variation in oxidation	Measured oxidation of NDMA[b] by HLM[c] from 20 donors	○ CYP2E1 protein correlated well (r = 0.87) with oxidation ○ One sample had no detectable CYP2E1; three samples had induced CYP2E1 from donor ethanol or isoniazid exposure ○ CYP2E1 protein appeared unrelated to age, sex or smoking ○ Immunoinhibition experiments implicate CYP2E1 as primary enzyme responsible for NDMA metabolism (70-80%)
Yoo *et al.*, 1988	16-fold variation in oxidation (range, 0.18-2.99 nmol/min/mg protein) 48-fold variation in CYP2E1 protein (range, 2.1-100, arbitrary units[d])	Measured CYP2E1 protein and oxidation of NDMA by HLM from 16 donors	○ CYP2E1 protein was highly correlated with oxidation rates (r = 0.971, $p < 0.001$) ○ One sample had unusually high CYP2E1 protein and activity relative to other samples, reasons unknown
Peter *et al.*, 1990	5.4-fold variation in V_{max} values (range, 1.1-5.9 nmol/nmol CYP/min)	Measured oxidation of CLZ[e] by HLM from 14 kidney donors, additional investigation based on 28 HLM samples	○ Report primary involvement of CYP2E1 in CLZ metabolism based on immunoinhibition and immunochemical and catalytic characterization [CLZ oxidation correlated well with NDMA oxidation (r = 0.94, $p < 0.00001$) and CYP2E1 protein (r = 0.86)] of 28 HLM samples
Guengerich and Turvy, 1991	Variation of two orders of magnitude in CYP2E1 protein	Measured CYP2E1 protein in HLM from 100 subjects, including 36 normal, healthy donors	○ Observation was the same regardless of donor health status (i.e., normal or liver disease) ○ Enzyme activity was not investigated

Reference	Observed variation[a]	Methodology	Other observations
Lucas *et al.*, 1993	22-fold variation in butanol oxidation 5.8-fold variation in CLZ hydroxylation 5.7-fold variation in CYP2E1 protein (range, 6-34, arbitrary units[f])	Measured CYP2E1 protein and activity in HLM from 42 patients undergoing liver biopsy	○ CYP2E1 protein correlated well with CYP2E1 activity (r = 0.75) ○ Two samples had no detectable CYP2E1 ○ Variance appeared unrelated to liver disease, cancer status, alcohol consumption or smoking ○ Patients remained alcohol- and tobacco-free for one month prior to surgery, and were unexposed to xenobiotics known to influence CYP2E1
Carriere *et al.*, 1996	34-fold variation in CYP2E1 protein (range, 1.21-41.2 µg/mg MSP)	Measured CYP2E1 protein and activity in HLM from 93 European Caucasian kidney donors Investigated association between CYP2E1 genotype and phenotype	○ CLZ hydroxylation correlated with expression of CYP2E1 (r = 0.59, p < 0.001) but not that of CYP3A or CYP1A2; contribution of other CYPs cannot be ruled out ○ No available information on pre-death diet or drug exposure ○ No clear association between polymorphisms and CYP2E1 protein or CLZ oxidation
Kim *et al.*, 1996	Japanese HLM had lower CYP2E1 protein (61%) and reduced CLZ hydroxylation activity (22%) than Caucasian HLM	Studied CLZ disposition following oral administration in 20 young healthy Caucasian men and 20 Japanese men Measured CYP2E1 protein and activity in HLM from donors representative of the two racial groups	○ Elevated plasma concentrations and reduced elimination rates in Japanese compared to Caucasians ○ No relationship was found between polymorphisms and CYP2E1 activity ○ Donors: aged 18-35 years, not tobacco or medicine users, alcohol-free for ≥ 3 days before study ○ Japanese (residents of Tokyo) had smaller body size than whites (residents of Tennessee)
Lipscomb *et al.*, 1997	10-fold variation in oxidation	Measured oxidation of TCE[g] by HLM from 23 donors	○ TCE metabolism largely depended (> 60%) on CYP2E1 activity, even under saturating conditions, with minimal contribution from CYP1A and 3A (based on incubations with microsomes selectively expressing individual CYP forms) ○ TCE metabolism correlated well with CYP2E1 protein (r^2 = 0.61, p < 0.05) and activity of CYP2E1 toward both dimethylnitrosamine (r^2 = 0.84, p < 0.05) and CLZ (r^2 = 0.51, p < 0.05) ○ TCE metabolism also correlated well with expression of CYP3A4 (r^2 = 0.56, p < 0.05) but not CYP1A2 ○ Donor alcohol and smoking history were provided, as well as K_m and V_{max} values for each sample

Reference	Observed variation[a]	Methodology	Other observations
Shimada *et al.*, 1999	CYP2E1 protein range: 1-31% of total CYPs	Measured oxidation of CLZ by HLM from 24 donors (8 Japanese, 16 Caucasian)	○ Lower CYP2E1 in Japanese (mean, 5 ± 2) than in Caucasians (mean, 13 ± 12) ○ CYP1A2 and 3A4 also oxidized CLZ, individual CYP contributions to oxidation depend on relative expression
Nedelcheva *et al.*, 1999	23-fold variation in rates of formation of metabolites covalently bound to MSP at non-saturating concentration of 0.2 mM benzene (vs. 6.7-fold variation at saturating concentration of 2.8 mM benzene)	Measured oxidation of benzene by HLM from 12 donors Performed genetic analysis of subset of 6 samples	○ Covalent binding of reactive metabolites to MSP correlated well with CYP2E1 protein and metabolic rates ○ Benzene oxidation rates were highly correlated with CYP2E1 protein and oxidation rates of other CYP2E1 substrates but not with expression or activity of CYP1A2, 2C9 or 3A4 ○ Benzene oxidation was almost completely inhibited by CYP2E1 inhibitor, supporting primary role for CYP2E1 in benzene metabolism ○ Genetic analysis suggests possible relationship between lowest K_m values for benzene oxidation and C/D and/or c_1/c_2 genotypes ○ Some donors were administered antibiotics or hormones 24-48 hours prior to death
Lipscomb *et al.*, 2003	4.3-fold variation in CYP2E1 protein (range, 23-100 pmol/mg MSP)	Measured CYP2E1 protein in HLM from 20 donors	○ Enzyme activity not measured
Yang *et al.*, 2011	Lower CLZ hydroxylation in Chinese HLM than Caucasian HLM (range, 0.5-34.1 µL/min/mg MSP for Chinese; 4.1-96.3 µL/min/mg MSP for Caucasians)	Measured oxidation of CLZ by HLM from Chinese (n = 30) and Caucasian (n = 30) donors	○ Median value of Chinese HLM was 35% that of Caucasians ○ Inter-ethnic difference was associated with different K_m ○ Donor history was unknown for some HLM

[a] Since study authors generally provide limited information on storage and handling conditions, a potential source of reported variation for CYP2E1 *in vitro* could be inter-laboratory variation;
[b] N-nitrosodimethylamine
[c] Human liver microsomes;
[d] Units are arbitrary and relative to the sample with the highest CYP2E1 protein; expression, which is set at 100;
[e] Chlorzoxazone;
[f] Relative concentrations of CYP2E1 were determined using arbitrary units by a Gemini image analyzer (Joyce Loebl Ltd., England);
[g] Trichloroethylene

Age-Dependent Variability in CYP2E1

Evidence suggests age-related changes in CYP2E1 activity. In a review of the ontogeny of xenobiotic metabolizing enzymes, Hines (2013) classifies CYP2E1 as group 3, negligible activity in fetus and increasing activity postnatally.

Studies of human fetal liver suggest that CYP2E1 protein is either not expressed or is expressed at levels lower than in adults. A study of CYP2E1 protein content in various fetal stages showed that the enzyme was immunodetectable in approximately 37 percent of samples taken during the second trimester and in almost all samples taken during the third trimester (Johnsrud *et al.*, 2003). Protein content increased to levels comparable to that of adults by postnatal day 90. 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 at 10 weeks gestational age, but expression was confirmed in a fetal liver sample at 19 weeks gestational age. The catalytic capability of CYP2E1 in human fetal microsomes varied from 12 to 27 percent of that in adult microsomes.

Vieira *et al.* (1996) detected small amounts of *CYP2E1* mRNA in fetal liver samples (approximately 5 to 10 percent of the levels in adult liver) collected from fetuses aged 14 to 40 weeks, but did not detect immunoreactive CYP2E1 protein.

Probe drug studies conducted in various age groups indicate that CYP2E1 activity undergoes a rapid increase after birth to a level maintained until the age of 20 years, followed by a gradual decrease until age 64 years, and a rapid decrease thereafter (Tanaka, 1998). 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.

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 transcription in the liver of rats is activated at birth and that the amount of transcript 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).

Other Genes Potentially Involved in THM Metabolism

The closely related CYP isoforms CYP2B1 and CYP2B2 are also believed to participate in the metabolism of chloroform in rats, though the contribution of these enzymes may be modest at low environmental exposures in comparison to other enzymes (ILSI, 1997; US EPA, 1997, 1998c). As well, 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).

Cantor *et al.* (2010) evaluated polymorphisms in *GSTT1*, a gene involved in the metabolism of brominated THMs. Their study indicated significantly stronger associations between THM exposure and bladder cancer among subjects with functioning *GSTT1* (+/+ or +/– genotypes) than among subjects with deletions in both alleles (–/–).

Kogevinas *et al.* (2010), in a study of genotoxic effects of exposure to DBPs in swimming pool water, examined genetic variants, including 13 single-nucleotide polymorphisms (SNPs) and three copy-number variants (CNVs), in three genes involved in DBP metabolism (*GSTT1*, *CYP2E1*, and *GSTZ1*), four additional genes that may play a minor role in the metabolism of DBPs (*GSTT2B*, *GSTM1*, *CYP1A2*, and *CYP2D6*), and four DNA repair genes (*APEX1*, *ERCC2*, *OGG1*, *XRCC1*). SNPs with the functional variants judged most likely to influence gene expression or function were selected. Results from 45 adult volunteers who swam for 40 minutes were evaluated for relationships between the polymorphisms and changes in micronuclei (MN) and DNA damage (comet assay) in peripheral blood lymphocytes before and 1 hour after swimming; urine mutagenicity (Ames assay) before and 2 hours after swimming; and MN in exfoliated urothelial cells before and 2 weeks after swimming. Statistically significant interactions between the polymorphisms and genotoxicity markers were noted for exposure to bromoform, for polymorphisms in *GSTT1*, *GSTZ1*, and *CYP2E1*. Two *GSTZI* variants showed effects on the numbers of MN in lymphocytes, and two *CYP2E1* variants affected the MN in exfoliated urothelial cells in urine. No statistically significant interactions were found between bromoform and any of the gene variants associated with DNA damage and repair as assessed with the comet assay for the other THMs.

A preliminary Canadian study (Infante-Rivard *et al.*, 2002) of 170 children aged zero to 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. The risk was elevated among children homozygous for *GSTT1* deletion null genotype. The interaction odds ratio (IOR) for a postnatal average of TTHM above the 95th percentile with *GSTT1* null genotype was 9.1 (95 percent CI 1.4-57.8). Risks for subjects carrying the *CYP2E1**5 variant (G-1259C) were elevated for exposure during pregnancy and the postnatal period to TTHM ≥ the 75th percentile; the interaction OR was 9.7 (95 percent CI 1.1-86.0). Note the wide confidence intervals for these OR. These results contrast with 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.

Infante-Rivard (2004) investigated the influence of polymorphisms in the *CYP2E1* gene G1259C and the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene C677T in mothers and newborns on effects of exposure to THMs on fetal growth. Exposure to THMs from drinking water did not result in an overall increased risk of intrauterine growth restriction, but significant

effect modification was observed between newborns with and without the *CYP2E1* variant. Among newborns with the variant, the adjusted OR for intrauterine growth restriction associated with exposure to average TTHMs above the 90th percentile (29.4 µg/L) was 13.2 (95 percent CI 1.2-146.7). The large confidence limits reflect the wide uncertainty and small number of subjects involved.

CYP2E1 Regulation, Induction and Inhibition

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), solvents (acetone, benzene, carbon tetrachloride, trichloroethylene), and ethanol (Koop *et al.*, 1985; Raucy, 1995).

The CYP2E1 enzyme metabolizes ethanol in humans and animals. A 4-fold increase in CYP2E1 has been observed in alcoholics (Lieber, 1997), while moderate drinking increased CYP2E1 by 25 percent (Snawder and Lipscomb, 2000). Chronic alcohol consumption has even been reported to result in up to 10-fold induction of this enzyme (Lieber, 1997). This observation suggests that concurrent exposure to ethanol and 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) has been shown to be 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.

Some 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 (Hong *et al.*, 1987; Miller and Yang, 1984; O'Shea *et al.*, 1994; Teschke *et al.*, 1981; Yun *et al.*, 1992; Salazar *et al.*, 1988; Raucy *et al.*, 1991; O'Shea *et al.*, 1994; Dong *et al.*, 1988; Song *et al.*, 1987, 1990; Bolt *et al.*, 2003). One study found that body weight accounted for as much as 43% of variation in oral clearance of chlorzoxazone among 50 Japanese subjects (Le Marchand *et al.*, 1999). 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.

CYP2E1 is also subject to inhibition. Naturally occurring compounds, such as those found in garlic, red peppers, cruciferous vegetables, and black and green tea, have been demonstrated to inhibit CYP2E1 *in vivo* or *in vitro* (Brady *et al.*, 1991; Gannet *et al.*, 1990; Ishizaki *et al.*, 1990; Shi *et al.*, 1994; Leclercq *et al.*, 1998). Other CYP2E1 inhibitors include pyrazole, 4-methyl pyrazole, diethyldithiocarbamate, and the therapeutic drug, disulfiram (Wu *et al.*, 1990; Guengerich *et al.*, 1991; Brady *et al.*, 1991).

Excretion

The four THMs are eliminated from the body via expired breath, urine and feces, with a substantial amount eliminated through the lungs without undergoing metabolic transformation. Carbon dioxide is the predominant end product of the portion metabolized prior to elimination. Thus, quantification of metabolite elimination has largely been restricted to measurements of ^{14}C-carbon dioxide in expired breath after administration of radiolabeled THMs.

Data on chloroform excretion are provided by studies in humans, mice and rats exposed to chloroform via ingestion or inhalation. In humans given a single oral dose of 500 mg of ^{13}C-chloroform in olive oil, chloroform and carbon dioxide were excreted in expired breath as early as 20 to 30 minutes following ingestion, with peak concentrations measured 40 minutes and two hours after administration, and chloroform detected in the breath of some subjects up to 24 hours after dosing (Fry *et al.*, 1972). Substantial inter-individual variability was observed in the amount of non-metabolized chloroform recovered in expired air: 17.8 to 66.6 percent in males and 25.6 to 40.4 percent in females. An observed 48.5 to 50.6 percent of the dose was respired as carbon dioxide. In contrast, another study found that 38.2 percent of radiolabel was recovered as carbon dioxide and only 0.6 percent was recovered as chloroform in the expired breath or urine of eight human volunteers exposed to 7 to 25 ppb of ^{14}C-chloroform in air for two hours (Raabe, 1988). The much lower percentage of exhaled chloroform observed in the latter study may be due to the great difference in dose between studies, with possible metabolic saturation in the study of Fry *et al.* (1972), and a difference in route of exposure. 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.

In mice exposed to chloroform at up to 366 ppm for six hours via inhalation, carbon dioxide represented approximately 85 percent of recovered metabolites (Corley *et al.*, 1990). Mice 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 (Brown *et al.*, 1974). An additional 4.0 to 5.0 percent of the dose was recovered in the urine, feces, and carcass, and 5.2 to 7.1 percent was exhaled as chloroform. Similar patterns of excretion were observed for rats. For example, 66.1 to 68.0 percent of a single oral dose of ^{14}C-chloroform (0.1 or 0.3 mmoles/kg) to rats was recovered as ^{14}C-carbon dioxide in respired air over 24 hours, and 7.6 percent of the dose was recovered in urine and feces (Reynolds *et al.*, 1984; Brown *et al.*, 1974). Whereas Brown *et al.* (1974) reported that 5.2 to 6.0 percent of a single oral dose underwent pulmonary excretion as chloroform, Reynolds *et al.* (1984) found dose-dependent elimination via exhalation of chloroform, with 5 and 12 percent of the dose exhaled as chloroform at 0.1 or 0.3 mmoles/kg, respectively. Utilizing a linear, two-compartment model, Reynolds *et al.* (1984) estimated that in animals given 11.9 mg/kg of

chloroform, the half-times 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.

Data on elimination of bromoform, BDCM and DBCM come from studies in mice and rats exposed via gavage. Although expired air was the principal route of excretion for these three THMs, Mink *et al.* (1986) observed marked differences between species in the proportions of excretory products recovered eight hours post-dosing. For each THM, rats excreted a greater percentage of the dose as non-metabolized parent compound whereas mice eliminated a greater percentage as carbon dioxide. Further, the half-life of the parent compound was shorter for rats than mice and, despite low (< 5 percent) urinary excretion, mice had higher urinary excretion than rats.

Mathews *et al.* (1990) quantified not only expired carbon dioxide but also carbon monoxide excreted by rats gavaged with BDCM. F344/N rats were given either a single oral dose of 1, 10, 32, or 100 mg/kg of ^{14}C-BDCM in corn oil or repeated doses of 10 or 100 mg/kg-day over a 10-day period. Following a single-dose exposure, 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 was excreted 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 from 30 percent of the dose on day one to 60 percent of the dose on days three and ten. 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 administered to pregnant rats by gavage in either aqueous solution or corn oil at 75 mg/kg on GD 6. BDCM concentrations were measured in whole blood collected at 0.5, 1.5, 4.5, and 24 hours after dosing, and animals were sacrificed at the 24-hour time point. 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.

5. TOXICOLOGICAL PROFILE: CHLOROFORM

The toxicological effects of chloroform have been comprehensively reviewed by a number of public health organizations and regulatory agencies (DHS, 1990; US EPA, 2001a; WHO, 1994, 2008; ATSDR, 1997; IARC, 1999b; Health Canada, 2006; NTP, 2016). The present document is based in part on information obtained from these reviews, and from an updated literature review. The database for toxicological effects of chloroform in humans is not large. Hence, most of the chloroform toxicological data presented in this document come from experimental animal studies.

Acute Toxicity

Effects in Humans

Most human experience with acute exposure to chloroform alone derives from its use as an inhalation anesthetic (Whitaker and Jones, 1965). Concentrations used to induce anesthesia were in the range of one to three percent (10,000 to 30,000 ppm). Higher concentrations (40,000 ppm) could be lethal. Concentrations lower than 1,500 ppm produce central nervous system depression but not anesthesia.

Deaths occurring during chloroform anesthesia have been attributed to respiratory and cardiac failure. Chloroform sensitizes the heart muscle to epinephrine, resulting in arrhythmias and, in some cases, ventricular tachycardia and fibrillation, and directly depresses the myocardium, potentially resulting in asystole and cardiac failure (Adriani, 1970).

Chloroform anesthesia has also been known to result in delayed deaths. Symptoms include progressive weakness, prolonged vomiting, jaundice, enlarged liver, hemorrhage, delirium, coma, and death secondary to hepatic dysfunction (Wood-Smith and Stewart, 1962).

Poisoning caused by chloroform ingestion was reported in a human case report (Jayaweera *et al.*, 2016). A 30-year-old female ingested around 20-30 milliliters (ml) of 99% chloroform solution. In addition to central nervous system (CNS) and respiratory depression, delayed hepatotoxicity, severe gastrointestinal injury and dermatitis were observed. Plasma chloroform concentration was measured at 2 µg/mL, 4 hr 20 min after ingestion.

Chloroform is irritating to the skin and eyes upon direct contact. Repeated brief daily applications have 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). Direct chloroform exposure to the eye has produced burning pain, conjunctival redness, and in some cases mild damage to the cornea (Winslow and Gerstner, 1978).

Effects in Animals

Acute lethal levels have been identified for chloroform in laboratory animals. Oral LD_{50} values in rats and mice are summarized in Table 5.1

Table 5.1. Acute Oral LD_{50} Values for Chloroform

Species	Sex	Age	LD_{50} (mg/kg)	Reference
Rat	M	14 day	446	Kimura *et al.*, 1971
	M	young adult	1,337	
	M	old adult	1,188	
	M, F	Adult	908 - 2,180	Torkelson *et al.*, 1976 Chu *et al.*, 1982a,b
Mouse	M	Adult	36 - 460	Pericin and Thomann, 1979
	F	Adult	353 - 1,366	
	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; Hill, 1978; Bowman *et al.*, 1978). The age at which animals were tested can also contribute to variability in LD_{50} results, as demonstrated in studies in rats (Kimura *et al.* 1971; Chu *et al.*, 1982a,b).

As in humans, prominent effects of acute high exposures to chloroform observed in animal studies include CNS and respiratory depression, cardiac arrhythmia, and liver and kidney damage, all of which are potential causes of lethality in exposed animals (Whitaker and Jones, 1965; Smith *et al.*, 1973; Kimura *et al.*, 1971; Bowman *et al.*, 1978; Balster and Borzelleca, 1982; Taylor *et al.*, 1976; Müller *et al.*, 1997;). Evidence suggests chloroform directly depresses the heart, sensitizes the heart to endogenous catecholamines, and depresses the CNS (Müller *et al.*, 1997; Dutta *et al.*, 1968).

Liver toxicity resulting from acute intoxication with chloroform is characterized by pathological changes in the liver such as 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; Wang *et al.* 1994, 1995, 1997a; Brondeau *et al.*, 1983).

Short-Term Toxicity

Effects in Humans

No published experimental or epidemiological data were located on short-term toxicity of chloroform exposure in humans.

Effects in Animals

Liver

In mice, 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).

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).

Kidney

Degenerative or necrotic lesions in the kidney tubules are characteristic of chloroform toxicity in acute and short-term animal studies. Larson *et al.* (1994a) reported a significant increase in renal labeling index in male F344/N 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/N rats exposed to 30 or 90 ppm for four days, six hours/day, but there were no significant difference from controls. At 300 ppm, the investigators reported minimal (average score of 1) vacuolation of proximal tubule in all (5/5) tested rats and significantly increased labeling index. 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 increased from 30 ppm to 90 ppm. Extension of the exposure period to two weeks resulted in severe kidney toxicity and 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). 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).

Severe renal lesions were reported at oral doses of 35 to 200 mg/kg in rats and mice (Gemma *et al.*, 1996b; Larson *et al.*, 1993, 1994b, 1995a,b; Moore *et al.*, 1982). Larson *et al.* (1995a) reported degeneration of renal proximal tubules at 34 mg/kg and above in male F344/N 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).

The increased sensitivity of males to renal toxicity is a result of differences in renal cytochrome P450-mediated reactive intermediate formation as chloroform requires biotransformation to produce nephrotoxicity (Van Vleet and Schnellmann, 2003). Castration before exposure decreased cytochrome P450 levels and afforded resistance to male mice, while pretreatment of female mice with testosterone increased both general cytochrome P450 content and renal susceptibility to chloroform. CYP2E1 is implicated in the activation of chloroform in the liver and kidney of mice (Boobis, 2009). Chloroform apparently can be metabolized by CYP2E1 to phosgene, or undergo reductive bioactivation to initiate renal cell injury (Bailie *et al.*, 1984; Gemma *et al.*, 1996a, 2004). 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.

Kidney effects have also been found following acute dermal exposure to chloroform in rabbits at levels that did not produce toxicity to the liver (Torkelson *et al.*, 1976).

Strain Differences in Liver and Kidney Toxicity Following Short-term Oral Exposure

Strain-related differences have also been reported for oral exposure. Templin *et al.* (1996c) found that male F344/N rats administered 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 showed neither signs of liver or kidney lesions nor increase in liver labeling index at any dose; however, they did display 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 administered a single dose of chloroform via gavage in oil, but found that renal toxicity, as indicated by glucosuria or proteinuria, differed notably among the three strains (Effective Dose for half of the population or 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.

Influence of Vehicle on Kidney and Liver Toxicity

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 produced 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 administered chloroform via 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 (increased serum sorbitol dehydrogenase, alanine aminotransferase, or aspartate aminotransferase at all doses) and nephrotoxicity (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/N rats treated with 0, 89, 119, 179, 239, or 358 mg/kg of chloroform by gavage in aqueous 10 percent Emulphor® solution. A follow-up 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). Greater renal toxicity of chloroform was observed when administered in corn oil in male rodents. 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 or above 298 mg/kg in the Emulphor® experiment. At the highest doses, the decrease resulting from corn oil vehicle administration was significantly greater than that of the aqueous vehicle.

Several studies have compared the effects of chloroform administered by gavage in oil versus in drinking water. Larson *et al.* (1995a) treated male F344/N 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, where mice were dosed with chloroform either by gavage in corn oil (0, 3, 10, 34, 90, 238, 477 mg/kg-day) or in the drinking water (0, 60. 200, 400, 900, or 1800 ppm) for either 4 days or 3 weeks. Administration in corn oil for four days 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. After three weeks, macroscopic liver effects were noted at 34 mg/kg-day and were more severe at all doses than after four days of exposure. Labeling index in the liver was increased at doses of 238 and 477 at four days and at or above 90 mg/kg-day after three weeks. Administration in drinking water for four days (doses from 16 to 105 mg/kg-day) or for three weeks (up to 329 mg/kg-day) produced no evidence of necrosis or proliferation in the liver, but some evidence of increased

proliferation in the kidney. Pale cytoplasmic eosinophilic staining of centrilobular hepatocytes was observed at doses ≥ 53 mg/kg-day at four days of exposure. However, the authors report that after 3 weeks of drinking water exposure the livers were not much different from the controls.

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, in which severity of hepatic lesions was lower in B6C3F_1 female mice treated at a reported chloroform 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) does not appear to reflect any decrease in the animals' drinking water intake, which is at odds with the findings of other investigators (e.g., Larson *et al.* (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 weaker toxicity through drinking water.

Nasal Tissue Toxicity

Short-term 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/N 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; Méry *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/N rats treated with 34 to 400 mg/kg-day chloroform by gavage in corn oil for four days (Larson *et al.*, 1995b).

Subchronic Toxicity

Effects in Humans

Phoon *et al.* (1983) reported two outbreaks of toxic jaundice associated with subchronic chloroform exposure in workers. In the first outbreak, 13 of 102 workers at a Singapore electronics factory 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 400 ppm maximum range of the detector. 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.

Acute liver injuries were reported in two women (Kang *et al.*, 2014), aged 34 and 41 years, working in a medical endoscopic device manufacturer as cleanroom workers. The women were working for 40-45 days before suffering severe liver damage. The average chloroform concentrations in the air of the cleanroom were 82.74 and 64.24 ppm, respectively.

Effects in Animals

The primary target organs for chloroform with subchronic exposure are the liver, kidney, and nasal epithelium.

Subchronic Inhalation Studies

Several studies have investigated chloroform toxicity in animals exposed by inhalation. The lowest-dose effects are summarized by study in Table 5.2 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.

Table 5.2. Summary of Low-Dose Subchronic Effects of Chloroform via Inhalation

Species Strain	Sex	Duration	Doses	Effects	Reference
Rats, rabbits, guinea pigs, dogs	M, F	7 hrs/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/N	M, F	6 hrs/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
Rat Wistar	M	6 hrs/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
Mouse $B6C3F_1$	M, F	6 hrs/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

Species Strain	Sex	Duration	Doses	Effects	Reference
Mouse BDF_1	M, F	6 hrs/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

In the early 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 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 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.

Templin *et al.* (1996a) studied a broader range of exposure concentrations in rats and mice to identify threshold levels for hepatic, renal, and nasal lesions, and also evaluated several time points to assess progression of the lesions. In the rat study, male and female F344/N rats were exposed to 0, 2, 10, 30, 90, or 300 ppm of chloroform six hours/day, seven days/week for three, six, or thirteen 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 at ≥ 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 at ≥ 30 ppm after three, six and 13 weeks of exposure. In females, renal lesions appeared to be increased in all groups (2 ppm and above) after three and 13 weeks (no data for 6 weeks presented). Kidney labeling index was significantly increased at ≥ 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 at ≥ 10 ppm after three weeks and at ≥ 2 ppm after six or thirteen weeks. Labeling index in the turbinates (measured only in males) was significantly increased at ≥ 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 at ≥ 30 ppm in male and female mice at three to thirteen weeks. Hepatocyte labeling index was significantly increased in females at ≥ 30 ppm, but only at 90 ppm in males. Renal lesions were increased in male mice at ≥ 30 ppm at three to thirteen 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 thirteen weeks. 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 thirteen 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.

Subchronic Oral Exposure Studies

Table 5.3 summarizes subchronic effects of chloroform in animals exposed via oral ingestion.

Table 5.3. Summary of Subchronic Effects of Chloroform via Oral Exposure

Species Strain	Sex	Duration	Doses	Effects	Reference
Dog Beagle	NS[a]	In capsules, 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	M, F	By gavage in toothpaste, 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
	M	In drinking water, 28 day, 7 days/week	"up to 41 mg/kg-day"	no treatment related effects	Chu *et al.*, 1982a

Species Strain	Sex	Duration	Doses	Effects	Reference
Rat Sprague-Dawley	M, F	In drinking water, 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
Rat F344/N	M	Drinking water, 5 days/week for 3 weeks	0, 6, 17, 62, 106 mg/kg-day	mild hepatocyte vacuolation at 106 mg/kg, NOAEL 62 mg/kg	Larson *et al.*, 1995a
	M	Corn oil gavage, 5 days/week for 3 weeks	0, 3, 10, 34, 90, 180 mg/kg-day	dose-related liver and kidney toxicity, NOAEL 34 mg/kg	Larson *et al.*, 1995a
	F	Corn oil gavage, 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
Rat	NS	Gavage, for 3 weeks[b]	400 mg/kg-day	nasal lesions	Dorman *et al.*, 1997
Mouse $B6C3F_1$	F	By corn oil gavage, 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
	M	Corn oil gavage, 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
	F	Corn oil gavage, 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
	F	Oil gavage, 5 days/week for 3 weeks	0, 55, 110, 238, 477 mg/kg-day	dose-related hepatic toxicity ≥ 55 mg/kg	Melnick *et al.*, 1998
	F	Drinking water, 5 days/week for 3 weeks	0, 16, 43, 82, 184, 329 mg/kg-day	liver weight increased at 82 mg/kg, LOAEL 43mg/kg; no renal effects	Larson *et al.*, 1994c
	F	In drinking water, 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

Species Strain	Sex	Duration	Doses	Effects	Reference
Mouse $B6C3F_1$	F	Drinking water, unknown days/week for 33 days	"up to 475 mg/kg"	"no histopathological lesions in liver"	Pereira and Grothaus, 1997
Mouse Swiss	M, F	By gavage in toothpaste, 6 days/week for 6 weeks	60 - 425 mg/kg-day	decreased weight gain at 60 mg/kg	Palmer *et al.*, 1979

[a] not stated; [b] number of days per week not stated.

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 at ≥ 60 mg/kg-day. Marked body weight losses occurred in dogs at ≥ 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. At 410 mg/kg-day, increased organ weight with fatty change and necrosis in the liver, atrophy of the gonads (both sexes), and increased cellular proliferation in bone marrow were observed. Roe *et al.* (1979) treated outbred Swiss mice with chloroform by gavage in toothpaste for six weeks (six days/week). Moderate decreases were found 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, at doses of 0, 55, 110, 238, and 477 mg/kg-day. They found dose-related hepatotoxicity at doses ≥ 55 mg/kg-day, as indicated by increases in incidence and

severity of hydropic degeneration of hepatocytes, 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/N 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 at ≥ 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/N 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 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 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 at ≥ 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 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 adult female F344 rats following corn oil gavage exposure to 400 mg/kg-day chloroform for 5 days/week for three weeks (Dorman *et al.*, 1997). This study found decreased body weight throughout dosing and nasal lesions in the form of degeneration and regenerative hyperplasia of the olfactory mucosal epithelium in the ethmoid turbinates. In spite of the fairly moderate chloroform-induced olfactory mucosal degeneration, areas of morphologically normal olfactory mucosal epithelia were found in the nose and no functional olfactory system deficit was detected by the avoidance behavior paradigm used.

Müller *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.

DeAngelo *et al.* (2002) demonstrated that while brominated THMs administered in the drinking water significantly induced the incidence of preneoplastic aberrant crypt foci (ACF), a type of early preneoplastic lesion, in the colon of male F344/N rats, no statistically significant increase was observed with chloroform treatment. 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. Consistent with these findings, no statistically significant increase was observed with chloroform treatment when exposure was extended to 26 weeks by Geter *et al.* (2004c) (although in this study there were 4 total ACF in chloroform treated animals, 0.67 ± 0.33 ACF per colon, versus zero in water vehicle controls).

In another study of THM-related induction of aberrant crypt foci, McDorman *et al.* (2003) exposed male Eker rats for ten months to DBPs administered in drinking water, including chloroform (0.4 or 1.8 g/L) and BDCM (0.07 or 0.7 g/L). Animals administered chloroform and BDCM had elevated aberrant crypt foci in the colon relative to untreated rats, with statistically significant increases in the total number of aberrant crypt foci in high-dose chloroform-exposed animals and the number of crypts in aberrant crypt foci in low-dose chloroform-exposed animals. These results differ somewhat from other studies in that the increase in aberrant crypt foci formation induced by chloroform was statistically significant and the observed BDCM-induced formation of aberrant crypt foci in the colon was not significant. It should be noted that Eker rats used in this study have a genetic predisposition to tumors, although not in the colon, which could possibly have influenced the difference in results. The urinary bladders were also examined for transitional cell hyperplasia, which was not induced in chloroform- or BDCM-treated animals.

Genetic Toxicity

Extensive genotoxicity tests have been conducted on chloroform, with mixed results reported. There are some important considerations regarding the database on genetic toxicity of chloroform. 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 in which the metabolites are formed inside the test organism. Highly reactive products of exogenous activation outside the test organism might not reach the genetic material inside to generate genotoxicity. Because chloroform genotoxicity studies have met these conditions to varying degrees, the possibility exists for false negative results. The positive results that have been found have typically been mild, occurring at high or cytotoxic concentrations. The apparent genotoxic effect in such cases may be secondary to the cytotoxic damage (Brusick, 1986). Further, there is 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 carbonate or diethyl carbonate, which are potent alkylating agents (US EPA, 2001a). Nevertheless, available evidence indicates that chloroform is capable of inducing genetic toxicity under various experimental conditions as discussed in the following sections.

In Vitro Assays

DNA Damage and Covalent Binding

Mirsalis *et al.* (1989) observed that chloroform was inactive in the *in vivo/in vitro* hepatocyte DNA repair assay, but increased S-phase synthesis in mouse hepatocytes, the latter reflecting the observed hepatocarcinogenicity of chloroform in the mouse.

Larson *et al.* (1994d) evaluated chloroform-induced DNA repair *in vitro* in female mouse hepatocytes. 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 a series of experiments, Beddowes *et al.* (2003) found that 2-hour chloroform treatment of primary female rat hepatocytes resulted in statistically significant increases in DNA strand breaks (at 8 and 20 mM) and M_1dG adducts (at 4, 8 and 20 mM) but not 8-oxodG (at 4-20 mM). These results suggest that chloroform induced DNA damage directly, as well as indirectly as a consequence of lipid peroxidation, but not via ROS-mediated genotoxicity, respectively.

Geter *et al.* (2004a) 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 4 hours. Ribeiro *et al.* (2006, 2007) similarly reported negative results of *in vitro* DNA strand breaks in Chinese hamster ovary (CHO K-1) cells and L5178Y mouse lymphoma cells exposed to a non-cytotoxic concentration of chloroform (1.25 µL/mL) for 3 hours. In contrast, Zhang *et al.* (2012) reported that incubation of human HepG2 hepatoma cells for 4 hours with chloroform induced DNA strand breakage at 10,000 µM (10 mM), but had no effect at concentrations from 1 to 1000 µM.

Although formation of adducts to nuclear protein or lipid is not a direct-acting genotoxic effect, it is worthy of evaluation within the context of genotoxicity for several reasons. Nuclear protein and lipid are in close proximity to DNA and are more abundant than DNA, and DNA adducts are likely to be more short-lived because of elimination via DNA repair processes. Thus, it is plausible that nuclear protein adducts may serve as a potential marker for DNA adducts and hence genotoxicity. Moreover, adducts to histones could potentially disrupt DNA replication and transcription, and thereby alter gene expression. 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. However, the authors found no evidence of appreciable binding to DNA in a separate experiment in which radiolabeled chloroform was incubated with microsomes or mouse liver DNA for 30 minutes (Diaz Gomez and Castro, 1980a).

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). Rosenthal (1987) 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 difficult.

Fabrizi *et al.* (2003) reported that phosgene ($COCl_2$), the major active metabolite of chloroform, is able to form irreversible adducts with the N-terminus of human histone H2B *in vitro*. Histone H2B (0.25 mM) was reacted with or without phosgene (0.03-36 mM) for 30 minutes and analyzed by different methods of mass spectrometry. The experimental data showed that adduction occurred primarily at lysine residues, with all seven lysine residues being subject to adduction, and that N-terminal serine and proline residues were also susceptible to adduction by phosgene. Further, up to three CO moieties could form adducts with histone H2B, and the presence of glutathione partially attenuated adduction, allowing production of a minor adducted species that contains glutathione.

Gemma *et al.* (2004) investigated covalent binding of chloroform metabolites to phospholipids following *in vitro* bioactivation in the liver and kidney of F344 and Osborne-Mendel rats. Phospholipid polar head adducts (formed by electrophilic intermediates) were predominant in hepatic microsomes, with very low or not significant levels of fatty acyl chain adducts measured. In contrast, only fatty acyl chain adducts (formed by radical intermediates) were detected in renal microsomes from both rat strains *in vitro*.

Gene Mutation

Gene Mutation in Bacteria

Assays for mutagenicity of chloroform in *S. typhimurium* and *Escherichia coli* bacteria have been almost universally negative, with or without metabolic activation or sealed conditions (Greim *et al.*, 1977; Simmon *et al.*, 1977; Uehleke *et al.*, 1977; San Agustin and Lim-Sylianco, 1978; Nestmann *et al.*, 1980; Rapson *et al.*, 1980; Gocke *et al.*, 1981; Kirkland *et al.*, 1981; Van Abbé *et al.*, 1982; Varma *et al.*, 1988; Roldán-Arjona *et al.*, 1991; Roldán-Arjona and Pueyo, 1993; Le Curieux *et al.*, 1995; Pegram *et al.*, 1997; Kargalioglu *et al.*, 2002). Uehleke *et al.* (1977) 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 Abbé *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 in these tests, 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 non-transfected 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 $\pm$ 75 revertants/plate versus 23 $\pm$ 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 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.

Khallef *et al.* (2018) studied the mutagenic potential of chloroform and bromoform with the Ames test using Salmonella typhimurium strains TA98 and TA100. Both chloroform and bromoform increased the number of revertant colonies in a dose-dependent manner with all concentrations tested. These effects were observed both in the absence and presence of S9 metabolic activation. In the same paper, these same investigators also demonstrated DNA damage in onion tissues exposed to chloroform and bromoform.

Gene Mutation in Yeast

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; 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 chromosomal 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.

Chromosomal Damage

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

DNA Damage and Covalent Binding

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; Geter *et al.*, 2004a). Studies of DNA binding have generally shown negative results (Diaz Gomez and Castro, 1980a,b; Reitz *et al.*, 1982; Pereira *et al.*, 1982). Diaz Gomez and Castro (1980a) studied binding of ^{14}C-chloroform to nucleic acid and nuclear proteins in mice 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. No appreciable binding was observed. However, histones and other nuclear proteins were radiolabeled, suggesting that chloroform or a metabolite is able to cross the nuclear membrane and make contact to form adducts. 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.

Vittozzi *et al.* (2000) conducted a single-dose study in B6C3F1 mice, Osborne-Mendel and Sprague-Dawley rats to evaluate covalent binding of chloroform reactive metabolites produced *in vivo* in the kidney and liver, two target organs of chloroform carcinogenicity. Male animals

were treated intraperitoneally with radiolabeled chloroform in corn oil at doses ranging from 6 to 740 mg/kg, and sacrificed 5 hours later. Hepatic and renal microsomal preparations were analyzed using an assay that differentiates between adducts of phospholipid polar heads (formed by electrophilic intermediates) and phospholipid fatty acyl chains (formed by radical intermediates). Polar head adducts were found in all samples tested but did not correlate with species or organ susceptibility to chloroform carcinogenicity, in contrast with levels of fatty acyl chain adducts which, although lower than those of polar head adducts, were found to be statistically significant in the B6C3F1 mouse liver.

Similarly, phospholipid adducts due to oxidative and reductive metabolites were detected in renal microsomes from F344 and Osborne-Mendel rats treated with a single intraperitoneal injection of radiolabeled chloroform (0, 6, 15, 60 or 180 mg/kg) in corn oil (Gemma *et al.*, 2004). However, only phospholipid polar head adducts were detected in the liver.

DNA damage followed by DNA repair in the form of unscheduled DNA synthesis 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). Mirsalis *et al.* (1982) dosed F344/N rats with 40 or 400 mg/kg chloroform by gavage; hepatocytes were isolated from perfused livers and incubated with ^{3}H-thymidine 2 or 12 hours after treatment. They found no evidence that chloroform induced unscheduled DNA synthesis. Similarly, Larson *et al.* (1994d) reported no induction of unscheduled DNA synthesis at either dose or time point in hepatocytes isolated 2 and 12 hours post-treatment from female B6C3F$_1$ mice administered 238 or 477 mg/kg chloroform in corn oil, which is consistent with the *in vitro* assay of that study.

Mixed results have also been reported in studies using the alkaline comet assay to assess genetic damage in the form of DNA strand breaks. Wada *et al.* (2015) reported negative results in both stomach and liver tissue from male Sprague-Dawley rats administered chloroform at doses of 125, 250 or 500 mg/kg-day by gavage three times over a 48-hour period prior to sacrifice. In contrast, Teixidó *et al.* (2015), using zebrafish embryos, observed statistically significant induction of DNA damage following 72-hour exposure to chloroform (0.85 mM, median effective concentration or EC_{50} value for teratogenic effects) relative to control. This positive response was weak (< 5% mean DNA damage) compared to DNA damage produced by the positive control (methyl methansesulfonate (MMS)), which was dose-dependent and ranged between 4 and 37%. According to the authors, no data are available regarding the capacity of zebrafish embryos to metabolize chloroform. Testing was conducted in hermetically-sealed glass vials to minimize chemical loss by volatilization.

Gene Mutation

Gene Mutation in Invertebrates

Assays for sex-linked recessive lethal mutation in *Drosophila* were negative (Gocke *et al.*, 1981; Vogel *et al.*, 1981).

Gene Mutation in Mammalian Cells

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). 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.

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. Topham (1980) tested chloroform, among other compounds, for the ability to induce morphological abnormalities in the sperm of mice. No effect of chloroform administered intraperitoneally was reported at 371 mg/kg (Topham, 1980 as cited in US EPA, 2001a).

Mice exposed to chloroform by inhalation at 0.04 or 0.08 percent in air for four hours/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.

Chromosomal Damage

Chromosomal Aberrations

Fujie *et al.* (1990) analyzed chromosomal aberrations consisting of breaks and gaps in bone marrow from Long-Evans rats following oral (males only) or intraperitoneal (males and females) exposure to chloroform at 1.2 to 119.4 mg/kg. Oral doses were administered by gavage in saline for five consecutive days. Relative to untreated controls, significant increases in chromosomal aberrations were observed 12 hours following intraperitoneal exposure to chloroform at ≥ 1.2 mg/kg and 18 hours following the fifth day of oral exposure at 119.4 mg/kg.

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 intraperitoneal 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 intraperitoneal injections of chloroform (238, 476, or 952 mg/kg) at 0 and 24 hours, they found that chloroform caused a slight but non-significant increase in the number of micronuclei.

Micronucleus tests showed both positive (San Agustin and Lim-Sylianco, 1978; Robbiano *et al.*, 1998; Shelby and Witt, 1995) and negative (Gocke *et al.*, 1981; Salamone *et al.*, 1981; Tsuchimoto and Matter, 1981; Le Curieux *et al.*, 1995) results. Doses in the positive studies were high and associated with liver and kidney toxicity.

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.

Developmental and Reproductive Toxicity

Classification of Developmental Toxicity Potential

Chloroform has been on the California Proposition 65 list on the basis of reproductive toxicity (developmental endpoint) since 2009, based initially on a determination by the American Conference of Governmental Industrial Hygienists. California's Developmental and Reproductive Toxicant Identification Committee (DARTIC) reconsidered this identification in 2016, and determined that chloroform should remain on the Proposition 65 list (OEHHA, 2016).

Effects in Humans

The most relevant human data on chloroform exposure are from epidemiological studies on reproductive outcomes associated with intake of disinfected water.

Chloroform also has been observed to affect development in studies in animals and humans. Relevant studies were considered by the DARTIC, as discussed in the OEHHA document on Evidence of Developmental and Reproductive Toxicity of Chloroform (OEHHA, 2016). The committee determined that chloroform is a developmental toxicant, and that chloroform should remain on the Proposition 65 list. The DARTIC specifically identified effects of chloroform exposure on birth weight, including decreases in birth weight in humans and animals and an increased risk of low birthweight in humans (infants born weighing less than 2,500 grams).

As noted in OEHHA (2016) concerning low birth weight:

> "There are a number of well-conducted epidemiologic studies with extensive exposure assessment that examined the risk for low birth weight in association with chloroform exposure. Statistically significant increased risks were observed in studies by Toledano *et al.* (2005), Lewis *et al.* (2006)[3], Iszatt *et al.* (2014), Danileviciute *et al.* (2012), and Grazuleviciene *et al.* (2011)."

The DARTIC found that the weight of evidence from epidemiologic and animal studies was strong for birthweight-related effects of exposure to chloroform. Findings in four well-designed and well-conducted epidemiologic studies are particularly noteworthy as they include dose response assessment, showing dose-response associations as a function of water concentration, change in water concentration, or estimated internal dose (Grazuleviciene *et al.*, 2011; Iszatt *et al.*, 2013; Lewis *et al.,* 2006; Toledano *et al.*, 2005).

Many epidemiological studies have investigated potential links between exposure to THMs (as disinfection byproducts in drinking water) and adverse reproductive or developmental outcomes in humans, as discussed in OEHHA (2016). Studies of human effects of exposures to chloroform alone 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 an increase, albeit just short of statistical significance, in risk of spontaneous abortion of pregnancies in women who had worked with chloroform with an OR adjusted for mother's age and previous miscarriages of 2.3 (95 percent confidence interval 0.9 to 5.9), based on 13 cases in 86 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 showed any relationship to spontaneous abortion in this cohort. The OR 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.

Effects in Animals

Major developmental and reproductive toxicity studies in laboratory animals exposed to chloroform by inhalation and oral routes are summarized in Table 5.4.

[3] Lewis *et al.,* 2006 measured total trihalomethanes (TTHM), of which chloroform constituted ~90%.

Table 5.4. Summary of Developmental and Reproductive Toxicity Studies on Chloroform

Route	Species Strain	Sex	Duration	Doses	Effects	Reference
Inhalation	Rat Sprague-Dawley	F	7 hrs/day on GD[a] 6–15	0, 30, 100, or 300 ppm	300 ppm: decreased food consumption, body weight gain, decreased pregnancies, live fetuses/ litter, and pup size. 30 and 100 ppm: increased anomalies	Schwetz *et al.*, 1974
			1 hr/day on GD 7–14	0, 942, 2,232, or 4,117 ppm	4,117 ppm: increased resorptions and decreased fetal body weight	Newell and Dilley, 1978
			Two 10-min period/d on GD 17-21	Not provided, saturated hermetic exposure chamber	decreased pup body weight and cranial diameter at 0, 24, 48 and 72 h postnatally (PN); decreased Purkinje cell number at 24, 48 and 72 h PN; no abortions or resorptions	García-Estrada *et al.*, 1990
	Rat Wistar	F	7 hrs/day on GD 7–16	0, 30, 100, or 300 ppm	dose-related decrease in food consumption and body weight, decreased live fetuses and fetal length at all doses, and in fetal body weight at 300 ppm	Baeder and Hoffman, 1988
			7 hrs/day on GD 7–16	0, 3, 10, or 30 ppm	dose-related decrease in food consumption and body weight, decreased ossification at 3 and 10 ppm, decreased fetal size at 30 ppm	Baeder and Hoffman, 1991
	Mouse CF-1	F	7 hrs/day on GD 1-7, 6-15, or 8-15	0, 100 ppm	pregnancies decreased in the early exposures, decreased food consumption and body weight gain in all exposures, decreased fetal body weight and size	Murray *et al.*, 1979
	Mouse strain not specified	M	4 hrs/day for 5 days	0, 400, or 800 ppm	increased abnormal sperm, 28 day after exposure	Land *et al.*, 1981
Oral, gavage in corn oil	Rat Sprague-Dawley	F	GD 6-15	0, 20, 50, 126 mg/kg-day, in 2 divided doses	two higher doses: decreased food consumption and body weight gain; fatty liver; 126 mg/kg: decreased fetal body weight	Thompson *et al.*, 1974
				0, 100, 200, or 400 mg/kg-day	3/15 dams died at 400 mg/kg; hemoglobin decrease, increased relative liver weight at all doses; fetal decreased body weight, increased variations at 400 mg/kg	Ruddick *et al.*, 1983

Route	Species Strain	Sex	Duration	Doses	Effects	Reference
Oral, gavage in corn oil	Rabbit Dutch-belted	F	GD 6-18	0, 20, 35, or 50 mg/kg-day	4/15 does died of hepatic toxicity, decreased fetal body weight all doses	Thompson *et al.*, 1974
	Mouse CD-1	M, F	7 days /week multi-generation, from before mating through F_2 birth	0, 6.6, 16, or 41 mg/kg-day	no effects on reproductive performance; at 41 mg/kg-d, increased epididymis weight and epithelial degeneration in F_1 males, increased liver weight and liver lesions in F_1 females	NTP, 1988
Oral, in toothpaste in capsule	Dog Beagle	M, F	6 days/week for over 7 years	0, 15, or 30 mg/kg-day	ectopic testes with inhibition of spermatogenesis in 2 males at 30 mg/kg-day, 1 male at 15 mg/kg-day and 1 untreated control;	Heywood *et al.*, 1979
Oral, gavage in vegetable oil/ Emulphor/ saline	Mouse ICR	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 decreased postnatal day 7 - 22, decreased pup forelimb placement postnatal day 5 and 7 but not 9	Burkhalter and Balster, 1979
Oral, drinking water	Rat Osborne-Mendel	M	7 days/week for 90 days	0, 20, 38, 57, 81, 160 mg/kg-day	decreased body weight on all days at 160 mg/kg-day (also in water-matched controls) as well as during first week of treatment at 81 mg/kg-day); one case each of testicular hyperplasia and interstitial cell hyperplasia (unclear whether in the same animal) at 160 mg/kg-day on day 30	US EPA, 1980c
	Mouse ICR	M, F	7 days/ week multi-gen, from 5 week before F_0 mating through sacrifice of F_{2b} pups	est. 0, 19, 190, 950 mg/kg-day	deaths and decreased body weight gain in F_0 at 950 mg/kg; decreased body weight in F_{1b} females at 190 mg/kg; decreased reproductive performance in F_1 and F_2	Borzelleca and Carchman, 1982
Whole embryo exposure	Zebrafish	M, F	4 to 76 hrs post-fertilization	< 2.1 mM[b]	malformation of eyes, heart and tail; delayed growth, movement and hatching	Teixidó *et al.*, 2015

[a] gestation day

[b] for teratogenic effects: effective concentration 20% (EC_{20}) value of 0.7 mM and median effective concentration (EC_{50}) value of 0.85 mM; median lethal concentration (LC_{50}) value of 2.1 mM

Chloroform inhalation studies in rats and mice found evidence for developmental effects. 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 GDs 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.

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 GDs 1 to 7, 6 to 15, or 8 to 15, and sacrificed on day 18 (Murray *et al.* 1979). 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 GDs 1 to 7 group (44 percent) and GDs 6 to 15 group (43 percent), compared to controls (74 and 91 percent, respectively), but not in the group exposed only later in gestation. Resorptions were also significantly increased in the GDs 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 GDs 1 to 7 and GDs 8 to 15 groups. The incidence of cleft palate was significantly increased in the GDs 8 to 15 group. Cleft palate was typically found in fetuses with low body weight.

Mice exposed to 400 or 800 ppm of chloroform vapor 4 hours/day for 5 days had 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 for 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. Reductions in food consumption and body weight gain, and mild fatty changes in the liver were reported in dams treated with 50 or 126 mg/kg-day. 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 pregnant 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 GDs 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 7 hours/day on GDs 7 to 16, and sacrificed them on GD 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.

Female Wistar rats (N = 12 for each of three groups) were 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 of pups (*in utero* and lactational exposure from birth to postnatal day 21). Offspring 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.

A screening study on the effect of chloroform on behavior of developing mice treated groups of five ICR mice of each sex 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 (Burkhalter and Balster, 1979). Offspring were treated with the same doses 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.

Multigenerational 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 multigenerational 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 starting five weeks before mating in the F_0 generation and continuing through sacrifice of F_{2b} pups. These exposures equate to estimated doses of 0, 19, 190, or 950 mg/kg-day, using US EPA (1987a) reference values for water consumption of 0.0057 L/day and body weight of 0.03 kg. 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 GD 18 in fetuses from CD-1 mice given municipal tap water from Durham, North Carolina, compared to a 21.1 percent incidence in fetuses from dams given distilled water, in an eight-month study with

approximately 500 pregnant mice. F344/N rats (N = 60 for each group) fed tap water from Davis, California from GDs 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).

Heywood *et al.* (1979) examined reproductive endpoints in male and female beagle dogs administered chloroform in toothpaste via capsule at 0, 15, or 30 mg/kg-day for 6 days/week for over 7 years. Ectopic testes with inhibition of spermatogenesis was observed in two males at 30 mg/kg-day, one male at 15 mg/kg-day and one untreated control; nodular hyperplasia of mammary gland was found in three females at 15 mg/kg-day, five vehicle controls and one untreated control. No treatment-related changes were observed in ovaries or uteri. No statistical analysis was reported.

US EPA (1980c) conducted studies in male Osborne-Mendel rats and female B6C3F1 mice administered chloroform in drinking water 7 days/week for 90 days. Male rats were exposed to chloroform at 0, 20, 38, 57, 81 or 160 mg/kg-day. Decreased body weight was reported on all days for males at 160 mg/kg-day and for water-matched controls, as well as during the first week of treatment for animals at 81 mg/kg-day. One case each of testicular hyperplasia and interstitial cell hyperplasia was reported at 160 mg/kg-day on day 30 (unclear whether in the same animal). Female B6C3F1 mice were treated with 0, 20, 40, 60, 90, 180 or 270 mg/kg-day; no pathological changes were noted for any group in mammary tissue, ovaries or uteri.

García-Estrada *et al.* (1990) exposed female Sprague-Dawley rats via inhalation in a hermetically-sealed exposure chamber saturated with chloroform from soaked cotton; the concentration of chloroform in the chamber was not reported. Animals were exposed for two 10-minute periods/day on gestation days 17-21. Decreased pup body weight and cranial diameter were observed at 0, 24, 48 and 72 hours postnatally, as well as decreased Purkinje cell numbers at 24, 48 and 72 hours postnatally.

Teixidó *et al.* (2015) investigated the teratogenic potential of THMs in zebrafish. Exposure of zebrafish embryos to chloroform at 4 to 76 hours post-fertilization resulted in adverse developmental effects, including most notably malformation of the eyes, heart and tail, and delayed growth, movement and hatching (Teixidó *et al.*, 2015). Chloroform ranked fourth in terms of potency among the four THMs in this study, with 20% and 50% effective concentration (EC_{20} and EC_{50}) values for teratogenic effects of 0.7 mM and 0.85 mM, respectively. Testing was conducted in hermetically-sealed glass vials to minimize chemical loss by volatilization.

Immunotoxicity

Effects in Humans

No published experimental or epidemiological data were located on immunotoxicity through exposure to chloroform by humans.

Effects in Animals

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

Effects in Humans

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.

Effects in Animals

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

Effects in Humans

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 neurological symptoms 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 likely exposed to 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 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 indicating 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 study, 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 study, 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).

Effects in Animals

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 non-cancer target organ effects observed in these studies as summarized in Table 5.5.

In order to minimize repetition, methodological details and gross non-specific effects (e.g., body weight, clinical signs, and survival) are presented in more detail along with the cancer results in the following carcinogenicity section.

The chronic inhalation toxicity data are from inhalation cancer studies by Nagano *et al.* (1998) and Yamamoto *et al.* (2002) and a study of multi-route exposures via inhalation and drinking water by Nagano *et al.* (2006).

Table 5.5. Summary of Non-Cancer Effects in Chronic Toxicological Studies on Chloroform

Route	Species Strain	Sex	Duration	Doses	Effects	Reference
Inhalation	Rat F344/N	M, F	6 hrs/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; US EPA, 2001a; report on this study
	Mouse BDF1			0, 5, 30, 90 ppm	metaplasia of olfactory epithelium, respiratory goblet cell hyperplasia, ossification of nasal septum	
	Rat F344		6 hrs/day, 5 days/week for 104 weeks	0, 10, 30, 90 ppm	renal proximal tubule nuclear enlargement, renal tubular lumen dilation, hepatic vacuolated cell foci	Yamamoto *et al.*, 2002
	Mouse BDF1			0, 5, 30, 90 ppm	hepatic fatty change and altered cell foci, renal proximal tubule nuclear enlargement, renal cytoplasmic basophilia, atypical renal tubule hyperplasia	
Oral, gavage in corn oil	Rat Osborne-Mendel	M, F	5 days/week for 78 weeks, sacrificed at 111 weeks	M - 0, 90, 180 F - 0, 100, 200 TWAs[a]	hepatic necrosis, urinary bladder hyperplasia, splenic hematopoiesis, decreased body weight gain and survival	NCI, 1976
					above changes plus testicular atrophy and in F heart thrombosis	Reuber, 1979; re-evaluation of NCI, 1976
	Mouse $B6C3F_1$	M, F	5 days/week for 78 weeks, sacrificed at 92 or 93 weeks	M - 0, 138, 277 F - 0, 238, 477 TWAs	hepatic hyperplasia, some hepatic necrosis	NCI, 1976
					no increased non-cancer findings noted	Reuber, 1979; re-evaluation of NCI, 1976
Oral, gavage in toothpaste	Rat Sprague-Dawley	M, F	7 days/week for 52 weeks	0, 15, 75, 165	no treatment related effects	Palmer *et al.*, 1979
Oral, gavage in toothpaste	Rat Sprague-Dawley	M, F	7 days/week for 95 weeks	0, 60	decreased body weight, serum butyryl ChE and relative liver weight in F	Palmer *et al.*, 1979

Route	Species Strain	Sex	Duration	Doses	Effects	Reference
Oral, gavage in toothpaste	Mouse ICI	M, F	unknown days/week for 80 weeks	0, 17, 60	no non-cancer effects (kidney tumors)	Roe *et al.*, 1979
	Mouse CFLP	M	unknown days/week for 80 weeks	0, 60	no non-cancer effects (kidney tumors)	
	Mouse CBA and CF/1	NS[b]	unknown days/week for 80 weeks	0, 60	moderate to severe kidney lesions (but no increased kidney tumors)	
Oral, gavage in arachis oil	Mouse ICI	M	unknown days/week for 80 weeks	0, 60	moderate to severe kidney lesions (increased kidney tumors)	
Oral, in toothpaste in a capsule	Dog Beagle	M, F	6 days/week for over 7 years	0, 15, 30	increased fatty cysts in liver, plus increased markers of liver damage	Heywood *et al.*, 1979
Oral, in drinking water	Mouse B6C3F$_1$	F	7 days/week 104 weeks	0, 34, 65, 130, 263 [0, 200, 400, 900, 1800 ppm]	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	M		0, 19, 38, 81, 160 [0, 200, 400, 900, 1800 ppm]	longer survival in 2 higher-dose groups (and increased renal tumors)[c]	
					renal lesions (faint basophilia, vacuolation, nuclear crowding, or karyomegaly)	Hard *et al.*, 2000; *re-evaluation of slides* of Jorgenson *et al.*, 1982, 1985
	Rat F344/N	M	7 days/week 100 weeks	0, 45, or 90 [0, 900, 1800 ppm]	proliferative liver lesions, no increase in kidney lesions	DeAngelo, 1995; as reported by US EPA, 2001a
Mixed: Inhalation; oral, in drinking water	Rat F344	M	for 104 weeks: inhalation for 6 hrs/day, 5 days/week; *ad libitum in water*	0, 25, 50, or 100 ppm via inhalation; 0 or 1000 ppm in drinking water	renal nodules, cytoplasmic basophilia, dilatation and nuclear enlargement of proximal tubular lumen, positive urinary glucose	Nagano *et al.*, 2006

[a] time-weighted averages
[b] not stated
[c] re-evaluation of slides by Hard *et al.* (2000) reported renal lesions

In the Nagano *et al.* (1998) study, groups of 50 male and female F344/N 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, US EPA (2001a) 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 concentrations tested. Respiratory tumors were not increased (Nagano *et al.*, 1998). US EPA (2001b,d,e) and IARC (1999b) also reported that the 30 and 90 ppm exposures 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 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).

Yamamoto *et al.* (2002) exposed groups of 50 male and female F344 rats and BDF1 mice to chloroform vapor six hours/day, five days/week for 104 weeks. Chloroform concentrations were 0, 10, 30, or 90 ppm in rats and 0, 5, 30, or 90 ppm in mice. Statistically significant increases were observed in incidences of atypical tubule hyperplasia and nuclear enlargement in the kidneys of male mice exposed to 30 and 90 ppm. Incidence of cytoplasmic basophilia in the kidney was significantly elevated in male mice exposed to chloroform at 30 and 90 ppm (at severity level 2 but not 3) and in female mice exposed to 90 ppm (at severity level 1 only). In the liver of mice, chloroform exposure significantly increased incidences of altered cell foci in females and in fatty change in both sexes at 90 ppm. In the kidneys of male and female rats, dose-dependent increases were found in incidences of nuclear enlargement of proximal tubule and dilation of tubular lumen. There was also a significantly increased incidence of hepatic vacuolated cell foci in the 90 ppm-exposed female rats.

In the Nagano *et al.* (2006) study, groups of 50 male F344 rats were exposed by inhalation to 0, 25, 50, or 100 ppm of chloroform vapor for 6 hours/day, 5 days/week for 104 weeks, with each inhalation group receiving drinking water containing 0 or 1000 ppm chloroform *ad libitum* for 24 hours/day, 7 days/week during the same inhalation exposure time period. Macroscopic evaluation showed increased incidence of renal nodules in the combined-exposure groups, with incidence increasing with the inhalation concentration as follows: 2/50 cases for the low-dose combined-exposure group (25 ppm via inhalation), 4/50 for the mid-dose combined-exposure group (50 ppm via inhalation), and 13/50 for the high-dose combined-exposure group (100 ppm via inhalation). A significant increase was observed in the incidences of cytoplasmic basophilia and dilatation of the lumen in the proximal tubule in all chloroform treatment groups relative to the untreated group. Notably, these incidences were markedly increased in the combined-exposure groups than in the single-route exposure groups with matched concentration. Nuclear enlargement did not occur in the oral-alone group, but its incidence was increased in both the inhalation-alone and combined-exposure groups, with a significantly greater increase in the combined-exposure groups relative to the inhalation-alone groups with matching concentrations. There was a high incidence of positive urinary glucose (> 80%) in all three combined-exposure groups compared with a low incidence (< 15%) in the single-route exposure groups, with increased severity of positive urinary glucose in the three combined-exposure groups.

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 non-neoplastic 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 animals with heart thrombosis also had liver carcinomas.

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 follow-up 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. Non-neoplastic 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 non-neoplastic 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 non-neoplastic 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 non-neoplastic 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. Non-tumor 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 non-neoplastic observations were reported. Renal tumors were increased in the male rats.

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 non-neoplastic lesions in the mice. Tumors were not increased.

Hard *et al.* (2000) re-evaluated the pathology data from this study to investigate non-neoplastic effects in the kidney in more detail. The researchers distinguished 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 middle 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 exposure duration 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.

US EPA (2001a) summarized the results of an unpublished study by DeAngelo (1995) that found midzonal vacuolation in livers of male F344/N 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

Classification of Carcinogenic Potential

Chloroform has been listed under California's Proposition 65 since 1987 as a carcinogen. The Toxic Air Contaminant Program within the California Department of Health Services (1990), IARC (1999b), US EPA (1998e) and NTP (2016a) all found sufficient evidence of carcinogenicity for chloroform in studies on experimental animals. IARC concluded that chloroform was possibly carcinogenic to humans (Group 2B). NTP concluded it was reasonably anticipated to be a human carcinogen, and US EPA classified it as a Group B2 probable human carcinogen.

The rest of this chapter lays out data from carcinogenicity bioassays on chloroform, after noting the relatively sparse epidemiological data on exposures to chloroform alone, i.e., chloroform exposures that are not in conjunction with other disinfection byproducts.

Effects in Humans

Linde *et al.* (1981) performed an historical cohort mortality study of anesthesiologists presumed to have been occupationally exposed to chloroform vapor from 1880 to 1890 who died from 1930 to 1946. There was no clear evidence of increased cancer mortality.

US EPA (1997, 1998e) reviewed the earlier epidemiological studies, examining the relationship between exposure to THMs in chlorinated drinking water and cancer mortality. The most notable findings of these studies were weak but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon and rectum. None of these studies 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 were found to be inconclusive with regard to the potential carcinogenicity of THMs in drinking water in humans by IARC (1999b), ATSDR (1997), and US EPA (2001a).

More than a dozen subsequent studies have added to the evidence of associations of THM exposure with cancer of the bladder or lower GI tract. Several meta-analyses (Villanueva *et al.*, 2003, 2004, 2006; Costet *et al.*, 2011) have reaffirmed the increased risk of bladder cancer with THM exposure. US EPA (2005a) acknowledged that the bladder cancer studies appear to provide the strongest evidence of increased cancer risk from THMs. However, these studies cannot ascribe the increased risk to exposure to chloroform alone or any other specific chemical. Epidemiological studies on associations of DBP/THM exposures with cancer are reviewed in Appendix C and IARC (2013).

Effects in Animals

Chloroform produces kidney and liver tumors following oral exposures in rats and mice (NCI, 1976; Jorgenson *et al.*, 1985; Roe *et al.*, 1979), kidney tumors in mice following inhalation exposures (Nagano *et al.*, 1998) and kidney tumors in rats following combined inhalation and oral exposures (Nagano *et al.*, 2006). These and other relevant studies are summarized below.

Major results of chloroform administered orally to mice and rats are shown in Table 5.6 and Table 5.7, respectively. Results of the combined inhalation and oral exposure study are provided in Table 5.8.

Oral Carcinogenicity Studies for Chloroform

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 for males and 400 mg/kg-day for 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.

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, and 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 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 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 5.6), 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 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 hepatocellular tumors, compared to 18/50 low-dose males and 44/45 high-dose males. Similarly, total tumor incidence in vehicle-control, low- and high-dose females was 0/20, 36/45 and 39/41, respectively. These tumors were also significantly elevated when compared with colony controls (males: 5/77; females: 1/80). 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.

The NCI (1976) histopathological slides in mice were re-evaluated by Reuber (1979) who also found highly significant increases in liver tumors in treated male and female mice, with a few additional treated animals observed with tumors (Table 5.6); further discussed later in this section).

Jorgenson *et al.* (1985) conducted studies to examine the carcinogenicity of chloroform administered in drinking water at doses similar to those used by NCI (1976) in order to address the role of the corn oil vehicle in the induction of hepatic carcinomas in $B6C3F_1$ mice. 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. 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[4]. 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. These concentrations equated to estimated time-weighted average doses of 0, 19, 38, 81 and 160 mg/kg-day in male rats, and 34, 65, 130, and 263 mg/kg-day in female mice. All tissues were examined. In $B6C3F_1$ mice in the Jorgenson *et al.* (1985) study, 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 6 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. In notable contrast to the NCI (1976) bioassay, this study found no evidence of chloroform carcinogenicity to female $B6C3F_1$ mice (Table 5.6).

A re-evaluation of the pathology data from this study by Chiu *et al.* (1996) reported the lack of effect on liver tumor incidence. The re-evaluation 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.

[4] The chloroform used in the study was pesticide-quality chloroform. The chloroform was distilled at 61°C using a steam bath twice a week and the distillate was used to prepare drinking water solutions. The purpose of the distillation was to separate chloroform from diethylcarbonate that had been found in chloroform preserved with ethanol.

Roe *et al.* (1979) administered chloroform to three strains of male and female mice by gavage in toothpaste or arachis oil for 80 weeks at doses up to 60 mg/kg-day. An increased incidence of renal epithelial tumors was observed at the 60 mg/kg-day dose level, but only in male ICI mice (Table 5.6).

Table 5.6. Carcinogenicity Studies of Chloroform Administered Orally to Mice

Sex	Applied dose (mg/kg-day)	Tumor site and type	Incidence
NCI (1976), B6C3F$_1$ mice, by gavage			
M	0 (colony[c]), 0 (vehicle [v]), 138, 277	Liver, hepatocellular carcinoma	5/77, 1/18, 18/50(***)*, 44/45(***)***
F	0 (c), 0 (v), 238, 477		1/80, 0/20, 36/45(***)***, 39/41(***)***
Reuber (1979), B6C3F$_1$ mice (re-examination of slides of NCI (1976))			
M	0 (untreated), 0 (vehicle), 138, 277	Liver, hyperplastic nodule	1/17, 2/17, 11/46, 0/44
		Liver, hepatocellular carcinoma	1/17, 0/17, 20/46(**)***, 44/44(***)***
		Liver, hyperplastic nodule or carcinoma	3/17, 2/17, 31/46(***)***, 44/44(***)***
		Hematopoietic system, malignant lymphoma	0/17, 0/17, 14/46(**)**, 10/44(*)*
F	0 (untreated), 0 (vehicle), 238, 477	Liver, hyperplastic nodule	0/20, 0/19, 1/45, 0/40
		Liver, hepatocellular carcinoma	0/20, 0/19, 40/45(***)***, 40/40(***)***
		Liver, hyperplastic nodule or carcinoma	0/20, 0/19, 41/45(***)***, 40/40(***)***
		Hematopoietic system, malignant lymphoma	0/20, 0/19, 9/45(*)*, 4/40
Roe *et al.* (1979), ICI mice, by gavage in toothpaste			
M	0, 17, 60[a]	Kidney, adenoma	0/72, 0/37, 5/38**
		Kidney, hypernephroma	0/72, 0/37, 3/38*
		Kidney, adenoma or hypernephroma	0/72, 0/37, 8/38***
M	0 (untreated), 0 (vehicle), 60[b]	Kidney, adenoma	1/45, 6/237, 7/49(*)**
M	0 (untreated), 0 (vehicle), 60[b]	Kidney, hypernephroma	0/45, 0/237, 2/49
		Kidney, adenoma or hypernephroma	1/45, 6/237, 9/49(*)***
	0 (untreated), 0 (vehicle), 60[c]	Kidney, adenoma or hypernephroma	0/83, 1/49, 5/47**

Sex	Applied dose (mg/kg-day)	Tumor site and type	Incidence
M	0, 60[d]	Kidney, adenoma or hypernephroma	1/50, 12/48***
Jorgenson *et al.* (1985), female B6C3F1 mice, in drinking water			
F	0, 0 (matched control), 34, 65, 130, 263 [0, 200, 400, 900, 1800 ppm]	Hepatocellular adenoma plus carcinoma	21/415, 0/47, 15/410, 9/142, 0/47, 1/44

[a] six days/week for 80 weeks, killed at 96 weeks; [b] as in a, killed at 104 weeks; [c] as in a, killed at 97 or 99 weeks; [d] chloroform by gavage in oil six days/week for 80 weeks, terminal sacrifice at 97 or 99 weeks.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, Fisher exact comparison between treated and control group. p-values within parentheses denote significance to the respective colony, untreated or match controls, p-values without parentheses denote significance to the respective vehicle controls.

Treatments of Osborne-Mendel rats by NCI (1976) began when animals were seven weeks old, and continued for 78 weeks, five days/week Table 5.7). 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. Doses administered to female rats were initially 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 control animal died before week 90.

Treatment with chloroform was associated with a statistically significant increase ($p < 0.05$, Fisher exact test) in renal tubular cell adenomas and carcinomas in male but not female rats

The incidence of thyroid gland tumors (Follicular cell and C-cell adenomas and carcinomas) was statistically higher in treated female rats compared to controls. In contrast, the incidence in male rats was reversed and not significant. NCI (1976) did not find these tumors biologically significant because of contrasting trends in incidence between male and female rats, and because evaluation of total thyroid tumors was not considered valid given that the two thyroid epithelial tumor cell types have different embryonic cell origins and physiological functions. When evaluated separately, the incidence of follicular cell adenoma or carcinoma was found to be significantly elevated in high-dose female rats, but only when compared to colony controls. Similarly, the incidence of C-cell tumors was significantly increased in low- and high-dose females relative to colony controls, but not to match controls. It should also be noted that mortality in high-dose rats weakened the study's ability to observe treatment-related effects overall.

In his re-evaluation of the NCI (1976) data, Reuber (1979), consistent with NCI (1976), concluded that chloroform exposure resulted in hepatocellular carcinoma in male and female mice (Table 5.6), and kidney adenoma and carcinoma in female rats (Table 5.7).

Similar to NCI (1976), Reuber also reported thyroid tumors in chloroform-exposed female rats. However, unlike NCI, Reuber also reported hepatic hyperplastic nodule and hepatocellular carcinoma in exposed male and female mice (Table 5.6) and rats (Table 5.7), as well as malignant lymphoma in male and female mice (Table 5.6). Reuber's analysis also differed from that of NCI in that he included incidence of cholangiocarcinoma in exposed rats, which is rare in rats (Hailey *et al.,* 2014), as well as increased incidence of total liver tumors in exposed rats of both sexes compared to controls (Table 5.7). NCI separately reported thyroid C-cell and follicular cell tumors in rats; Reuber's analysis combined the two tumors. Reuber also combined hepatocellular carcinoma and hyperplastic nodules in mice and rats.

Table 5.7. Carcinogenicity Studies of Chloroform Administered Orally to Rats

Sex	Applied dose (mg/kg-day)	Tumor site and type	Incidence
NCI (1976), Osborne-Mendel rats, gavage			
M	0 (colony), 0 (vehicle), 90, 180[a]	Kidney, carcinoma or adenoma	0/99, 0/19, 4/50*, 12/50(***)*
F	0 (colony), 0 (vehicle), 100, 200[a]	Thyroid, Follicular cell adenoma or carcinoma	1/98, 1/19, 2/49, 6/46(**)
		Thyroid, C-cell adenoma or carcinoma	0/98, 0/19, 6/49(**), 4/46(**)
Reuber (1979), Osborne-Mendel rats (re-examination of slides of NCI (1976))			
M	0 (untreated), 0 (vehicle), 90, 180	Liver, hyperplastic nodule	0/20, 1/19, 5/50, 8/49
		Liver, hepatocellular carcinoma	0/20, 0/19, 0/50, 2/49
		Liver, hyperplastic nodule or hepatocellular carcinoma	0/20, 1/19, 5/50, 10/49(*)
		Liver, all tumors	0/20, 2/19, 5/50, 12/49(*)
		Kidney, adenoma or carcinoma	0/20, 0/19, 6/50, 12/49(*)*
F	0 (untreated), 0 (vehicle), 100, 200	Liver, hyperplastic nodule	1/20, 2/20, 7/39, 12/39(*)
		Liver, hepatocellular carcinoma	0/20, 0/20, 2/39, 2/39
		Liver, hyperplastic nodule or hepatocellular carcinoma	1/20, 2/20, 9/39, 14/39(**)*
		Liver, cholangiofibroma or cholangiocarcinoma	0/20, 0/20, 3/39, 11/39(**)**
		Liver, all tumors	1/20, 2/20, 10/39, 20/39(***)**
F	0 (untreated), 0 (vehicle), 100, 200	Thyroid, adenoma or carcinoma	3/20, 1/20, 11/39*, 12/39*
Tumasonis *et al.* (1985), Wistar rats, drinking water			
M	0, 200[b]	Liver, hepatic adenofibrosis[c]	0/22, 17/28***
F	0, 240 [b]	Liver, hepatic adenofibrosis[c]	0/18, 34/40***

Sex	Applied dose (mg/kg-day)	Tumor site and type	Incidence
F	0, 240 [b]	Liver, neoplastic nodule	0/18, 10/40*
Jorgenson *et al.* (1985), Osborne-Mendel rats, drinking water			
M	0 (untreated), 0 (match), 19, 38, 81, 160[d]	Hematopoietic system, lymphoma and leukemia	5/303, 1/50, 19/316(**), 5/148, 2/48, 3/50
		Kidney, tubular cell adenoma	4/301, 0/50, 2/313, 3/148, 2/48, 5/50(**)*
		Kidney, tubular cell adenoma or adenocarcinoma	4/301, 1/50, 4/313, 4/148, 3/48, 7/50(***)/*

[a] gavage five times/week for 78 weeks, sacrificed at 111 week.
[b] in drinking water for 180 weeks, sacrifice time assumed 180 week, doses calculated as time-weighted averages.
[c] proliferative lesion of the bile duct
[d] in drinking water for 104 weeks, sacrificed at 104 weeks; controls matched for water intake of highest dose; time-weighted averages from calculation of Jorgenson *et al.* (1985). *p $\leq$ 0.05, ** p $\leq$ 0.01, ***p $\leq$ 0.001, Fisher exact comparison between treated and control group. For studies having multiple control groups, p-values within parentheses denote significance to the respective colony, untreated or match controls, p-values without parentheses denote significance to the respective vehicle controls.

In Jorgenson *et al.* (1985), described above, 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 inversely 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.

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. However, 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 5.7). 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). Non-tumor renal pathology was high in all groups, including controls; the incidence of nephropathy was over 90 percent in all groups. No other non-neoplastic observations were reported.

Chiu *et al.* (1996) re-evaluated the pathology data from this study and also reported 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.

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. One experiment 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) 6 days/week for 80 weeks 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. 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. Eight high-dose male mice of 38 examined had renal tumors. Of these, five were adenomas and three were hypernephromas (carcinomas) (Table 5.6) 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 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 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 6 days/week 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. 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 5.6). The incidence of these tumors was significantly different from the controls when evaluated by individual tumor type ($p < 0.05$, adenomas; $p < 0.01$, hypernephromas), or in combination ($p < 0.001$).

In the third experiment, Roe *et al.* (1979) administered chloroform in toothpaste at 0 or 60 mg/kg-day to C57BL, CBA, CF/1, and ICI mice; for each strain, the treated and control groups

consisted of 52 males each. Additional groups of ICI mice were either untreated (100 animals), received chloroform in arachis oil (52 animals), or received arachis oil alone (52 animals). Survival was poor for both treated and control CF/1 mice and ICI mice. This is in contrast to relatively good survival of the other two strains. 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 increase in malignant renal tumors in animals given chloroform in arachis oil was significantly different from the controls ($p < 0.01$), as was the combined incidence of benign and malignant renal tumors ($p < 0.001$).

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 that of 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 5.7). 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" 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) 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.*, 1979; Heywood *et al.*, 1979). Chloroform was administered daily by gavage in a toothpaste base to 25 male and 25 female rats at 15, 75, and 165 mg/kg; the

concurrent vehicle-control group had 75 rats of each sex. However, due to "severe" respiratory and renal disease, the experiment was terminated after one year.

A second experiment involved Caesarian-derived, specific pathogen free SD rats. In this study, 50 male and 50 female rats (age unspecified) were gavaged 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, and then sacrificed. Survival of both treated and control animals was very poor.

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. Palmer *et al.* (1979) concluded that chloroform did not "significantly influence the age of onset, malignancy, or location of tumors." However, the poor survival provided low sensitivity for detecting any effects.

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 post-treatment 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. The seven year dosing period is less than the average 13 to 14-year lifespan of a beagle. Consequently, the treatment regimen is 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. There was a statistically significant increase by Cochran-Armitage Trend Test ($p = 0.0378$) in the total (combined) incidence of neoplasms in females from both treatment groups.

US EPA (2001a) reported the results of an unpublished drinking water study by DeAngelo (1995). Groups of 50 male F344/N 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. US EPA (2001a) 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.

Chernichenko *et al.* (2009) exposed male and female $F_1(CBAxC_{57}Bl_6)$ mice to chloroform in sunflower seed oil via gavage for 85 weeks. Groups of 60-70 animals were administered chloroform at 0, 45 or 180 mg/kg-day alone or in combination with other drinking water chlorination by-products. Significant increases were observed in the incidences of total tumors in animals of both sexes exposed to 180 mg/kg-day. However, the authors did not specify the tumor site or type observed for each experimental group.

Inhalation Carcinogenicity Study for Chloroform

Only one study was located regarding the carcinogenicity of chloroform via inhalation exposure alone. Nagano *et al.* (1998) exposed groups of 50 male and 50 female F344/N rats and BDF1 mice to chloroform vapor 6 hours/day, 5 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 non-neoplastic 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, US EPA (2001a) and IARC (1999b) 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 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, 30 ppm for two weeks, before reaching the intended level).

Combined Oral and Inhalation Carcinogenicity Study for Chloroform

Nagano *et al.* (2006) conducted a multi-route chloroform exposure study in rats involving combined ingestion and inhalation. Groups of 50 male F344 rats were exposed by inhalation to 0, 25, 50, or 100 ppm of chloroform vapor for 6 hours/day, 5 days/week for 104 weeks, with each inhalation group receiving drinking water containing 0 or 1000 ppm chloroform *ad libitum* for 24 hours/day, 7 days/week for 104 weeks. No difference was observed in the survival rate between the untreated and chloroform-exposed animals, although body weights were significantly reduced in the treated animals relative to the controls. Water consumption was markedly decreased in the oral-alone and combined-exposure groups compared with the untreated or inhalation-alone groups with matching concentrations.

Renal tumors were observed only in the three combined-exposure groups, except for one small, renal-cell adenoma in the inhalation-alone group exposed to 100 ppm chloroform (Table 5.8). Incidences of renal cell adenomas and carcinomas and their combined incidences in the combined-exposure groups showed a significant positive trend as calculated by Peto's test in the study. The incidence of renal-cell carcinomas and the combined incidence of renal cell adenomas and carcinomas in the combined-exposure group exposed to 100 ppm via inhalation were both significantly increased relative to those of the untreated control group, the oral-alone group, or the inhalation-alone group with matched concentrations. Although not statistically significant, the incidence of tumors in the combined-exposure groups administered 25 and 50 ppm chloroform via inhalation exceeded the upper range of the historical control data for the animals. The observed renal cell tumors in the combined-exposure groups were characterized by large size, high incidence, and malignancy; 18/20 renal cell carcinomas exhibited marked cellular pleomorphism indicative of malignancy, and 8 tumors larger than 25 mm in size metastasized to other organs, including the lung, lymph node, liver, and peritoneum. A significant dose-dependent increase was observed in the incidence of atypical renal-tubule hyperplasia, a pre-neoplastic lesion, in the combined-exposure groups in the same dose-related manner as in the incidence of renal cell tumors.

Table 5.8. Tumor Data for Male F344 Rats Administered Chloroform via Inhalation and Drinking Water for 104 Weeks (Nagano *et al.*, 2006)

Exposure group[a]	Estimated chloroform uptake[b] (mg/kg-day) - Inhalation	Estimated chloroform uptake (mg/kg-day) - Water	Estimated chloroform uptake (mg/kg-day) - Total	Incidence of renal cell tumors - Adenoma	Incidence of renal cell tumors - Carcinoma	Incidence of renal cell tumors - Adenoma or carcinoma
Inh-0 + Orl-0	0	-	0	0/50	0/50	0/50
Inh-25 + Orl-0	20	-	20	0/50	0/50	0/50
Inh-50 + Orl-0	39	-	39	0/50	0/50	0/50
Inh-100 + Orl-0	78	-	78	1/50	0/50	1/50
Inh-0 + Orl-1000	0	45	45	0/49	0/49	0/49

Exposure group[a]	Estimated chloroform uptake[b] (mg/kg-day) - Inhalation	Estimated chloroform uptake (mg/kg-day) - Water	Estimated chloroform uptake (mg/kg-day) - Total	Incidence of renal cell tumors - Adenoma	Incidence of renal cell tumors - Carcinoma	Incidence of renal cell tumors - Adenoma or carcinoma
Inh-25 + Orl-1000	20	53	73	2/50	2/50	4/50
Inh-50 + Orl-1000	39	54	93	0/50	4/50	4/50
Inh-100 + Orl-1000	78	57	135	4/50	14/50*	18/50*

[a] exposure values are in ppm; Inh, inhalation; Orl, oral
[b] provided by study authors
* $p<0.0001$, by Fisher Exact comparison between treated animals and control.

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 DEN 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 DEN 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 chloroform dose of 1.5 mmol/kg was given by gavage twice weekly for eight weeks to DEN-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 the controls, but was not significantly different compared to rats that received DEN 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). Treatment with chloroform (1,800 ppm) or sodium phenobarbital (500 ppm) in drinking water began at 5 weeks 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 ENU had significantly ($p \leq 0.01$, Student's t test) fewer liver tumors than ENU controls. Treatment with chloroform had no significant effect on lung tumor incidence in ENU-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 DEN for four weeks; animals then received water containing 600 or 1,800 mg/L of chloroform for 51 weeks. In DEN-initiated animals, chloroform significantly decreased the

average size and number of liver tumors, compared to animals given DEN 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 DEN, 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 DEN, that concurrent treatment with chloroform and DEN 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/N rats initiated with DEN and promoted with 200, 400, 900, or 1,800 ppm chloroform in the drinking water for 12 weeks. Similarly, Daniel *et al.* (1989) observed a decrease in the incidence of gastrointestinal tumors in male F344/N rats initiated with 1,2-dimethylhydrazine followed by exposure to 900 or 1,800 ppm chloroform in drinking water for 39 weeks.

6. TOXICOLOGICAL PROFILE: BROMOFORM

Literature on the toxicological effects of bromoform in humans is limited. Because the industrial production and use of bromoform are limited, toxicity data are not available from occupational studies. However, there is some information on acute effects stemming from its historical use as a sedative for children with whooping cough. No published experimental or epidemiological data were located for subchronic toxicity, chronic toxicity, immunotoxicity, or carcinogenicity through oral or inhalation exposure to bromoform in humans. Hence, the vast majority of the toxicological data on bromoform presented in this document come from experimental animal studies.

The toxicological effects of bromoform have been reviewed by US EPA (1980a, 1987) and ATSDR (2005), and the evidence on the carcinogenicity has been reviewed by IARC (1991, 1999a).

Acute Toxicity

Effects in Humans

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 (US EPA, 1994a).

Effects in Animals

Selected studies on the acute oral toxicity of bromoform are summarized in Table 6.1 below.

Table 6.1. Summary of Selected Acute, Single-Dose Oral Toxicity Studies on Bromoform

Species Strain	Route	Sex	N	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Mouse ICR Swiss	Gavage (water)	M, F	10	500	1,000: central nervous system	Bowman *et al.* (1978)
Rat Sprague-Dawley	Gavage (corn oil)	M, F	10	-	546: central nervous system, gross pathology	Chu *et al.* (1980)

Selected acute oral toxicity studies and estimated oral lethal dose (LD_{50}) of bromoform in rats and mice are summarized in Table 6.1 and 6.2. 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 doses of bromoform solubilized in a solution of Emulphor:alcohol:saline (1:1:8), ranging from 500 to 4,000 mg/kg. The post-treatment observation period was 14 days. LD_{50}s were 1,400 and 1,550 mg/kg for males and females, respectively. Ataxia, sedation, and anesthesia occurred within 60 minutes of treatment at doses of 1,000 mg/kg and above.

Chu *et al.* (1980, 1982a) exposed male and female SD rats (10/sex/dose) to bromoform by gavage in corn oil at doses ranging from 546 to 2,100 mg/kg. The post-treatment observation period was 14 days. The LD_{50}s in male and female rats were 1,388 and 1,147 mg/kg, respectively. Sedation, flaccid muscle tone, ataxia, piloerection, hypothermia, and liver and kidney congestion in treated animals were observed.

Table 6.2. Oral LD_{50} Values for Bromoform

Species	Strain	Route (vehicle)	Sex	Number per dose group	LD_{50} in mg/kg (95% Confidence Interval)	Reference
Mouse	ICR Swiss	Gavage (aqueous)	M F	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	1,388 (1,167-1,693) 1,147 (890-1,524)	Chu *et al.* (1980)
	F344/N		M F	5	933 (669-1,301) 933 (669-1,301)	NTP (1989a)
Mouse	$B6C3F_1$		M F	5	707 (404-1,239) 1,072 (768-1,495)	

NTP (1989a) reported an LD_{50} of 933 mg/kg for both male and female F344/N rats, and LD_{50} values of 707 mg/kg and 1,072 mg/kg for male and female B6C3F1 mice, respectively.

Short-Term Toxicity

Effects in Humans

No published experimental or epidemiological data were located on short-term toxicity of bromoform exposure in humans.

Effects in Animals

Selected studies on the short-term toxicity of bromoform are summarized in Table 6.3 below.

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. The authors estimated the daily doses to be 0, 0.7, 8.5, or 80 mg/kg-day. There were no treatment-related deaths. The authors reported no dose-related biochemical, hematological, or histologic changes in the treated animals, and identified a NOAEL of 80 mg/kg-day.

Table 6.3. Summary of Selected Short-Term Toxicity Studies on Bromoform

Species Strain	Route	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Rat Sprague-Dawley	Drinking water	M	10	28 days	80	--	Chu *et al.* (1982a)
Rat F344/N	Gavage (corn oil)	M, F	5	14 days	200	400: Decreased body weight gain in males	NTP (1989a)
	Gavage (water)	M	4	1, 3, or 7 days	379	--	Potter *et al.* (1996)
Rat Wistar	Diet	M	7	1 month	62	187: Hepatic vacuolization	Aida *et al.* (1992a)
		F	7	1 month	56	208: Hepatic vacuolization	
Mouse CD-1	Gavage (water)	M, F	6-12	14 days	125	250: Increased serum enzyme activity, decreased immune response in males	Munson *et al.* (1982)
	Gavage (corn oil)	M	8-16	14 days	145	289: Kidney, liver histopathology	Condie *et al.* (1983)
Mouse $B6C3F_1$		F	10	3 weeks (5 days/ week)	-	200: Liver histopathology, increased labeling index	Melnick *et al.* (1998)
		M, F	5	14 days	200	400: Stomach nodules in males	NTP (1989a)
		F	10	11 days	-	200: Increased labeling index	Coffin *et al.* (2000)
	Drinking water	F	10	11 days	-	301: Increased labeling index	

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 (see section on immunotoxicity below) were evaluated. Body weights were significantly decreased in high-dose females, while body weights in males were significantly increased at the mid dose and high dose. Absolute and relative liver weights were significantly increased in males at the mid dose and high dose and in females at the high dose. A NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day were identified based on statistically significant changes in clinical chemistry including decreased glucose levels, increased aspartate aminotransferase activity, and decreased blood urea nitrogen.

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. No treatment-related clinical signs were reported, and 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. 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 five rats/sex 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. These rats exhibited lethargy, labored breathing, ataxia, and lacrimation. 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.

Five female mice/group were dosed with 0, 100, 200, 400, 600, or 800 mg/kg-day of bromoform in corn oil by gavage for 14 days. Five male mice/group were given doses of 0, 50, 100, 200, 400, or 600 mg/kg-day. Ataxia, lethargy and death were noted at ≥ 600 mg/kg-day. 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, 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 feed with 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.

High-dose animals of both sexes exhibited slight piloerection and emaciation, and mean final body weight was significantly reduced 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. Observed dose-related changes in serum chemistry included significant decreases in serum glucose, triglycerides, cholinesterase activity, lactate dehydrogenase, and blood urea nitrogen. Gross and histopathological findings were limited to the liver. The incidence and severity of liver cell vacuolization and swelling were dose-related. 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/N rats. Four rats/dose received 0.75 or 1.5 mmol/kg (190 or 379 mg/kg-day) of bromoform in four percent Emulphor® by gavage for one, three, or seven days. 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 (0, 200 or 500 mg/kg-day) or in drinking water (about 300 mg/kg-day) for 11 days on liver toxicity, cell proliferation and DNA methylation in female B6C3F$_1$ mice. The high dose was selected because it had been demonstrated to cause cancer in female mice. 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 corn oil gavage but not drinking water when compared to the control. 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 or in drinking water caused a significant, dose-dependent increase in the proliferating cell nuclear antigen-labeling index.

Subchronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on subchronic toxicity of bromoform exposure in humans.

Effects in Animals

Selected studies on the subchronic toxicity of bromoform are summarized in Table 6.4 below. Several published studies have addressed the subchronic oral toxicity of bromoform. 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 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, but non-significantly, at the high dose.

Table 6.4. Summary of Selected Subchronic Toxicity Studies on Bromoform

Route	Species Strain	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley	M	20	90 days	57	218: hepatic vacuolization and other lesions	Chu *et al.* (1982b)
		F	20	90 days	55	283: hepatic vacuolization and other lesions	
	Rat F344/N	M	6	13 weeks	N/A[a]	73: Colon aberrant crypt foci	DeAngelo *et al.* (2002)
			6	26 weeks	N/A	73: Colon aberrant crypt foci	Geter *et al.* (2004c)
			12	26 weeks	N/A	56: Colon aberrant crypt foci	Geter *et al.* (2005)
Gavage (corn oil)		M, F	10	13 weeks (5 days/ week)	25	50: hepatic vacuolization in males	NTP (1989a)
	Mouse $B6C3F_1$	M, F	10	13 weeks (5 days/ week)	100	200: hepatic vacuolization in males	

[a] not applicable

Lymphocyte counts were significantly decreased in high-dose males and females 90 days after cessation of treatment. The only change 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 the controls. Although the authors noted that histologic changes were mild and similar to controls after the 90-day recovery, males in the high-dose group continued to exhibit an increased incidence of hepatic lesions with greater severity than the controls. 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 on hepatocellular vacuolation 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.

DeAngelo *et al.* (2002) demonstrated that brominated THMs administered in the drinking water significantly induced the incidence of preneoplastic aberrant crypt foci, a type of early putative preneoplastic lesion, in the colon of male F344/N rats. Rats were exposed to 1.1 g/L bromoform for 13 weeks (calculated as 73 mg/kg-day); the other THMs were also tested. Deionized water and 0.25 percent Alkamuls EL-620[5] dissolved in deionized water were the negative and vehicle controls, respectively. A single intraperitoneal injection of azoxymethane (30 mg/kg) served as the positive control. In bromoform-treated animals, 67 percent of the animals had aberrant crypt foci. The number of aberrant crypt foci/colon was 1.17 ± 0.40 which was statistically significant ($p < 0.01$ compared to combined controls). The positive control animals treated with azoxymethane, had 27.17 ± 6.28 ACF per colon ($p < 0.01$ relative to bromoform treated animals). THM-induced aberrant crypt foci occurred primarily (92 percent) in the rectal segment of the colon.

To evaluate whether THM-induced aberrant crypt foci could be promoted by a diet high in saturated animal fat, Geter *et al.* (2004c) exposed male F344/N rats to isomolar concentrations of the THMs with the bromoform exposure of 1.1 g/L in drinking water for 26 weeks; the dose of

[5]Alkamuls EL-620 (formerly emulphor) is a polyethoxylated vegetable oil, Adventis Corporation (Research Triangle Park, NC).

bromoform was calculated as 73 mg/kg-day. Half the animals were fed the normal 4.5% fat Purina 5001 diet and the other half receied the feed supplemented with 19% animal fat. Compared to control animals, all groups administered brominated THMs showed significant increases in aberrant crypt foci induction; no increase was observed with chloroform treatment, consistent with their earlier findings (DeAngelo *et al.*, 2002). There was a statistically significant association between the number of aberrant crypt foci per colon and the number of bromine atoms on the THM molecule. In rats administered bromoform and a high fat diet, a significant and near 2-fold increase in aberrant crypt foci was observed relative to those administered bromoform and the 4.5% fat diet (2.83 $\pm$ 1.05 for the 4.5% fat diet versus 5.33 $\pm$ 1.17 aberrant crypt foci/colon for the high fat diet). Consistent with the findings of DeAngelo *et al.* (2002), THM-induced aberrant crypt foci were found primarily in the medial and distal segment of the colon, the observed site of THM-induced neoplasia.

Geter *et al.* (2005) then demonstrated that dietary folate was protective against bromoform-induced aberrant crypt foci in the colon of rats, for a statistically significant increase was observed in the number of aberrant crypt foci in bromoform-exposed rats administered a folate-free diet compared to those given a normal diet containing folate (2.75 $\pm$ 0.45 versus 1.75 $\pm$ 0.3 aberrant crypt foci/colon, respectively). In this study, male rats were exposed to 0.5 g/L bromoform in drinking water and fed a normal diet or a folate-free diet for 26 weeks. Animals on the folate-free diet had significantly reduced serum folate levels and elevated serum homocysteine levels relative to those on the normal diet. It should be noted that, although there was not a significant difference in bromoform dose between rats fed the normal and folate-free diets (56 $\pm$ 6.4 versus 60 $\pm$ 6.4 mg/kg-day), the folate-free group was lighter and drank more water, which could have contributed somewhat to the observed increase in their induction of aberrant crypt foci.

Genetic Toxicity

Although the overall data are mixed, positive results have been reported for mutagenicity in several test strains of *S. typhimurium*; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosomal 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.

Effects Measured in Humans In Vivo

Kogevinas *et al.* (2010) evaluated 49 volunteers 18-50 years of age for associations between swimming in chlorinated pools and biomarkers of genotoxicity. Among the participants, 33 were women, 48 were Caucasian, 45 had post-secondary education, all were nonsmokers with 14 being ex-smokers and 34 being exposed regularly to second-hand smoke, and 27 played regular sports (at least once per week) with 11 being regular swimmers (at least once per month). An increase was reported in micronuclei in peripheral blood lymphocytes one hour after swimming for 40 minutes in an indoor chlorinated pool. The increase in micronuclei was associated with exhaled concentrations of bromoform (p = 0.01), BDCM (p = 0.03), and DBCM

(p = 0.05), but not chloroform. Increased urine mutagenicity (Ames assay) two hours after swimming was associated with the concentration of bromoform in exhaled breath (p = 0.004). DNA damage in peripheral blood lymphocytes (comet assay) and micronuclei in exfoliated urothelial cells 2 weeks after swimming were not associated with THM exposures.

In Vitro Assays with Human Cells

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 (*GSTT1-1*$^+$) and negative (*GSST1-1*$^-$) 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 *GSTT1-1*$^+$ or *GSTT1-1*$^-$ individuals. The study authors noted that glutathione *S*-transferase theta 1-1 is expressed in the red blood cells that were co-cultured 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 *GSST1-1*$^+$ and *GSST1-1*$^-$ 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 *GSST1-1*$^+$ and *GSST1-1*$^-$ 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.

Other *In Vitro* Assays

Genotoxicity of bromoform has been evaluated in many *in vitro* assays with bacteria and eukaryotic cells (Table 6.5). Relevant studies are briefly described by endpoint below. 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 loss of bromoform from the incubation mixture. Use of a sealed test system is noted in cases where this information was provided.

Table 6.5. Summary of *In Vitro* Mutagenicity and Genotoxicity Studies on Bromoform

Endpoint	Assay system	Results: with/without metabolic activation	References
Gene mutation – Bacteria *S. typhimurium*	TA100, TA1535	Not tested (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[c] TA100 TA97, TA98	-/- -/±[c] ±[c]/-	NTP (1989a)
	TA100	-/+	Ishidate *et al.* (1982)
	TA98 TA100, TA1538	+/+ -/-	Zeiger (1990)
	TA100 (fluctuation test)	-/+	Le Curieux *et al.* (1995)
	Arabinose resistance assay (*Ara*) BA13/BAL13	-/+[a]	Roldán-Arjona and Pueyo (1993)
	RSJ 100	NT/+	DeMarini *et al.* (1997)
Gene mutation – Mammalian cells	Mouse lymphoma cells	+/+	NTP (1989a)
Chromosomal aberrations	Chinese hamster fibroblasts	+/-	Ishidate *et al.* (1982)
	Chinese hamster ovary cells	-/±	Galloway *et al.* (1985), NTP (1989a)
DNA damage	*Escherichia coli* PQ37, SOS chromotest (indirect measure of DNA damage by induction of SOS function)	+/+	Le Curieux *et al.* (1995)
	Human lymphocytes in whole blood culture (single cell gel electrophoresis, or comet assay)	NT/+	Landi *et al.* (1999b)
	Rat hepatocytes (DNA alkaline unwinding assay)	NT/-	Geter *et al.* (2004a)

Endpoint	Assay system	Results: with/without metabolic activation	References
	Human lymphoblastic leukemia cells (DNA alkaline unwinding assay)	NT/+	Geter *et al.* (2004a)
	Human HepG2 hepatoma cells (single cell gel electrophoresis)	NT/+	Zhang *et al.* (2012)
Sister chromatid exchange	Toadfish leukocytes	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)

[a] equivocal results

DNA Damage

Mixed results have been reported in *in vitro* tests of DNA damage induction. Le Curieux *et al.* (1995) obtained positive results for the SOS chromotest conducted 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*, as assessed 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 the order of activity as bromoform the highest, followed by DBCM, then BDCM (Geter *et al.*, 2004a). However, Geter *et al.* (2004a) reported negative results of *in vitro* DNA strand breaks in primary rat hepatocytes exposed to bromoform at 5 or 10 mM for four hours. Zhang *et al.* (2012) reported that DNA strand breakage was induced in human HepG2 cells by incubation for 4 hours with bromoform at 1000 µM, but not at lower concentrations (1 to 100 µM).

Gene Mutation in Bacteria

Multiple studies have reported positive results for mutagenicity in *S. typhimurium* strain TA100 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 TA100 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. Le Curieux *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 TA98, TA100, TA1535, and TA1537. A slight increase in revertants (230 versus 130 spontaneous revertant colonies) was observed for TA100 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 TA100 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 TA97 and TA98 in the absence of metabolic activation.

NTP (1989a) evaluated the mutagenic potential of bromoform in *S. typhimurium* strains TA100, TA1535, and TA1537. Concentrations of 0.04 to 13 µmol/plate (10 to 3,333 µg/plate) produced no evidence of mutagenicity in strains TA1535 or TA1537 with or without exogenous metabolic activation by rat or hamster liver S9 fraction. Equivocal evidence of mutagenicity was noted in strain TA100 without activation, and in strains TA97 and TA98 in the presence of liver microsomes prepared from Aroclor-induced Syrian hamsters. Haworth *et al.* (1983) concluded that bromoform is mutagenic in the absence of metabolic activation, based on results obtained in test strain TA100.

Zeiger (1990) reported positive mutagenicity results in *S. typhimurium* strain TA98 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 TA100 and TA1538 with or without rat or hamster liver S9 fraction.

Roldán-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.

Khallef *et al.* (2018) studied the mutagenic potential of chloroform and bromoform with the Ames test using Salmonella typhimurium strains TA98 and TA100. Both chloroform and bromoform increased the number of revertant colonies in a dose-dependent manner with all concentrations tested. These effects were observed both in the absence and presence of S9 metabolic activation. As well, these same investigators demonstrated DNA damage in onion tissues exposed to chloroform and bromoform.

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.

Chromosomal Aberrations

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 contrasts with the mutagenicity results 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 chromosomal aberrations in Chinese hamster ovary cells in the absence of exogenous metabolic activation and negative results in the presence of S9 fraction. Benigni *et al.* (1993) obtained positive results for induction of aneuploidy in the mold *Aspergillus nidulans*. The lowest effect concentration was reported to be 3.43 mmol.

Micronuclei Induction

The newt micronucleus assay detected a clastogenic effect on the peripheral blood erythrocytes of *Pleurodeles waltl* larvae from BDCM and bromoform (Le Curieux *et al.*, 1995).

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 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.

Effects in *In Vivo* Assays in Animals

In vivo data for bromoform genotoxicity are available for chromosomal 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 6.6

Table 6.6. Summary of *In Vivo* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Result	References
Micronuclei induction	Mouse, bone marrow cells	-	Hayashi *et al.* (1988)
	Mouse, bone marrow cells	-	Stocker *et al.* (1997)
	Mouse, bone marrow cells	+	NTP (1989a)
Micronuclei induction	Newt, peripheral erythrocytes	+	Le Curieux *et al.* (1995)

Endpoint	Assay system	Result	References
Micronuclei induction	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
Chromosomal 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*	-	
Aneuploidy	*Aspergillus nidulans*	+	Benigni *et al.* (1993)
Initiation	Rat, liver	-	Herren-Freund and Pereira (1986)

DNA Damage

Potter *et al.* (1996) evaluated the effect of bromoform on the incidence of DNA strand breaks in the kidney. Male F344/N 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.*, 2004a).

Stocker *et al.* (1997) assessed *in vivo* genotoxicity of bromoform in rats by measuring unscheduled DNA synthesis in their livers after bromoform treatment. Male SD rats received single doses of 324 or 1,080 mg/kg bromoform by gavage in aqueous one percent methylcellulose. These doses were estimated as 30 and 100 percent of the maximum tolerated dose. Unscheduled DNA synthesis was evaluated in hepatocytes 2 and 14 hours after treatment; results were negative.

Bromoform, unlike chloroform and DBCM, did not induce DNA damage in zebrafish embryos exposed to 0.2 mM (EC_{50} value for teratogenic effects) at 4 to 76 hours post-fertilization (Teixidó *et al.*, 2015). Testing was conducted in hermetically-sealed glass vials to minimize chemical loss by volatilization.

Sex-Linked Recessive Lethal Mutation

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).

Chromosomal Aberrations

Chromosomal 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 chromosomal 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 chromosomal breaks for both administration routes.

Micronuclei Induction

Micronuclei induction has been measured in several *in vivo* studies. NTP (1989a) tested bromoform in B6C3F_1 mice, obtaining positive results following two 800 mg/kg doses via intraperitoneal injection. Hayashi *et al.* (1988) obtained negative results following intraperitoneal 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 micronuclei induction in bromoform-treated animals at 24 and 48 hours after dosing. Le Curieux *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.

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 doses each of 25 mg/kg. NTP (1989a) evaluated sister chromatid exchange in bone marrow cells of B6C3F_1 mice administered 200 to 800 mg/kg doses of bromoform via intraperitoneal injection. Positive results were obtained at the 800 mg/kg dose.

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.

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 RSJ100, which has been engineered to express the rat glutathione *S*-transferase theta 1-1 (*GSTT1-1*) gene, and TPT100, which has the rat *GSTT1-1* gene inserted in a non-functioning orientation. Exposure to 1,600 ppm bromoform induced a 95-fold increase in revertant colonies in the RSJ100 compared to background revertant formation. Bromoform-induced mutations at the *his*G46 allele in strain RSJ100 were analyzed using the colony probe hybridization method, a DNA colony-hybridization method for the rapid characterization of Salmonella typhimurium hisG46 revertants. DeMarini *et al.* (1997) also studied the glutathione *S*-transferase mediated induction of GC → AT transitions by dichloromethane, BDCM, and DBCM in RSJ100. All four halomethanes were mutagenic in RSJ100. Molecular analysis showed that 96 to 100 percent of the base pair substitutions induced by the halomethanes at the *hisG46* allele in RSJ100 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 TA100 (which does not express *GSST1-1*) for comparison. In contrast to the bromoform-induced mutations in RSJ100, only 15 percent of the dichloromethane-induced mutations in TA100 were GC → AT. This suggests that overexpression of *GSTT1-1* in RSJ100 mediated bromoform mutagenicity and induced a specific type of mutational lesion in *Salmonella*. The proposed pathway for bromoform bioactivation is illustrated in Figure 4.1 in Chapter 4.

Developmental and Reproductive Toxicity

Effects in Humans

Many epidemiological studies have investigated potential links between exposure to THMs (as disinfection byproducts in drinking water) and adverse reproductive or developmental outcomes in humans, summarized in OEHHA (2016). Bromoform is one of the DBPs considered in the epidemiology studies. Although many of the studies measure individual THMs and not just total THMs or other DBPs, it is difficult to attribute observed associations to any one of the DBPs given the mixed exposures.

Effects in Animals

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 over two generations using a continuous breeding protocol. The methodology used in each study was generally consistent with standard guidelines for reproductive or developmental studies (US EPA, 1998a,b). The significant developmental and reproductive toxicity effects of bromoform in experimental animals, as judged in other reviews (IARC, 1991, 1999a; US EPA, 1980a, 1987, 2005a; ATSDR, 2005) are summarized in Table 6.7.

Ruddick *et al.* (1983) gave pregnant SD rats (14-15/dose group) 0, 50, 100, or 200 mg/kg-day of bromoform in corn oil by gavage on GDs six to 15. Body weights were measured on GD one, GDs six through 15, and before and after pups were delivered by caesarean section on GD 22. Maternal blood samples were evaluated for standard hematology and clinical chemistry parameters. The dams were sacrificed on GD 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. Each dam was evaluated 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, two-thirds were examined for skeletal alterations and one third for visceral abnormalities. The study methods appear to be consistent with prenatal developmental toxicity guidelines (US EPA, 1998a), except for lack of information on food and water consumption by test animals and absence of information on the sex and sex ratio of fetuses.

Table 6.7. Reproductive and Developmental Studies on Bromoform

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

* gestation day

In the Ruddick *et al.* (1983) study, maternal weight gain, organ weights, hematology, and clinical chemistry were unaffected by bromoform treatment. No significant differences between exposed and control groups were observed for resorption sites, fetuses per litter, fetal weights, fetal gross malformations, or visceral abnormalities. No treatment-related histopathological effects were noted in either dams or fetuses. However, an increased incidence of several skeletal variations was reported, including the presence of a 14th rib, wavy ribs (high dose only), interparietal deviations, and sternebral aberrations (Table **6.**8). 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 sternebral 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.

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 (Table **6.**8). The incidence of sternebral 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.

Table 6.8. 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	Sternebral 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

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 40 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 pre-cohabitation and 98 days 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 US EPA's two-generation reproductive and developmental toxicity protocol (US EPA, 1996).

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.

Bromoform has tested positive for teratogenic effects in studies recently conducted in aquatic organisms. Among 20 DBPs in chlorinated saline sewage effluent, bromoform ranked 13 in developmental toxicity to embryos of the marine polychaete *P. dumerilii* (Yang and Zhang, 2013). This was based on a median effective concentration (EC_{50}, the concentration at which half of the embryos developed abnormally) of 0.73 mM for a 12-hour exposure period. In another study, this time of zebrafish embryos exposed at 4 to 76 hours post-fertilization, bromoform (0.2 mM, EC_{50}) ranked second in potency among the four THMs in inducing adverse developmental effects, which included malformation of the eyes, heart and tail, as well as delayed growth, mobility and hatching (Teixidó *et al.*, 2015).

Immunotoxicity

Effects in Humans

No published data were located on immunotoxicity through exposure to bromoform by humans.

Effects in Animals

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. This effect was evident when results were 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. No treatment-related effects on immune system function were observed in the females (data not reported in the publication). 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.

Haematological Toxicity

Lodhi *et al.* (2017) studied the effect of chloroform and bromoform on human blood in vitro at 10, 30 or 50 µg/mL. Bromoform caused damage at all concentrations and was more potent than chloroform

Neurotoxicity

Effects in Humans

No data on the neurotoxicity of bromoform in controlled human exposure studies were available. Clinical observations are consistent with central nervous system depression (summarized in US EPA, 1994a).

Effects in Animals

Bromoform is a central nervous system toxicant at high 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) (see Table 6.1). 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 non-specific anesthetic effect, similar to that produced by various other volatile halocarbons (ATSDR, 2005).

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 (described in the next paragraph). The study included 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 9.2 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 non-gavaged 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 different 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 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 vehicle pretreatment 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 non-progressive decrease (with development of partial tolerance) in response rates in a test of operant conditioning.

Chronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on chronic toxicity through exposure of humans to bromoform.

Effects in Animals

Studies on the chronic toxicity of bromoform are summarized in Table 6.9 below.

Table 6.9. Summary of Chronic Toxicity Studies on Bromoform

Route	Species Strain	Sex	N	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Diet	Rat Wistar SPF	M	40[a]	2 years	19	85: decreased body weight; increased relative liver weight, serum enzyme change, altered liver appearance	Tobe *et al.* (1982)
		F			15	68: decreased body weight; increased relative liver weight, serum enzyme change, altered liver appearance	
Gavage (corn oil)	Rat F344/N	M, F	50	103 weeks (5 days/ week)	-	100: decreased terminal body weight, liver histopathology	NTP (1989a)
	Mouse $B6C3F_1$	M			100	--	
		F			-	100: decreased body weight, liver fatty change	

[a] Tobe used 12 animals of each sex from the control group and 7 animals of each sex for the 24-month sacrifice to examine relative organ weight and body weight

The chronic oral toxicity of bromoform has been assessed in three studies: a dietary study conducted in rats and two 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 six 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 at sacrifice and reported food consumption (average of reported range for each sex) the dietary levels administered in this experiment correspond to doses of about 0, 19, 85, and 514 mg/kg-day for males and 0, 15, 68, and 407 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 increased relative liver weight was also observed. Dose-related decreases in non-esterified fatty acids were observed in all treated males and in females at the mid-dose 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-dose 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, NOAELs and LOAELs of 19 and 85 mg/kg-day, respectively, for males and 15 and 68 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 five days/week for 103 weeks at 0, 100, or 200 mg/kg-day (Table 6.10). 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 the controls. Survival of the high-dose males was significantly decreased relative to the control. Treatment-related clinical signs included lethargy in males and females and aggressiveness in males. Treatment-related lesions included fatty change, active chronic inflammation, and necrosis in the liver (Table 6.10). 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.

NTP (1989a) gavaged groups of male or female B6C3F$_1$ mice (50/sex/dose) with bromoform 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).

Table 6.10. Selected Hepatic Lesions in Fischer 344/N Rats Administered Bromoform by Gavage in Corn Oil for Two Years (NTP, 1989a)

Lesion	Dose (mg/kg-day)		
	0	100	200
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

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. Non-neoplastic 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

Classification of Carcinogenic Potential

Since 1991, bromoform has been listed as a carcinogen under California's Proposition 65. IARC (1999a) concluded that there were limited animal data and inadequate human data on bromoform carcinogenicity, and therefore classified bromoform as a Group 3 carcinogen, not classifiable as to its carcinogenicity to humans. US EPA classified bromoform as a probable human carcinogen, Group B2, in 1993 based on inadequate human data and sufficient evidence of carcinogenicity in animals, namely an increased incidence of tumors following oral administration of bromoform in rats and intraperitoneal administration in mice (US EPA, 2018a). The WHO (2008) drinking water guideline for bromoform is based on the IARC Group 3 classification. Health Canada (2006) classifies bromoform as Group IIID, possibly carcinogenic to humans, based on limited evidence for carcinogenicity in one species of experimental animals and no data in humans.

The rest of this chapter lays out data from carcinogenicity bioassays on bromoform, after noting the lack of epidemiological data on exposures to bromoform alone, i.e., bromoform exposures that are not in conjunction with other disinfection byproducts.

Effects in Humans

No published epidemiological data were located on carcinogenicity through exposure to bromoform alone by humans.

US EPA (1997, 1998e) reviewed early epidemiological studies, examining the relationship between exposure to THMs in chlorinated drinking water and cancer mortality. The most notable findings of these studies were weak but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon and rectum. None of these studies 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 were found to be inconclusive with regard to the potential carcinogenicity of THMs in drinking water in humans by IARC (1999b), ATSDR (1997), and US EPA (2001a).

More than a dozen subsequent studies have added to the evidence of associations of THM exposure with cancer of the bladder or lower gastrointestinal (GI) tract. Several meta-analyses (Villanueva *et al.*, 2003, 2004, 2006; Costet *et al.*, 2011) have reaffirmed the increased risk of bladder cancer with THM exposure. US EPA (2005a) acknowledged that the bladder cancer studies appear to provide the strongest evidence of increased cancer risk from THMs. However, these studies cannot ascribe the increased cancer risk to exposure to any specific THM or other chemical. Epidemiological studies on associations of DBP/THM exposures with cancer are reviewed in Appendix C and IARC (2013).

Effects in Animals

Bromoform has been found to produce tumors in the colon of male and female F344/N rats following administration by corn oil gavage. No studies were identified that examined carcinogenicity in animals exposed to bromoform by inhalation.

Theiss *et al.* (1977) gave bromoform by intraperitoneal 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. This was followed by a 6-week observation period. 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 at the intermediate dose.

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 five days/week for 103 weeks; females (50/group) received doses of 0, 100, or 200 mg/kg-day bromoform by the same protocol. 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 non-neoplastic 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 parallel experiments, the NTP (1989a) evaluated the carcinogenicity of bromoform in male and female F344/N rats (50/sex/dose) (Table 6.11). 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 **6.**11) ($p<0.001$ by logistic regression trend in female rats; $p = 0.03$ for logistic regression trend for the male rat) . 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.

OEHHA also conducted a statistical analysis of the results by Fisher Exact test. To adjust for early mortality, OEHHA used the standard approach of excluding animals dying before observation of the first tumor at a given site. This was done for all studies reporting individual animal data (NTP 1985, 1987, 1989a), and the week of the first recorded tumor for a specific site is indicated in the appropriate tables for each study.

Although the number of tumors in the male rat was small, the occurrence was considered to be treatment-related by NTP because it was consistent with the findings in the female rat, and intestinal tumors are rare in male F344/N rats administered corn oil by gavage. NTP reported the historical control incidences 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 historical control incidences for female F344/N rats were 0/282 for the contract laboratory and 0/1,888 overall.

Table 6.11. Incidence of Large Intestine Tumors in F344/N Rats Gavaged with Bromoform in Corn Oil 5 days/week for 103 weeks (NTP, 1989a)

Tumor	Tumor frequency[a]		
Male rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/43	0/49	1/39
Polyp (adenomatous)	0/43	0/49	2/39
Adenomatous polyp or adenocarcinoma	0/43	0/49	3/39
Female rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/43	0/4	2/41
Polyp (adenomatous)	0/43	1/4	6/41*
Adenomatous polyp or adenocarcinoma	0/43	1/4	8/41**

[a] To adjust for early mortality, OEHHA excluded animals dying before observation of the first tumor at a given site for studies reporting individual animal data. First tumor of the large intestine was recorded at week 76 for males and week 91 for females in this study. *$p \leq 0.05$, ** $p \leq 0.01$ Fisher exact comparison between treated and control group.

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. Because the majority of the lesions observed in females did not meet the NTP morphological criteria for classification as hepatocellular adenomas, 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.

7. TOXICOLOGICAL PROFILE: BROMODICHLOROMETHANE

The toxicological effects of BDCM in experimental animals have been comprehensively reviewed in documents prepared by US EPA (1980b, 1987) and ATSDR (2018). Additional information is available on IRIS (US EPA, 20012b, last reviewed 3/01/1993) and in IARC monographs (1991, 1999a). Individual summary tables of toxicological data are provided below by exposure duration (e.g., subchronic or chronic) or toxicity category (e.g., reproductive or developmental toxicity).

Toxicity information 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 cancer and reproductive outcomes associated with intake of disinfected water are summarized in OEHHA (2016). Because the industrial production and use of BDCM are limited, there are no toxicity data available from occupational studies and no clinical or case reports. Therefore, the majority of the toxicological data on BDCM presented in this document come from experimental animal studies.

Acute Toxicity

Effects in Humans

No published experimental or epidemiological data were located on acute toxicity of BDCM exposure in humans.

Effects in Animals

Summaries of the acute oral toxicity studies on BDCM in rats and mice are provided in Table 7.1 and Table 7.2 below. Bowman *et al.* (1978) assessed the acute toxicity of single doses of BDCM solubilized in a 1:1:8 solution of Emulphor®, alcohol, and saline, by gavage in groups of 10 male and female fasted ICR Swiss mice. The post-treatment observation period was 14 days. The LD_{50}s were 450 mg/kg and 900 mg/kg for males and females, respectively. Sedation and anesthesia occurred within 30 minutes of treatment at doses of 500 mg/kg and above.

Table 7.1. Oral LD_{50} Values for BDCM

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

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

Chu *et al.* (1980, 1982a) dosed SD rats with BDCM by corn oil gavage and reported LD_{50} values for male and female rats of 916 mg/kg and 969 mg/kg, respectively. The post-treatment observation period was 14 days. 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. Chu *et al.* (1982a) reported significantly decreased food intake and body weight gain in surviving males from their earlier study (Chu *et al.*, 1980) at doses of 765 and 1,071 mg/kg. Other observations in surviving high dose animals include significant reductions in relative liver and kidney weights, and in lactate dehydrogenase activity. BDCM had no significant effect on any measured biochemical parameter in females. No clearly dose-related histological effects were noted in the liver or kidney of either sex.

NTP (1987) reported LD_{50}s for males and females of 651 and 751 mg/kg, respectively, following gavage treatment with single doses of BDCM in corn oil ranging from 150 to 2,500 mg/kg. The rats were observed for 14 days post-treatment and at least one male and one female in each dose group were necropsied at study termination. Clinical signs at 1,250 or 2,500 mg/kg included lethargy and labored breathing. At necropsy, the liver from rats dosed with 1,250 or 2,500 mg/kg appeared pale.

NTP (1987) also gave 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, and observed the

animals for 14 days post treatment. A necropsy was performed on at least one male and one female in each dose group. The male survival pattern prevented the calculation of an LD_{50}. The LD_{50} for females was 651 mg/kg. At necropsy, the liver appeared pale and the cranial cavity contained blood in mice dosed with 1,250 or 2,500 mg/kg.

Table 7.2. Summary of Acute Studies on BDCM

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

Lilly *et al.* (1997b) administered single doses of 0, 123, 164, 246, 328, or 492 mg/kg of BDCM to male F344/N rats (five/dose) by gavage in aqueous 10 percent Emulphor®. Urine was 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 ≥ 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 ≥ 246 mg/kg. At 48 hours, only relative kidney weight at the high dose was significantly increased. Serum enzyme activities indicative of hepatotoxicity were statistically significantly increased at all dose levels at 24 hours relative to controls. Statistical significance was lost for the lowest dose at 48 hours. In the groups evaluated at 48 hours, statistically significant elevations relative to controls were noted ≥ 246 mg/kg for aspartate aminotransferase and alanine aminotransferase and ≥ 123 mg/kg for sorbitol dehydrogenase. No changes in urinary markers of renal toxicity were found at either 123 or 164 mg/kg. Lactate dehydrogenase, N-acetylglucosaminidase, and aspartate aminotransferase activity in urine were significantly elevated (with some exceptions) at 24, 36 and 48 hours post-treatment at ≥ 246 mg/kg. 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). Rather, in this study, the liver was more sensitive based on serum enzyme levels. 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) administered a single dose of 0, 20.5, 30.7, 41.0, 81.9, 123, 164, or 246 mg/kg BDCM dissolved in a 10 percent aqueous solution of Alkamuls EL-620 to 6 male F344 rats/group, and sacrificed the animals 24 hours later. 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.

Other acute studies on BDCM have examined effects of different vehicles (Lilly *et al.*, 1994) and pretreatment with a corn oil vehicle (Lilly *et al.*, 1996). Lilly *et al.* (1994) administered a single dose of 0, 200, or 400 mg/kg BDCM to male F344/N 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. At 400 mg/kg, but not 200 mg/kg, renal toxicity was greater in animals administered BDCM in corn oil. 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 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/N 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 pre-treatment 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,and the rats were sacrificed at 48 hours. All animals were necropsied and organ weights were collected. Serum levels of bile acids, triglycerides, cholesterol, and albumin, and urinary levels of N-acetylglucosaminidase and gamma glutamyl transpeptidase activity were measured. 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 pre-treatment group at the high dose. Kidney weight was reduced in both pre-treatment groups at the high dose. Activities of serum aspartate aminotransferase and lactate dehydrogenase were significantly elevated in both pre-treatment groups at 400 mg/kg. Alanine aminotransferase activity increased in a dose-dependent manner in the water pre-treatment group, but significant elevations were noted only at the 400 mg/kg dose in rats pre-treated with corn oil. Activities of urinary aspartate aminotransferase and lactate dehydrogenase in both pre-treatment groups were greater than the control at all time points. Alkaline phosphatase levels were significantly increased in both pre-treatment groups at 24 hours, but only in water-pretreated animals at 36 and 48 hours. High incidences of renal tubular necrosis occurred at 200 and 400 mg/kg in both pre-treatment 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 pre-treated rats compared to the water controls. The weight of evidence indicates that six weeks of pre-treatment 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.

Short-Term Toxicity

Effects in Humans

No published experimental or epidemiological data were located on short-term toxicity of BDCM exposure in humans.

Effects in Animals

The short-term toxicity of BDCM has been investigated in more than a dozen studies ranging from three to 35 days in duration, as summarized Table 7.3.

Table 7.3. Summary of Short-Term Toxicity Studies on BDCM

Route	Species Strain	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	References
Drinking water	Rat Sprague-Dawley	M	10	28 days	0, 0.8, 8, 68	68	-	Chu *et al.* (1982a)
		M, F	6	2 weeks	0, 11, 45, 91, 124	124	-	NTP (1998)
			5-13	35 days	Group A males 0, 9, 38, 67	9	38: single cell hepatic necrosis	
	Rat F344/N	M	10	3 weeks	0, 6, 12, 20, 38, 71	71	-	NTP (2006)
	Mouse $B6C3F_1$	F	10	3 weeks	0, 6, 10, 16, 29, 51	51	-	NTP (2006)
			10	11 days	0, 138	-	138: liver hydropic degeneration	Coffin *et al.* (2000)
Diet	Rat Wistar	M	7	1 month	0, 21, 62, 189	62	189: liver histopathology	Aida *et al.* (1992a)
		F	7	1 month	0, 21, 66, 204	66	204: liver histopathology	
Gavage (corn oil)	Rat F344/N	M, F	5	14 days	0, 38, 75, 150, 300, 600	150	300: decreased final body weight	NTP (1987)

Route	Species Strain	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	References
Gavage (corn oil)	Mouse B6C3F$_1$	M, F	5	14 days	0, 19, 38, 75, 150, 300	75	150: mortality, gross renal pathology	NTP (1987)
		F	10	3 weeks (5 days/ week)	0, 75, 150, 326	75	150: hepatocyte degeneration; increased labeling index; serum hepatotoxicity markers	Melnick *et al.* (1998)
		F	10	11 days (9 doses)	0, 150, 300	-	150: liver hydropic degeneration; increased proliferating cell nuclear antigen-labeling index	Coffin *et al.* (2000)
	Mouse CD-1	M	8-16	14 days	0,37,74,148	74	148: liver, kidney histopathology	Condie *et al.* (1983)
Gavage (aqueous)	Rat F344/N	M	4	1, 3, or 7 days	0, 123, 246	246[a]	-	Potter *et al.* (1996)
		F	6	5 days	0, 75, 150, 300	75	150: liver, renal histopathology; serum hepatotoxicity markers	Thornton-Manning *et al.* (1994)
	Mouse C57BL/6J	F	6	5 days	0, 75, 150	75	150: serum hepatotoxicity markers	Thornton-Manning *et al.* (1994)
	Mouse CD-1	M,F	8-12	14 days	0, 50, 125, 250	50	125: decreased immune function; organ weights	Munson *et al.* (1982)

[a]Study of hyaline droplet formation and cell proliferation, neither of which were observed.

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. Body and organ weights, hematology, serum enzyme levels, and immune system functions (discussed in the Immunotoxicity section of this document) were evaluated. No compound-related deaths occurred. Body weights were significantly decreased in males and females at the high dose. Significant effects observed in mid- and/ or high-dose males and females included increased relative liver weight, decreased absolute spleen weight, decreased relative spleen weight, and decreased fibrinogen levels. Significant alterations in clinical chemistry occurred at the high dose in males and/or females including decreased glucose levels increased alanine aminotransferase and aspartate aminotransferase activities, and increased blood urea nitrogen levels. A NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day were identified based on organ weight changes.

Chu *et al.* (1982a) found no compound-related effects on growth rate or food consumption, clinical signs of toxicity, biochemical alterations, or histological changes in male SD rats administered BDCM in drinking water for 28 days at concentrations of 0, 5, 50, or 500 ppm (equivalent to average daily doses of 0, 0.8, 8.0, or 68 mg/kg-day, as calculated by the authors). 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. Significant changes at the mid- and/or high dose included elevated alanine aminotransferase activity, decreased *para*-aminohippurate uptake by kidney slices (an indication of kidney damage), and decreased blood urea nitrogen. Dose-related hepatic lesions at the high dose 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 liver and kidney histopathological changes.

NTP (1987) gave 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. 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. 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.

Aida *et al.* (1992a) exposed Slc:Wistar rats (7/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 (in mid- and high-dose males and females) and hepatic swelling (in mid- and high-dose males, high-dose females). Other observed lesions included swelling of hepatocytes (in high-dose males, mid- and high-dose females), single cell necrosis (in high-dose males), hepatic cord irregularity (in high-dose males), and bile duct proliferation (in 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 liver histopathology. The NOAELs are 62 mg/kg-day for males and 66 mg/kg-day for females.

Thornton-Manning *et al.* (1994) gave 0, 75, 150, or 300 mg/kg-day of BDCM to groups of six female F344/N rats by gavage for five consecutive days, in an aqueous vehicle containing 10 percent Emulphor®. Two animals in the high-dose group died during the exposure period. Final body weights were significantly decreased at the high dose compared to controls, and absolute and relative kidney and liver weights were significantly increased at 150 and/or 300 mg/kg-day. Increases in lactate dehydrogenase, aspartate aminotransferase, sorbitol dehydrogenase, creatinine, and blood urea nitrogen at 300 mg/kg-day were suggestive of renal and hepatic toxicity. Dose-related centrilobular vacuolar degeneration was observed in the liver at 150 and 300 mg/kg-day. 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 high-dose animals. Significant decreases in the hepatic microsomal activity towards 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 cytochrome P450 levels were reduced at mid-dose and high dose. A NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day were identified, based on the kidney and liver lesions.

Thornton-Manning *et al.* (1994) conducted an analogous experiment with female C57BL/6J mice. Six mice/group were given doses of 0, 75 or 150 mg/kg-day of BDCM suspended in aqueous 10 percent Emulphor® by gavage for five consecutive days. 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. Alanine aminotransferase was significantly increased at 150 mg/kg-day, and there was a significant dose-related increase in sorbitol dehydrogenase activity. 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/N rats treated with BDCM (4/dose) gavaged with 0, 123 or 246 mg/kg-day BDCM in four percent Emulphor® for one, three, or seven days. No exposure-related increase in hyaline droplet formation and no statistically significant effects on tubular cell proliferation were observed following exposures of up to seven days. In this study, 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. Significant dose-related increases in absolute liver weight and liver weight to body weight ratio occurred at 150 and 326 mg/kg-day. Serum alanine aminotransferase activity was significantly increased at the mid-dose and high dose. Serum sorbitol dehydrogenase activity was increased at all doses. Hydropic degeneration was evident in the livers of animals treated with the mid-dose and high dose. The labeling index was significantly increased at the mid-dose and high dose, indicating hepatocyte proliferation. A NOAEL and LOAEL of 75 and 150 mg/kg-day, respectively, based on histopathology.

NTP (1998) 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 6 rats/sex/dose at nominal concentrations of 0, 100, 500, 1,000, and 1,500 ppm in drinking water for two weeks, yielding average BDCM doses of 11, 45, 91 and 124 mg/kg-day estimated by the study authors. Feed and water consumption were measured twice weekly. 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, possibly related to decreased food and water consumption at concentrations of 500 ppm and up. 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.

NTP (1998) 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 or 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[6]-treated, 5 or 8 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). 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, and 126 mg/kg-day for Group C females. Test animals were dosed for 25 to 30 days, except for Group B females, which were dosed from GD 6 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.

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. 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 at 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. Mild kidney necrosis was observed in Group A males in the 1,300 ppm group. 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. 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. 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 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.

[6] Bromodeoxyuridine, BrdU, animals are pretreated with this compound for determination of labeling index.

A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage; but not 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. The histopathology findings for animals receiving BDCM in the drinking water were similar to those observed in the low-dose gavage group. BDCM given by gavage, but not by drinking water, caused a dose-dependent increase in the proliferating cell nuclear antigen-labeling index, significant at each dose, compared to the control. 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.

NTP (2006) conducted a three-week exposure of rats and mice to BDCM in drinking water as a range-finding study for a two-year carcinogenesis bioassay. Groups of ten male F344/N rats were exposed to target concentrations of 0, 43.7, 87.5, 175, 350, or 700 mg/L BDCM (equivalent to average daily doses of about 0, 6, 12, 20, 38, or 71 mg/kg-day) in drinking water for three weeks. All rats survived to the end of the study. The mean body weight gains at the two highest dose levels were significantly less than that of the controls. Dose-related decreases in water consumption were observed during the first week. Relative kidney weights of rats in the 20, 38, and 71 mg/kg groups were significantly greater than controls. No significant chemical-related histopathological changes were observed.

Groups of ten female B6C3F$_1$ mice were exposed to target concentrations of 0, 43.7, 87.5, 175, 350, or 700 mg/L BDCM (equivalent to average daily doses of about 0, 6, 10, 16, 29 or 51 mg/kg-day) in drinking water for 3 weeks. All mice survived to the end of the study. Final mean body weights of the mice at the three higher dose levels and mean body weight gains at the two highest doses were significantly less than those of the controls. The authors attributed these decreases to decreased water consumption at 87.5 mg/L and higher, related to poor palatability of the water. Relative liver, kidney, and thymus weights of mice at the two highest dose levels were significantly greater than those of the controls. Absolute lung weights of mice in these two groups were significantly less than the controls. No significant chemical-related histopathological changes were observed.

Subchronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on subchronic toxicity of BDCM exposure in humans.

Effects in Animals

Two published studies have addressed subchronic oral toxicity of BDCM (Table 7.4). 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 currently available for BDCM.

Table 7.4. Summary of Subchronic Toxicity Studies on BDCM

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

[a] non dose-dependent hepatic and thyroid lesions were observed.

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 in males 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. 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 in the 150 and 300 mg/kg-day dose groups was reduced by 30 and 55 percent, respectively. Final mean body weight of female rats in the 150 and 300 mg/kg-day dose groups was reduced by 12 and 21 percent, respectively. 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 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 both males and females.

NTP (1987) also assessed the toxicity of BDCM in male and female B6C3F$_1$ mice (10/sex/dose) with gavage 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, 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 lesion, 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. Azoxymethane (AOM) served as a positive control. Aberrant

crypt foci incidence (percent of animals tested) and number (aberrant crypt foci/colon) for the rat were: combined controls, 0%, 0; AOM 100 %, 27.17 ± 6.28 ($p < 0.01$); chloroform, 16.7%, 0.17 ± 0.17; BDCM, 83.3%, 1.50 ± 0.56 ($p < 0.01$); DBCM, 50%, 1.17 ± 0.65 ($p < 0.01$); bromoform, 66.7%, 1.17 ± 0.40 ($p < 0.01$). THM-induced aberrant crypt foci occurred primarily (92 percent) in the rectal segment of the colon.

Geter *et al.* (2004b) went on to show that formation of aberrant crypt foci in the colon of male F344/N rats was independent of the vehicle of BDCM exposure, with drinking water and corn oil gavage administration over 26 weeks producing similar values of 1.33 ± 0.49 and 1.5 ± 0.51 aberrant crypt foci/colon, respectively. It should be noted, however, that the authors report significant increases in aberrant crypt foci were observed for BDCM administered in water only relative to the corresponding control, which had no aberrant crypt. BDCM in corn oil induced 1.5 ± 0.51 aberrant crypt foci per colon vs 0.16 ± 0.16 for the corn oil vehicle, which the authors reported as not statistically significant.

To evaluate whether THM-induced aberrant crypt foci could be promoted by a diet high in saturated animal fat, Geter *et al.* (2004c) exposed male F344/N rats 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 in drinking water for 26 weeks, with half the animals fed the normal 4.5% fat Purina 5001 diet and the other half receiving the feed supplemented with 19% animal fat. Compared to control animals, all groups administered brominated THMs showed significant increases in aberrant crypt foci induction; no increase was observed with chloroform treatment, consistent with their earlier findings (DeAngelo *et al.*, 2002). There was a statistically significant association between the number of aberrant crypt foci per colon and the number of bromine atoms on the THM molecule. Among animals exposed to chloroform, BDCM and DBCM, no difference was observed in the number of aberrant crypt foci between animals fed the normal diet and those fed the high-fat diet. In contrast, rats administered bromoform and a high fat diet exhibited a significant and near 2-fold increase in aberrant crypt foci relative to animals administered bromoform and the 4.5% fat diet (5.33 ± 1.17 aberrant crypt foci/colon versus 2.83 ± 1.05, respectively). Consistent with the findings of DeAngelo *et al.* (2002), THM-induced aberrant crypt foci were found primarily in the medial and distal segment of the colon, the observed site of THM-induced neoplasia.

In another study of THM-related induction of aberrant crypt foci, McDorman *et al.* (2003) exposed male Eker rats for ten months to DBPs administered in drinking water, including BDCM (0.07 or 0.7 g/L). Animals administered BDCM had elevated aberrant crypt foci in the colon relative to untreated rats. It should be noted that Eker rats used in this study have a genetic predisposition to tumors, although not in the colon, which could possibly have influenced the difference in results. The urinary bladders were also examined for transitional cell hyperplasia, which was not induced in chloroform- or BDCM-treated animals.

Genetic Toxicity

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

chromosomal aberrations *in vivo* and *in vitro*. Positive or equivocal results were obtained for mutagenicity in cultured mouse lymphoma cells. Thus, the weight of evidence suggests that BDCM is mutagenic and genotoxic.

In Vitro Assays

Robbiano *et al.* (2004) observed statistically significant dose-dependent increases in DNA fragmentation, as evaluated by the comet assay, following 20-hour exposure of primary human kidney cells to 0.5 to 4 mM BDCM. Consistent with these results, statistically significant dose-dependent increases were also observed in the frequency of micronucleated cells following 48-hour exposure to BDCM at 1 to 4 mM.

The genotoxicity of BDCM has been evaluated in numerous *in vitro* assays in bacteria and eukaryotic cells (Table 7.5). 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 when the study authors provided this information.

Table 7.5. Summary of *In Vitro* Mutagenicity and 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)	-/+	Le Curieux *et al.* (1995)
	TA1535[a]	NT/+	Pegram *et al.* (1997)
	TA97, TA98, TA100, TA1535, TA1537	-/-	NTP (2006)
Gene mutation – Mammalian	Mouse lymphoma cells	+/-	NTP (1987, 2006),
	Mouse lymphoma cells	?/-	Sofuni *et al.* (1996)
Chromosomal aberrations	Chinese hamster fibroblast cells	+/-	Ishidate *et al.* (1982); Ishidate (1987)

Endpoint	Assay System	Results (with/without metabolic activation)	References
	Chinese hamster FAF cells	NT/+	Strobel and Grummt (1987)
	Chinese hamster ovary cells	-/- -[b]/-	NTP (1987), Anderson *et al.* (1990) NTP (2006)
	Chinese hamster lung fibroblasts[a]	(+)/(+)	Matsuoka *et al.* (1996)
	E. coli PQ37, SOS chromotest	-/-	Mersch-Sundermann *et al.* (1989)
DNA damage	*E. coli* PQ37, SOS chromotest (induction of SOS genes)	+/+	Le Curieux *et al.* (1995)
	Human lymphocytes	NT/+	Morimoto, Koizumi (1983)
	Human lymphoblastic leukemia cells (DNA alkaline unwinding assay)	NT/- NT/+	Mersch-Sundermann *et al.* (1989) Geter *et al.* (2004a)
	Rat hepatocytes DNA alkaline unwinding assay)	NT/-	Geter *et al.* (2004a)
	Human HepG2 hepatoma line (single cell gel electrophoresis assay)	NT/+	Zhang *et al.* (2012)
	Primary rat kidney cells (comet assay)	NT/+	Robbiano *et al.* (2004)
	Primary human kidney cells (comet assay)	NT/+	Robbiano *et al.* (2004)
Induction of micronuclei	Primary rat kidney cells	NT/+	Robbiano *et al.* (2004)
	Primary human kidney cells	NT/+	Robbiano *et al.* (2004)
Sister chromatid exchange	Human lymphocytes Rat liver cells	+/NT (+)/NT	Sobti (1984)
	Chinese hamster ovary cells	-[b]/- -/-	NTP (1987), Anderson *et al.* (1990) NTP (2006)
	Chinese hamster FAF cells	NT/-	Strobel and Grummt (1987)
Sister chromatid exchange	Rat erythroblastic leukemia K_3D cells	?/+	Fujie *et al.* (1993)

Endpoint	Assay System	Results (with/without metabolic activation)	References
Sister chromatid exchange	*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 conducted in closed system; [b] Equivocal results.

DNA Damage and Covalent Binding

Le Curieux *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.*, 2004a). In contrast, Mersch-Sundermann *et al.* (1989) found no induction of DNA damage by BDCM using the same assay. Geter *et al.* (2004a) reported negative results for DNA strand breaks in primary rat hepatocytes exposed to BDCM at 5 or 10 mM for four hours. Zhang *et al.* (2012) reported DNA strand breaks with 1-1000 µM DBCM incubated 24 hours with cells from the human HepG2 hepatoma line. Robbiano *et al.* (2004) observed statistically significant dose-dependent increases in DNA fragmentation, as evaluated by the comet assay, following 20-hour exposure of primary rat kidney cells to 0.5 to 4 mM BDCM.

Ross and Pegram (2004) demonstrated through a series of *in vitro* experiments the DNA covalent binding activity of reactive intermediates generated by GSTT1-1-mediated metabolism of BDCM. Concentration-dependent increases were found in bacterial DNA-associated radioactivity when GSTT1-1-expressing *S. typhimurium* were exposed to radiolabeled BDCM, versus little binding to S. typhimurium strain TPT100, which does not express GST1-1. Further, rat or mouse liver cytosol preparations incubated with radiolabeled BDCM, glutathione and calf thymus DNA resulted in 3-fold to 7-fold elevated levels of radioactivity associated with purified DNA relative to control DNA (incubated with radiolabeled BDCM and glutathione but without cytosol). The DNA labeling levels were consistent with conjugation activity of the cytosols toward BDCM. Similarly, incubations containing recombinant GSTT1-1 enzyme with glutathione and radiolabeled BDCM in the presence of either calf thymus DNA or deoxyguanosine also demonstrated covalent binding of radiolabeled BDCM-derived metabolites to DNA and deoxyguanosine (about 0.02% of metabolized radiolabeled BDCM formed DNA adducts).

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 measure 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 non-mutagenic in

strains TA1535, TA1537, TA98, or TA100 when tested in the presence or absence of metabolic activation using a pre-incubation 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. Le Curieux *et al.* (1995) obtained negative results in TA100 in the fluctuation test modification of the reverse mutation assay. NTP (2006) found negative results with and without activation with *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537; no protection against volatilization is noted.

Gene Mutation in Yeast

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.

Gene Mutation in Mammalian Cells

Two studies have examined mutagenicity in cultured mammalian cells. NTP (1987) 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. NTP (2006) also tested BDCM for mutation induction in mouse lymphoma L5178Y/TK$^{+/-}$cells. Results were positive in the presence of induced rat liver S9 and negative without S9.

Chromosomal Aberrations

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 Chinese hamster ovary cells with up to 5,000 µg/mL BDCM with or without exogenous metabolic activation. Matsuoka *et al.* (1996) found weak induction of chromosomal 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. NTP found no induction of chromosomal aberrations in BDCM-treated Chinese hamster ovary cells, with or without S9.

Benigni *et al.* (1993) evaluated induction of aneuploidy and toxicity in *Aspergillus nidulans* treated with the THMs, as well as other halomethanes. Among the THMs, the most active compound in inducing whole chromosome segregants (non-disjunctional 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 of the other halomethanes but one (bromotrichloromethane, 3.11 percent at 0.015 percent) exhibited less aneuploid induction than the THMs. All the values cited above were statistically significant with the X^2 test ($p < 0.001$). The authors also estimated doses able to block mitotic growth, the dose with 37 percent of survival, and the lowest efficient concentration in aneuploidy induction (LEC). For the THMs, the observed LECs were DBCM, 1.47 mM, bromoform, 3.43 mM, and BDCM, 6.00 mM, respectively. A 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.

Micronuclei Induction

Consistent with results of the DNA fragmentation assay by Robbiano *et al.* (2004), statistically significant dose-dependent increases were observed in the frequency of micronucleated cells following 48-hour exposure of primary rat kidney cells to BDCM at 1 to 4 mM.

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 Chinese Hamster Ovary 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. NTP (2006) reported that in cultured Chinese hamster ovary cells, BDCM induced a small increase in SCEs in one of four trials with induced rat liver S9 enzymes, and no significant increases without S9.

In Vivo Assays

In vivo data for BDCM genotoxicity are available for chromosomal 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 7.6.

Effects Measured in Humans In Vivo

Kogevinas *et al.* (2010) reported an increase in micronuclei in peripheral blood lymphocytes one hour after swimming for 40 min in an indoor chlorinated pool. The increase in micronuclei was associated with exhaled concentrations of BDCM (p = 0.03), DBCM (p = 0.05), and bromoform (p = 0.01), but not chloroform. Increased urine mutagenicity (Ames assay) two hours after swimming was associated with the concentration of bromoform in exhaled breath (p = 0.004). DNA damage in peripheral blood lymphocytes (comet assay) and micronuclei in exfoliated urothelial cells 2 weeks after swimming were not associated with THM exposures

Table 7.6. Summary of *In Vivo* Genotoxicity Studies on BDCM

Endpoint	Assay System	Result	References
Chromosomal aberrations	Rat, bone marrow cells	+	Fujie *et al.* (1990)
	Mouse, bone marrow cells	-	NTP (1987)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
Micronuclei induction	Mouse, rat, bone marrow cells	-	Ishidate *et al.* (1982)
	Mouse, bone marrow cells	-	Hayashi *et al.* (1988), NTP (2006)
	Mouse, peripheral erythrocytes	-	Hayashi *et al.* (1992), NTP (2006, 2007)
	Newt, peripheral erythrocytes	(+)	Le Curieux *et al.* (1995)
	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
	Rat, renal cells	+	Robbiano *et al.* (2004)
	Mouse, bone marrow and peripheral blood erythrocytes	(+)	Torti *et al.* (2002)
DNA damage (strand breakage)	Rat, renal cells (DNA alkaline unwinding assay)	-	Potter *et al.* (1996)
	Rat, liver, renal, duodenum cells (DNA alkaline unwinding assay)	-	Geter *et al.* (2004a)
DNA damage (strand breakage)	Zebrafish, whole embryos (comet assay)	-	Teixidó *et al.* (2015)
	Rat, renal cells (comet assay)	+	Robbiano *et al.* (2004)

Endpoint	Assay System	Result	References
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al.* (1997)
Sex-linked recessive lethal mutation	*D. melanogaster*	-	Foureman *et al.* (1994)
Aneuploidy Induction	*Aspergillus nidulans*	+	Benigni *et al.* (1993)

(+) = weakly positive.

DNA Damage

Potter *et al.* (1996) examined the effect of BDCM on DNA strand breakage in kidney cells of male F344/N 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 effects of DNA strand breaks were observed at either dose (Potter *et al.*, 1996). No DNA strand breaks in liver, kidney, or duodenum epithelial cells were observed in male F344/N rats administered 0.3 or 0.6 mM/kg as a single oral gavage dose of BDCM in 0.5 percent Emulphor® for four hours (Geter *et al.*, 2004a). 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.*, 2004a).

Robbiano *et al.* (2004) observed a statistically significant increase in DNA fragmentation, as evaluated by the comet assay, in the kidney of male Sprague-Dawley rats sacrificed 20 hours following administration of a single oral dose of 458 mg/kg BDCM (1/2 LD_{50}). BDCM did not induce DNA damage in zebrafish embryos exposed to 0.26 mM (EC_{50} value for teratogenic effects) at 4 to 76 hours post-fertilization (Teixidó *et al.*, 2015).

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.

Gene Mutation

Foureman *et al.* (1994) found DBCM to be non-mutagenic in the sex-linked recessive lethal mutation 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.

Chromosomal Aberrations

Fujie *et al.* (1990) assessed chromosomal 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 chromosomal breaks. A more pronounced response was observed following a single intraperitoneal dose with statistically significant effects occurring at 16.4 mg/kg. A chromosomal aberration test with BDCM (NTP, 1987) did not show clastogenicity in mouse bone marrow.

Micronuclei 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 intraperitoneal (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.

Torti *et al.* (2002) also reported weakly positive results for induction of micronuclei following inhalation exposures of mice to BDCM daily for 6 hours/day. At the one-week exposure time point, the only statistically significant effect was an increase in micronuclei frequency in bone marrow erythrocytes in the FVB/N wild-type mice at the 100 ppm exposure, but not at 150 ppm or in the FVB/N $p53^{+/-}$ mice; no effect was observed in either the C57BL/6 wild-type or $p53^{+/-}$ mice at any dose at this time point. At the 3-week exposure time point, no significant effect was observed in bone marrow erythrocytes in any animal group tested. At the 13-week exposure time point, a small but significant increase in micronuclei induction was observed only at the highest concentration tested (15 ppm) in peripheral blood erythrocytes in both animal groups tested (C57BL/6 $p53^{+/-}$ and FVB/N $p53^{+/-}$ mice). In contrast, Robbiano *et al.* (2004) reported a statistically significant increase in the frequency of micronucleated cells in the kidney of male Sprague-Dawley rats sacrificed 20 hours following administration of a single oral dose of 458 mg/kg BDCM (1/2 LD_{50}). These results are consistent with those of primary cultures of human and rat kidney cells treated with BDCM *in vitro* in that study.

NTP (2006) found no increases in the frequency of micronucleated erythrocytes in bone marrow of male $B6C3F_1$ mice administered 200 to 500 mg/kg BDCM i.p. for 3 days. In a second test, NTP (2006) found no induction of micronuclei in peripheral blood normochromatic erythrocytes of female $B6C3F_1$ mice administered up to 700 mg/L BDCM in drinking water for 3 weeks.

NTP (2007) conducted peripheral blood micronucleus tests on male and female Tg.AC hemizygous and p53 haploinsufficient mice exposed to BDCM by dermal application, in drinking water, and by gavage for 26 weeks. Results in Tg.AC hemizygous mice were judged to be equivocal in males and negative in females treated by dermal application, equivocal for both males and females in the drinking water study, and negative in males and females treated by

gavage. In the p53 haploinsufficient mice, the drinking water route gave equivocal results in males and negative results in females, while gavage administration gave negative results in both males and females.

Le Curieux *et al.* (1995) obtained weakly positive results for induction of micronuclei in *Pleurodeles waltl* larvae.

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.

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 studies are 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 4.1 in Chapter 4.

Developmental and Reproductive Toxicity

Effects in Humans

Many epidemiological studies have investigated potential links between exposure to THMs (as disinfection byproducts in drinking water) and adverse reproductive or developmental outcomes in humans, summarized in OEHHA (2016). BDCM is one of the DBPs considered in the epidemiology studies. Although many of the studies measure individual THMs and not just total THMs or other DBPs, it is difficult to attribute observed associations to any one of the DBPs given the mixed exposures. Chen *et al.* (2003, 2004), in studies spurred by the epidemiological associations of BDCM and other THMs in drinking water with adverse reproductive outcomes (Dodds and King, 2001; OEHHA, 2016), suggested that the 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 (SCTBs) 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 mono-nucleated cytotrophoblast cells undergoing morphological differentiation to multinucleated SCTB-like colonies inhibited the formation of multinucleated colonies in a dose-dependent manner. Secretion of chorionic gonadotropin was significantly inhibited in a dose-dependent manner, and cellular levels of gonadotropin were also reduced. These results indicated that BDCM disrupts SCTB formation and inhibits gonadotropin secretion *in vitro*.

Effects in Animals

The experimental database for the reproductive and developmental toxicity of BDCM consists of at least ten oral exposure studies conducted in rats, mice, or rabbits (Table 7.7). These studies include prenatal and two-generation studies and experiments designed to collect information on the mode of action for induction of full litter resorption in F344/N rats.

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

No differences were reported for fetal weights, gross malformations, or visceral anomalies. However, 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 the treated groups differ significantly from control, however, the Cochran-Armitage trend test showed a significant dose-related trend for increased sternebral aberrations in treated animals ($p < 0.01$). A developmental toxicity NOAEL of 200 mg/kg-day was identified in this study. The maternal NOAEL and LOAEL are 100 and 200 mg/kg-day, respectively, based on decreased maternal body weight gain.

Table 7.7. Summary of Developmental and Reproductive Toxicity Studies on BDCM

Route	Species Strain	Sex	N	Dose (mg/kg-day)	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley (SD)	F	25	0.0, 2.2, 18.4, 45.0, 82.0 (0, 50, 150, 450, 900 ppm in water)	GD 6-21	Maternal: 18 Dev: 45[a]	Maternal 45: Reduced BW gain Dev 82: reduced ossification	Christian *et al.* (2001a)
		M, F	10-13	M: 0, 8, 41, 68 F: 0, 14, 72, 116	25-30 days	M: 68 F: 116	--	NTP (1998)
			10	(0, 50, 150, 450, or 1350 ppm in drinking water)	14 days before cohabit-ation through lactation	16.3 - 41.4[b] (50 ppm in water)	23.5 - 40.3[b] (150 ppm): Decreased pup body weight. No change in reproductive parameters	Christian *et al.* (2001b)

Route	Species Strain	Sex	N	Dose (mg/kg-day)	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley (SD)		30	(0, 50, 150 or 450 ppm in drinking water)	Two generation	4.1-12.6[a] (50 ppm in water)	11.6-40.2[a] (150 ppm): delayed sexual maturation with reduced body weight	Christian *et al.* (2002)
	Rat F344/N	M	7	0, 22, 39	52 weeks	22	39: decreased sperm velocities	Klinefelter *et al.* (1995)
	Rabbit NZ White	F	5	0, 4.9, 13.9, 32.3, 76.3	GD 6-29	76	--	Christian *et al.* (2001b)
			25	0, 1.4, 13, 36, 55		55		Christian *et al.* (2001a)
Gavage (water)	Rat F344/N	F	12-14	0, 75	GD 6-10	--	75: 62% litter resorption	Bielmeier *et al.* (2001)
			8-11	0, 75	GD 9	--	75: full litter resorption	
	Rat SD	F	13-14	0, 75, 100	GD 6-10	100	No effect	
	Rat F344/N	F	13-14	0, 25, 50, or 75	GD 6-15	25	50: full litter resorption	Narotsky *et al.* (1997)
Gavage (oil)	Rat SD	F	9-14	0, 50, 100, 200	GD 6-15	200	--	Ruddick *et al.* (1983)
	Rat F344/N	F	13-14	0, 25, 50, or 75	GD 6-15	25	50: full litter resorption	Narotsky *et al.* (1997)

[a]maternal NOAEL (reduction in BW gain) of 18 mg/kg-day was lower than developmental NOAEL. [b] The daily dose varied widely, depending on the age of the animal.

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.

Klinefelter *et al.* (1995) evaluated the effects of BDCM on male reproduction during a cancer bioassay study. Seven F344/N 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 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 in which sperm measures including motility endpoints were not altered by BDCM exposure in drinking water for up to 35 days (NTP, 1998).

Narotsky *et al.* (1997) evaluated developmental toxicity of BDCM in F344/N 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 GD 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 GD 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-dose and high dose 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 GDs 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 higher BMDL than the aqueous vehicle, reflecting the different CIs around the estimated five percent response levels.

NTP (1998) conducted a short-term reproductive and developmental toxicity screen in SD rats of 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 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. Non-reproductive 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/group) and three groups of female rats designated as Groups A (periconception exposure, 10 rats/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 GD six to birth (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 (1998) 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/N 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 GDs six to 10. F344/N 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/N rats at 75 mg/kg-day. 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/N rats that resorbed their litters. F344/N 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/N 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/N 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/N 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 GD

six to 10 (which includes the luteinizing hormone-dependent period of pregnancy) or on GD 11 to 15 (a luteinizing hormone-independent period). Additional groups of 8-10 rats dosed with 0 or 75 mg/kg-day on GD six to 15 served as negative and positive controls, respectively. Full litter absorption occurred only in rats treated during the luteinizing hormone-dependent period (GD six to 10 or six to 15). In contrast, all rats treated with BDCM on GD 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 experiment, 8 to 10 F344/N rats/dose were gavaged with 100 mg/kg BDCM in 10 percent Emulphor® on GD eight or nine. Progesterone and luteinizing hormone concentrations were measured in tail blood samples collected once daily on GD nine through 12. Full litter resorption occurred in 0, 60, and 100 percent of the control, GD eight and GD 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 experiment, Bielmeier *et al.* (2001) gave 0, 75, or 100 mg/kg-day of BDCM to F344/N rats (8-11/dose) on GD nine by gavage in 10 percent Emulphor®. Blood samples were collected at 0, 6, 12, and 24 hours after dosing for progesterone and luteinizing hormone assay. Full litter resorption in the three dose groups was 0, 64, and 90 percent, respectively. Peak progesterone levels occurred at six hours post-treatment 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 (not statistically significant). 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. 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/N rats. A NOAEL could not be identified.

Bielmeier *et al.* (2007) studied the effect of BDCM on luteal responsiveness to luteinizing hormone, using *ex vivo* and *in vitro* approaches. For the *ex vivo* study, dams were dosed at 0 or 100 mg/kg/d by gavage on gestation days (GD) 6-9, killed one hour after the last exposure and the corpora lutea evaluated for their response to hCG, a leuteinizng hormone agonist (degree of secretion of progesterone). For the *in vitro* study, corpora lutea were pooled from untreated F344 rats on GD 9, cultured with BDCM at 0, 0.01, 0.10 or 3.0 mM, and evaluated for responsiveness to hCG. The results suggest that BDCM disrupts pregnancy in F344 rats via

two modes: disruption of luteinizing hormone secretion, and disruption of the corpora lutea's ability to respond to luteinizing hormone.

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 GD 10. F344/N rats were gavaged with 75 mg/kg-day BDCM on GDs 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. 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 GD six to 21. These concentrations were based on the NTP (1998) results and a 14-day range-finding study conducted by Christian *et al.* (2001b), which found 1,350 ppm to cause excessive maternal toxicity. Effects on viability, clinical signs, body weight, water consumption, and feed consumption were investigated. All study animals were sacrificed on GD 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.

The average BDCM 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, calculated by the study authors from measured water consumption and body weights. 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 occurred in the 150, 450, and 900 ppm groups. In the 150 ppm group, the effects were statistically significant only on GD 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 GD 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 non-effect 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 GD 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 GD 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 GD 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 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 GD 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 GD 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 in SD rats. BDCM was provided to 30 rats/sex/dose group 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 doses for each 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 at 150 and 450 ppm 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 secondary to 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.

BDCM tested positive for teratogenic effects in a recent study conducted in aquatic organisms, similar to the other three THMs. Exposure of zebrafish embryos to BDCM (0.26 mM, EC_{50} value) at 4 to 76 hours post-fertilization resulted in adverse developmental effects, including malformation of the eyes, heart and tail, and delayed growth, movement and hatching (Teixidó *et al.*, 2015).

Immunotoxicity

Effects in Humans

No published experimental or epidemiological data were located on immunotoxicity through exposure to BDCM by humans.

Effects in Animals

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 spleen and decreased hemagglutination titers (antibody titer in plasma). 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 mouse 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 mouse 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/N rats in two experiments, examining the immunological parameters of 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, yielding 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 were reported. These data suggest NOAEL and LOAEL values of 5 and 49 mg/kg-day, respectively.

In the second rat study, French *et al.* (1999) administered deionized water, 10 percent Emulphor®, or 75, 150, or 300 mg/kg BDCM in 10 percent Emulphor® by gavage to six female F344/N 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 at 150 mg/kg-day. Concanavalin A responses were significantly depressed in both spleen and mesenteric lymph node cells at 300 mg/kg-day. 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

Effects in Humans

Villanueva *et al.* (2018) evaluated the association between DBP exposure during pregnancy and child neuropsychological outcomes at one and four to five years of age using a cohort of Mother-Child pairs in Spain (INMA project 2003-2008). Tap water concentrations of trihalomethanes were modeled for each month of pregnancy, based on measurements at the

tap and reports from water agencies. These concentrations were then combined with ingestion, showering and bathing habits to estimate multi-route exposures. Modeled concentrations were multiplied by daily use and uptake factors for the different routes of exposure to estimate a daily blood concentration of the trihalomethanes. The investigators separately analyzed the associations between chloroform, combined brominated trihalomethanes, and total trihalomethanes and measures of neuropsychological development based on the Bayley Scales of Infant Development in one year olds and the McCarthey Scales of Children's Abilities in four to five year olds. Linear regression was used to estimate associations adjusting for a number of covariables (e.g., maternal age, height, weight, intelligence, SES, smoking and alcohol consumption, etc) in 1855 subjects at one year, and 1453 subjects at four to five years of age. Most of the evaluated associations were null. However, the association between a doubling of all-route total THM exposure and cognitive score was significant ($p<0.05$) with a decrease in cognitive score of -0.54 (95%CI -1.03 to -0.05) points. The investigators also found a statistically significant decrease in cognitive score of −0.64 (−1.16 to −0.12) points associated with a doubling of all-route total brominated THMs. The investigators acknowledge that the results should be cautiously interpreted and that chance cannot be ruled out given the small magnitude of the association and the large number of tests performed.

Effects in Animals

BDCM is a central nervous system toxicant at high 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 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 non-specific anesthetic effect, similar to that produced by various other volatile halocarbons (ATSDR, 2018).

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, with six to eleven mice/treatment. They 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 treatment at 100 mg/kg-day; and 60 days 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 non-gavaged control group was included in the 14 and 90 day experiments in addition to a vehicle-control group.

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 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).

The results of this study indicate 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 non-progressive decrease (with development of partial tolerance) in the operant conditioning response rates.

Moser *et al.* (2007) examined the potential neurotoxicity of BDCM and dibromoacetonitrile in F-344 rats. BDCM and dibromoacetonitrile were given to male and female F-344 rats via drinking water for 6 months. The average intakes were approximately 9, 27, and 72 mg/kg-day for BDCM, and 5, 12, and 29 mg/kg-day for dibromoacetonitrile, respectively. Few neurobehavioral changes were observed, and those were not considered toxicologically relevant. Treatment-related neuropathological changes were absent. The authors concluded that neurotoxicity may not be a toxicity concern for halomethanes or haloacetonitriles.

Chronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on chronic toxicity through exposure to BDCM by humans.

Effects in Animals

The chronic oral toxicity of BDCM has been assessed in several studies: a dietary study conducted in male and female rats, two gavage studies in male and female rats and mice, and a drinking water study in male rats and female mice (Table 7.8). No data are available on the chronic inhalation toxicity of BDCM.

Table 7.8. Summary of Chronic Toxicity Studies on BDCM

Route	Species Strain	Sex	N	Dose (mg/kg-day)	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Gavage (oil)	Rat F344/N	M, F	50	0, 50, 100	102 weeks	-	50: kidney and liver lesions	NTP (1987)

Route	Species Strain	Sex	N	Dose (mg/kg-day)	Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Gavage (oil)	Mouse $B6C3F_1$	M, F	50	0, 25, 50	102 weeks	-	25: liver, kidney, and thyroid lesions	NTP (1987)
Diet	Rat Wistar	M, F	40	M: 0, 6, 26, 138 F: 0, 8, 32, 168	24 months	-	6: increased relative liver weight; liver fatty degeneration and granuloma	Aida *et al.* (1992b)
Drinking water	Rat F344/N	M	50	0, 6, 12, 25	105 weeks	25	--	NTP (2006)
	Mouse $B6C3F_1$	F	50	0, 9, 18, 36	105 weeks	36	--	

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 investigators estimated average doses as 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 the first month until study termination. Relative liver weight was significantly increased at the mid-dose and high dose at each time point in both males and females. 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 7.9. Dose-related fatty degeneration and granuloma were observed in males and females at 24 months. Cholangiofibrosis was observed at the high dose. A LOAEL of 6 mg/kg-day for histopathological changes was identified in both sexes.

Table 7.9. 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 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 the vehicle controls. Mean body weights of high-dose male and female rats were decreased compared to the vehicle controls starting at 15 and 32 weeks, respectively. Final mean body weights in high-dose males and females were 88 and 79 percent of the controls, respectively. No compound-related clinical signs were reported. In males, compound-related non-neoplastic 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 liver and kidney histopathology.

NTP (1987) also assessed chronic effects of BDCM in male and female $B6C3F_1$ mice (50/sex/dose) gavaged in corn oil 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 the vehicle controls. 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 controls, respectively. No compound-related clinical signs were reported. Compound-related non-neoplastic 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.

NTP (2006) exposed groups of 50 male F344/N rats to target concentrations of 0, 175, 350, or 700 mg/L BDCM in drinking water for two years. These exposures were equivalent to average doses of about 0, 6, 12, or 25 mg/kg-day. There were no reported differences in survival of exposed groups compared to the controls. Mean body weights of all exposed and control groups were similar, although water consumption was less than that of the controls throughout the study in all exposed groups. Decreased water consumption was attributed by the authors to poor palatability of the treated water. The incidences of chronic inflammation in the livers of the 12 and 25 mg/kg dose groups were significantly greater than that in the controls. Although the authors considered the biological significance of the elevated chronic liver inflammation uncertain, OEHHA considers the elevated incidence of chronic liver inflammation as a possible sign of toxicity. The NTP (2006) report notes that the chronic inflammation is considered consistent with spontaneous inflammatory foci observed in aged rats and considered a result of “bacterial showering from the intestinal tract”. Nonetheless, the incidence is significantly increased with dose. Thus, a NOAEL of 6 mg/kg-day can be inferred based on this endpoint.

NTP (2006) exposed groups of 50 female B6C3F_1 mice to target concentrations of 0, 175, 350, or 700 mg/L BDCM in drinking water for two years. These concentrations equate to average doses of 0, 9, 18, or 36 mg/kg-day. Survival of the exposed and control groups was similar. From week 4 on, the mean body weights of all exposed groups were less than the controls. Water consumption by the exposed mice was less than that of the controls throughout the study, which was attributed by the authors to poor palatability of the water. The authors reported no non-neoplastic effects on tissues, resulting in a NOAEL of 36 mg/kg-day.

NTP (2007) conducted toxicology studies of BDCM in genetically modified (FVB Tg.AC hemizygous) mice by dermal, drinking water, and gavage administration. This strain of mice are hemizygous for a mutant v-Ha-*ras* transgene, and prone to cancer especially of the skin. In the

dermal studies, groups of 15 Tg.AC hemizygous mice of both sexes were dermally administered 0, 64, 128, or 256 mg/kg BDCM in acetone, 5 days/week for 26 weeks, and groups of 10 mice of each sex were dermally administered the same doses 5 days/week for 39 weeks. There were no reported differences in survival and mean body and organ weights between the controls and all dosed groups of males and females. The investigators did not observe statistically or biologically significant increases in the incidences of non-neoplastic lesions or neoplasms.

In the drinking water studies, groups of 15 Tg.AC hemizygous mice of both sexes were exposed to drinking water containing 0, 175, 350, or 700 mg/L BDCM for 26 weeks, yielding estimated average daily doses of 20, 36, or 61 mg/kg for males and 31, 61, or 130 mg/kg for females. No differences in survival of exposed and control mice were observed. Males exposed at the two highest doses weighed less than controls, which may have been due to decreased water consumption due to poor palatability. Decreased absolute heart and right kidney weights of exposed males relative to the controls was observed. Increased incidences of hepatocyte fatty change and hypertrophy at 350 and 700 mg/L and cytoplasmic vacuolization at 700 mg/L in females were reported. In males, the investigators observed increased incidences of renal tubule dilatation at exposures of 175 mg/L or greater, increased renal tubule hypertrophy at the two highest doses, and increased nephropathy and renal tubule degeneration at the highest dose.

Groups of 10 Tg.AC hemizygous mice of both sexes were also exposed to drinking water containing 0, 175, 350, or 700 mg/L BDCM for 42 weeks, yielding average daily doses of about 18, 33, or 64 mg/kg for males and 28, 49, or 111 mg/kg for females. No differences in survival between control and treated animals were observed. Males in the 350 and 700 mg/L groups weighed less than the controls at the end of the study. Water consumption decreased with increasing exposure concentration, which the investigators believed was due to poor palatability. The investigators reported increased incidences of hepatocyte fatty change in all exposed groups of females, renal tubule dilatation in all exposed groups of males, and nephropathy in the high-dose males.

In the gavage studies, groups of 15 Tg.AC hemizygous mice of both sexes were given 0, 25, 50, or 100 mg BDCM/kg in corn oil 5 days/week for 26 weeks. No differences in survival between the controls and treated animals were observed. The incidence of multiple squamous cell papilloma of the forestomach in 100 mg/kg females was significantly greater than that in the vehicle controls. The investigators reported increased incidences of hepatocyte fatty change in all dosed groups of females, hepatocyte cytoplasmic vacuolization in low and mid-dose females, renal tubule hypertrophy in high dose females, and renal tubule degeneration in high-dose males.

Groups of 10 Tg.AC hemizygous mice of both sexes were also gavaged with 0, 25, 50, or 100 mg/kg BDCM in corn oil 5 days/week for 41 weeks. No differences in survival were noted. In females, increased incidences of multiple squamous cell papillomas of the forestomach were observed relative to the controls at 25 and 100 mg/kg, and of all squamous cell papillomas of the forestomach at 100 mg/kg. The investigators reported increases incidences of hepatocyte

cytoplasmic vacuolization at 50 mg/kg and hepatocyte fatty change at 50 and 100 mg/kg. The incidence of renal tubule degeneration in 100 mg/kg males was also significantly greater than that in the vehicle-control group.

NTP (2007) also conducted toxicology studies of BDCM in genetically modified p53 haploinsufficient mice through drinking water and gavage routes. This genetically modified mouse strain contains a mutant allele for the p53 gene, which codes for the p53 protein that acts as a tumor suppressor. Groups of 15 p53 haploinsufficient mice of both sexes were exposed to drinking water containing 0, 175, 350, or 700 mg/L BDCM for 26 weeks, yielding estimated average daily doses of 16, 31, or 65 mg/kg for males and 26, 50, or 100 mg/kg for females. No differences in survival between the controls and treated mice were noted. The investigators observed increased incidences of renal tubule dilatation in all exposed groups of males, renal tubule degeneration in mid- and high-dose males. The incidence of fatty change in hepatocytes in high-dose females was significantly greater than in controls.

Groups of 10 p53 haploinsufficient mice of both sexes were also exposed to drinking water containing 0, 175, 350, or 700 mg/L for 42 weeks, yielding estimated doses of 14, 30, or 55 mg/kg for males and 22, 43, or 98 mg/kg for females. No differences in survival between control and treated mice were noted. Significantly increased incidences of renal tubule degeneration in 350 and 700 mg/L males relative to control were observed.

In the gavage studies, groups of 15 p53 haploinsufficient mice of both sexes were administered 0, 25, 50, or 100 mg/kg BDCM in corn oil for 26 weeks. No differences in survival were reported. Decreased absolute heart, right kidney, and right testis weights and increased renal tubule degeneration in 100 mg/kg males relative to controls were reported. The absolute liver weight and incidence of fatty changes in the liver of 100 mg/kg females was significantly greater than in controls.

Groups of 10 p53 haploinsufficient mice of both sexes were also administered 0, 25, 50, or 100 mg/kg in corn oil by gavage for 41 weeks. Increased absolute liver weight and incidences of fatty liver changes in 100 mg/kg females relative to vehicle controls were reported. Significantly increased incidences of hepatocyte fatty change and renal tubule degeneration and nephropathy in 100 mg/kg males relative to vehicle controls were observed.

Carcinogenicity

Classification of Carcinogenic Potential

BDCM has been listed since 1990 as a carcinogen under Proposition 65. IARC (1991, 1999a) concluded there was sufficient evidence in experimental animals for BDCM carcinogenicity but inadequate human data, thereby classifying BDCM as possibly carcinogenic to humans (Group 2B). In 1993, US EPA (2018b) classified BDCM as a probable human carcinogen, Group B2, on the same basis as IARC. NTP (2016b) classified BDCM as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. The WHO (2008) drinking water guideline for BDCM cites the IARC Group 2B classification. Health

Canada (2006) considered BDCM to be a probable carcinogen in humans, with sufficient evidence in animals and inadequate evidence in humans.

The rest of this chapter lays out data from carcinogenicity bioassays on BDCM, after noting the lack of epidemiological data on exposures to BDCM alone, i.e., BDCM exposures that are not in conjunction with other disinfection byproducts.

Effects in Humans

No published epidemiological data were located on carcinogenicity through exposure to BDCM alone by humans.

US EPA (1997, 1998e) reviewed early epidemiological studies, examining the relationship between exposure to THMs in chlorinated drinking water and cancer mortality. The most notable findings of these studies were weak but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon and rectum. None of these studies 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 were found to be inconclusive with regard to the potential carcinogenicity of THMs in drinking water in humans by IARC (1999b), ATSDR (1997), and US EPA (2001a).

More than a dozen subsequent studies have added to the evidence of associations of THM exposure with cancer of the bladder or lower gastointestinal tract. Several meta-analyses (Villanueva *et al.*, 2003, 2004, 2006; Costet *et al.*, 2011) have reaffirmed the increased risk of bladder cancer with THM exposure. US EPA (2005a) acknowledged that the bladder cancer studies appear to provide the strongest evidence of increased cancer risk from THMs. However, these studies cannot ascribe the increased cancer risk to exposure to any specific THM or other chemical. Epidemiological studies on associations of DBP/THM exposures with cancer are reviewed in Appendix C and IARC (2013).

Effects in Animals

The database on the carcinogenic potential of BDCM consists of oral, dermal, and intraperitoneal injection bioassays (Table 7.10). No studies were identified that examined carcinogenicity in animals exposed to BDCM by inhalation.

Table 7.10. Summary of Carcinogenicity Studies on BDCM

Route	Species, strain, sex	N	Dose (mg/kg-day)	Exposure duration	Response	Reference
Drinking water	Rat	50	0, 3.9, 20.6, 36.3	104 weeks	significant increase in hepatocellular adenomas and carcinomas at low and mid-dose	George *et al.* (2002)
	Rat F344/N M	50	0, 6, 12, 25	105 weeks	no evidence of neoplastic effects	NTP (2006)

Route	Species, strain, sex	N	Dose (mg/kg-day)	Exposure duration	Response	Reference
Drinking water	Rat Wistar M, F	58	M - 100 F - 175	185 weeks	significant increases in hepatic neoplastic nodules, hepatic adenofibrosis, and lymphosarcoma in females	Tumasonis *et al.* (1987)
	Mouse	50	0, 8.1 27.2, 43.4	100 weeks	no significant increase in any tumor type	George *et al.* (2002)
	Mouse $B6C3F_1$ F	50	0, 9, 18, 36	105 weeks	no evidence of neoplastic effects	NTP (2006)
	Mouse CBA × C57B1/6 M, F	50-75	0, 0.0076, 0.76, 76	104 weeks	no significant increase in any tumor type	Voronin *et al.* (1987b)
Gavage (corn oil)	Rat F344/N M, F	50	0, 50, 100	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	102 weeks	significantly increased tumors in male kidneys and female livers	NTP (1987)
Diet	Rat Wistar M, F	40	M - 0, 6, 26, 138 F - 0, 8, 32, 168	24 months	low incidence of tumors in liver and kidney	Aida *et al.* (1992b)
IP Injection (up to 3 times/wk)	Mouse Strain A M	20	0, 20, 40, 100	8 weeks	increased incidence of lung tumors at high dose, not statistically significant	Theiss *et al.* (1977)

Oral Studies

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 females and on high-dose and vehicle-control males. Histopathology was also conducted on low-dose males in instances of premature death, on all gross lesions, and on tissues and organs where chemically-related lesions were found in the high-dose males (adrenal glands, colon, heart, kidney, liver, lung, pancreas, preputial gland, and rectum). Non-cancer effects are discussed under chronic toxicity. Statistically significant increases in incidences of adenomatous polyps and adenocarcinoma of the large intestine and renal tubular cell adenoma and carcinoma were observed in both sexes (Table 7.11). The neoplasms of the large intestine and kidney were considered biologically significant because they are uncommon tumors in F344/N rats. Historical incidence of large intestine tumors 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.

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 7.12). NTP (1987) considered these tumors to be clear evidence of carcinogenic activity under these experimental conditions.

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 (about 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 the 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 non-significantly 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.

Table 7.11. Incidence of Tumors in F344/N Rats Gavaged with BDCM in Corn Oil for 102 weeks (NTP, 1987)

Sex	Site	Tumor Type	Applied Dose (mg/kg-day)		
			0	50	100
Male	Large intestine [a,b]	Adenomatous polyp	0/50	3/49	33/50***
		Adenocarcinoma	0/50	11/49***	38/50***
		Combined adenoma and adenocarcinoma[f]	0/50	13/49***	45/50***
	Kidney [a,c]	Tubular cell adenoma	0/42	1/43	3/42

Sex	Site	Tumor Type	Applied Dose (mg/kg-day)		
			0	50	100
Male	Kidney [a,c]	Tubular cell adenocarcinoma	0/42	0/43	10/42***
		Combined adenoma and adenocarcinoma[g]	0/42	1/43	13/42***
Female	Large intestine [d]	Adenomatous polyp	0/41	0/39	7/45**
		Adenocarcinoma	0/41	0/39	6/45*
		Combined adenoma and adenocarcinoma[h]	0/41	0/39	12/45***
Female	Kidney[e]	Tubular cell adenoma	0/36	1/30	6/42*
		Tubular cell adenocarcinoma	0/36	0/30	9/42**
		Combined adenoma and adenocarcinoma[i]	0/36	1/30	15/42***

To adjust for early mortality, OEHHA excluded animals dying before observation of the first tumor at a given site for studies reporting individual animal data. If an animal died before observation of a tumor, that information is provided below.

[a] One low-dose rat died accidentally at week 33 and was dropped from the calculation.
[b] First tumor of the large intestine was recorded at week 49 for males.
[c] First tumor of the kidney was recorded at week 89 for males.
[d] The intestine was not examined in four vehicle controls and three high-dose rats. First tumor of the large intestine was recorded at week 88 for females.
[e] First tumor of the kidney was recorded at week 103 for females.
[f] Historical incidence at study laboratory (mean): 0/250; historical incidence in NTP studies: 3/1,390 (0.2 percent).
[g] Historical incidence at study laboratory (mean): 1/250 (0.4 percent); historical incidence in NTP studies: 8/1,448 (0.6 percent).
[h] Historical incidence at study laboratory: 0/236; historical incidence in NTP studies: 0/1,400.
[i] Historical incidence at study laboratory (mean): 0/250; historical incidence in NTP studies: 2/1,447 (0.1 percent).
*$p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$, Fisher exact comparison between treated and control group.

Table 7.12. Incidence of Tumors in B6C3F$_1$ Mice Gavaged with BDCM in Corn Oil for 102 weeks (NTP, 1987)

Sex	Site	Tumor type	Applied Dose (mg/kg-day)		
			0	**25**	**50**
Male	Kidney[a]	Tubular cell adenoma	1/33	2/32	6/42
		Tubular cell adenocarcinoma	0/33	0/32	4/42
		Combined adenoma and adenocarcinoma[c]	1/33	2/32	9/42*
			0	**75**	**150**
Female	Liver[b]	Hepatocellular adenoma	1/45	13/44***	23/37***
		Hepatocellular carcinoma	2/45	5/44	10/37**
		Combined adenoma and carcinoma[d]	3/45	18/44***	29/37***

To adjust for early mortality, OEHHA excluded animals dying before observation of the first tumor at a given site for studies reporting individual animal data. If an animal died before observation of a tumor, that information is provided below.
[a] Mice not included in the tumor incidence were two controls and one low-dose that died in week one, and one control that died during week nine and one that escaped in week 79.
[b] First tumor of the liver was recorded at week 62 for females.
[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).
*$p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$, Fisher exact comparison between treated and control group.

Voronin *et al.* (1987b, as cited in US EPA, 2005a) 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 US 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.

George *et al.* (2002) investigated carcinogenicity of BDCM in male F344/N rats and male B6C3F$_1$ mice. Groups of 50 animals/dose were provided with BDCM in drinking water at 0.05, 0.25 or 0.5 g/L for mice and 0.07, 0.35 or 0.70 g/L for rats, plus 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 mg/kg-day 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.

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 non-neoplastic 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, marginal at the mid-dose, and with no effect at the high dose. The authors speculated 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 cytochrome 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/N 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/N rat. Cytochrome P450 levels mirrored effects on CYP2B1 levels.

NTP (2006) exposed groups of 50 male F344/N rats to target concentrations of 0, 175, 350, or 700 mg/L BDCM (equivalent to average doses of about 0, 6, 12, or 25 mg/kg-day) in drinking water for two years (105 weeks). Survival of exposed groups was similar to that of the controls. Mean body weights of all exposed groups were similar to those of the controls throughout the study, although water consumption was less than that of the controls throughout the study in all groups. The decreases were attributed by the authors to poor palatability of the treated water. No increased incidences of neoplasms that could be attributed to BDCM were observed. The incidences of chronic liver inflammation in the 12 and 25 mg/kg dose groups were significantly greater than that in the controls. The biological significance of these increases was considered uncertain. The authors concluded that there was no evidence of carcinogenic activity of BDCM in male F344/N rats under the conditions of this two-year drinking water study, with BDCM doses up to 25 mg/kg-day.

NTP (2006) exposed groups of 50 female B6C3F_1 mice to target concentrations of 0, 175, 350, or 700 mg/L BDCM (equivalent to average doses of about 9, 18, or 36 mg/kg-day) in drinking water for two years. Survival of exposed groups was similar to that of the controls. Mean body weights of all exposed groups were generally less than those of the controls from week 4 through the end of the study. Water consumption by the exposed mice was less than that of the controls throughout the study. The authors attributed the decreases to poor palatability of the water. There was a dose-related negative trend in incidence of hepatocellular adenoma or carcinoma (combined). The incidence in the 36 mg/kg group was significantly decreased compared to controls, and the incidence of hemangiosarcoma in all organs was significantly decreased in the 18 mg/kg group. The authors concluded that there was no evidence of carcinogenic activity of BDCM in female B6C3F_1 mice under the conditions of this two-year drinking water study, with doses up to 36 mg/kg-day.

Intraperitoneal Injection Study

Theiss *et al.* (1977) examined the carcinogenic potential of BDCM in male Strain A mice. Mice (20/group) were given 20, 40, or 100 mg/kg BDCM by intraperitoneal injection up to three times/week 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 per mouse was increased at the highest dose of 100 mg/kg (0.85 ± 0.27) compared to the controls (0.27 ± 0.15), but did not reach statistical significance ($p = 0.067$).

Studies using Transgenic Animals

NTP (2007) exposed male and female Tg.AC heterozygous mice to DBCM for 6 or 9 months dermally, by drinking water or by gavage, and exposed p53 haploinsufficient mice to BDCM by drinking water or by gavage for the same treatment durations. These two mouse strains have been shown to be susceptible to the rapid development of cancer; these studies were part of an evaluation by the National Institute of Environmental Health Sciences (NIEHS) and the NTP of these strains as models for identifying chemical toxicity and/or chemical carcinogenic processes with less-than-lifetime studies. An additional study objective was to determine whether the use of genetically modified mice could provide more insight on the apparent discrepancy in carcinogenicity results between studies in which BDCM was given in the drinking water compared to by gavage.

The Tg.AC strain of mice is hemizygous for a mutant v-Ha-*ras* transgene and is regarded as a genetically initiated model. Point mutations in the Ha-*ras* gene are believed to be early events in the induction of skin papillomas and malignancies. The heterozygous B6.129-*Trp53* $(N12)^{tm1Brd(+/-)}$ mice have a null mutation in one p53 allele, which provides a target for mutagens because the p53 cancer suppressor gene therefore has only a single functional wild-type p53 allele. The heterozygous $p53^{(+/-)}$ mice develop normally but develop increased cancer (primarily lymphomas or sarcomas) with age, often with decreased latency.

For the 26-week exposures of Tg.AC hemizygous mice, groups of 15 males and 15 females were dermally administered BDCM at 0, 64, 128, or 256 mg/kg in acetone, five days/week, given drinking water containing 0, 175, 350, or 700 mg/L BDCM (equivalent to average daily doses of about 20, 36, or 61 mg/kg to males and 31, 61, or 130 mg/kg to females), or were administered 0, 25, 50, or 100 mg/kg BDCM in corn oil by gavage, five days/week. Groups of 10 male and 10 female Tg.AC hemizygous mice were treated for 39 to 42 weeks by all three routes with the same doses and treatment regimens; the drinking water exposure yielded average daily doses of approximately 18, 33, or 64 mg/kg to males and 28, 49, or 111 mg/kg to females.

For the 26-week exposures of p53 haploinsufficient mice, groups of 15 males and 15 females were given drinking water containing 0, 175, 350, or 700 mg/L BDCM (equivalent to average daily doses of about 16, 31, or 65 mg/kg to males and 26, 50, or 100 mg/kg to females), or gavaged with 0, 25, 50, or 100 mg/kg body BDCM in corn oil five days/week. Groups of 10 male and 10 female p53 haploinsufficient mice were exposed to the same concentrations of BDCM in drinking water for 42 weeks (equivalent to approximately 14, 30, or 55 mg/kg to males and 22, 43, or 98 mg/kg to females), or to the same gavage doses described above for 41 weeks.

Tissues from 15 sites were examined for every animal. The dermal and drinking water exposures of Tg.AC hemizygous mice produced no increases in neoplasms. The gavage exposures produced increases in squamous cell papillomas of the forestomach in females which were not considered as tumors by the authors. The drinking water and gavage exposures of p53 haploinsufficient mice also produced no increases in neoplasms. Kidney and

liver non-neoplastic effects are described above in the chronic toxicity section. NTP (2007) concluded that BDCM did not cause cancer in the genetically modified mice in these studies.

8. TOXICOLOGICAL PROFILE: DIBROMOCHLOROMETHANE

This chapter presents the toxicological profile for dibromochloromethane. The database for toxicological effects of DBCM in humans is limited. The primary DBCM exposure is through ingestion of tap water, where DBCM 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, described in OEHHA (2016), as well as cancer epidemiology studies of disinfection byproducts (reviewed in IARC, 2013). The industrial production and use of DBCM are limited, and there are no toxicity data from occupational studies. Therefore, the majority of the toxicological data on DBCM presented in this chapter come from experimental animal studies.

The toxicological effects of DBCM in experimental animals have been comprehensively reviewed in documents prepared by public health organizations and regulatory bodies (US EPA, 1980b, 1987, 2018d; ATSDR; 2005, and IARC, 1991, 1999a). This chapter relies on these reviews and on updated literature identified by OEHHA through searches.

Acute Toxicity

Effects in Humans

No published experimental or epidemiological data were located on acute toxicity of DBCM exposure in humans.

Effects in Animals

The acute oral toxicity of DBCM has been studied in rats and mice. Acute lethality results are summarized in Table 8.1. Bowman *et al.* (1978) administered DBCM solubilized in a 1:1:8 solution of Emulphor:alcohol:saline to groups of 10 male and female fasted ICR Swiss mice, at dose levels from 500 to 4,000 mg/kg. LD_{50} values were 800 mg/kg and 1,200 mg/kg for males and females, respectively. Ataxia, sedation, and anesthesia occurred within 30 minutes of treatment at 500 mg/kg and above. Necropsy on animals that died revealed apparent fatty infiltration of the liver, pale kidneys, and hemorrhage in the adrenals, lungs, and brain.

Table 8.1. Oral LD_{50} Values for DBCM

Species	Strain	Route (vehicle)	Sex	Number per dose group	LD_{50} in mg/kg (95 percent CI)	Reference
Rat	Sprague-Dawley	Gavage (corn oil)	M	10	1,186 (997-1,421)	Chu *et al.* (1980)
			F	10	848 (576-1,090)	
	F344/N	Gavage (corn oil)	M	5	Not reported	NTP (1985)
			F	5		

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

Chu *et al.* (1980, 1982a) administered DBCM by gavage to 10 SD rats of both sexes at doses of 546 to 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 and 848 mg/kg , respectively. Gross pathological examination revealed liver and kidney congestion in treated animals.

The NTP (1985) administered DBCM at doses from 160-2,500 mg/kg in corn oil by gavage to male and female F344/N rats and $B6C3F_1$ mice, but did not compute LD_{50}s. All rats at the highest dose died, and four male rats and one female rat died at 1,250 mg/kg. 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. 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 an LD_{50} of 145 mg/kg in male golden hamsters, but did not provide experimental details. 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 and the recovery time course. Male Wistar rats received 0, 83, 167, 333, or 667 mg/kg DBCM by gavage in olive oil. Heart rate, blood pressure, body temperature, and physical activity were monitored in six conscious rats/dose group by telemetry. Observations included premature ventricular contractions following 667 mg/kg DBCM, decreased heart rate and body temperature in all treatment groups, with variable recovery, increases in blood pressure initially in all treatment groups with recovery toward control values or decreases below control (high dose) within 48 hours, and depressed physical activity.

Short-Term Toxicity

Effects in Humans

No published epidemiological data were located on short-term toxicity of DBCM exposure in humans.

Effects in Animals

The short-term toxicity of DBCM has been evaluated in nine studies ranging from three days to one month in duration (Table 8.2).

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 (estimated daily doses of DBCM were 0, 0.7, 8.5, or 68 mg/kg-day, calculated by the authors). 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. Mean final body weight and absolute and relative spleen and thymus weights was significantly decreased in high-dose males. Relative liver weight was significantly increased in males and females at the mid-dose and high dose. Statistically significant changes in high-dose males and females were decreased fibrinogen and serum glucose, and increased serum aspartate aminotransferase and alanine aminotransferase activities. The NOAEL and LOAEL are 50 and 125 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. No treatment-related clinical signs were reported, and body weight was unaffected by DBCM 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. 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 NOAEL and LOAEL were 74 and 147 mg/kg-day, respectively, based on kidney and liver histopathological changes.

Table 8.2. Summary of Short-Term Toxicity Studies on DBCM

Route	Species	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley	M	10	28 days	0, 0.7, 8.5, 68	68	--	Chu *et al.* (1982a)
	Mouse $B6C3F_1$	F	10	11 days	0, 171	--	171: liver histopathology	Coffin *et al.* (2000)
Diet	Rat Wistar	M	7	1 month	0, 18, 56, 173	18	56: liver histopathology	Aida *et al.* (1992a)
		F	7	1 month	0, 34, 101, 333	34	101: liver histopathology	
Gavage (water)	Mouse CD-1	M, F	8-12	14 days	0, 50, 125, 250	50	125: decreased body weight and immune function; increased relative liver weight	Munson *et al.* (1982)
	Rat F344/N	M	4	1, 3, or 7 days	0, 156, 312	156	312: decreased body weight after 7 days	Potter *et al.* (1996)
Gavage (corn oil)	Rat F344/N	M, F	5	14 days	0, 60, 125, 250, 500, 1,000	125	250: reduced male body weight	NTP (1985)
	Mouse CD-1	M	8-16	14 days	0, 37, 74, 147	74	147: liver, kidney, and stomach histopathology	Condie *et al.* (1983)
	Mouse $B6C3F_1$	M, F	5	14 days	0, 30, 60, 125, 250, 500	60	125: liver and kidney gross pathology	NTP (1985)
		F	10	11 days	0, 100, 300	--	100: increased labeling index, liver histopathology	Coffin *et al.* (2000)
			10	3 weeks (5 d/wk)	0, 50, 100, 192, 417	100	192: liver histopathology	Melnick *et al.* (1998)

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) administered DBCM by gavage in corn oil in B6C3F$_1$ mice (5/sex)at doses of 0, 30, 60, 125, 250, or 500 mg/kg-day in corn oil for 14 days. Mice given the high dose exhibited ataxia, lethargy, and labored breathing, and four males and three females at the high dose died before study termination. 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 capsules. 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. 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.

Statistically significant and dose-related changes in serum chemistry observed in males included decreased non-esterified fatty acids (high dose), triglycerides (high dose), alkaline phosphatase activity (mid-dose and high dose) and cholinesterase (high dose) and significantly increased total cholesterol (mid-dose and high dose). Significant, dose-related changes in females included total triglycerides (high dose), total cholesterol (all doses), alkaline phosphatase (mid-dose 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 and females. Liver cell vacuolization appeared to be the most sensitive response. The NOAELs reported by the investigators in this study are 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. Note that increased relative liver weight compared to controls was statistically significant in females at all doses; thus, a NOAEL based on relative liver weight is not identified and 34 mg/kg-day would be a LOAEL for this effect.

Potter *et al.* (1996) evaluated the effect of DBCM on hyaline droplet formation, testosterone levels, and cell proliferation in the kidney of male F344/N 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. 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.

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. No compound-related clinical signs of toxicity were observed. 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 for these effects. However, relative liver weight was slightly but statistically significantly elevated at 100 mg/kg-d relative to controls; thus, 100mg/kg-day is a LOAEL for increased relative liver weight.

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. A statistically significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage, but not by drinking water, compared to the control. 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.

Subchronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on subchronic toxicity of DBCM exposure in humans.

Effects in Animals

Four published studies have addressed the subchronic toxicity of DBCM through oral exposure in rats or mice (Table 8.3). 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.

Table 8.3. Summary of Subchronic Toxicity Studies on DBCM

Route	Species	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley	M	20	90 days	0, 0.57, 6.1, 49, 224	49	224: hepatic lesions	Chu *et al.* (1982b)
		F	20	90 days	0, 0.69, 7.5, 59, 237	59	237: hepatic lesions	
Gavage (corn oil)	Rat F344/N	M, F	10	13 weeks	0, 15, 30, 60, 125, 250	30	60: hepatic lesions in males	NTP (1985)
	Rat Sprague-Dawley	M, F	10	90 days	0, 50, 100, 200	-	50: hepatic lesions in males and renal lesions in females	Daniel *et al.* (1990)
	Mouse $B6C3F_1$	M, F	10	13 weeks	0, 15, 30, 60, 125, 250	125	250: hepatic and renal lesions in males	NTP (1985)

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 rats were sacrificed at the end of the exposure period and the rest 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.

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 controls. The response was weakly dose-related in males (trend test, $p<0.1$) (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 2500 ppm females. Although the authors noted that histologic changes were mild and similar to the 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 controls. 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), statistically significant by the Fisher exact test at ≥ 60 mg/kg-day. 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 were not significantly affected at any dose. Necrosis and vacuolar change (interpreted by the study authors as fatty metamorphosis) were observed in 5/10 males receiving the high dose. In addition, toxic nephropathy characterized by tubular degeneration or mineralization was observed in 5/10 high-dose males. No similar hepatic or renal lesions were 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 were gavaged with 50, 100, or 200 mg/kg-day DBCM in corn oil for 90 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.

DeAngelo *et al.* (2002) demonstrated that brominated THMs administered in the drinking water significantly induced the incidence of preneoplastic aberrant crypt foci, a type of early putative preneoplastic lesion, 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. Azoxymethane served as a positive control. Aberrant crypt foci incidence (percent) and number (aberrant crypt foci/colon) were: combined controls, 0%, 0; Azoxymethane, 100%, 27.17 $\pm$ 6.28 ($p < 0.01$); chloroform, 16.7%, 0.17 $\pm$ 0.17; BDCM, 83.3%, 1.50 $\pm$ 0.56 ($p < 0.01$); DBCM, 50%, 1.17 $\pm$ 0.65 ($p < 0.01$); bromoform, 66.7%, 1.17 $\pm$ 0.40 ($p <$

0.01). THM-induced aberrant crypt foci occurred primarily (92%) in the rectal segment of the colon.

To evaluate whether THM-induced aberrant crypt foci could be promoted by a diet high in saturated animal fat, Geter *et al.* (2004c) exposed male F344/N rats 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 in drinking water for 26 weeks, with half the animals fed the normal feed, 4.5% fat Purina 5001, and the other half receiving the feed supplemented with 19% animal fat. Compared to control animals, all groups administered brominated THMs showed significant increases in aberrant crypt foci induction. There was a statistically significant association between the number of aberrant crypt foci per colon and the number of bromine atoms on the THM molecule. Among animals exposed to DBCM, no difference was observed in the number of aberrant crypt foci between animals fed the normal diet and those fed the high-fat diet. Consistent with the findings of DeAngelo *et al.* (2002), THM-induced aberrant crypt foci were found primarily in the medial and distal segment of the colon, the observed site of THM-induced neoplasia.

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 chromosomal 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 is mutagenic and genotoxic.

In Vitro Assays

The genotoxicity of DBCM has been evaluated in numerous *in vitro* assays in bacteria and eukaryotic cells (Table 8.4). 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 8.4. Summary of *In Vitro* Mutagenicity and Genotoxicity Studies on DBCM

Endpoint	Assay system	Results (with/without activation)	References
Gene mutation – Bacteria *S. 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	+/+	Varma *et al.* (1988)

Endpoint	Assay system	Results (with/without activation)	References
Gene mutation – Bacteria *S. typhimurium*	TA98, TA100	+/-	Varma *et al.* (1988)
	TA97, TA98, TA100, T102	-/-	Mersch-Sundermann (1989)
	TA100 (fluctuation test)	-/-	Le Curieux *et al.* (1995)
Gene mutation – Mammalian	Mouse lymphoma cells[a]	NT/+	McGregor *et al.* (1991)
	Mouse lymphoma cells	+/(+)	Sofuni *et al.* (1996)
Chromosomal 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)
	Chinese hamster lung fibroblast aneuploidy	-/+	Matsuoka *et al.* (1996)
	Aspergillus nidulans aneuploidy	NT/+	Benigni *et al.* (1993)
DNA damage	*E. coli* PQ37, SOS chromotest (induction of SOS genes)	-/-	Mersch-Sundermann *et al.* (1989)
	E. coli PQ37, SOS chromotest (induction of SOS genes)	+/+	Le Curieux *et al.* (1995)
	Human lung epithelial cells (comet assay)	NT/-	Landi *et al.* (2003)
	Human lymphoblastic leukemia cells (DNA alkaline unwinding assay)	NT/+	Geter *et al.* (2004a)
	Human HepG2 hepatoma cells (single cell gel electrophoresis)	NT/+	Zhang *et al.* (2012)
Sister chromatid exchange	Human lymphocytes	NT/+	Morimoto and Koizumi (1983)
	Human lymphocytes	+/NT	Sobti (1984)
	Rat liver cells	+/NT	
	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)	-/-	

NT = Not Tested; NA = Not Applicable.
(+) weakly positive response.
[a] Assay was conducted in a closed system.

DNA Damage

Le Curieux *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.*, 2004a). In contrast, Mersch-Sundermann *et al.* (1989) found no induction of DNA damage by BDCM using the same assay. Geter *et al.* (2004a) reported negative results for DNA strand breaks in primary rat hepatocytes exposed to 5 or 10 mM DBCM for four hours. Zhang *et al.* (2012) reported that DBCM increased DNA strand breakage in human HepG2 hepatoma cells incubated with 10, 100, or 1000 µM DBCM for 4 hours.

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 measure a mutagenic response was 57 µmol in these studies. 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 non-mutagenic 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. Le Curieux *et al.* (1995) obtained negative results with and without metabolic activation in TA100 in the fluctuation test modification of the reverse mutation assay.

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 RSJ100 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 that contains the plasmid pKM101 and which does not express GSST1-1) for comparison. In contrast to the DBCM induced mutations in RSJ100, 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 RSJ100 mediated the mutagenicity of DBCM and induced a specific type of mutation in *Salmonella*.

Gene Mutation in Yeast

Nestmann and Lee (1985) investigated mutagenicity of DBCM in *S. cerevisiae* strains D7 and XV185-14C at 11 to 5,700 µM with and without exogenous metabolic activation. The results for conversion in strain D7 were weakly positive without metabolic activation. Results for strain XV185-14C were negative with and without activation.

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.

Chromosomal 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 chromosomal aberrations in Chinese hamster lung fibroblast (CHL/IU) cells with but not without metabolic activation when exposed to DBCM in tightly capped flasks.

Positive results for aneuploidy were obtained in Chinese hamster lung fibroblasts without, but not with exogenous metabolic activation (Matsuoka *et al.*, 1996). Benigni *et al.* (1993) obtained positive results for aneuploidy in *Aspergillus nidulans*.

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 presence and absence of metabolic activation.

Effects in Humans In Vivo

Kogevinas *et al.* (2010) reported an increase in micronuclei in peripheral blood lymphocytes one hour after swimming for 40 minutes in an indoor chlorinated pool. The increase in micronuclei was associated with exhaled concentrations of DBCM (p = 0.05), BDCM (p = 0.03), and

bromoform (p = 0.01), but not chloroform. DNA damage in peripheral blood lymphocytes (comet assay) and micronuclei in exfoliated urothelial cells 2 weeks after swimming were not associated with THM exposures.

In Vivo Assays

In vivo data for DBCM genotoxicity are available for chromosomal 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 8.5.

Table 8.5. Summary of *In Vivo* Genotoxicity Studies on DBCM

Endpoint	Assay system	Result	References
Micronuclei induction	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
	Mouse, rat, bone marrow cells	-	Ishidate *et al.* (1982)
	Mouse, bone marrow cells	-	Hayashi *et al.* (1988)
	Newt, peripheral erythrocytes	-	Le Curieux *et al.* (1995)
Chromosomal 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 (comet assay)	Rat, renal cells	-	Potter *et al.* (1996)
	Zebrafish, whole embryos	+	Teixidó *et al.* (2015)
	Mouse liver, brain	+	Sekihashi *et al.* (2002)
	Mouse stomach, colon, liver, kidney, lung, bladder, bone marrow	-	
	Rat stomach, colon, liver, kidney, bladder, lung	+	
	Rat brain, bone marrow	-	
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al.* (1997)
Sex-linked recessive lethal mutation	Drosophila	+	Foureman *et al.* (1994)

DNA Damage

Potter *et al.* (1996) examined the effect of DBCM on DNA strand breakage in kidney cells of male F344/N 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 four hours following the administration of 0.3 or 0.6 mM/kg of DBCM as a single oral gavage dose in 0.5 percent Emulphor® (Geter *et al.*, 2004a).

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.

Teixidó *et al.* (2015), using zebrafish embryos, observed statistically significant (though weak) induction of DNA damage following 72-hour exposure to DBCM (0.16 mM, EC_{50} value for teratogenic effects) relative to control. It should be noted that the response resulted from a concentration several orders of magnitude greater than the reported blood levels of THMs in exposed humans.

Chromosomal Aberrations

Fujie *et al.* (1990) assessed chromosomal 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 chromosomal breaks. A more pronounced response was observed following a single ip dose, with statistically significant effects occurring at 20.8 mg/kg.

Micronuclei 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. Le Curieux *et al.* (1995) obtained negative results for induction of micronuclei in *Pleurodeles waltl* larvae.

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.

Developmental and Reproductive Toxicity

Effects in Humans

Many epidemiological studies have investigated potential links between exposure to THMs (as disinfection byproducts in drinking water) and adverse reproductive or developmental outcomes in humans, summarized in OEHHA (2016). DBCM is one of the DBPs considered in the epidemiology studies. Although many of the studies measure individual THMs and not just total THMs or other DBPs, it is difficult to attribute observed associations to any one of the DBPs given the mixed exposures.

Effects in Animals

The database for DBCM reproductive and/or developmental toxicity in animals consists primarily of three oral studies, summarized in Table 8.6.

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.

Table 8.6. Summary of Developmental and Reproductive Toxicity Studies on DBCM

Route	Species Strain	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Mouse ICR Swiss	M F	10 30	During premating, mating, gestation, and lactation	0, 17, 171, 685	--	17: marginal - reduced F/2b body weight on postnatal days 14 and 21	Borzelleca and Carchman (1982)

Route	Species Strain	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Drinking water	Rat Sprague-Dawley	M	10	29 days	4.2, 12.4, 28.2	28.2	--	NTP (1996)
		F	10	35 days	6.3, 17.4, 46.0	46.0	--	
		F	13	16 days GD 6 - parturition	7.1, 20.0, 47.8	47.8	--	
Gavage (corn oil)	Rat Sprague-Dawley	F	10-12	GD 6-15	0, 50, 100, 200	200	--	Ruddick *et al.* (1983)

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 GD 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 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 GD 6 to 15. Body weights were measured on GD 1 through 15, and before and after caesarean section on GD 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 GD 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 weighed. Tissues from five control and high-dose dams 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 at any dose. 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.

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 6-34. Group A females (periconception, 10/group) were treated on study days 1-34. Group A females were mated to treated males on study days 13-18 and necropsied on study day 34. Group B females (gestational exposure, 13/group) were mated on study day one to untreated males, treated from GD 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. Mean absolute body weights, feed consumption, and gross findings were comparable across all 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-dose 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 were 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.

Narotsky *et al.* (2011) assessed the combined toxicity of DBPs in F344 rats. F344 rats were treated with a mixture of four THMs (chloroform, bromodichloromethane, chlorodibromomethane, bromoform) daily by gavage on gestation days 6-20. Litters were examined postnatally. THM mixture caused pregnancy loss at ≥ 613 µmol/kg-day. However, the study used a THM mixture, and thus the design does not allow attribution of the observed effect to any particular THM.

In a study of zebrafish embryos, DBCM (0.16 mM, EC_{50} value) ranked first among the four THMs in inducing adverse developmental effects following 72-hour exposure in hermetically sealed glass vials for prevention of loss by volatilization (Teixidó *et al.*, 2015). Teratogenic

effects included malformation of the eyes, heart and tail, as well as delayed growth, movement and hatching.

Immunotoxicity

Effects in Humans

No published experimental or epidemiological data were located on immunotoxicity through exposure to DBCM by humans.

Effects in Animals

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-dose and 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 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

Effects in Humans

No published experimental or epidemiological data were located on neurotoxicity through exposure to DBCM by humans.

Effects in Animals

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 non-specific anesthetic effect like that produced by other volatile halocarbons (ATSDR, 2005).

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 non-gavaged 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 for three days pretreatment (after vehicle only), then 30 minutes after treatment with DBCM for 60 days, and for three days after the end of the DBCM dosing. 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 study period, but presented no specific data 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 non-progressive 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 DBCM doses. The animals were observed during a 10 day pretreatment period (days −9 to zero). For the acute exposure experiment, 6-7 animals/group received a single gavage dose of 0 or 50 mg/kg. For the repeated dose experiment, nine hamsters/group were gavaged with 0 or 5 mg/kg in olive oil for 14 days. Home cage activities, wheel running, locomotion, and contact with the water bottle teats were monitored continuously. 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 non-behavioral parameters differed significantly from the control. The repeated-dose animals significantly increased water bottle contacts on 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 to 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 controls. 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 the repeated-dose results.

Chronic Toxicity

Effects in Humans

No published experimental or epidemiological data were located on chronic toxicity through exposure to DBCM by humans.

Effects in Animals

The chronic oral toxicity of DBCM has been assessed in three studies: a dietary study in rats and one gavage study each in rats and mice (Table 8.7). 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.

Based on body weights at terminal sacrifice and food consumption, the doses were about 0, 10, 43, and 230 mg/kg-day for males and 0, 9, 39, and 220 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.

Relative liver weights were also increased in a dose-dependent fashion. 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-dose and high dose, and roughening of the

liver surface in high-dose males. The results suggest NOAELs of 10 and 9 mg/kg-day and LOAELs of 43 and 39 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 histopathology 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 8.8), including fatty change and "ground glass" appearance of the cytoplasm. Ground glass appearance was described in the NTP report as pink staining cytoplasm in enlarged liver cells, or pink staining hyaline droplets in the cytoplasm. A dose-related increased nephrosis was observed in females (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 8.7. Summary of Chronic Studies on DBCM

Route	Species Strain	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Diet	Rat Wistar SPF	M	40[a]	2 years	0, 10, 43, 230	10	43: decreased body weight gain; increased relative liver weight	Tobe *et al.* (1982)
		F	40[a]	2 years	0, 9, 39, 220	9	39: decreased body weight gain; increased relative liver weight	
Gavage (corn oil)	Rat F344/N	M, F	50	2 years	0, 40, 80	--	40: hepatic lesions	NTP (1985)
		M, F	50	105 weeks	0, 50, 100	--	50: hepatic and thyroid lesions	

[a] Tobe used 12 animals of each sex from the control group and 7 animals of each sex for the 24 month sacrifice to examine relative organ weight and body weight.

Table 8.8. Selected Hepatic Lesions Observed in Fischer 344/N Rats Gavaged with DBCM 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	8/50	22/50	34/50
Females			
Fatty change	12/50	23/49	50/50
Ground glass cytoplasmic change	0/50	1/50	12/50

NTP (1985) also conducted a chronic oral 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 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 remaining data for this group are inadequate for risk assessment. No compound-related clinical signs were observed. Mean final body weight was decreased in high-dose males and 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 thyroid follicular cell hyperplasia at both doses (Table 8.9). Thyroid lesions were not observed in high-dose males. The LOAEL is 50 mg/kg-day, the lowest dose, based on histopathological changes in female liver and thyroid.

Table 8.9. Selected Non-neoplastic Lesions Observed in Female B6C3F$_1$ Mice Gavaged with DBCM 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

Classification of Carcinogenic Potential

Dibromochloromethane was delisted in 1999 as a chemical known to the State of California to cause cancer after being listed in 1990 under the Safe Drinking Water and Toxic Enforcement Act of 1986, also known as Proposition 65. IARC (1999a) classified DBCM a Group 3 carcinogen, not classifiable as to its carcinogenicity to humans, based on limited animal carcinogenicity data and inadequate human data on DBCM carcinogenicity. In 1992, US EPA (2018d) classified DBCM as a possible human carcinogen, Group C, 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. The WHO (2008) drinking water guidelines are based on the IARC classification. Health Canada (2006) classified DBCM to Group IIID, possibly carcinogenic to humans, based on limited evidence for carcinogenicity in one experimental animal species and no data in humans.

Effects in Humans

No published epidemiological data were located on carcinogenicity through exposure to DBCM alone by humans.

US EPA (1997, 1998e) reviewed early epidemiological studies, examining the relationship between exposure to THMs in chlorinated drinking water and cancer mortality. The most notable findings of these studies were weak but fairly consistent associations between exposure to chlorination byproducts in drinking water and cancers of the bladder, colon and rectum. None of these studies provided a definitive conclusion about the relationship between THM exposure and cancer, due to the presence of other potentially carcinogenic chemicals in the chlorinated drinking water. IARC (1999b), ATSDR (1997), and US EPA (2001a) found these data to be inconclusive with regard to the potential carcinogenicity of THMs in drinking water in humans.

More than a dozen subsequent studies have added to the evidence of associations of THM exposure with cancer of the bladder or lower GI tract. Several meta-analyses (Villanueva *et al.*, 2003, 2004, 2006; Costet *et al.*, 2011) have also associated bladder cancer with THM exposure. US EPA (2005a) acknowledged that the bladder cancer studies appear to provide the strongest evidence of increased cancer risk from THMs. However, these studies cannot ascribe the increased cancer risk to exposure to any specific THM or other chemical. Epidemiological studies on associations of DBP/THM exposures with cancer are reviewed in Appendix C and IARC (2013).

Effects in Animals

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 102 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 non-neoplastic lesions (fatty metamorphosis and ground glass 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 102 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 differ 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 non-neoplastic lesions in the livers and thyroids are discussed above in the Chronic Toxicity section. A statistically significant increase in incidence of hepatocellular adenomas and combined adenomas and carcinomas was observed in high-dose females (Table 8.10). In male mice, a statistically significant increased incidence of hepatocellular carcinomas and combined adenomas and carcinomas was observed in the high-dose group. A negative trend in incidence of malignant lymphomas was evident in high-dose male mice compared to vehicle controls. The study authors concluded that there was equivocal evidence for carcinogenicity in males and some evidence of carcinogenicity in females.

Table 8.10. Incidence of Liver Tumors in B6C3F$_1$ Mice Gavaged with DBCM in Corn Oil 5 days/week for 105 weeks (NTP, 1985)

Liver Tumor	Applied Dose (mg/kg-day)		
Males[b]	**0**	**50[a]**	**100**
Adenoma	14/50	--	10/48
Carcinoma	10/50	--	19/48*
Adenoma or carcinoma	23/50	--	27/48
Females[c]	**0**	**50**	**100**
Adenoma	2/46	4/49	11/48**
Carcinoma	4/46	6/49	8/48
Adenoma or carcinoma	6/46	10/49	19/48**

To adjust for early mortality, OEHHA excluded animals dying before observation of the first tumor at a given site for studies reporting individual animal data. If an animal died before observation of a tumor, that information is provided below.

[a] At week 58, 35/50 male mice in the low-dose (50 mg/kg) group were accidentally killed. Thus, survival was too poor for tumor quantification in males in this group.

[b] First tumor of the liver was recorded at week 58 for males.

[c] First tumor of the liver was recorded at week 69 for females.

*$p \leq 0.05$, ** $p \leq 0.01$, Fisher exact comparison between treated and control group.

9. MECHANISMS OF ACTION OF CARCINOGENICITY

Animal studies have demonstrated that each of the THMs can produce tumors in liver or kidney. The main area of dispute involves the mechanism(s) responsible for these tumors, and whether human risk should be estimated using linear or non-linear extrapolation. A summary of the mechanistic considerations is provided here first for the brominated THMs and then for chloroform, the best-studied of these chemicals. Recent cancer risk estimates for exposure to the THMs in drinking water have assumed they do not operate through a threshold mechanism and have thus applied a linear dose-response model (Lee *et al.*, 2004; Uyak, 2006; Wang *et al.,* 2007a,b; Panyakapo *et al.*, 2008; Viana *et al.,* 2009; Pardakhti *et al.,* 2011; Basu *et al.,* 2011; Chowdhury *et al.,* 2011; Legay *et al.,* 2011; Yamamoto, 2011). OEHHA concurs with this approach in estimating cancer risks of the THMs, based both on available scientific evidence and as a matter of prudence for public health protection.

The potential role of epigenetic alterations induced by THMs and their involvement in THM-induced carcinogenesis was discussed in some studies. THMs are associated with increased urothelial bladder cancer (UBC) risk, and changes to DNA methylation may contribute to urothelial cancer. In a case-control study of UBC conducted in Spain, Salas *et al.* (2014) evaluated the relationship between LINE-1 5-methylcytosine levels (LINE-1%5mC) in granulocyte DNA and UBC risk during long-term THM exposure. LINE-1 is a highly repeated retrotransposon in the human genome, and measurement of CpG methylation in LINE-1 is a surrogate measure of global DNA methylation status. Methylation of LINE-1 limits expression of this retrotransposon and inhibits genomic damage. Salas et al (2014) measured the methylation status of LINE-1 and exposure to THM in the drinking water of 548 bladder cases and 550 controls. The results suggest a positive association between lower LINE-1%5mC levels and THM levels and between UBC risk and THM exposure. Salas *et al.* (2015) studied the association between lifetime THM exposure and DNA methylation. The authors selected 138 individuals separated into two groups with an average lifetime THM exposure at ≤85 µg/L vs. >85 µg/L (n = 68 and n = 70, respectively). The average lifetime THM levels in the two groups were 64 and 130 µg/L, respectively. The results indicated that THM exposure may affect DNA methylation in genes related to tumors, including colorectal and bladder cancers. Kuppusamy *et al.* (2015) investigated the relationship between the points of departure (PODs) for cancer incidence and DNA methylation changes, both modeled as $BMDL_{10}$, in laboratory animals exposed to environmental toxicants bromodichloromethane, dibromochloromethane, chloroform, hydrazine, trichloroethylene, benzidine, trichloroacetic acid, and di(2-ethylhexyl) phthalate. The results indicated that the $BMDL_{10s}$ for cancer incidence and altered DNA methylation are similar. The high degree of correlation between PODs for cancer incidence and DNA methylation suggests that DNA methylation may potentially be used as a screening tool in predicting the potential carcinogenicity.

Brominated THMs

It is clear, as discussed in the toxicology profiles (Chapter 6-8), that BDCM, DBCM, and bromoform have produced carcinogenic effects in animal studies. The data overall provide strong support for using a non-threshold model for the brominated trihalomethanes.

Genotoxicity

As noted in the corresponding toxicological profile chapters (Chapters 6-8), the weight of evidence suggests that bromoform, BDCM and DBCM are mutagenic and genotoxic. Positive genotoxicity results have been obtained for bromoform (Chapter 6) in *in vitro* and/or *in vivo* assays for micronuclei induction, sister chromatid exchange, chromosomal aberrations including DNA damage, sex-linked recessive lethal mutations, and aneuploidy. For BDCM (Chapter 7), positive results were obtained for mutagenicity in several *S. typhimurium* strains, induction of sister chromatid exchange in at least three studies, and chromosomal aberrations *in vivo* and *in vitro*. Positive or equivocal results were obtained for mutagenicity in cultured mouse lymphoma cells. For DBCM, 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 chromosomal aberrations *in vivo* and *in vitro*, DNA damage in bacteria, and aneuploidy in mammalian cells and *Aspergillus nidulans*.

Formation of Adducts

As discussed in Chapter 7 (Toxicological Profile: Bromodichloromethane), evidence *in vitro* suggests that a potential mechanism of carcinogenicity of BDCM may involve covalent binding to DNA of reactive intermediates generated by GSTT1-1-mediated metabolism of BDCM (Ross and Pegram, 2004). The authors suggest that this may occur in the kidney and large intestine (target organs of BDCM carcinogenicity in rats), which were demonstrated in this study to have a much lower detoxification rate to bioactivation rate (CYP/GST ratio) compared to the liver (a non-cancer target in rats). This may result in an enhanced relative production of reactive intermediates with the capacity to covalently modify DNA in target tissues, ultimately contributing to BDCM carcinogenicity.

Development of Preneoplastic Lesions

Several *in vivo* studies in rats suggest a plausible mechanism of carcinogenicity of brominated THMs in the colon involving formation of putative early preneoplastic lesions in the colon called aberrant crypt foci. As discussed in Chapter 7 (Toxicological Profile: Bromodichloromethane), DeAngelo *et al.* (2002) reported that the brominated THMs, particularly BDCM, administered in drinking water significantly induced the incidence of aberrant crypt foci in the colon of male F344/N rats, between 50 percent (DBCM) and 83 percent (BDCM). Geter *et al.* (2004b) then showed that formation of aberrant crypt foci in the colon of male F344/N rats was independent of the method of BDCM administration, with drinking water and corn oil gavage administration producing similar values of aberrant crypt foci per colon. It should be noted, however, that the authors indicated in their table 3 that the increases in aberrant crypt foci per colon was significant only for drinking water administration (1.33 ± 0.49 vs 0 for controls), but not for corn oil administration (1.5 ± 0.51 vs 0.16 ± 0.16 for corn oil vehicle). OEHHA staff reanalyzed the mean incidence of aberrant crypt foci per colon of BDCM treated rats using a Student's t test. Based on our analysis, the number of aberrant crypt foci per colon for BDCM given in water or in corn oil are highly statistically significantly different from their corresponding control values at $P < 0.005$. Further, the authors state that the total number of ACF induced by BDCM in corn oil

was not different than that produced by corn oil controls. However, the total number of ACF in corn oil controls was reported as 1 while that in animals treated with BDCM in corn oil was 9.

Geter *et al.* (2004c) evaluated the influence of a diet high in saturated animal fat on THM-induced ACF. Consistent with their earlier findings (DeAngelo *et al.*, 2002), the authors report that all groups administered brominated THMs showed significant increases in ACF induction compared to control animals, whereas no statistically significant increase was observed with chloroform treatment (although there were four total ACF in chloroform treated animals and 0.67 ± 0.33 ACF per colon in chloroform treated animals with a regular diet versus zero in water vehicle controls with a regular diet). A statistically significant association was found between the number of ACF per colon and the number of bromine atoms on the THM molecule. In animals exposed to chloroform, BDCM and DBCM, no difference was observed in the number of ACF between animals fed the normal diet and those fed the high-fat diet. However, in rats administered bromoform, a significant and near two-fold increase in ACF was observed in the high-fat group relative to those fed the normal diet. According to the authors, this observed effect of bromoform may be due to the higher octanol/water partition coefficient (log P_{ow}) of bromoform which makes it more lipophilic, allowing it to more readily cross cell membranes and enter the lymphatic circulation, thereby bypassing detoxification in the liver. Consistent with the findings of DeAngelo *et al.* (2002), THM-induced ACF were found primarily in the medial and distal segment of the colon, the observed site of THM-induced neoplasia.

Geter *et al.* (2005) then demonstrated that dietary folate was protective against bromoform-induced ACF in the colon of male rats. A statistically significant increase was observed in the number of ACF in bromoform-exposed male rats administered a folate-free diet compared to those given a normal diet containing folate. It should be noted that, although no significant difference in bromoform dose was observed between rats fed the normal and folate-free diets, the folate-free group was lighter and drank more water, which could have contributed somewhat to the observed increase in their induction of ACF.

Other supportive evidence *in vivo* of THM-related induction of ACF comes from a study by McDorman *et al.* (2003) in male Eker rats exposed via drinking water to DBPs, including chloroform and BDCM. Animals administered chloroform and BDCM had elevated ACF in the colon relative to untreated rats, with statistically significant increases in the total number of ACF in high-dose chloroform-exposed animals and the number of crypts in ACF in low-dose chloroform-exposed animals. These results are somewhat different than other studies in that the chloroform-induced ACF formation was statistically significant (as opposed to non-significant increases reported in other studies) and the observed BDCM-induced formation of ACF in the colon was not significant. It is noteworthy that Eker rats used in this study have a genetic predisposition to tumors, although not in the colon, which could possibly have influenced the difference in results.

Studies *in vitro* provide further evidence in support of the carcinogenic potential of THMs toward the colon. DeAngelo *et al.* (2007) reported that treatment of human NCM460 colonocytes with bromoform, as well as azoxymethane, the classic colon carcinogen, enabled them to grow in

media lacking serum and other growth factors, compared to untreated control cells or cells treated with other DBPs, suggesting that these cells had undergone transformation.

Overall, these studies demonstrate that brominated THMs in drinking water induce ACF in the colon of rats, with bromoform exerting the greatest potency. Further, they illustrate how dietary factors, particularly fat and folate, can influence promotion of THM-induced lesions that could potentially progress to colon cancer.

Cytotoxicity and Cellular Regeneration

Melnick *et al.* (1998) investigated the relationship between liver toxicity and tumorigenicity of BDCM and DBCM. Female B6C3F$_1$ mice (10/group) were gavaged with BDCM or DBCM in corn oil five days/week for three weeks at BDCM doses of 0 (vehicle only), 75, 150, or 326 mg/kg-day, or at DBCM doses of 0 (vehicle), 50, 100, 192, or 417 mg/kg-day. For BDCM, 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. For DBCM, 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. For both BDCM and DBCM, serum alanine aminotransferase activity was significantly increased in the two highest-dose groups. For BDCM, serum sorbitol dehydrogenase activity was elevated at all doses tested, whereas for DBCM the serum sorbitol dehydrogenase activity was significantly elevated at all doses above 50 mg/kg-day, with the increases being small relative to the control at 100 and 192 mg/kg-day. For both compounds, there was clear evidence of hepatocyte hydropic degeneration in animals treated at the two highest doses. BrdU was administered to the animals during the last six days of the study, and hepatocyte labeling index was analyzed. For BDCM, the two highest doses resulted in significantly elevated hepatocyte proliferation, whereas this effect was observed only at the highest dose of DBCM. For both compounds, the study authors compared the 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 authors therefore concluded that their results do not support a causal relationship between liver toxicity with subsequent reparative hyperplasia and tumor development.

Chloroform

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), and neoplastic nodules and hepatic adenofibrosis in female Wistar rats as well as 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), in male F344 rats exposed via both inhalation and drinking water (Nagano *et al.*, 2006), in male ICI mice treated with chloroform by gavage in toothpaste or arachis oil (Roe *et al.*, 1979), in male Osborne-Mendel rats treated by gavage in corn oil (NCI, 1976), and in male Osborne-Mendel rats exposed to chloroform in drinking water (Jorgenson *et*

al., 1982, 1985). Together, these data provide sufficient evidence of the carcinogenicity of chloroform in experimental animals (IARC, 1991a, 1999b; US EPA, 2001a).

It has been suggested that tumor formation may be secondary to cytotoxicity and tissue regeneration that have been observed at high doses associated with liver and kidney tumors (ILSI, 1997; US EPA, 1998c). It is also plausible that formation of tumors is not dependent on cytotoxicity and tissue regeneration. Alternative modes of action that have not been thoroughly investigated may be involved. The role of genotoxicity, for example, in chloroform-induced tumors remains unclear. The rest of this chapter addresses the potential roles of genetic toxicity and cytotoxicity/regenerative cell proliferation in chloroform-induced carcinogenicity.

Genotoxicity and Formation of Adducts

Although mixed results have been reported, available evidence indicates that chloroform is capable of inducing genetic toxicity under various experimental conditions as discussed in Chapter 5 (Toxicological Profile: Chloroform). One unanswered yet important question is the level to which genotoxicity is involved in chloroform-induced tumors. Although chloroform does not appear to be a strong genotoxic agent, there is some evidence of genotoxicity of metabolites. Shortcomings of some of the older genotoxicity studies in the chloroform database are discussed in Chapter 5. It is plausible that genotoxicity of chloroform metabolites contributes to the carcinogenicity of chloroform in conjunction with other modes of action, as alluded to by the US EPA's Science Advisory Board (2000). For example, repeated rounds of cytotoxicity and regenerative proliferation could work in concert with genetic toxicity, ultimately resulting in growth of tumors in target organs. However, further investigation of this hypothesis is warranted.

Studies *in vitro* provide evidence that phosgene ($COCl_2$), the major active metabolite of chloroform, is able to form irreversible adducts with the N-terminus of human histone H2B (Fabrizi *et al.*, 2003). The experimental data showed that adduction occurred primarily at lysine residues, with all seven lysine residues being subject to adduction, and that N-terminal serine and proline residues were also susceptible to adduction by phosgene. Further, up to three CO moieties could form adducts with histone H2B, and the presence of glutathione partially attenuated adduction, allowing production of a minor adducted species that contains glutathione. An earlier study showed that in the liver of rats treated *in vivo* with radiolabeled chloroform, histones and other nuclear proteins, but not DNA, became radiolabeled (Diaz Gomez and Castro, 1980a), suggesting that the metabolite phosgene is able to cross the nuclear membrane and make contact with histones to form adducts.

The *in vivo* data of Vittozzi *et al.* (2000) and Gemma *et al.* (2004) indicate formation of adducts of chloroform-derived reactive metabolites that are presumably different from phosgene. Vittozzi *et al.* (2000) conducted a single-dose study in B6C3F1 mice, Osborne-Mendel and Sprague-Dawley rats to evaluate covalent binding of chloroform reactive metabolites produced *in vivo* in two target organs of chloroform carcinogenicity in these animals, the kidney and liver. Whereas the levels of phospholipid fatty acyl chain adducts were statistically significant only in the B6C3F1 mouse liver, levels of phospholipid polar head adducts did not correlate with

species or organ susceptibility to chloroform carcinogenicity. Further, polar head adducts were observed in all samples tested, including the kidney of rats.

The presence of polar head adducts in the kidney of rats contrasts with earlier *in vitro* data from Gemma *et al.* (1996a) showing that renal microsomes from rats of the same two strains produced only phospholipid fatty acyl chain adducts when incubated with radiolabeled chloroform, which indicates activation of chloroform only through reductive metabolism. These findings are further substantiated by data from F344 and Osborne-Mendel rats showing that renal microsomes from both rat strains were unable to produce any oxidative metabolite *in vitro*, although adducts due to both oxidative and reductive metabolites were detected *in vivo* (Gemma *et al.*, 2004). These results indicate the presence in the rat kidney of electrophilic metabolites that are different from phosgene, representing either electrophilic metabolites that are produced secondary to reductive radicals or the formation at a different tissue site of oxidative metabolites that are stable enough for transport to the kidney. More studies involving prolonged chloroform exposures are needed to identify the specific metabolite(s) involved. Taken together, these data help elucidate a plausible genotoxic mechanism of chloroform carcinogenicity that involves covalent binding of chloroform-derived reactive metabolites to nucleic acid, nuclear protein or phospholipid.

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 administration via air, drinking water, or corn oil gavage to rats or mice. In general, cytotoxicity in the liver and kidney appears to increase with dose. The effects of vehicle, administration route, and exposure duration on cytotoxicity are ambiguous. In many studies, the severity of toxicity appears to diminish with time. Histopathology and other effects observed initially are absent or much less evident with continual exposure, suggesting development of tolerance to chloroform during chronic exposure (Nagano *et al.*, 1998; Yamamoto *et al.*, 1999).

Although chloroform's toxicity and carcinogenicity have been attributed by some to use of a corn oil vehicle (since these effects have not been observed when drinking water is employed), other factors may be involved (Bull *et al.*, 1986; Condie *et al.*, 1986; 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 tended to initially drink substantially less water (presumably due to taste aversion) and therefore initially received a lower dose. With time, water consumption increased and the dose of chloroform they consumed increased. The reduced toxicity, histopathology or cellular regeneration observed after several weeks in these animals may simply reflect induction of 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 labeled cells in S phase), has been extensively studied following chloroform administration. DNA synthesis is measured usually with either H3-thymidine followed by autoradiography or with bromodeoxyuridine followed by

methods to detect labeled DNA via monoclonal antibodies. DNA synthesis precedes cell division and thus is a good marker for tissue regeneration (Madhavan, 2007). Increases in labeling index tended to occur at high doses of chloroform but often did not reflect the observed pattern of toxicity including carcinogenicity. Studies reported:

- toxicity occurring without an apparent increase in labeling index;
- toxicity increasing with continual exposure while the labeling index diminished;
- increased labeling index without apparent toxicity;
- cytotoxicity and increases in the labeling index without any tumorigenic response evident.

Discussion of noteworthy studies on possible links among cytotoxicity, labeling index and increased tumors in the liver or kidney of rats or mice administered chloroform follows.

Reitz et al. (1980, 1982)

Reitz *et al.* (1980, 1982) examined chloroform induction of cell regeneration as measured by ^{3}H-thymidine uptake. Oral chloroform doses (15, 60, or 240 mg/kg) induced a dose-dependent increase in replicative DNA synthesis 48 hours post-exposure in liver and kidney of B6C3F$_1$ mice. Similarly, an oral dose of 180 mg/kg chloroform to Osborne-Mendel rats induced a 160 percent increase in cell regeneration in liver and a 40 percent increase in kidney. Forty-eight hours after a single oral dose of 240 mg/kg chloroform to mice (n = 2), there was evidence of induced tissue damage and increased mitosis in liver. The liver was not measurably affected by doses of 15 or 60 mg/kg. The kidneys of these animals exhibited necrotic lesions after 240 mg/kg and epithelial regeneration after 60 or 240 mg/kg of chloroform.

Moore et al. (1982)

Moore *et al.* (1982) used gross tissue uptake of ^{3}H-thymidine at 96 hours post-exposure as a measurement of cell regenerative activity. They observed that single doses of about 18, 60, or 200 mg/kg chloroform in corn oil or toothpaste induced a dose-related increase in cell regeneration in liver and kidney of male Swiss albino mice.

Pereira et al. (1984)

Pereira *et al.* (1984) studied whether chloroform induces regenerative hyperplasia after 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 18 hours after treatment. The magnitude of this effect was species-dependent. The maximum 10-fold increase in mice occurred after a dose of 375 mg/kg, whereas in rats the maximum 52-fold increase was found after 750 mg/kg. In rats, hepatic DNA synthesis was inversely related to chloroform dose. In the rat kidneys, DNA synthesis also decreased with increasing chloroform dose, but less than in the liver. DNA synthesis was stimulated by 3.1-fold in liver and by 6.7-fold in kidney by 50 mg/kg chloroform, while at doses over 50 mg/kg, DNA synthesis decreased.

Larson et al. (1993)

Larson *et al.* (1993) investigated the dose-response for hepatotoxicity, and nephrotoxicity in male F344/N rats (0, 34, 180, 477 mg/kg), and female B6C3F$_1$ mice (0, 34, 238, 477 mg/kg) given one dose by gavage in corn oil, and necropsied 24 hours later. They also investigated the time course of labeling in the liver and kidney in additional groups of mice receiving 350 mg/kg and rats receiving 180 or 477 mg/kg, and necropsied 0.5, 1, 2, 4, or 8 days after administration and given radiolabeled BRDU two hrs prior to euthanasia. Doses were chosen to include the doses given for the carcinogenicity bioassay conducted by NCI (1976). Changes in plasma enzymes were measured in three rats per dose, while labeling index (LI) was measured in two to five rats or mice. In the male rat, hepatotoxicity (as indicated by histopathology and increased plasma enzyme activity) was most prominent at 477 mg/kg, but mild histopathologic changes occurred at lower doses. An increase in LI of the rat liver was only observed at the highest dose in the time course study (477 mg/kg), while hepatotoxicity was noted at the lower dose (180) in the time course study. Nephrotoxicity was observed at 34 mg/kg or more in a dose-related manner. In the time course study, nephrotoxicity was most severe after one day and tubular epithelial regeneration was obvious at Day 4, diminishing by Day 8. Increased LI in the rat kidney was reported at 180 and 477 mg/kg two days after exposure (N = 2 at 477 and N = 5 at 180 mg/kg-d). While toxicity in the kidney became more severe at 477 mg/kg, the increase in LI was less than in animals given 180 mg/kg-day.

These findings (increase in toxicity and LI) are consistent 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, which induced prominent hepatotoxicity and increased LI in the liver, is well above the doses tested by NCI in the rat (90 and 180 mg/kg-d for male rats). However, this study was limited to effects following a single dose.

Hepatotoxicity was reported in female mice given a single dose of 238 mg/kg and was clearly evident in mice that received 350 or 477 mg/kg. The authors reported that the increase in LI was most marked in animals two days after receiving 350 mg/kg in the time course study, declining at four days and back to background at eight days. No renal lesions were detected in the mice, but significant increases in LI were observed only at day 2 in the time course study, and the increased labeling was much smaller than observed in the liver. The effects in the liver were at doses comparable to those that resulted in tumors in female mice in the NCI (1976) study (238 and 477 mg/kg-day for female mice). However, Larson *et al.* (1993) administered chloroform only once to the animals. Hepatotoxicity and increases in LI in the mouse may occur at lower doses in animals chronically exposed to chloroform.

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. If this was the only mechanism involved in tumorigenicity of chloroform, 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 in Larson *et al.* (1993) occurred at a dose(s) below the high dose, although increased LI was only observed at the high dose. The high dose (477 mg/kg) is much higher than the doses used in the NCI study in male rats (90 and

180 mg/kg-d), so the lack of rat liver tumors in the NCI (1976) study is consistent with the increase in LI only at the high-dose level in Larson et al (1993). However, the increase in LI only in the high-dose group is inconsistent with appearance of toxicity in this tissue at lower doses.

Effects observed in the mouse in Larson *et al.* (1993) are also difficult to interpret with respect to their hypothesis of the mechanism of chloroform carcinogenicity. Since only one dose was used in the time course study examining labeling, it is unclear at what doses increases in LI might occur in the mouse liver. No pathological lesions of the kidney were observed while increased LI was reported at 350 mg/kg-day. This suggests that LI may not be directly related to tumors, given that significant increases in kidney tumors were not observed in female mice in the NCI (1976). Neither nephrotoxicity nor increased LI should have been observed according to the hypothesis, because tumors did not occur in mouse kidney at the doses (238 and 477 mg/kg-d) used in the NCI (1976) study.

Larson et al. (1994b)

Larson *et al.* (1994b) gave chloroform 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 livers of all dose groups after four days of exposure but by three weeks the liver appeared normal in the 34 mg/kg-day dose groups and in three of five mice in the 90 mg/kg-day groups. Liver histopathology was evident at the two highest doses at three weeks. The liver LI was significantly increased in all dose groups after four days. Labeling index at three weeks was increased only at the two highest doses, with LI being comparable to the level observed at four days in the highest-dose group. In this study the changes in LI 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 LI 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. In the NCI (1976) study, chloroform did not induce renal tumors in B6C3F1 mice. Thus, the presence of renal lesions and the increased LI at the two higher doses in Larson *et al.* (1994b), which are the same doses given in the NCI study, is inconsistent with their hypothesis of tumor induction by chloroform.

Larson et al. (1994c)

Larson *et al.* (1994c) administered chloroform in corn oil (0, 3, 10, 34, 90, 238, or 477 mg/kg-day) or drinking water (0, 60, 200, 400, 900, or 1,800 ppm) to groups of female mice for four days or three weeks (five mice/group). Hepatotoxicity and nephrotoxicity as well as changes in LI 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 *et al.*, 2013). 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 ≥ 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 for the first four days of exposure, and 82.5, 184 and 329 mg/kg-day averaged over three weeks of exposure. 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).

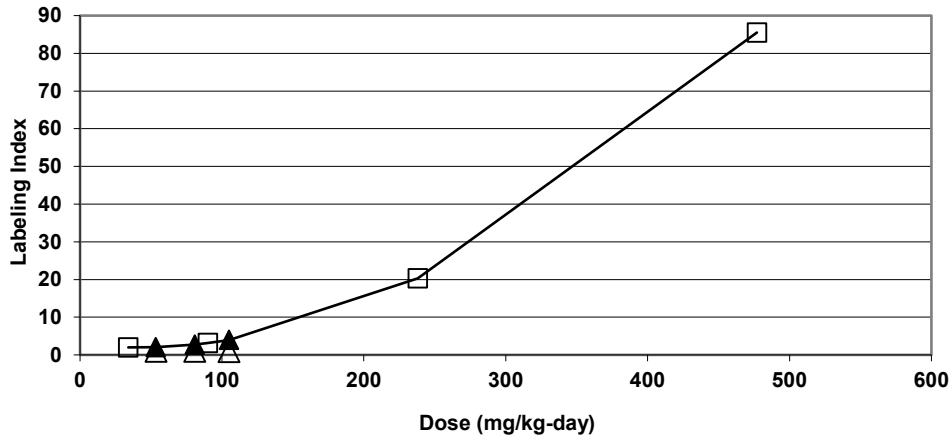
Liver – Labeling Index

In the Larson *et al.* (1994c) study, chloroform given to female mice in corn oil for four days or three weeks at doses of 238 and 477 mg/kg-day markedly increased LI in the liver. Labeling index was increased at three weeks in the 90 mg/kg-day dose group. Interestingly, LI was not increased at the lower dose (34 mg/kg-day) in animals that displayed hepatotoxicity at three weeks. While hepatotoxicity was more severe after three weeks than after four days, the increase in LI was considerably greater at four days than at three weeks, although the LI was still significantly elevated at three weeks exposure.

Increases in LI 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 *et al.* (2013), the doses at which increased LI was observed in corn oil were considerably greater, and not “comparable,” to the high doses of chloroform administered in drinking water during the first four days (see Figure 9.1). Thus, at the lower doses received by the female mice in drinking water at four days, the lack of response (increase in LI) is consistent with the lack of response in animals receiving comparable doses in corn oil.

OEHHA evaluated the dose-response for LI for mice given chloroform in corn oil for four days by fitting curves to define the relationship between the four highest doses and the resulting labeling index using SPSS software. A cubic relationship [LI = 2.8375 - 0.042475(dose) + $0.000521(dose)^2$ - $0.0000001439(dose)^3$] yielded the best fit (Figure 9.1). Using the dose-response relationship observed in mice given chloroform in corn oil for four days, the LI for doses of chloroform in drinking water for four days (provided by the study authors) were predicted. Various doses of chloroform given to 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 LI responses are shown in Figure 9.1. The LI in the liver of mice given chloroform in drinking water for four days were consistent with predicted LI in mice given an equivalent dose in corn oil for four days.

Figure 9.1. Liver Labeling Index after Four Days of Exposure (data from Larson *et al.*, 1994c)



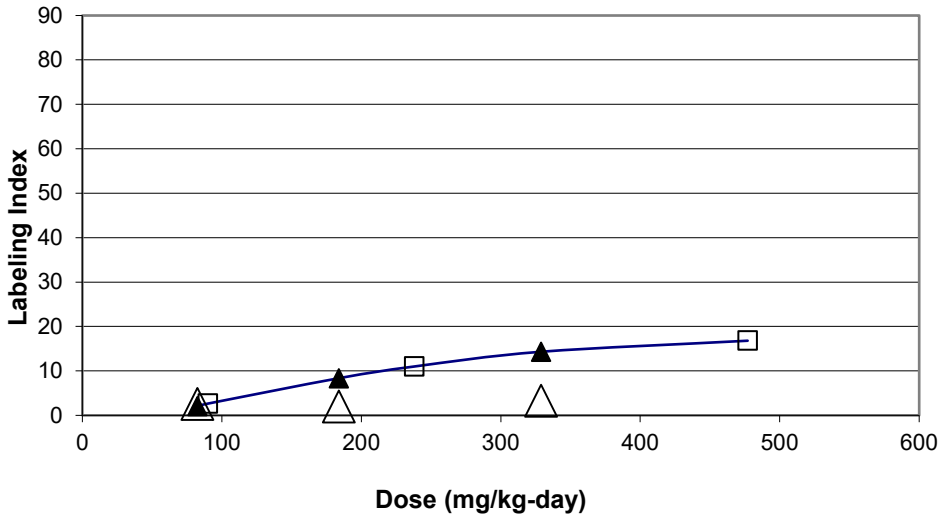
Legend:
Open square: observed labeling index in mice given chloroform in corn oil
Solid line: dose-response curve based on doses given in corn oil
Black triangle: predicted labeling index if doses were given in corn oil
Open triangle: observed labeling index when doses were given in drinking water

After three weeks, chloroform administered to female mice in corn oil by gavage yielded a considerably lower LI response compared to that observed at day four (Figure 9.2). 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, average daily doses were 53.5, 80.9 and 105 mg/kg-day for four days of exposure and 82.5, 184 and 329 mg/kg-day for three weeks of exposure, respectively.

OEHHA fit various relationships to the three highest doses and the resulting LI in mice exposed to chloroform in corn oil for three weeks using SPSS software. A quadratic relationship [LI = $-4.20 + 0.083668(dose) - 0.000083(dose)^2$] yielded the best fit (Figure 9.2). Using the dose-response relationship for mice receiving chloroform in corn oil for three weeks, we predicted the

LI associated with the doses of chloroform in drinking water as if the dose had been delivered in corn oil. Various doses of chloroform received by the mice in their drinking water and the predicted and observed LI responses are shown in Figure 9.2.

Figure 9.2. Liver Labeling Index after Three Weeks Exposure (data from Larson *et al.*, 1994c).



Legend:
Open square: observed labeling index in mice given chloroform in corn oil
Solid line: dose-response curve based on doses given in corn oil
Black triangle: predicted labeling index if drinking water doses were in corn oil
Open triangle: observed labeling index when doses were in drinking water

A small increase in the LI was predicted to occur but was not observed in the liver of mice receiving the highest dose of chloroform in drinking water. The predicted LI assumes equivalent bioavailability at comparable doses of chloroform administered in corn oil or drinking water. The lack of observed response could perhaps reflect the loss of a portion of the chloroform dose from the drinking 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 LI in mice receiving chloroform in corn oil were substantially reduced from levels observed at four days. Therefore, the difference between the predicted and observed LI at three weeks was not marked. It is unclear if there is a real difference in response due to the vehicle at three weeks.

Chloroform given in corn oil resulted in hepatotoxicity 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. However, the increase in LI did not correlate with hepatotoxicity. LI was only increased at the two highest doses,

although toxicity occurred at lower doses. The increase in LI was most notable at four days post-administration and had markedly decreased by three weeks, although it was still statistically elevated relative to the corresponding controls. Mice receiving lower doses of chloroform in corn oil should have displayed increased LI at three weeks, given the evidence of hepatotoxicity in these animals. The authors reported a NOAEL for hepatotoxicity of 10 mg/kg-day and a NOAEL for increased LI of 34 mg/kg-day. Thus, these data do not entirely support a direct link between hepatotoxicity and measureable cell proliferation and the hypothesis that tissue regeneration following toxicity is responsible for tumor formation by chloroform.

Kidney

No increase in kidney weights, macroscopic, or microscopic lesions were observed in mice treated by Larson *et al.* (1994c) with chloroform in corn oil. Increased LI was observed in the kidney (cortex and medulla) at the high dose 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 in drinking water did statistically significantly increase LI in the renal medulla after four days (900 and 1,800 ppm) and three weeks (200, 400, 900 and 1,800 ppm), although the increase was not dramatic (around two-fold for medulla). Increases in LI in the kidney were observed in mice receiving chloroform in corn oil or drinking water without evidence of renal toxicity. Thus, this study did not provide much evidence of a direct link between toxicity and tissue regeneration.

The administration of chloroform resulted in increased tumors in female mouse liver in the NCI (1976) study, but not in the kidney. In this study, increases in LI were observed in the female mouse kidney. Thus, this study does not provide clear support for a mechanism whereby cell proliferation resulting from toxicity is necessary for tumor formation.

Larson et al. (1995a)

Liver

Chloroform was administered to male F344/N rats in drinking water (0, 60, 200, 400, 900, or 1,800 ppm) ad libitum for four days or three week, or in corn oil (0, 3, 10, 34, 90, or 180 mg/kg-day) by gavage (five rats/dose group) for four days or five days/week for three weeks (Larson *et al.*, 1995a). Changes in LI and evidence of toxicity were evaluated in the liver and kidney.

After four days of administration of chloroform in corn oil, increases in liver weight were reported for all but the lowest dose, and increases in serum alanine aminotransferase and sorbitol dehydrogenase activity were observed for the two highest doses. Liver histopathology was observed at the three highest doses. No microscopic alterations were observed at 10 mg/kg-day or less. After three weeks increased liver weight was observed at the two highest doses, and elevated serum enzymes were reported only in the high-dose group. Liver histopathology was evident at 180 mg/kg-day at three weeks.

When chloroform was administered in drinking water for four days, mild histopathology in the liver was observed after four days at 900 and 1,800 ppm (average daily doses of 68.1 and 57.5 mg/kg-day, respectively). After three weeks, changes were only observed at 1,800 ppm in the

drinking water (equivalent to 106 mg/kg-day). No changes in serum enzyme activities were evident at four days or three weeks following drinking water administration.

At four days, the LI 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 LI in the liver. No increase in LI was observed when chloroform was given in drinking water, but reduced water consumption resulted in an average daily dose at four days of 68.1 or 57.5 mg/kg-day for 900 and 1,800 ppm, respectively.

After three weeks, based on drinking water consumption, the doses of chloroform with 900 and 1,800 ppm were 62.3 and 106 mg/kg-day, respectively. At three weeks, an increased LI was observed at 180 mg/kg-day chloroform in corn oil but not at 90 mg/kg-day, and no increase was observed with chloroform in drinking water. However, no increase would necessarily be expected at three weeks for chloroform in water (all other things being equal), given that the highest dose of chloroform administered in water (106 mg/kg-day) was only slightly larger than a dose administered in corn oil (90 mg/kg-day) that did not yield a significant increase in LI in the liver. Therefore, as in the mouse, this paper does not provide support that giving chloroform in corn oil to rats results in a greater increase in LI 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 in corn oil. The changes were greatest at four days. Administration of chloroform in corn oil resulted in an increase in LI in the kidney only at the highest dose at four days, but not at three weeks. Thus cytotoxicity did not necessarily trigger a measurable regenerative response in the kidney in this study.

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 more after three weeks. The authors report that this did not seem to be dose-dependent. No increase in LI in the kidney was detected at four days or three weeks when the compound was given in drinking water.

Note that the NCI study on carcinogenicity used Osborne-Mendel rats. These investigators chose F344/N rats. Thus, strain differences may influence the results.

Larson et al. (1995b)

Larson *et al.* (1995b) gave female F344/N rats 0, 34, 100, 200 or 400 mg/kg-day of chloroform in corn oil by gavage, at five rats/dose group for either four days or three weeks. At termination of treatment, the rats were sacrificed and the liver and kidney were examined for histopathology and changes in labeling index. Four days or three weeks of treatment resulted in slight liver histopathological changes in rats exposed to 100 or 200 mg/kg-day, with mild effects in rats receiving 400 mg/kg-day. The LI 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 and three weeks at 100 mg/kg-day or more. Unlike the toxicity, LI appeared to be greater after three weeks in the 100 and 200 but not the 400 mg/kg-day dose groups.

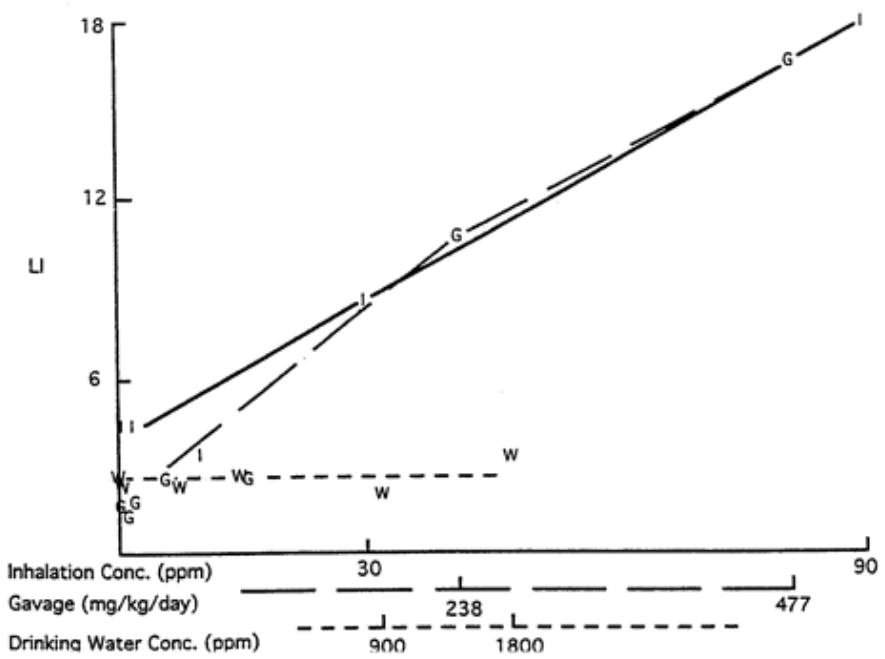
Larson et al. (1996)

Larson *et al.* (1996) exposed male and female B6C3F1 mice 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, or 7 days/week for three, six or 13 weeks. Groups of male mice were exposed 7 days/week for for three or seven weeks. In addition, separate groups were exposed for 5 days/week for 13 weeks. Based on standard body weights of 0.0316 kg and 0.0246 kg and inhalation rates of 0.053 and 0.04 m^3/day for male and female $B6C3F_1$ mice, respectively (US EPA, 1988), OEHHA estimated the doses based on an assumed pulmonary absorption of 50 percent of the inhaled chloroform.

In females, 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 LI 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. At 90 mg/kg-day, the LI increased after four days and remained elevated through 13 weeks. The LI pattern was not consistent with the cytotoxicity in the liver. While hepatotoxicity was observed at 10 mg/kg-day, an increased LI 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 LI was much higher at three weeks and then declined. At 90 mg/kg-day, both hepatotoxicity and LI were elevated from four days to 13 weeks of exposure. Neither pathology nor increase in LI was detected in the kidney at any of the doses.

In male mice, liver weights were significantly increased only at absorbed doses of 31 and 94 mg/kg-day for 13 weeks, but not three weeks. Liver histopathology was also evident in males at 31 and 94 mg/kg-day and was equal or more severe after 13 weeks versus three weeks of exposure. Figure 9.3 from Larson *et al.* 1996 provides information on the liver labeling indices. Labeling indices are on the Y-axis and doses (concentration in water (ppm), air (ppm), or corn oil (mg/kg-day)) are on the X-axis. Increased LI in the liver was only evident at 94 mg/kg-day; increases in LI peaked at three weeks (no data for four days) and were lower at six and 13 weeks. As with females, the change in LI did not appear to reflect hepatotoxicity. While hepatotoxicity was observed in males at 31 and 94 mg/kg-day dosed for three or 13 weeks, increases in LI were only associated with the highest dose. Whereas LI was much higher after three weeks at 94 mg/kg-day, hepatotoxicity was equal or more severe after 13 weeks.

Figure 9.3. Labeling Index in Female Mice Given Chloroform for Three Weeks



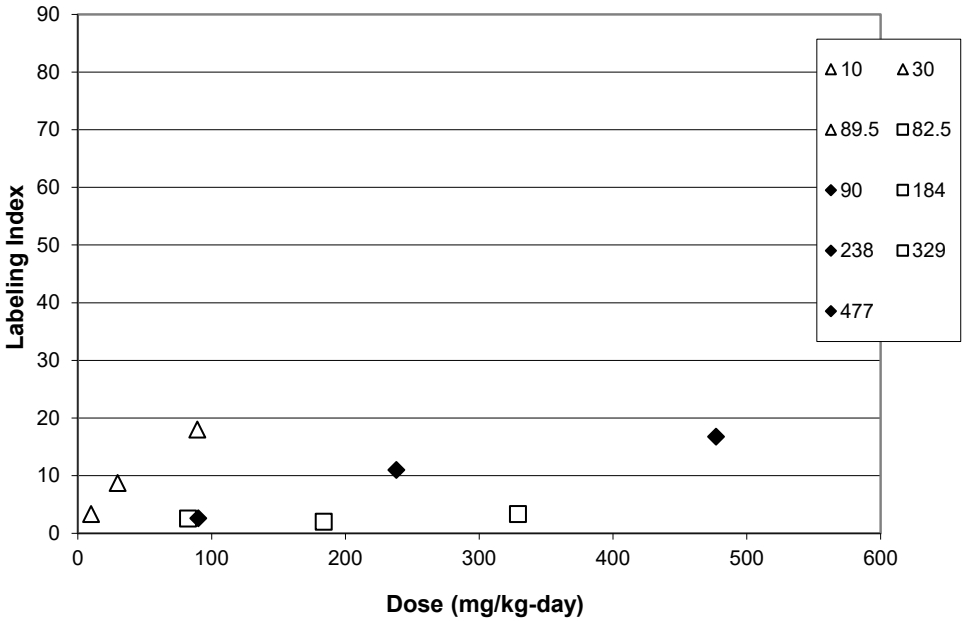
From Larson *et al.*, 1996. I = Inhalation; W = Drinking water; G = Gavage in corn oil.

The male mice had minimal to mild kidney pathology at absorbed doses of 31 and 94 mg/kg-day after three and 13 weeks of exposure. Labeling index in the kidney was also elevated in both doses, and unlike the histopathology, LI was much higher after three weeks. Interestingly, in animals only exposed for five days/week, LI was elevated at 10.5 mg/kg-day (no data for 31 mg/kg-day).

In the Larson *et al.* (1996) plot on the influence of administration route on hepatic LI after three weeks of chloroform exposure (Figure 9.3), it is very difficult to make a meaningful comparison of the different metrics plotted on the X-axis. Changes in LI based on administered dose yield a much more meaningful comparison. OEHHA plotted LI 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 9.4. The LIs in females after three weeks (and not four days) were selected because males were not included in Larson *et al* (1994b,c), while LI 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 LI 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 LI 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 LI in these animals.

Figure 9.4. Labeling Index in Female Mice Exposed to Chloroform for Three Weeks



Legend:
Black diamond: observed labeling index in mice given chloroform in corn oil
Open triangle: observed labeling index in mice exposed to chloroform in air
Open square: observed labeling index in mice given chloroform in drinking water

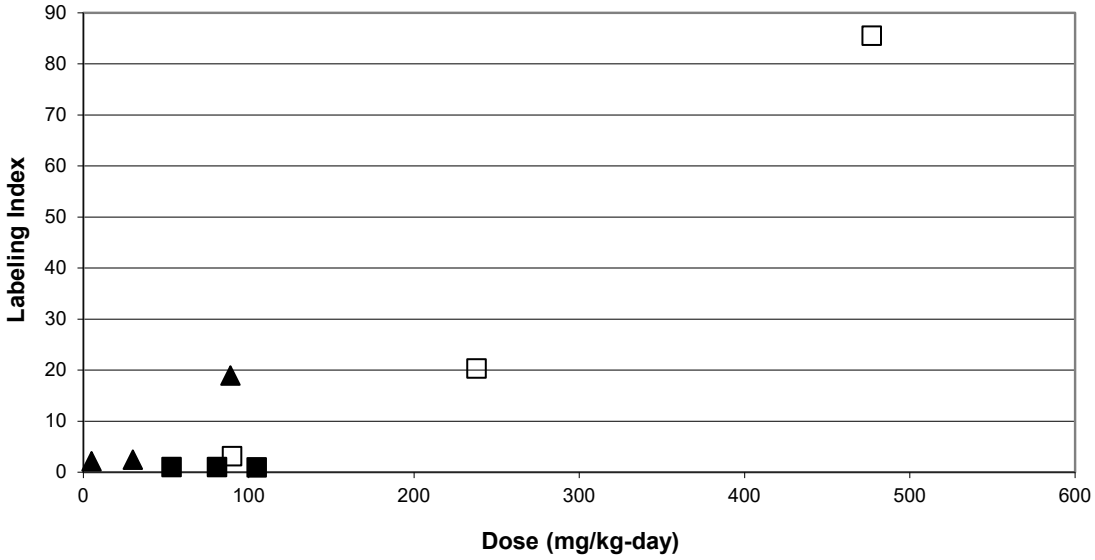
Templin et al. (1996b)

Eleven-week-old male or female BDF_1 mice were exposed by Templin *et al.* (1996b) 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 chloroform inhalation cancer bioassay. Eighteen hours after the exposure, the animals were sacrificed and the liver and kidney were examined for histopathology and LI. Separate groups of male mice were exposed to chloroform for five days/week for two weeks. OEHHA estimated doses based on standard mouse body weights of 0.0223 kg and 0.0204 kg and inhalation rates of 0.037 and 0.033 m^3/day for male and female BAF_1 mice, respectively (US EPA, 1988), and an assumed 50 percent absorption).

No significant liver lesions were observed in either male or female mice exposed at or below 30 ppm. Substantial histopathology was observed in both male and female mice exposed to 90 ppm for four days. The severity of the lesions in the female mice was reported to be less than that observed in male mice. LI after four days was markedly increased in both males and females exposed to 90 ppm and in males exposed to 30 ppm of chloroform. The increase in LI at 30 ppm (31 mg/kg-day) in males was not consistent with the lack of toxicity in the liver at this dose. Cytotoxicity as well as increased LI was observed in the kidney of male mice exposed to 31 or 93 mg/kg-day for four days. No toxicity or increase in LI was observed in the kidney of female mice.

Although a different mouse strain was employed in this study than in Larson *et al.* (1994b,c), LIs at four days in livers of female mice were compared (using an absorbed dose metric) for chloroform administered in corn oil or drinking water (Figure 9.5). The two lower doses in the inhalation study, which did not result in increases in LI, are consistent with what was observed when the compound was administered by the oral route. The LI associated with the high dose in the inhalation study is apparently greater than the LI associated with chloroform administered in corn oil or drinking water.

Figure 9.5. Labeling Index in Liver of Female Mouse Exposed for Four Days



Legend:
Open square: observed labeling index after chloroform given in corn oil
Black triangle: observed labeling index after chloroform in the air
Black square: observed labeling index after chloroform in drinking water

Templin et al. (1996a)

Nine-week-old male or female F344/N rats were exposed to chloroform by Templin *et al.* (1996a) 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 resulting from 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/N rats, respectively, and an assumption of 50 percent absorption (Table 51).

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. LI in the liver was significantly increased only in male and female rats exposed to 300 ppm. The LI in the liver peaked at 13 weeks in the females and

at 6 weeks in the males. In the liver, the increase in LI was consistent with the increase in toxicity in rats exposed to 300 ppm. However, toxicity observed in male and female rats exposed to 90 ppm was not associated with an increase in LI. In addition, in the male rat, LI peaked at six weeks while the toxicity was more severe at 13 weeks. The increased toxicity and LI 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 9.1. Absorbed Doses from Inhalation Exposure in Fischer 344 Rats (Templin *et al.*, 1996a)

Concentration (ppm)	Absorbed dose (mg/kg-day)	
	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 (200 mg/kg-day for males and 220 mg/kg-day for females), with 90 ppm greater than 30 ppm. The increases in LI in the kidney in this study were not consistent with the occurrence of tumors in the rat. Yamamoto *et al.* (2002) 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)

Templin *et al.* (1996c) gave male Osborne-Mendel or F344/N rats 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 LI. 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/N rats. Unlike the histopathology, LI was increased in kidneys of both strains. Statistically significant increases were observed in Osborne-Mendel rats at ≥34 mg/kg, and in F344/N rats at doses of 90, 180 or 477 mg/kg. No pathology or increased LI was observed in the liver at any dose in the

Osborne-Mendel rats, while increased liver LI and hepatotoxicity was observed in F344/N rats only at 477 mg/kg-day.

This study does not provide support for a clear link between hepatotoxicity and LI.

Constan et al. (2002)

Female B6C3F$_1$ mice were exposed to chloroform in air by Constan *et al.* (2002) at 10, 30 or 90 ppm from two to 18 hours for seven days (five mice/dose group). OEHHA estimated absorbed doses based on a standard body weight of 0.0246 kg, an inhalation rate of 0.04 m^3/day (US EPA, 1988), and a pulmonary absorption of 50 percent of inhaled chloroform. Changes in body and relative liver weight, and histopathology and LI in liver were investigated. While an increased LI was observed in animals exposed to 30 ppm for 6 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 (from 8 to 190 animals/dose group), were exposed by Pereira (1994) to 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. Groups of control mice received corn oil containing no chloroform or no treatment. Body weight, drinking water consumption, liver LI, and organ weight and histopathology were determined. In a separate study, fasted mice were treated with labeled chloroform in drinking water or corn oil by gavage, and the uptake and binding of chloroform to liver and kidney macromolecules were determined at up to four hours post-administration.

Chloroform in the drinking water markedly reduced drinking water consumption between days 0 and 7. Water consumption then increased, but remained significantly below control through days 134 to 140, the last days it was monitored. The average chloroform 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 mice receiving chloroform in their drinking water had no effect on their water consumption. Mice 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 mice 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. Changes in the hepatocytes were observed in mice 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 mice receiving chloroform in corn oil by gavage. The LI peaked at day five through day 12 post-administration and then declined to half-peak values on day 33. At day 159, LI was one-tenth peak levels. Vehicle-dependent differences in LI after 12 days of exposure may reflect the initial reduced dose that the mice received from the chloroform in their drinking water. The administration of a smaller dose of chloroform has been observed to foster 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 mice treated 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 given in water to liver macromolecules was higher for the first 40 minutes post-administration and then was comparable for the rest of the study. After gavage 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 given in the corn oil vehicle. Binding to macromolecules was also markedly higher throughout the study when chloroform was given in water.

The much higher peak chloroform levels in liver and more binding to macromolecules after gavage in water, compared to gavage in corn oil, is not consistent with other investigators' reporting lack of hepatotoxicity when given in water or increase in LI in the liver when administered in corn oil but not in water.

Melnick et al. (1998)

Groups of eight- to nine-week-old female B6C3F$_1$ mice (ten/dose group) were gavaged by Melnick *et al.* (1998) with various doses of one of the four THMs in corn oil 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 LI and hepatotoxicity were investigated (as indicated by changes in liver weight, histopathology and changes in serum enzymes).

Hepatotoxicity as indicated by increased liver weight, serum alanine aminotransferase and sorbitol dehydrogenase activity, and histopathology appeared to be dose-dependent and equivalent on a mmol/kg-day basis for chloroform, BDCM and DBCM but not bromoform. Changes in LI were both dose- and compound-related with increased LI highest for chloroform followed by BDCM, DBCM, and bromoform. The dose-related change in LI 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, LI was not equivalent at the same dose for the various THMs. These findings suggest that the increase in LI is not directly related to the increase in hepatotoxicity.

Relationships Among Cytotoxicity, Cellular Regeneration, and Tumors

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, 2005c).

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 to males and 238 or 477 mg/kg-day to females (NCI, 1976). Statistically significant increases in tumors in Osborne-Mendel rat kidney were also observed by Jorgenson *et al.* (1985) at 160 mg/kg-day. Statistically significant increases in renal tumors were observed in male ICI mice given 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).

Toxic effects have been observed in various tissues (in addition to the liver and kidney) within the range of chloroform doses used in the cancer bioassays. This multiplicity of effects is typical of many toxicants. Chloroform has been associated with a wide range of effects, including changes in body and organ weights, water consumption, hematological changes, suppressed immunity, and sedation, which are documented in chapter 5 in this document. 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. 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 (US EPA, 1998d; ILSI, 1997). If true, no tumors would occur at exposure levels at which no cytotoxicity and tissue regeneration occur, and a dose-response assessment would focus on determining the highest dose at which cytotoxicity, tissue regeneration and therefore tumor formation do 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 also occur at the dose levels associated with tumors. Thus, observed cytotoxicity and tissue regeneration at the dose(s) of chloroform associated with tumors are not 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; this includes consistency in the dose-response relationship for these effects and a consistent temporal relationship for the effects (Tables 9.2 and 9.3).

Consistency

If tumors are secondary to cytotoxicity and tissue regeneration, then neither tumors nor regeneration should occur without the occurrence of cytotoxicity. Likewise, cytotoxicity should be followed by tissue regeneration and then tumors. However, increased LI (a measure of tissue regeneration) without cytotoxicity was observed in the kidneys of animals administered chloroform (Larson *et al.*, 1993; Templin *et al.*, 1996c). Conversely, cytotoxicity was observed

in the kidney (Larson *et al.*, 1995a) and liver (Larson *et al.*, 1994c, 1995a) without an increase in LI.

Following chloroform administration, increased cytotoxicity or LI 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.

Table 9.2. Relationships Among Changes in Cytotoxicity, Labeling Index, and the Occurrence of Tumors in the Livers of Rats and Mice

Study	Species/Sex/ Vehicle	Relationships Among Cytotoxicity, Labeling Index and Tumor Occurrence	Comment
Larson *et al.*, 1993	Rat/M/Corn oil	Differences in dose-response	Cytotoxicity at lower doses without comparable increase in labeling index
	Mouse/F/Corn oil	Inadequate data	
Templin *et al.*, 1996c	Rat F344/N/M/Corn oil	Consistent changes in cytotoxicity and labeling index	
	Rat Osborne-Mendel/M/Corn oil	Consistent changes in cytotoxicity and labeling index	
Larson *et al.*, 1995b	Rat F344/N/F/Corn oil	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/M/Corn oil	Consistent changes in cytotoxicity and labeling index	
Larson *et al.*, 1994c	Mouse/F/Corn oil	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/F/Drinking water	Divergent responses	Mild cytotoxicity with no increase in labeling index
Larson *et al.*, 1995a	Rat/M/Corn oil	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/M/Drinking water	Divergent responses	Cytotoxicity with no increase in labeling index
		Divergent responses	Cytotoxicity with no tumors at this site
Larson *et al.*, 1996	Mouse/M/Air	Differences in dose-response	Cytotoxicity with no increase in labeling index at mid-dose (30 ppm)

Study	Species/Sex/ Vehicle	Relationships Among Cytotoxicity, Labeling Index and Tumor Occurrence	Comment
Larson *et al.*, 1996	Mouse/M/Air	Temporal differences when responses occurred	Labeling index peaked at 3 weeks, cytotoxicity remained elevated at 13 weeks in high-dose group
	Mouse/F/Air	Differences in dose-response	Cytotoxicity but no increase in labelling 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/M/Air	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/F/Air	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/M/Air	Differences in dose-response	Little cytotoxicity but increased labeling index at the mid-dose
	Mouse/F/Air	Consistent changes in cytotoxicity and labeling index	
Constan *et al.*, 2002	Mouse/F/Air	Differences in dose-response	No cytotoxicity but increased labeling index in one dose group

Table 9.3. Relationships Among Changes in Cytotoxicity, Labeling Index, and the Occurrence of Tumors in the Kidneys of Rats and Mice

Study	Species/Sex/ Vehicle	Relationships Among Cytotoxicity, Labeling Index and Tumor Occurrence	Comments
Larson *et al.*, 1993	Rat/M/Corn oil	Differences in dose-response	Cytotoxicity increased with dose, labeling index decreased at higher dose
	Mouse/F/Corn oil	Divergent responses	No cytotoxicity but increased labeling index
		Divergent responses	Increased labeling index but no tumors at site
Templin *et al.*, 1996c	Rat F344/N/M/Corn oil	Differences in dose-response	Dose-dependent increase in labeling index at all doses, cytotoxicity only at high dose
	Rat Osborne-Mendel/M/Corn oil	Divergent responses	No cytotoxicity but dose-dependent increase in labeling index
Larson *et al.*, 1995b	Rat F344/N/F/Corn oil	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/M/Corn oil	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/F/Corn oil	Divergent responses	No cytotoxicity but increased labeling index at high dose
		Divergent responses	Increased labeling index but no tumors at site
	Mouse/F/ Drinking water	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/M/Corn oil	Differences in dose-response	Dose-dependent cytotoxicity, increase labeling index only at high dose
	Rat/M/Drinking water	Divergent responses	Mild cytotoxicity, no increase in labeling index

Study	Species/Sex/ Vehicle	Relationships Among Cytotoxicity, Labeling Index and Tumor Occurrence	Comments
Larson *et al.*, 1996	Mouse/M/Air	Divergent response (perhaps)	Marked increased labeling index at 3 weeks but minimal cytotoxicity at three or 13 weeks in mid-dose (30 ppm) group
	Mouse/F/Air	No cytotoxicity or changes in labeling index	
Templin *et al.*, 1996a	Rat/M/Air	Differences in dose-response	Cytotoxicity but no increase in labeling index at low dose
	Rat/F/Air	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/M/Air	Consistent changes in cytotoxicity and labeling index	
	Mouse/F/Air	Consistent changes in cytotoxicity and labeling index	

Dose-Response Relationship

Comparable (parallel) dose-response relationships for cytotoxicity, tissue regeneration and tumor formation would provide more compelling evidence that cytotoxicity followed by tissue regeneration is necessary for tumor formation. Because tumors were observed at most at two doses, dose-response information for this effect is very limited. However, there is considerable information on cytotoxicity and tissue regeneration in the liver and kidney. Increased LI was seen at chloroform doses at which no cytotoxicity was seen in 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 seen at doses at which increased LI or comparable increased LI was not seen in the liver or kidney (Larson *et al.*, 1993, 1994b,1995a,b, 1996; Templin *et al.*, 1996a). The dose-response relationship for LI for various THMs did not appear to parallel the hepatotoxicity dose-responses (Melnick *et al.*, 1998). Suggestions that LI is a more sensitive measure of cytotoxicity is not supported by studies in which toxicity was seen at doses with no increase in LI in the liver (Templin *et al.*, 1996; Larson *et al.*, 1995a) or the 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 tumor development should be observed. Whereas toxicity preceded or accompanied increases in LI in certain studies (Larson *et al.*, 1995b, 1993), increases in LI preceded toxicity or peaked and then were reduced or returned to near background levels, while toxic effects persisted or

became more severe in the liver (Larson *et al.*, 1994c, 1996; Templin *et al.*, 1996a). Levels of serum alanine aminotransferase and sorbitol dehydrogenase peaked at three weeks at 238 and 477 mg/kg-day while hepatic LI peaked at 34 days and was markedly reduced at these dose levels at three weeks. Suggestions that the LI decreased because increased toxicity prevented regeneration were not supported by the findings that LI was not increased at lower doses at which toxicity was not as severe (Larson *et al.*, 1994a).

Vehicle Effects

Because of test compound volatility, palatability, and 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. Whereas 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). Corn oil given 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 following chloroform administration in drinking water (Science Advisory Board, 2000). However, our re-evaluation of the findings of Larson and coworkers (1994a,b, 1996a,b, 1996) does not fully support this conclusion. As shown above, the doses of chloroform administered in drinking water were lower than those administered in corn oil. Thus, the initial increases in cytotoxicity and LI following corn oil delivery but not water administration may be attributed to the difference in the doses applied. Interestingly, equivalent doses administered by the inhalation route appear to yield a greater increase in LI response. Further study of this effect is warranted.

Any vehicle-dependent differences (drinking water vs. corn oil) in toxicity and LI 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 LI (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 following chloroform administration in corn oil has been attributed to higher tissue levels of chloroform in the kidney and liver (Bruckner *et al.*, 2013). However, more rapid chloroform uptake into the liver and kidney and more binding to macromolecules have been observed when chloroform was administered by gavage in water versus in corn oil in a study by Pereira (1994); this is not consistent with the hypothesis that administration of chloroform in corn oil leads to higher tissue levels and thus more toxicity. The findings of Pereira (1994) are not unexpected, given that lipids delay gastric emptying.

Carcinogenic Mechanism of Action: Conclusion

Taken together, the time course and pattern of toxicity and tissue regeneration following chloroform administration raise doubts that these effects are solely responsible for tumors in the rat or mouse. In most instances, toxicity and LI peaked after only a few days or, at most, a few weeks of daily treatments. Given that toxicity and increases in LI usually occur immediately after the animals are exposed to chloroform, and then rapidly diminish, the importance to cellular proliferation of continued exposure to chloroform appears unclear. Tumors might be expected after a brief or repeated short-term exposures, which is a pattern more reminiscent of a strong initiator, not a chemical that acts by a non-genotoxic mechanism. However, the data are insufficient to distinguish among the various mechanistic possibilities, or to attribute a dominant role to any specific mechanism.

In rodents, chloroform is both a hepatic and renal toxin and a carcinogen. There is little evidence that chloroform or its metabolites directly interact with or damage DNA *in vivo*. As a consequence, it has been hypothesized that the carcinogenic activity of chloroform may be due to a non-genotoxic process (Reitz *et al.*, 1980, 1982; Pereira *et al.*, 1982, 1984, 1985). This hypothesis is based on the theory that chloroform may act as a promoter of previously initiated cells by virtue of regenerative hyperplasia that occurs in response to renal and hepatic toxicity (i.e., chloroform-induced cell death).

However, studies of single-dose chloroform exposures have found neither a consistent relationship between induction of hepatic ornithine decarboxylase activity and DNA synthesis (two markers of hyperplasia) nor any clear dose-response relationship for induction of DNA synthesis alone in the liver or kidney of mice or rats (Reitz *et al.*, 1980, 1982; Moore *et al.*, 1982; Pereira *et al.*, 1984). Overall, these data do not provide a clear association between administration of chloroform and stimulation of cell proliferation in the liver or kidney. There is evidence that chloroform metabolites bind covalently to macromolecules including histones, and therefore may be indirectly influencing DNA expression. Further, THMs may result in changes in methylation patterns in DNA, which could be involved in epigenetic modes of action for carcinogenicity. Thus, OEHHA concludes that the strength of the evidence is insufficient to support that chloroform acts solely via a non-genotoxic mechanism of action secondary to cytotoxicity and tissue regeneration for chloroform.

10. DOSE-RESPONSE ASSESSMENT

This chapter describes the dose-response assessments for both the non-carcinogenic and carcinogenic effects of each THM for use in the calculation of the PHG.

Non-Cancer Dose-Response Analyses and Acceptable Daily Dose Calculations

This section analyzes the non-cancer dose-response information presented in the Toxicological Profiles (Chapters 5-8) to establish acceptable daily doses (ADDs), in units of milligrams per kilogram of body weight per day (mg/kg-day), for each THM. ADD is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without any anticipated toxic effects. It takes into account sensitive populations and uncertainties in the analysis. This is similar to the term "reference dose" used by US EPA. ADDs derived in this chapter are used in Chapter 11 to calculate non-cancer health-protective concentrations for each THM in drinking water.

Method for Calculating ADDs

The derivation of ADDs involves the following steps:

- determining the critical study and critical endpoint;
- conducting a dose-response analysis to establish a point of departure (POD), as explained below;
- estimating an ADD from the POD by taking into account variability in human susceptibility as well as uncertainties in the underlying data.

Dose-Response Analysis and Selection of POD

The POD is a dose of a chemical (in units of mg/kg-day) from a study in animals or humans that is used as a starting point for ADD calculation. The POD is typically established by fitting a dose-response model to the dose-response data for the selected endpoint and study. We use US EPA's Benchmark Dose (BMD) Software (BMDS) when the dose-response data are amenable to such an analysis. The BMDS software is publicly available online[7]. Available models in BMDS (Version 2.4) are then fitted to the dose-response data according to the BMDS program criteria (see e.g., US EPA, 2012; Appendices D and E). Model selection criteria (comparing outputs of different models for the same dataset) are generally: the lowest Akaike's information criterion (AIC), goodness of fit p-value ≥ 0.05, scaled residual ≤ absolute value of 2, and visual inspection of the dose-response curve. When using BMD modeling, the POD is the 95 percent lower confidence limit on the dose associated with a pre-determined level of response (benchmark response, or BMR) above background (typically 5 percent for dichotomous responses, or 1 standard deviation or 10% change for continuous data). This POD is referred to as the BMDL ("L" stands for lower confidence limit). Thus, the BMD_{05} is an

[7] http://www.epa.gov/ncea/bmds/

estimate of the dose associated with a 5 percent increased risk of the endpoint being analyzed, and the $BMDL_{05}$ is the lower 95 percent confidence limit on the BMD_{05}.

A no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) can also serve as the POD. This approach is used when data are not amenable to BMD modeling (for example, when the responses at the lower doses in the data set are considerably higher than the BMR). Application of BMD modeling for non-cancer effects is generally preferred as it mitigates some of the limitations of the NOAEL/LOAEL approach, including:

- dependence of NOAEL/LOAEL on study dose selection and sample size;
- inability to account for variability of experimental results due to the characteristics of the study design;
- the need to use an additional uncertainty factor when a NOAEL cannot be determined in a study; and
- inability to account for the shape of the dose-response curve.

Calculating Average Daily Dose

Inputs into BMD modeling include the dose levels used in the study. For studies exposing animals less frequently than daily, adjustments are made to the administered dose to calculate an average daily dose during the exposure period. The average daily dose is calculated from the nominal dose with adjustments for days of dosing during the week and total dosing weeks during the experimental period. The nominal dose is indicated followed by the average daily dose within parentheses for all incidence tables presented in this chapter.

To determine the ADD, the POD of the chemical is divided by uncertainty factors that account for variabilities and uncertainties, such as differences between animals and humans, variability among humans, and various database deficiencies.

Uncertainty Factors

When developing health-protective levels for non-cancer effects, default uncertainty factors are used. Default uncertainty factors for ADD derivation are presented in Table 10.1. When scientific evidence is compelling, these defaults can be supplanted by alternative factors or modeled results.

Table 10.1. Default Uncertainty Factors for Deriving Non-Cancer Health-Protective Concentrations[1]

Uncerntainty Factor	Value
LOAEL uncertainty factor (UF_L)	
Values used:	10 LOAEL, any effect 1 NOAEL or BMDL
Inter-species uncertainty factor (UF_A)	
Combined inter-species uncertainty factor (UF_A):	1 human observation √10 animal observation in non-human primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 where animal and human PBPK models are used to describe inter-species differences √10 non-primate studies with no chemical- or species-specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 where animal and human mechanistic data fully describe inter-species differences. (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 non-primate studies with no data on toxicodynamic inter-species differences
Intra-species variability factor (UF_H)	
Toxicokinetic component (UF_{H-k}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured inter-individual variability √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data
Toxicodynamic component (UF_{H-d}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children) √10 studies including human studies with normal, healthy adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)

Uncerntainty Factor	Value
Subchronic uncertainty factor (UF_S)[2]	
Values used:	1 study duration > 12% of estimated lifetime √10 study duration 8 - 12% of estimated lifetime 10 study duration < 8% of estimated lifetime
Database deficiency factor (UF_D)	
Values used:	1 no substantial data gaps √10 substantial data gaps, including, but not limited to, developmental toxicity

[1] Adapted from (OEHHA, 2008).
[2] Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008).

The combined uncertainty/variability factor is referred to as a total uncertainty factor (UF). UF is the product of the uncertainty factors:

$$UF = UF_L \times UF_A \times UF_H \times UF_S \times UF_D$$

When based on observed endpoints in standard chronic animal studies, OEHHA generally applies a UF of 300: 10 for inter-species extrapolation, consisting of √10 for pharmacodynamics and √10 for pharmacokinetics; and 30 for intra-species variability, consisting of √10 for pharmacodynamics and 10 for pharmacokinetics, which is meant to account for diversity in these factors among humans (OEHHA, 2008). In particular, as outlined in our risk assessment guidelines (OEHHA, 2008), there is wide variability in toxicokinetics in humans by age at exposure for many environmental chemicals. Thus, OEHHA's guidelines recommend using a full 10 fold uncertainty factor to account for the toxicokinetic component of the UF_H.

The ADD is calculated using the following equation:

$$ADD = \frac{POD}{UF}$$

Chloroform

Chapter 5 provides the toxicological profile for chloroform. OEHHA relies on data from studies described in the toxicological profile in the evaluation of dose-response relationships below.

Critical Studies and Endpoints

Human data identified the central nervous system, liver, and kidney as the primary targets of concern after long-term occupational exposure to chloroform at sub-anesthetic levels (Bomski *et al.*, 1967; Phoon *et al.*, 1983; Li *et al.*, 1993). The dose-response information in these studies was determined to be insufficient for use in quantifying dose-response relationships. In animal studies, liver and kidney toxicity were also seen, following repeated exposure to relatively low levels of chloroform (Torkelson *et al.*, 1976; Condie *et al.*, 1983; Heywood *et al.*, 1979; Hard *et al.*, 2000; Nagano *et al.*, 2006). Central nervous system effects have not been well studied in repeated-exposure animal studies; thus, there is limited evidence regarding the occurrence in

animals of subtle neurological effects, such as those reported in chronically-exposed workers (Balster and Borzelleca, 1982). Obvious central nervous system depression is associated with acute high-level exposure in animals (Whitaker and Jones, 1965; Bowman *et al.*, 1978). Animal studies also found the nasal epithelium to be a sensitive target of chloroform (Templin *et al.*, 1996a; Larson *et al.*, 1995b; Dorman *et al.*, 1997). Nasal lesions have been observed after inhalation and oral (gavage) exposures. 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.

Chloroform also has been observed to affect fetal development in animal and human studies. A comprehensive compilation of such studies was provided to the Developmental and Reproductive Toxicant Identification Committee (DARTIC) under Proposition 65 in the OEHHA document on Evidence of Developmental and Reproductive Toxicity of Chloroform (OEHHA, 2016). In addition, the DARTIC was provided the original study reports. The committee determined that chloroform is a developmental toxicant, and that determination is the current basis for chloroform's inclusion on the Proposition 65 list. The DARTIC specifically identified effects of chloroform exposure on birth weight, including decreases in birth weight in humans and animals and an increased risk of low birthweight in humans (infants born weighing less than 2,500 grams).

Animal studies of reproductive and developmental toxicity provide effect levels that can be compared to other types of toxicity. Based on the available data, liver and kidney effects in animals appear to occur at lower exposure levels than reproductive and developmental toxicity. Thus, effects on these organs provide sensitive endpoints for protection against the non-carcinogenic effects of chloroform.

In subchronic studies, liver and kidney effects from chloroform were observed in rats, mice, guinea pigs, rabbits, and dogs (Torkelson *et al.*, 1976; Heywood *et al.*, 1979; Templin *et al.*, 1996a; Larson *et al.*, 1994b and c, 1995b, 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.

Most chronic studies have emphasized carcinogenesis findings, but several have reported on non-cancer kidney and liver toxicity. We evaluated the chronic studies listed in Table 10.2 below for the determination of a POD for chloroform. The studies are described in more detail in chapter 5.

Table 10.2. Candidate Chronic Studies for Dose-Response Assessment of Non-Carcinogenic Effects of Chloroform

Reference	Species, Strain, Sex	N	Dose (mg/kg-day)	Route	Exposure Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Heywood *et al.,* 1979	Dog Beagle M, F	7	0, 15, or 30	Oral, tooth-paste in capsule	6 days per week for over 7 years	None	15: Increased fatty cysts in liver, plus increased markers of liver damage
Hard *et al.*, 2000; re-evaluation of slides of Jorgenson *et al.*, 1982, 1985	Rat Osborne-Mendel M	50 - 300	0, 19, 38, 81, or 160	Oral, drinking water	104 weeks	38	81: Renal lesions (faint basophilia, vacuolation, nuclear crowding, or karyomegaly)
NCI, 1976	Rat Osborne-Mendel M,F	48-50	M - 0, 90, 180 mg/kg F - 0, 100, 200 mg/kg	Oral, gavage, corn oil	5 days per week for 78 weeks, sacrificed at 111 week	None	90 (M) 100 (F) Hepatic necrosis
Yamamoto *et al.*, 2002	F344/DuCrj Rat M, F	50	0, 10, 30, 90 ppm	Inhalation	6hr per day, 5 days per week for 104 weeks	10 ppm	30: Kidney nuclear enlargement; dilatation of tubular lumen
	Crj:BDF1 Mice M, F	50	0, 5, 30, 90 ppm	inhalation		5 ppm	30: Kidney atypical tubule hyperplasia; cytoplasmic basophlia; nuclear enlargement
Nagano *et al.*, 2006	Rat F344 M	50	Air: 0, 25, 50 or 100 ppm; Water: 0 or 1000 ppm	Inhalation; Oral, drinking water [a]	104 weeks	None	Kidney cytoplasmic basophilia, dilatation of proximal tubular lumen, nuclear enlargement, atypical tubular hyperplasia

[a] Animals were exposed by inhalation to 0, 25, 50, or 100 ppm of chloroform vapor for 6 hours/day, 5 days/week for 104 weeks, with each group receiving drinking water containing 0 or 1000 ppm chloroform *ad libitum* for 104 weeks

Chloroform POD

The lowest adverse effect level observed 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 (see Table 10.3). 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 including 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 serum enzyme indicators of hepatotoxicity at 60 mg/kg-day, and jaundice and loss of general condition at 120 mg/kg-day.

Table 10.3. Incidence Data for Beagle Dogs Exposed to Chloroform in Toothpaste (Heywood *et al.*, 1979)

Hepatic fatty cysts	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	15 (12.9)	30 (25.7)	
Males	0/12	6/7***	6/7***	$p < 0.001$
Females	1/15	3/8	7/8***	$p < 0.001$

Animals were dosed 6 days/week for 376 weeks. The nominal dose is indicated followed by the average daily dose within parentheses.
*** $p \leq 0.001$, Fisher exact comparison between treated and control group, calculated by OEHHA.

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 20 to 35 mg/kg-day and up (Larson *et al.*, 1994b; Hard *et al.*, 2000; Nagano *et al.* (2006)).

No NOAEL was identified in Heywood *et al.* (1979). The NOAEL equivalent is 1.3 mg/kg-day, i.e., the lowest dose tested (15 mg/kg-d X [6 days/7 days] = 12.9 mg/kg-day) divided by a UF_L of 10.

As noted above, benchmark dose modeling is preferred to the LOAEL/NOAEL approach for deriving a POD where the data are amenable to curve fitting by the available models in BMDS, particularly where no NOAEL is identified. OEHHA performed BMD modeling of the data from female dogs in Heywood *et al.* (1979), which exhibited a more gradual response than males, to derive a possible POD. The Logistic model gave the best fit of the data because it generated the lowest AIC and highest p-value, producing BMD_{05} and $BMDL_{05}$ estimates of 3.47 and 1.83 mg/kg-day, respectively.

OEHHA also conducted benchmark dose analyses of the data on renal lesions in male rats as reported in Hard *et al.* (2000), based on the data from the Jorgensen *et al.* (1982, 1985) study of chloroform in drinking water. Hard *et al.* (2000) report on the incidence of lesions that they

consider to be the result of chloroform toxicity, distinguishing it from chronic nephropathy observed in aged male rats. All models were run with default parameters and a benchmark response of 5 percent. The log-logistic model had the best fit to the data in Hard *et al.* (2000). The $BMDL_{05}$ from this modeling of the renal histopathology was 52 mg/kg-day, much higher than that from modeling liver pathology in female dogs in the Heywood *et al.* (1979) study.

OEHHA also considered the results of the inhalation and mixed drinking water and inhalation exposure studies of Yamamoto *et al.* (2002) and Nagano *et al.* (2006), conducted in the same laboratory, that provided extensive examination of toxicity in the kidney. In the dose response analysis OEHHA also examined the PBPK model published by Sasso *et al.* (2013) for chloroform. Sasso *et al.* (2013) applied PBPK modeling to estimate internal kidney dose in mice, rats, and humans. They then applied BMDS models to characterize dose-response based on the internal kidney metabolized dose. The investigators provided BMD analyses of five kidney toxicity endpoints for chloroform in male and female rats and male mice from Yamamoto *et al.* (2002) and Nagano *et al.* (2006) (see Sasso *et al.*, Table 7), studies that used inhalation or inhalation plus drinking water as the medium for exposure. The endpoints included in the Sasso analysis were: nuclear enlargement of proximal tubules; atypical tubule hyperplasia; dilation of the tubular lumen; cytoplasmic basophilia; and cellular proliferation. Sasso *et al.* (2013) used $BMDL_{10}$ values with a human PBPK model to estimate human equivalent doses (HEDs) ranging from 33.5 to 49.2 mg/kg-day for continuous dosage and 4.08 to 7.82 mg/kg-day for pulsed bolus dosage simulating 24 hour drinking water consumption. The authors observed that when using internal dose metrics, the differences in dose-response between mice and rats based on external dose metric, was substantially diminished.

Since the HEDs calculated by Sasso *et al.* (2013) were based on $BMDL_{10}$ values and OEHHA's BMD analyses use $BMDL_{05}$, OEHHA reanalyzed the data to obtain $BMDL_{05}$ values and revised HEDs based on these $BMDL_{05}$ values (Appendix D). OEHHA analyzed the four kidney toxicity endpoints presented in Sasso *et al.*, Table 6 for male F344 rats: nuclear enlargement and dilation of tubular lumen (from Yamamoto *et al.* (2002) and Nagano *et al.* (2006)), and atypical tubular hyperplasia and cytoplasmic basophilia (from Nagano *et al.* (2006)). Good agreement was obtained with the $BMDL_{10}$ values of Sasso *et al.* (2013). The $BMDL_{05}$ values for each of these endpoints were 42.46, 36.32, 30.25, and 73.71 mg/L-24 hr., respectively. HED values estimated for continuous exposure for each of these endpoints were 32.62, 29.90, 24.15, and 36.96, respectively, based on $BMDL_{05}/BMDL_{10}$ ratios. For pulsed exposure revised HEDs were 4.11, 4.23, 3.04, and 5.22, respectively. Results of the model are shown in Table D1 in Appendix D and summarized in Table 10.4 below.

Table 10.4. Dose-Response Analysis of Kidney Endpoints Based on Sasso *et al.* (2013) PBPK Dosimetry and Data for Chloroform Exposed Male Fisher 344 Rats from Nagano *et al.* (2006) and Yamamoto *et al.* (2002) Studies

Kidney Lesion	Model	AIC	GOF[a] *p*-value	Benchmark Dose (mg/kg-day)	
				$BMDL_{05}$	HED
Nuclear Enlargement	Weibull	210.15	0.998	32.62	4.11
Dilation of the Tubular Lumen	Log-Probit	198.41	0.5685	29.9	4.23
Cytoplasmic basophilia	Weibull	199.25	0.998	24.15	3.04
Tubular hyperplasia	Gamma	163.43	0.5983	36.96	5.22

[a] Chi-square Goodness of Fit (GOF) p-value

Chloroform Non-Cancer ADD Calculation

The analysis of Sasso *et al.* (2013) used data from relatively recent studies which administered chloroform to rodents via inhalation and drinking water (Yamamoto *et al.*, 2002 and Nagano *et al.*, 2006). These routes of exposure are more relevant for humans relative to the use of toothpaste as a medium by Heywood *et al.* (1979). Further, the sample size was considerably larger in the rodent studies (N = 50) than in the dog study (N=7). Thus, it is more appropriate to use these studies as the basis of the ADD. Using the lowest $BMDL_{05}$-based HED of 3.04 mg/kg-day for kidney toxicity in rodents (based on the model for cytoplasmic basophilia), the ADD is calculated as:

$$\text{ADD} = \frac{\text{POD}}{\text{UF}} = \frac{3.04 \text{ mg/kg-day}}{180} = 0.017 \text{ mg/kg-day}$$

A total UF of 180 is applied:

- 6 for inter-species extrapolation (UF_A), comprised of √10 for toxicodynamic uncertainty, and 2 for residual toxicokinetic (model) uncertainty (see Table 10.1), and
- 30 for intra-species variability (UF_H), comprised of √10 for toxicodynamic variability, and 10 for toxicokinetic variability, particularly to account for age and developmental-stage related differences among humans. (See Table 10.1 and OEHHA (2008))

Bromoform

Critical Studies and Endpoints

Chapter 6 describes the available data for the non-carcinogenic effects of bromoform in detail. The critical studies that are useful for establishing an ADD are two subchronic studies and one chronic study showing hepatotoxicity as well as changes in relative organ weights and decreased body weight in rats (**Table 10.5**). Several subchronic and chronic studies were not chosen as candidates. The reported NOAELs for two studies of developmental or reproductive toxicity (Ruddick *et al.*, 1983; NTP 1989b), exceeded doses showing hepatotoxicity in the subchronic and chronic studies. The NTP subchronic study in mice is not a candidate because

that species was less sensitive than rats (NTP, 1989a). Data from the chronic NTP (1989a) studies in rats are not particularly good for establishing an ADD because the studies employed high doses, considerably higher than doses in the subchronic NTP studies. In the case of hepatic vacuolization, a sensitive endpoint in the subchronic study, a near 100 percent response was observed at the doses tested (100 and 200 mg/kg-day) in the chronic study. However, the consistency in the findings of hepatic vacuolization in the chronic and subchronic studies is notable, and lends justification to modeling data from the subchronic portion of the NTP study. The chronic study by Chu *et al.* (1982b) did not have detailed reporting and was thus not chosen as a candidate for the POD.

Table 10.5. Candidate Studies for Dose-Response Assessment of Non-Carcinogenic Effects of Bromoform

Reference	Species, Sex	N	Dose (mg/kg-day)	Route	Exposure Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Subchronic Studies							
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
Chronic Studies							
Tobe *et al.* (1982)	Rat M, F	40[a]	M: 0, 22, 90, 364 F: 0, 38, 152, 619	Diet	24 months	19 (M) 15 (F)	85 (M); 67 (F): serum chemistry changes, gross liver lesions; decreased body weight gain; increased relative liver and brain weights

[a]Tobe used 12 animals of each sex from the control group and seven animals of each sex from the treated groups for the 24 month sacrifice to examine relative organ weight and body weight.

Bromoform POD

A NOAEL and a LOAEL of 25 and 50 mg/kg-day, respectively, were identified from the subchronic NTP study based on hepatic vacuolization in male rats (NTP, 1989a; see Table 10.6). Corresponding hepatic effects were not observed in the females. The NTP subchronic results were assessed using a benchmark dose approach. All models yielded similar results (Table 10.7). However, the Gamma, Weibull and Quantal-Linear models gave the best (and identical) fits of the data, producing the lowest AIC and highest p-value, and generating BMD_{05} and $BMDL_{05}$ estimates of 2.16 and 1.27 mg/kg-day, respectively.

Table 10.6. Incidence Data for Male F344/N Rats Gavaged with Bromoform in Corn Oil in the NTP, (1989a) Subchronic Study

Effect	Dose (mg/kg-day)						Fisher exact trend
	0 (0)	12 (8.6)	25 (17.9)	50 (35.7)	100 (71.4)	200 (142.9)	
Hepatic vacuolization	3/10	6/10	5/10	8/10*	8/10*	10/10**	$p < 0.001$

Animals were dosed 5 days/week for 13 weeks.
* $p < 0.05$, ** $p < 0.01$, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.7. Dose-Response Analysis of Data Reported in the NTP (1989a) Subchronic Study of Bromoform

Model	AIC	GOF[b] *p*-value	Benchmark Dose (mg/kg-day)	
			BMD_{05}	$BMDL_{05}$
Gamma, Weibull, Quantal-Linear[a]	65.9	0.7	2.16	1.27

[a]These models produced identical results and gave the best fit of the data, and were therefore used as the basis for the PHG calculation.
[b] Chi-square Goodness of Fit (GOF) p-value t

Note, however, that the control incidence was 30% and while bromoform treatment by corn oil gavage increased the incidence of hepatic vacuolization, the incidence was not significantly different from control until the third highest dose.

Given that there are data available from a dietary study, which more closely mimics intake from drinking water (the route of concern for the PHG) than corn oil gavage, and the high incidence of lesions in the control group, we modeled the data from the Tobe *et al.* (1982) dietary study using BMDS. OEHHA applied BMD models for continuous data to the observed increased relative liver weight in bromoform-exposed male and female rats at 24 months of exposure reported in the dietary study by Tobe *et al.* (1982). The best-fit model was the Hill model (Table 10.8 below and in Appendix D). This model yielded a $BMDL_{1SD}$ (lower 95% confidence limit of the dose corresponding to a 1 standard deviation increase in relative liver weight) for these data in female rats of 12.9 mg/kg-day. The model yielded a $BMDL_{1SD}$ of 32.5 mg/kg-day for male rats, indicating that females were more sensitive as measured by this indicator of hepatotoxicity. Thus, we chose the $BMDL_{1SD}$ for relative liver weight in female rats as the POD.

Table 10.8. BMD Analyses of relative liver weight in rats from Tobe *et al.* (1982) with Revised OEHHA Doses.

Experiment	Model	AIC	p-value[a]	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Male rats	Linear	14.03	0.1524	280.5	193.6
	Polynomial	12.90	0.4259	71.5	39.7
	Polynomial restricted to positive coefficients	14.03	0.1524	280.5	193.6
	Hill	14.3	NA	82.9	32.5
Female Rats	Linear	-8.05	<0.0001	82.7	65.9
	Polynomial	-27.75	0.2354	16.60	12.7
	Polynomial restricted to positive coefficients	-8.06	<0.001	82.7	65.9
	Hill	-27.16	NA	26.1	12.9

[a]Chi-square Goodness of Fit (GOF) p-value

Bromoform Non-Cancer ADD Calculation

Using the $BMDL_{1SD}$ of 12.9 mg/kg-day for increased relative liver weight in female rats derived from the chronic dietary study by Tobe et al (1982), the ADD is calculated as:

$$\text{ADD} = \frac{\text{POD}}{\text{UF}} = \frac{12.9 \text{ mg/kg-day}}{300} = 0.043 \text{ mg/kg-day}$$

A total UF of 300 is applied:

- 10 for inter-species extrapolation (UF_A), comprised of √10 for toxicodynamic uncertainty, and √10 for toxicokinetic uncertainty, and
- 30 for intra-species variability (UF_H), comprised of √10 for toxicodynamic variability, and 10 for toxicokinetic variability, particularly to account for age and developmental-stage related differences among humans. (See Table 10.1 and OEHHA (2008)).

Bromodichloromethane

Critical Studies and Endpoints

The findings of studies considered for dose-response assessment of non-carcinogenic effects of BDCM are summarized in Table 10.9. These are the most sensitive studies for BDCM, as discussed in Chapter 7. Included are chronic studies, and developmental or reproductive toxicity studies. Results from the subchronic study by Chu *et al.* (1982b) were not considered because of inadequate reporting of procedures for verifying the concentrations of BDCM in drinking water and inadequate histopathological data for the target tissues. In reproductive and developmental toxicity studies a variety of effects were observed, including full litter resorption

(Narotsky *et al.*, 1997; Bielmeier *et al.* 2001), growth retardation and effects on skeletal development (Christian *et al.* 2001a and b), and delayed sexual maturation (Christian *et al.* 2002). However, these effects were generally seen at levels higher than those in studies showing liver effects.

In chapter 7, we describe toxicity studies with genetically modified mice (NTP, 2007). However, these studies were not chosen as a basis for dose-response because the results based on these genetically modified mice are not readily amenable to extrapolation to the human population.

Table 10.9. Candidate Studies for Dose-Response Assessment of the Non-carcinogenic 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
	Mouse $B6C3F_1$ M, F	50	0, 25, 50			-	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
NTP (2006)	Rat F344/N M	50	0, 6, 12, 25	Drinking water	2 years	6	12: Chronic liver inflammation
	Mouse $B6C3F_1$ F	50	0, 9, 18, 36			36	--

[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.

BDCM POD

Results of the chronic studies conducted in male and female Wistar rats by Aida *et al.*, (1992b) were selected for quantification of non-carcinogenic effects. A LOAEL of 6 mg/kg-day was identified based on liver histopathological changes (fatty degeneration and granuloma) in male rats. In this study, BDCM was microencapsulated in gelatin-starch and mixed with the diet. The investigators noted that this method was used because of the difficulty of administering BDCM in drinking water due to low solubility and volatility. The various available developmental and reproductive toxicity studies indicate that liver endpoints exhibit greater sensitivity.

Dose-response relationships were assessed for hepatic fatty degeneration and granuloma in male and female rats (Table 10.10) using the BMD approach. Models providing the best fit of the data gave estimates of BMD_{05} between 0.6 and 2.4 mg/kg-day, and $BMDL_{05}$ estimates between 0.4 and 1.2 mg/kg-day and are presented in Table 10.11. The complete BMD modeling output profiles are presented in Appendix D.

Table 10.10. Incidence Data for Wistar Rats Exposed to Microencapsulated BDCM in the Diet for 24 Months (Aida *et al.*, 1992b)

Males	**Dose (mg/kg-day)**				**Fisher exact trend**
	0	**6**	**26**	**138**	
Hepatic fatty degeneration	0/24	5/14**	12/13***	19/19***	p < 0.001
Hepatic granuloma	0/24	4/14*	9/13***	19/19***	p < 0.001
Females	**Dose (mg/kg-day)**				**Fisher exact trend**
	0	**8**	**32**	**168**	
Hepatic fatty degeneration	2/32	8/19**	18/18***	18/18***	p < 0.001
Hepatic granuloma	0/32	8/19***	18/18***	18/18***	p < 0.001

$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.11. Dose-Response Analysis of Data Reported by Aida *et al.* (1992b) for BDCM

Hepatic Lesion	**Model**	**AIC**	**GOF[b] p-value**	**Benchmark Dose (mg/kg-day)**	
				BMD_{05}	**$BMDL_{05}$**
Fatty Degeneration in Males	Quantal-Linear	27.6	0.97	0.589	0.378
Granuloma in Males	Gamma, Weibull, Quantal-Linear[a]	35	0.99	1.05	0.674
Fatty Degeneration in Females	Probit	44.8	1	1.82	1.18
Granuloma in Females	Multistage	27.9	1	2.45	0.489

[a] These three models produced identical results.

[b] Chi-square Goodness of Fit (GOF) p-value

OEHHA also modeled data from NTP (2006) on chronic inflammation in the liver of male F344/N rats exposed to BDCM via drinking water. NTP reports significant increased incidence of liver inflammation relative to controls, while expressing concern about its biological significance. OEHHA views this endpoint as indicating BDCM toxicity. All models were run with default

parameters and a benchmark response of 5 percent. The log-logistic model gave the best fit to the data. The $BMDL_{05}$ from this modeling of 0.769 mg/kg-day is higher than the $BMDL_{05}$ of 0.378 mg/kg-day based on modeling liver toxicity in male rats in the study of Aida *et al.* (1992b). We chose the lowest BMDL05 from modeling the data of Aida *et al.* (1992b), which also had the lowest AIC.

BDCM Non-Cancer ADD Calculation

We calculated the ADD using the $BMDL_{05}$ of 0.378 mg/kg-day for liver toxicity in male rats derived from the chronic dietary study of Aida *et al.* (1992b). A total UF of 300 is applied:

- 10 for inter-species extrapolation (UF_A), comprised of √10 for toxicodynamic uncertainty, and √10 for toxicokinetic uncertainty, and
- 30 for intra-species variability (UF_H), comprised of √10 for toxicodynamic variability, and 10 for toxicokinetic variability, particularly to account for age and developmental-stage related differences among humans. (See Table 10.1 and OEHHA (2008)).

$$\text{ADD} = \frac{\text{POD}}{\text{UF}} = \frac{0.378 \text{ mg/kg-day}}{300} = 0.0013 \text{ mg/kg-day}$$

Dibromochloromethane

Critical studies and endpoints

The animal toxicity database for non-carcinogenic effects of DBCM consists of four subchronic studies and three chronic studies, which are summarized in Table 10.12. In addition, data from three studies of developmental or reproductive toxicity were reviewed for possible use in dose-response assessment.

These data demonstrate the kidney and liver as sensitive endpoints in the subchronic and chronic studies in male and female F344/N rats (NTP, 1985). The subchronic study identified NOAEL and LOAEL values of 30 mg/kg-day and 60 mg/kg-day, respectively, based on vacuolar change (fatty metamorphosis) in the liver of male rats. Adjusted to a continuous dose basis (i.e., multiplying by 5/7 to adjust for dosing occurring 5 days/week), the corresponding NOAEL and LOAEL values are 21.4 and 42.8 mg/kg-day, respectively. The kidney and liver effects seen in the NTP (1985) chronic and subchronic studies were more severe than those observed at similar dose levels in the chronic study of Tobe *et al.* (1982). The NTP studies had more thorough documentation of administered doses compared to Chu *et al.* (1982b), and were more sensitive. Thus, the chronic study by Chu *et al.* (1982b) was not considered further as a candidate for the POD.

Table 10.12. Candidate Studies for Dose-Response Assessment of the Non-Carcinogenic Effects of DBCM

Reference	Species Strain	Route	Sex	N	Duration	Dose (mg/kg-day)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)
Subchronic Studies								
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[a].	2 years	0, 10, 43, 230	10	43: decreased body weight gain; increased relative liver weight;
			F	40[a]	2 years	0, 9, 39, 220	9	39: Decreased body weight gain; increased relative liver weight
NTP (1985)	Rat F344/N	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

[a] Tobe used 12 animals of each sex from the control group and 7 animals of each sex from the treated groups for the 24 month sacrifice to evaluate clinical chemistry and body weight and relative organ weights.

DBCM POD

In the chronic NTP study, there was a high incidence of liver histopathology in the low-dose male rats (Chapter 8). Low-dose female rats had similar liver effects, although at relatively lower incidences, as well as kidney nephrosis. The gavage dose of 40 mg/kg-day for five days per week is equivalent to an average daily dose of 28.6 mg/kg-day (i.e., 40 mg/kg-day multiplied by 5/7). The NOAEL equivalent associated with this study is therefore 2.9 mg/kg-day (i.e., 28.6 mg/kg-day divided by UF_L of 10). NTP provided incidence data on histopathological changes in the liver. Those data are presented in Table 10.13 below.

Table 10.13. Selected Hepatic Lesions Observed in Fischer 344/N Rats Gavaged with DBCM in Corn Oil for Two Years (NTP, 1985)

Lesion	Vehicle control	40 mg/kg-day	80 mg/kg-day
Males			
Ground glass cytoplasmic change	8/50	22/50	34/50
Females			
Fatty change	12/50	23/49	50/50

We performed benchmark dose analyses on the data for ground glass cytoplasmic change in the liver in male rats and fatty change in the liver in female rats. The results are shown in Table 10.14 below and in Appendix D. Several models fit the data for ground glass cytoplasmic change in the livers of male rats well, including the gamma, multistage, and Weibull models, producing the same $BMDL_{05}$. The BMD_{05} ranged from 6.03 to 8.45 for the three models and the $BMDL_{05}$ was 3.4 mg/kg-day.

Table 10.14. Model Output Summary for DBCM – Ground Glass Cytoplasmic Change in Males

Model	AIC	p-value	Benchmark Dose (mg/kg-day)	
			BMD_{05}	$BMDL_{05}$
Gamma*	181.247	NA	8.45	3.40
Logistic	179.563	0.5743	8.86	7.14
LogLogistic*	181.247	NA	10.64	2.29
LogProbit*	181.247	NA	12.70	8.88
Multistage*	181.247	NA	6.03	3.40
Probit	179.492	0.6203	8.35	6.84
Weibull*	181.247	NA	7.66	3.40
Quantal-Linear[a]	179.461	0.6443	4.49	3.36

[a]This model yields the lowest AIC and highest p-value. However, the AIC values are similar for all models.
*Models yielding p-values of "NA" provide perfect curve fits to the data. Except for the LogProbit, they all yield similar BMDL values.

Liver pathology was also seen in the subchronic NTP study, and incidence data were provided for hepatic vacuolization. The dose-response relationship for hepatic vacuolar change in male rats in the subchronic NTP study was assessed using a BMD approach. BMDS models fit to the incidence data in Table 10.15 gave estimates of the BMD_{05} for hepatic vacuolar change of 0.74 to 4.25 mg/kg-day, all with acceptable fits to the data ($p > 0.05$). The $BMDL_{05}$ values generated by the models ranged from 0.2 to 1.08 mg/kg-day. The Probit model gave the best fit of the data, producing BMD_{05} and $BMDL_{05}$ estimates of 1.28 and 0.84 mg/kg-day, respectively.

Table 10.15. Incidence Data for Male F344/N Rats Gavaged with DBCM in Corn Oil for 13 Weeks (NTP, 1985)

Effect	Dose (mg/kg-day)						Fisher exact trend
	0	15 (10.7)	30 (21.4)	60 (42.9)	125 (89.3)	250 (178.6)	
Hepatic vacuolar change[a]	4/10	7/10	8/10	10/10**	10/10**	10/10**	$p < 0.001$

Animals were dosed 5 days/week for 13 weeks; [a] Interpreted by NTP as fatty metamorphosis.
** $p \leq 0.01$, Fisher exact comparison between treated and control group, calculated by OEHHA.

However, the control incidence of hepatic vacuolar change was 40% and while DBCM treatment by corn oil gavage increased the incidence of hepatic vacuolization, the incidence was not significantly different from control until the third highest dose, where the incidence was 100% (10/10). Thus, this subchronic exposure study was not further considered for derivation of a POD.

OEHHA also applied BMD models for continuous data to increased relative liver weight in DBCM-exposed male and female rats at 24 months of exposure reported by Tobe *et al.* (1982), which was a dietary study. None of the models fit the data on increased relative liver weight in females. One model, the linear model, had an adequate fit to the data in males. The BMD_{1SD} and $BMDL_{1SD}$ were 82.8 and 62.24 mg/kg-day respectively. This $BMDL_{1SD}$ is much higher than that modeled from the chronic NTP data.

We chose the $BMDL_{05}$ of 3.4 mg/kg-d for histopathologic changes in the liver of male rats in the chronic NTP study as the POD. Use of chronic data does not require using a subchronic uncertainty factor. The incidence data were clearly dose-related, and the difference between the BMD and BMDL was small. The sample size in the NTP chronic study (N= 50) was much larger than for the subchronic NTP study (10 per dose group) or the dietary study by Tobe (12 controls and 7 treated per dose group at the 24 month time point).

DBCM Non-Cancer ADD Calculation

Using the $BMDL_{05}$ of 3.4 mg/kg-day for liver histopathological changes in the liver of male rats derived from the chronic NTP study, the ADD is calculated as:

$$\text{ADD} = \frac{\text{POD}}{\text{UF}} = \frac{3.4 \text{ mg/kg-day}}{300} = 0.0113 \text{ mg/kg-day}$$

A total UF of 300 is applied:

- 10 for inter-species extrapolation (UF_A), comprised of √10 for toxicodynamic uncertainty, and √10 for toxicokinetic uncertainty, and
- 30 for intra-species variability (UF_H), comprised of √10 for toxicodynamic variability, and 10 for toxicokinetic variability, particularly to account for age and developmental-stage related differences among humans. (See Table 10.1 and OEHHA (2008)).

Cancer Dose-Response Analyses and Cancer Potency Derivation

This section analyzes the cancer dose-response information presented in the Toxicological Profiles (Chapters 5-8) to derive a cancer potency estimate for each THM. The cancer potency is a measure of the carcinogenic activity of the compound. It is often reported in units of 1/(mg/kg-day) (i.e., $(mg/kg\text{-}day)^{-1}$). For each THM, the cancer potency is combined with information on drinking water intake and early-life sensitivity of the young to calculate drinking water concentrations associated with a cancer risk of one per million. This later step is performed in Chapter 11.

Before presenting the cancer potency analysis for each THM, the method used to calculate cancer potency is described below. Epidemiological data indicate associations between exposure to drinking water DBPs and cancer development (reviewed in Appendix C and IARC, 2013), but are inadequate for use in estimating cancer potency of THMs due to the confounding presence of other carcinogenic DBPs in drinking water. Therefore, the cancer potency estimation of THMs relies on data from animal studies testing individual THMs for carcinogenic effects.

Method for Calculating Cancer Potency

Development of cancer potency estimates from animal bioassays includes consideration of:

- The quality, suitability, and sensitivity of the available animal bioassay studies; for example, the thoroughness of the experimental protocol, the temporal exposure pattern, the degree to which dosing resembles the expected manner of human exposure, the duration of study, the purity of test material, the number and size of exposed groups, and the extent of tumor occurrence.
- The cancer sites and types from the selected experiments most appropriate for characterizing the cancer potency. Where there are multiple sites with significant tumor findings in a selected experiment, a multi-site analysis is performed to describe the overall carcinogenic activity.
- Routes of exposure from tap water use. As shown in Chapter 3, significant THM exposures result from inhalation and oral routes. Dermal exposure levels are considered low.
- Whether a dose-response model that assumes the absence of a carcinogenic threshold dose should be used or whether there are compelling mechanistic data to support an alternative approach.
- Inter-species scaling of animal cancer potency to human cancer potency.
- Physiologic, pharmacokinetic and metabolic information for possible use in inter-species, inter-dose, or inter-route extrapolation.

Dose-Response Model

Data on the mechanisms of action involved in the carcinogenesis of THMs are evaluated to determine whether human risk should be estimated assuming low-dose linearity or otherwise.

This evaluation was conducted in the "Mechanisms of Action" chapter (see Chapter 9). The evaluation found that there is no sufficiently compelling mechanistic evidence to support the use of a non-default approach for dose-response analysis and thus the Multistage-Cancer model is used. This model is the default mathematical model used in the absence of compelling information that an alternative model is more appropriate. The form of this model used in cancer benchmark dose (BMD) model fitting, which calculates the lifetime probability of tumor (p) induced by an average daily dose (d), is assumed to be (US EPA, 2012):

$$p(d) = \beta + (1- \beta) \times \exp[-(q_1 d + q_2 d^2 + ... + q_i d^i)]$$

with constraints, $q_i \geq 0$ for all i. The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. With four dose groups, for example, the Multistage-Cancer model can have a maximum of four parameters, β, q_1, q_2, and q_3. When dose is expressed in units of mg/kg-day, q_1 is given in units of $(\text{mg/kg-day})^{-1}$. q_1 provides a measure of carcinogenic activity, with higher values indicative of stronger carcinogenicity. The parameter β provides the basis for estimating the background lifetime probability of the tumor (i.e., when dose d is zero, the probability of cancer, p, is equal to β).

Multistage-Cancer Model Fitting to Selected Bioassay Datasets to Estimate Cancer Potency

The Multistage-Cancer model is fit to the bioassay data for the THMs using the previously described US EPA BMDS (Version 2.4), and the dose associated with a benchmark response of 5 percent, that is the BMD_{05}, and its lower 95 percent confidence bound, the $BMDL_{05}$, are estimated. Goodness of fit is checked in three ways:

- The model p-value is ≥ 0.05 in a χ^2 goodness-of-fit test;
- The absolute value of the scaled residuals are all $\leq$ 2; and
- The dose-response curve is inspected visually for adequacy of fit.

The results presented here are for the acceptable fits to the datasets analyzed for each THM. Appendix E presents the complete output profiles from the BMD modeling and further details on the modeling.

Adjusting for Experimental Dose

The model is fit to dose-response data from animal studies. For studies that do not involve daily administration of a fixed mg/kg amount, an average daily dose "d" (in units of mg/kg-day) is calculated. This is done by adjusting the administered or nominal dose, accounting for days of dosing during the week and total dosing weeks during the experimental period. For studies using variable doses, the weighted mean dose is calculated considering the dosing frequency and duration of the various administered doses. For all incidence tables presented below, the nominal dose is indicated followed by the average daily dose, given in parentheses.

Adjusting for Experimental Duration

When the total experimental duration is at least the assumed natural lifespan of the animals (104 weeks for rats and mice), the $BMDL_{05}$ is used to estimate the cancer potency in animals, also called the "animal cancer slope factor" or CSF_{animal}. The CSF_{animal} is calculated by dividing the benchmark dose response value of 5 percent, or 0.05, by the $BMDL_{05}$. (The result is typically a value close to the upper 95 percent confidence bound on the parameter q_1.)

$$CSF_{animal} = 0.05 \div BMDL_{05}$$

However, when the total experimental duration is shorter than the natural lifespan of the animals, an adjustment is applied to account for the expected increased incidence of cancer with time. For experiments of duration T_e, rather than the natural lifespan of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$CSF_{animal} = [0.05 \div BMDL_{05}] \times (T/T_e)^3.$$

Adjusting for Human-Animal Differences

In the absence of reliable pharmacokinetic information, human cancer potency (CSF_{human}) is estimated by assuming that the chemical dose per body weight scaled to the three-quarters power produces the same degree of effect in different species. Under this assumption, the CSF_{animal} is multiplied by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-fourth power when animal cancer potency is expressed in units of $(mg/kg\text{-}day)^{-1}$:

$$CSF_{human} = CSF_{animal} \times (bw_h/bw_a)^{1/4}.$$

Reference body weights used in calculating CSF_{human} are provided in Table 10.16.

Table 10.16. Reference Body Weights Used in Calculating Cancer Potency

Species	Strain	Sex	Weight (kg)
Rat	F344/N	M	0.38
		F	0.25
	Osborne-Mendel	M	0.46
Mouse	$B6C3F_1$	F	0.035
	ICI	M	0.029
Human			70

Chloroform

Critical Studies and Endpoints

For dose-response determination, long-term studies were identified in which the administration of chloroform resulted in a statistically significant increase in liver tumors in male or female mice (NCI, 1976), kidney tumors in male rats (NCI, 1976; Jorgenson *et al.*, 1985; Nagano *et al.*, 2006), and kidney tumors in male mice (Roe *et al.*, 1979; Nagano *et al.*, 1998). The data of Nagano *et al.* (1998), although relevant, were not included in the dose-response assessment because the complete study details, including the dosing regimen which varied throughout the study, were not available to OEHHA; this left OEHHA reliant on a discussion of the study from IARC (1999b) and US EPA (2001a) and therefore unable to perform the analysis.

Dose-Response Analysis Using PBPK Model

We applied PBPK modeling to determine which measures of exposure to chloroform in the Nagano *et al.* (2006) study yielded more reliable exposure estimates to establish the dose-response relationship. Details of the modeling are in Appendix B.

We used the model in Sasso *et al.* (2013) applied to Nagano *et al.* (2006) for the dose-response analysis because it was thought that the improved treatment of kidney metabolism of this model might give improved model fit to to the renal cancer incidence data. There were PBPK simulated metabolized dose metrics that adequately fit the dose-response data from the Sasso model of the Nagano *et al.* (2006) study. However, the resulting cancer slope factor was not much different than using the applied dose. Hence, in the end, we did not use PBPK simulations of dose metrics in estimating the cancer slope factor for chloroform.

Dose-Response Analysis

For analysis of dose-response relationships for chloroform, BMD modeling was performed for the following datasets: incidence of hepatocellular carcinoma in female B6C3F_1 mice in the NCI (1976) study (see Table 10.17); combined incidences of renal adenoma and carcinoma in male Osborne-Mendel rats in both the NCI (1976) and Jorgenson *et al.* (1985) studies (see Table 10.18 and Table 10.19, respectively); combined incidences of renal adenoma and carcinoma in male ICI mice in the Roe *et al.* (1979) study (see Table 10.20); and combined incidences of renal adenoma and carcinoma in male F344 rats in the Nagano *et al.* (2006) study (see Table 10.21). Data for male B6C3F_1 mice from the NCI (1976) study are not shown because the females exhibited greater sensitivity in response to chloroform treatment and were therefore selected for use in the PHG calculation. Except for the Nagano *et al.* (2006) study in which chloroform uptake values were provided by the authors and the Jorgenson *et al.* (1985) study in which animals were administered chloroform daily via drinking water, adjustments were made to calculate the average daily dose in studies having less than daily exposures of animals, as outlined in the “Method for Calculating Cancer Potency” section above.

Table 10.17. Incidence Data for Female B6C3F1 Mice Gavaged with Chloroform in Corn Oil (NCI, 1976)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	200 or 250 (143.6)	400 or 500 (287.3)	
Hepatocellular carcinoma	0/20	36/45***	39/41***	p < 0.001

Animals dosed 5 days/week for 78 weeks and sacrificed at 92 to 93 weeks (averaged at 92.5 weeks). Doses were 0, 200 or 400 mg/kg-day for the first 18 weeks on study and increased to 0, 250 or 500 mg/kg-day for next 60. The nominal dose is indicated followed by the average daily dose in parentheses.
***p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.18. Incidence Data for Male Osborne-Mendel Rats Gavaged with Chloroform in Corn Oil (NCI, 1976)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	90 (45.2)	180 (90.4)	
Renal tubular cell adenoma or carcinoma	0/19	4/50*	12/50*	p < 0.01

Animals were dosed 5 days/week for 78 weeks and sacrificed at 111 weeks.
*p ≤ 0.05, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.19. Incidence Data for Male Osborne-Mendel Rats Administered Chloroform in Drinking Water (Jorgenson *et al.*, 1985)

Effect	Dose (mg/kg-day)					Fisher exact trend
	0	19	38	81	160	
Renal tubular cell adenoma or adenocarcinoma	4/301	4/313	4/148	3/48	7/50***	p < 0.001

*** p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.20. Incidence Data for Male ICI Mice Gavaged with Chloroform in Toothpaste (Roe *et al.*, 1979)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	17 (12.1)	60 (42.9)	
Renal adenoma or hypernephroma[a]	0/72	0/37	8/38***	p < 0.001

Animals were dosed 6 days/week for 80 weeks and sacrificed at 96 weeks. [a] Carcinoma;
***p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.21. Incidence Data for Male F344 Rats Administered Chloroform via Drinking Water and Inhalation (Nagano *et al.*, 2006)

Effect	Dose[a] (mg/kg-day)								Fisher exact trend
	0	20	39	78	45	73	93	135	
Renal adenoma or carcinoma	0/50	0/50	0/50	1/50	0/49	4/50	4/50	18/50***	p < 0.0001

Animals were exposed by inhalation to 0, 25, 50, or 100 ppm of chloroform vapor for 6 hours/day, 5 days/week for 104 weeks, with each group receiving drinking water containing 0 or 1000 ppm chloroform *ad libitum* for 104 weeks.
[a] Uptake doses (provided by study authors) corresponding to the nominal doses (in ppm) in the order: Inh-0 + Orl-0; Inh-25 + Orl-0; Inh-50 + Orl-0; Inh-100 + Orl-0; Inh-0 + Orl-1000; Inh-25 + Orl-1000; Inh-50 + Orl-1000; Inh-100 + Orl-1000. Inh, inhalation; Orl, oral.
***p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Chloroform Cancer Potency Estimate

BMD modeling results are summarized in Table 10.22 for the tumor data for mice and rats treated with chloroform. Of the five cancer datasets for chloroform, analysis of liver tumors in female mice (NCI, 1976) yielded the lowest BMD_{05} and $BMDL_{05}$ estimates with a CSF_{animal} of 0.128 $(mg/kg\text{-}day)^{-1}$. Analysis of renal tumors in male rats (Jorgenson *et al.*, 1985) yielded the highest BMD_{05} and $BMDL_{05}$ estimates with a CSF_{animal} of 0.003 $(mg/kg\text{-}day)^{-1}$ (Table 10.22). As outlined in the section "Method for Calculating Cancer Potency" earlier in this chapter, an adjustment was made to account for the less-than-lifetime experimental periods in both the NCI (1976) and Roe *et al.* (1979) studies. Converting each CSF_{animal} to a CSF_{human} and then deriving the geometric mean resulted in a mean CSF_{human} of 0.014 $(mg/kg\text{-}day)^{-1}$ (Table 10.22).

The geometric mean was calculated for the following reasons:

- the differences in species, strain, and sex of animals tested;
- the differences in route of administration, i.e., oral ingestion vs. inhalation;
- the differences in vehicle administration, i.e., gavage in corn oil or in toothpaste vs. drinking water;

- and the wide range of CSF_{human} estimates calculated, i.e., the forty-fold difference between the lowest value of 0.003 $(mg/kg\text{-}day)^{-1}$ and the highest value of 0.128 $(mg/kg\text{-}day)^{-1}$.

Therefore, the geometric mean will be used as the basis for the PHG calculation.

Table 10.22. Dose-Response Estimates for Cancer Potency Assessment of Chloroform

Study	Species	Sex	Tumor Site	Benchmark Dose (mg/kg-day)		Cancer Slope Factor $(mg/kg\text{-}day)^{-1}$	
				BMD_{05}	$BMDL_{05}$	CSF_{animal}	CSF_{human}
NCI (1976)	Mouse	F	Liver	4.69	3.71	0.0192	0.13
NCI (1976)	Rat	M	Kidney	33.4	14.1	0.00353	0.012
Jorgenson *et al.* (1985)	Rat	M	Kidney	90.2	61.1	0.000819	0.003
Roe *et al.* (1979)	Mouse	M	Kidney	20.8	11.4	0.00556	0.039
Nagano *et al.* (2006)	Rat	M	Kidney	79.6	68.3	0.000733	0.004
Geometric Mean CSF_{human}							**0.014**

Bromoform

Critical Studies and Endpoints

The database for bromoform carcinogenicity consists of three publications. The results of a well-conducted two-year gavage study in male and female F344/N rats (NTP, 1989a) were selected for quantification of carcinogenic effects. The two-year NTP (1989a) study in male and female $B6C3F_1$ mice 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 the injection route and lack of a clear tumorigenic dose-response relationship.

Bromoform Cancer Potency Estimate

BMD modeling was performed for the combined incidence of adenomatous polyps and adenocarcinoma in the large intestine of female F344/N rats (Table 10.23). These tumors were also observed in male rats, but they were less sensitive to treatment; thus, only data from females were selected for the dose-response assessment. Results of the BMD analysis are provided in Table 10.24. Converting the CSF_{animal} to the human equivalent cancer potency resulted in a CSF_{human} estimate of $0.0109\ (mg/kg\text{-}day)^{-1}$.

Table 10.23. Incidence Data for Female F344/N Rats Gavaged with Bromoform in Corn Oil (NTP, 1989a)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	100 (70.4)[a]	200 (140.8)	
Large intestinal Adenomatous polyps or adenocarcinoma	0/43	1/4	8/41**	$p < 0.01$

Animals were dosed 5 days/week for 103 weeks and sacrificed at 104 to 105 weeks (averaged at 104.5 weeks).

[a] This group of animals was not thoroughly examined histopathologically.

** $p \leq 0.01$, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.24. Dose-Response Analysis of Data Reported by NTP (1989a) for Bromoform

p-value	Benchmark Dose (mg/kg-day)		Cancer Slope Factor $(mg/kg\text{-}day)^{-1}$	
	BMD_{05}	$BMDL_{05}$	CSF_{animal}	CSF_{human}
0.66	31.0	18.7	0.00267	0.0109

Bromodichloromethane

Critical Studies and Endpoints

The experimental database for BDCM carcinogenicity consists of 13 oral bioassays, one intraperitoneal assay, and one dermal assay (Chapter 7). Significant increases in tumors were obtained in three of the five studies in rats and in one of the ten studies in mice. Data obtained in the NTP (1987) two-year gavage studies of BDCM in F344/N rats and $B6C3F_1$ mice were selected for quantification of carcinogenic effects because the observed statistically significant increases in historically uncommon tumors in the animals were considered clear evidence of carcinogenic activity under the experimental conditions of those studies.

BDCM Cancer Potency Estimate

BMD modeling was performed for the incidence of adenoma and adenocarcinoma in the large intestine of male F344/N rats (Table 10.25) and the incidence of hepatocellular adenoma and carcinoma in female $B6C3F_1$ mice (Table 10.26) because these two sites had the greatest tumor incidences per dose applied.

Results of the dose-response analyses for these data are provided in Table 10.27. Converting the CSF_{animal} for the large intestine tumor incidence in male rats to the human equivalent cancer potency resulted in a CSF_{human} estimate of $0.0255\ (mg/kg\text{-}day)^{-1}$. Analysis of the liver tumor incidence in female mice produced a CSF_{human} estimate of $0.087\ (mg/kg\text{-}day)^{-1}$. Because the mouse data yielded a higher cancer slope factor, it will be used as the basis for the PHG calculation.

Table 10.25. Incidence Data for Male F344/N Rats Gavaged with BDCM in Corn Oil (NTP, 1987)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	50 (34.9)	100 (69.7)	
Large intestine adenoma or adenocarcinoma	0/50	13/49***	45/50***	p < 0.001

Animals were dosed 5 days/week for 102 weeks and sacrificed at 104 to 105 weeks (averaged at 104.5 weeks). ***p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.26. Incidence Data for Female B6C3F$_1$ Mice Gavaged with BDCM in Corn Oil (NTP, 1987)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	75 (52.3)	150 (104.6)	
Hepatocellular adenoma or carcinoma	3/45	18/44***	29/37***	p < 0.001

Animals were dosed 5 days/week for 102 weeks and sacrificed at 104 to 105 weeks (averaged at 104.5 weeks). ***p ≤ 0.001, Fisher exact comparison between treated and control group, calculated by OEHHA.

Table 10.27. Dose-Response Estimates for Cancer Potency Assessment of BDCM Based on Data Reported by NTP (1987)

Species	Sex	Tumor site	Benchmark Dose (mg/kg-day)		Cancer Slope Factor (mg/kg-day)$^{-1}$	
			BMD_{05}	$BMDL_{05}$	CSF_{animal}	CSF_{human}
Rat	M	Large intestine	11.6	7.22	0.00693	0.0255
Mouse	F	Liver	11.1	3.86	0.0130	0.087

Dibromochloromethane

Critical Studies and Endpoints

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 adenoma and carcinoma in high-dose females (Table 10.28). Due to accidental death of males resulting from a dosing error, survival was too poor for tumor quantification in males in this study; thus, only data from females are presented here and used in the analysis.

Despite being delisted as a carcinogen in 1999 from the California Proposition 65 list (as

discussed in Chapter 8), DBCM is considered a carcinogen in this PHG document for the following reasons: DBCM is structurally similar to the other THM species, which are classified as either probable or possible carcinogens; the liver is a common target for THM-related tumors; DBCM has not been as thoroughly studied as the other THM species, resulting in much less available data to assess; and the data from female mice in the critical study employed for the dose-response analysis show positive association with liver tumors. As stated above, the data in males were compromised due to accidental death of the animals, which left only data from females available for the analysis.

Table 10.28. Incidence Data for Female B6C3F$_1$ Mice Gavaged with DBCM in Corn Oil (NTP, 1985)

Effect	Dose (mg/kg-day)			Fisher exact trend
	0 (0)	50 (34.9)	100 (69.8)	
Hepatocellular adenoma or carcinoma	6/46	10/49	19/48**	$p < 0.01$

Animals were dosed 5 days/week for 105 weeks and sacrificed at 107 to 108 weeks (averaged at 107.5 weeks). ** $p \leq 0.01$, Fisher exact comparison between treated and control group, calculated by OEHHA.

DBCM Cancer Potency Estimate

Dose-response relationships were determined for DBCM by performing BMD modeling of the combined incidence of liver adenoma and carcinoma in female B6C3F$_1$ mice (NTP, 1985). These results are provided in Table 10.29. Converting the CSF_{animal} to the human equivalent cancer potency yielded a CSF_{human} estimate of 0.0445 $(mg/kg\text{-}day)^{-1}$.

Table 10.29. Dose-Response Analysis of Data Reported by NTP (1985) for DBCM

p-value	Benchmark Dose (mg/kg-day)		Cancer Slope Factor $(mg/kg\text{-}day)^{-1}$	
	BMD_{05}	$BMDL_{05}$	CSF_{animal}	CSF_{human}
0.98	26.2	7.51	0.00666	0.0445

11. Health-Protective Drinking Water Concentrations

This chapter calculates health-protective concentrations of the regulated THMs to protect against both cancer and non-cancer effects of drinking water contaminated with THMs. As shown below, the water concentration associated with a one-in-one-million (10^{-6}) cancer risk from TTHMs is lower than the water concentration protecting against non-cancer effects from daily exposure to tap water. Thus, the PHG is based on cancer risk, since 10^{-6} is the *de minimis* risk threshold for establishing PHGs. Nonetheless, the non-cancer health-protective levels are presented to provide guidance for understanding potential risk for non-cancer effects at THM levels above the PHG.

Health-protective drinking water concentrations for the THMs are derived from both the ADDs for non-cancer effects and the cancer potency values presented in the previous chapters. The derivation considers intake from multiple routes of exposure (oral ingestion, inhalation, and dermal contact) to contaminants in tap water from household uses (e.g., drinking, cooking, bathing, and showering). This is necessary because exposure can occur from inhalation when a chemical volatilizes out of the water as well as from absorption of the chemical across the skin. Exposure is expressed as the daily water intake equivalent, in units of liters or liter equivalents per kilogram of body weight per day (L/kg-day or L_{eq}/kg-day, respectively). These liter equivalents represent the amount of tap water one would have to drink to account for the daily exposure to a chemical in tap water through the oral, inhalation, and dermal routes.

The calculation of non-cancer health protective concentrations also takes into account the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering) as part of total exposure from all sources (including food and air pollution), called the relative source contribution (RSC). RSC values typically range from 20 to 80 percent, and are based on available exposure data. RSC of 80 percent was selected for calculation of the health-protective concentration for each of the four THMs. 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.

Non-Cancer Health-Protective Drinking Water Concentrations

The approach for calculating the non-cancer health-protective concentration is to convert the ADD to a concentration level in drinking water, accounting for the extent of exposure to the chemical from tap water use, including major household uses. The concentrations here are expressed in units of milligrams/liter (mg/L) in drinking water. Using the non-cancer ADDs, the RSC of 0.8 described above, and the daily water intake (DWI) (as derived in Chapter 3, Exposure Assessment, for each respective THM), the Health-Protective Concentration for each THM is calculated:

$$\text{Health-Protective Concentration (HPC)} = \frac{\text{ADD} \times \text{RSC}}{\text{DWI}} = \frac{\text{ADD} \times 0.8}{\text{DWI}} \quad (1)$$

when the ADD is expressed in mg/kg-day. The Health-Protective Concentration can be expressed in units of µg/L by multiplying by 1000 (1000 µg/L = 1 mg/L). These concentrations are shown in Table 11. 1 below.

Table 11.1. Non-Cancer Health-Protective Concentrations for THMs

Chemical	Critical Study*	ADD (mg/kg-day)	DWI (L_{eq}/kg-day)	HPC (µg/L, ppb)
Chloroform	Nagano *et al.*, 2006; Yamamoto *et al.*,2002	0.017	0.080	170
Bromoform	Tobe *et al.*, 1982	0.043	0.081	430
BDCM	Aida *et al.*, 1992a	0.0013	0.081	13
DBCM	NTP, 1985	0.0113	0.083	110

*Critical endpoint for chloroform is nephrotoxicity and for the other three THMs is hepatotoxicity

Cancer Health-Protective Drinking Water Concentrations

The cancer health-protective level for each THM is set at a level where the cancer risk is estimated to be one per one-million persons exposed over a 70-year lifetime (based on the use of a 95% upper confidence limit of the extrapolated slope of the dose-response curve to estimate additional cancer risk; this does not include uncertainties beyond the statistical fit of the curve to the data). The calculation of health-protective concentrations for carcinogens addresses the potential additional sensitivity from exposure early in life. The calculation uses age sensitivity factors (ASFs) to account for the elevated risk in infants and children exposed to carcinogens (OEHHA, 2009). These factors are described in the OEHHA (2009) risk assessment guidelines, and are based on the OEHHA analysis of the influence of age at which exposure occurs on the potency of carcinogens. In that analysis, perinatal exposures of animals to a number of carcinogens results in a higher potency than when exposure occurs as mature organisms, with a median of approximately 10X. Potency is also higher when exposures occur in juvenile animals relative to fully mature animals (with a median of about 3X). The resulting weighting factors used by OEHHA are also supported by US EPA's analysis (US EPA 2005b, Barton *et al.*, 2005). The factors used are shown in Table 11.2. The sensitivity of the fetus/infant to carcinogens (from the third trimester until 2 years of age) is assumed to be 10 times that of adults; for children between the ages of 2 and 16 years, it is assumed to be 3 times that of adults. These assumptions are used in PHG calculations, unless reliable chemical-specific data indicate that an alternative approach or other factors should be used. Current practice is to use 70 years as the lifetime for humans.

Table 11.2. Age Sensitivity Factors (ASF) for Different Life Stages

Life Stage	Proportion of Life Spent in the Life Stage[a]	ASF[b]
3rd Trimester	0.25/70	10
Infant (0-2 years)	2/70	10
Child (2-16 years)	14/70	3
Adult (16-70 years)	54/70	1

[a] Time spent in the life stage divided by a lifetime of 70 years.
[b] Adopted by OEHHA (2009).

In addition to having greater sensitivity to carcinogens, the young drink more water relative to their body weight than do adults. The younger the child, the greater the proportionate amount of water he or she drinks. Table 11.3 shows water intake rates assumed for different ages. In addition, the amount of exposure – in terms of drinking water equivalents – occurring via inhalation and dermal routes during these different age windows is shown. The derivation of these estimates is provided in the Exposure Assessment, Chapter 3. Because infants neither take hot showers nor are subjected to significant exposure to volatiles from tap water use, their exposure is limited to ingestion and dermal contact; inhalation is assumed to be negligible.

Table 11.3. Daily Water Intake Equivalent Exposures Assumed for Different Ages[a]

Life Stage	Chloroform (L_{eq}/kg-day)	Bromoform (L_{eq}/kg-day)	BDCM (L_{eq}/kg-day)	DBCM (L_{eq}/kg-day)
Fetus	0.072	0.073	0.073	0.074
Infant (0-2 yr)	0.210	0.217	0.212	0.215
Child (2-16 yr)	0.110	0.109	0.110	0.113
Adult (16-70 yr)	0.067	0.068	0.067	0.069

[a] See Exposure Assessment, Chapter 3 for explanation.

This age-specific water intake is weighted by the age sensitivity factor (ASF_i) given in Table 11.2 above to account for the susceptibility to cancer during that age window. The life stage-susceptibility weighted drinking water intake over a lifetime - DWI_{life} - accounts for the duration spent in a life stage (d), the ASF during that life stage, and the daily water intake during that life stage (DWI_i):

$$DWI_{life} = ASF_i \times d_i \times DWI_i$$

for chloroform.

Table 11.4 shows the calculation of DWI_{life} for chloroform.

Table 11.4. Susceptibility-Weighted Daily Water Intake for Chloroform

Life Stage	ASF[a]	d[a]	DWI (L_{eq}/kg-day)[b]	ASF x d x DWI (L_{eq}/kg-day)
3rd trimester	10	0.25/70	0.072	0.0026
Infant (0-2 yr)	10	2/70	0.210	0.0600
Child (2-16 yr)	3	14/70	0.110	0.0662
Adult (16-70 yr)	1	54/70	0.067	0.0514
DWI_{life}: **Susceptibility-Weighted DWI (L_{eq}/kg-day)**				**0.180**

[a] Time spent in the life stage divided by a lifetime of 70 years.
[b] Adopted by OEHHA (2009)

Similarly, DWI_{life} was calculated for the three other THMs. The results for these are as follows:

Table 11.5. Susceptibility-Weighted Daily Water Intake for Bromoform

Life Stage	ASF[a]	d[a]	DWI (L_{eq}/kg-day)[b]	ASF x d x DWI (L_{eq}/kg-day)
3rd trimester	10	0.25/70	0.073	0.0026
Infant (0-2 yr)	10	2/70	0.217	0.0620
Child (2-16 yr)	3	14/70	0.109	0.0655
Adult (16-70 yr)	1	54/70	0.068	0.0522
DWI_{life}: **Susceptibility-Weighted DWI (L_{eq}/kg-day)**				**0.182**

[a] Time spent in the life stage divided by a lifetime of 70 years.
[b] Adopted by OEHHA (2009).

Table 11.6. Susceptibility-Weighted Daily Water Intake for BDCM

Life Stage	ASF[a]	d[a]	DWI (L_{eq}/kg-day)[b]	ASF x d x DWI (L_{eq}/kg-day)
3rd trimester	10	0.25/70	0.073	0.0026
Infant (0-2 yr)	10	2/70	0.212	0.0605
Child (2-16 yr)	3	14/70	0.110	0.0663
Adult (16-70 yr)	1	54/70	0.067	0.0517
DWI_{life}: **Susceptibility-Weighted DWI (L_{eq}/kg-day)**				**0.181**

[a] Time spent in the life stage divided by a lifetime of 70 years.
[b] Adopted by OEHHA (2009).

Table 11.7. Susceptibility-Weighted Daily Water Intake for DBCM

Life Stage	ASF[a]	d[a]	DWI (L_{eq}/kg-day)[b]	ASF x d x DWI (L_{eq}/kg-day)
3rd trimester	10	0.25/70	0.074	0.0027
Infant (0-2 yr)	10	2/70	0.215	0.0615
Child (2-16 yr)	3	14/70	0.113	0.0680
Adult (16-70 yr)	1	54/70	0.069	0.0531
DWI_{life}: **Susceptibility-Weighted DWI (L_{eq}/kg-day)**				**0.185**

[a] Time spent in the life stage divided by a lifetime of 70 years.
[b] Adopted by OEHHA (2009).

The lifetime dose (susceptibility-weighted) is calculated by multiplying the water concentration of chemical i, C_i, by the DWI_{life}.

$$Lifetime\ dose\ (susceptibility\ weighted) \quad = C_i \times\ DWI_{life}$$

Lifetime cancer risk of a chemical i is calculated by multiplying this lifetime dose by the cancer potency of the chemical, P_i:

$$Risk = C_i \times\ P_i\ \times DWI_{life}$$

For each THM, the concentration associated with a 10^{-6} risk of cancer can be calculated as follows:

$$C_i = Risk/(\ P_i\ \times DWI_{life})$$

$C_i = \ 10^{-6}/(\ P_i\ \times DWI_{life})$Table 11.8 shows the concentrations associated with a risk level of 10^{-6} for the four THMs.

Table 11.8. Concentrations associated with a cancer risk level of 10^{-6} for the THMs

Chemical	Study	Basis for Cancer Potency Estimate	Cancer Potency P_i in units (mg/kg-day)$^{-1}$	DWI_{life} (L_{eq}/kg-day)	C_i [a] (µg/L, ppb)
Chloroform	NCI (1976), Jorgenson *et al.* (1985), Roe *et al.* (1979), Nagano *et al.* (2006)	Male rat and male mouse kidney, female mouse liver	0.0137	0.180	0.4
Bromoform	NTP (1989a)	Female rat large intestine	0.0109	0.182	0.5
BDCM	NTP (1987)	Female mouse liver	0.087	0.181	0.06
DBCM	NTP (1985)	Female mouse liver	0.0445	0.185	0.1

[a] Rounded to one significant figure

Risk Characterization

Mechanistic Evidence and Other Considerations

Mechanistic evidence and some key uncertainties applicable to the PHGs and health protection concentrations for the THMs are summarized below:

- **Genotoxicity**. 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*. Further, phosgene is a major metabolite, and can react readily with cellular macromolecules. Thus, it is plausible that genotoxicity contributes to the carcinogenicity of chloroform. 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.

- **Chloroform mechanism of action and linear model of dose-response**. Several authors have interpreted the findings of numerous studies on the dose-response of chloroform with respect to hepatotoxicity and nephrotoxicity and the time course of regenerative hyperplasia 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 (e.g., binding of chloroform-derived reactive metabolites to nucleic acid, nuclear protein or phospholipid) based on reductive or conjugative metabolism of chloroform. Andersen *et al.* (1998) and Melnick and Kohn (1998) discussed the pros and cons of the cytotoxicity mode of action. If available studies clearly show that cytotoxicity and subsequent tissue regeneration are the sole mode of action of carcinogenicity of chloroform, then a threshold model for cancer risk assessment would be appropriate. However, OEHHA's re-evaluation of the studies (Chapter 9) found that the evidence is not entirely consistent with tumors in the kidney or in the liver being only secondary to cytotoxicity and tissue regeneration. The relationships among toxicity, tissue regeneration, and tumor formation are not clear. Thus, utilizing a threshold model with the assumption that cancer would only occur at doses causing measurable tissue regeneration is not a health-protective approach. OEHHA, therefore, applied the default approach of a linear extrapolation model to dose-response assessment of chloroform carcinogenicity as a prudent and public health protective method of cancer risk assessment for chloroform.

- **Relative Source Contribution (RSC) for THMs.** 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 50 percent pulmonary uptake, this represents an airborne exposure of about 1.5 to 3 μg/day. Waterborne exposure estimates from US EPA (1998f) would be 15 μg/L × 3 L_{eq}/day = 45 μg/day.

- **Interspecies Extrapolation**. To estimate the 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 between animals and humans that might result in differences in tumorigenic response to THM exposure. The difference between scaled and unscaled cancer slope values is about four-fold for rat-based and seven-fold for mouse-based values.

- **Interactions of THMs**. 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. Since human population exposures to THMs are

mostly to a mixture rather than to individual chemicals, potential synergistic and antagonistic effects among the individual THMs may influence the risk of cancer in humans from exposure to these chemicals. Studies have demonstrated that toxicokinetics changed when a mixture of THMs was given to male rats as opposed to when THMs were administered alone. These studies indicate that higher blood levels of THMs are achieved when given in a mixture than the same dose given alone, which the authors attributed to pharmacokinetic interactions among the THMs (Da Silva *et al.* 1999, 2000). Da Silva *et al.* (2000) suggested a role of mutual metabolic inhibition by the THMs. Wang *et al.* (1994) observed that ethanol consumption potentiated chloroform-induced hepatotoxicity, particularly via the oral route. However, this risk assessment is based on individual chemicals studied at relatively high doses under controlled conditions, while human exposures to THMs occur in complex mixtures containing hundreds of identified and unidentified compounds at low environmental concentrations. It is uncertain whether interactions among the THMs that alter toxic response occur at environmentally relevant THM 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 based on PBPK simulations did not result in substantially different risk estimates.

- **Early life exposure**. A growing body of scientific evidence indicates that children, infants, and the fetus may suffer disproportionately from some environmental health risks. Processes for uptake, distribution, metabolism, and elimination can vary considerably with age and developmental stage. For example, greater risk of health effects might occur if the enzymes responsible for bioactivation of THMs were more active or if detoxification processes were less active in fetuses, neonates, and/or children than in adults. Development of the fetus, infant and child involves complex physiological processes, including high rates of cell proliferation and differentiation. These processes present different target organs of toxicity relative to mature humans or animals. OEHHA (2009) provides detailed rationale for considering that early life exposures to carcinogens present higher risk than exposures that occur after maturity.

- **Correction for Early-in-Life Exposures to Carcinogens**. When determining cancer risk, OEHHA has adopted the use of age sensitivity factors (ASFs) to account for elevated risk in infants and children exposed to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2-16 years of age. These factors are applied regardless of the mechanism of action, unless chemical-specific data exist to better guide the risk assessment. In order to account more accurately for exposure during these sensitive periods, OEHHA adjusts for the greater drinking water consumption and inhalation rates during these early life periods using upper 95th percentile drinking water consumption and inhalation rates (see Calculation of the PHG).

Epidemiological findings. Epidemiological studies investigating potential associations between consumption of tap water containing THMs and incidences of cancer are discussed in IARC (2013) and in Appendix C. Associations between THMs and cancer rates are invariably complicated by the co-existence of other DBPs in tap water.

Chloroform

The health-protective level of 0.4 µg/L or ppb for chloroform was based on several carcinogenicity studies in experimental animals, calculated from liver and kidney tumors in mice and rats exposed to chloroform via corn oil gavage, in drinking water, or in both drinking water and via inhalation (Jorgenson *et al.*, 1985; NCI, 1976; Roe *et al.*, 1979, Nagano *et al.*, 2006). Human epidemiological studies suggest an association of exposure to DBPs with tumors.

Sources of uncertainty in the risk assessment for chloroform in drinking water are also the general issues of uncertainty in any risk assessment. These sources include incomplete knowledge of the mode of action, human variability, and uncertainties in intra-species extrapolation and dose-response extrapolation. Additional sources of uncertainty include the influence of other THMs present in drinking water on chloroform metabolism and toxicity, and the possible role of corn oil gavage versus drinking water methods of administration on carcinogenicity in the experimental animals.

Chloroform was listed in 1987 as a carcinogen under Proposition 65, and has an oral No-Significant Risk Level of 20 µg/day (OEHHA, 2018), based on the statute's risk level of 1 per 100,000.

IARC (1999b) 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. US EPA (2001a, 2018c) 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 (US EPA, 2018c) 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 DBPs; 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."

As noted above, OEHHA's re-evaluated the studies (see Chapter 9) investigating the hypothesis that tissue regeneration subsequent to toxicity is the mode of action for chloroform carcinogenicity. We found that the evidence is not entirely consistent with tumors in the kidney or in the liver being only secondary to cytotoxicity and tissue regeneration. The relationships among toxicity, tissue regeneration, and tumor formation are not clear. OEHHA considers use of the available cancer data on chloroform and a linear extrapolation in dose-response assessment for development of the health-protective concentration to be appropriate based on our evaluation of the strength of the evidence regarding a potential threshold mechanism.

To calculate the cancer health-protective level for chloroform, the cancer potency correction for early-in-life exposures to carcinogens was applied. We also incorporate data on water consumption rates by age and applied upper 95th percentile values for drinking water consumption and inhalation rates for various life stages (OEHHA, 2009, 2012, 2015).

Bromoform

The health-protective level of 0.5 µg/L or 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). 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). Sources of uncertainty in the risk assessment 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 include the influence of other THMs in drinking water on bromoform metabolism and toxicity and the uncertainties of the carcinogenicity database for estimating potency and risk.

Chlorinated tap water may contain the four THMs in varying proportions. These compounds are 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 at lower environmental exposures. Thus, the effects of concurrent exposures are unknown.

Bromoform has been listed since 1991 as a carcinogen under Proposition 65 (OEHHA, 2018). US EPA (2005a, 2018a) assigned bromoform to Group B2, probable human carcinogen, based on clear evidence in female rats and structural similarity to other THMs verified as probable or possible carcinogens. IARC (1991, 1999a) 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 (1999a) classification indicates some uncertainty regarding the carcinogenicity of bromoform,

OEHHA considers use of cancer data for estimation of the health-protective concentration to be appropriate based on the US EPA classification and OEHHA's prior assessment of the available data under Proposition 65.

To calculate the cancer health-protective level for chloroform, the cancer potency correction for early-in-life exposures to carcinogens was applied. We also incorporate data on water consumption rates by age and applied upper 95th percentile values for drinking water consumption and inhalation rates for various life stages (OEHHA, 2009, 2012, 2015).

Bromodichloromethane

The cancer health-protective level of 0.06 µg/L or ppb for BDCM was based on cancer 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 this was the most sensitive effect observed. Clear evidence of carcinogenicity was also obtained in male $B6C3F_1$ mice and in male and female F344 rats in the same study. Other studies have reported positive (Tumasonis *et al.*, 1987; George *et al.*, 2002 [rats]), negative (Voronin *et al.*, 1987b; Aida *et al.*, 1992b; George *et al.*, 2002 [mice]; NTP, 2006, 2007), or equivocal (Theiss *et al.*, 1977) results. Indications of carcinogenic potential are supported by reports of BDCM genotoxicity in some *in vitro* and *in vivo* assays. Therefore, use of cancer data for development of the health-protective concentration is considered appropriate. Sources of uncertainty in the risk assessment for BDCM in drinking water are the general issues of uncertainty in any risk assessment, including interspecies and dose-response extrapolations. Additional sources of uncertainty include the influence of other THMs in drinking water on BDCM metabolism and toxicity, the uncertainties of the carcinogenicity database for estimating potency and risk.

The database on human health effects of BDCM is limited. Epidemiologic studies have found associations between consumption of disinfected tap water containing BDCM and increased risk of cancer or adverse pregnancy outcomes. Such studies are confounded by co-exposures to a variety of identified and unidentified DBPs, including the other THMs discussed in this document.

To calculate the cancer health-protective level for chloroform, the cancer potency correction for early-in-life exposures to carcinogens was applied. We also incorporate data on water consumption rates by age and applied upper 95th percentile values for drinking water consumption and inhalation rates for various life stages (OEHHA, 2009, 2012, 2015).

Dibromochloromethane

The estimated health-protective level of 0.1 µg/L or 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. NTP (1985) found no evidence for carcinogenicity in male and female rats and equivocal evidence in male mice. Other relevant data include positive results in some mutagenicity tests and structural similarity to bromoform and BDCM, for which clear evidence of carcinogenic potential has been obtained in several well-conducted oral studies. Sources of uncertainty in the risk assessment 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 include the influence of other THMs in drinking water on DBCM metabolism and toxicity, the uncertainties of the carcinogenicity database for estimating potency and risk.

The specific database on human health effects of DBCM is very limited. Recent epidemiologic studies have found associations between consumption of disinfected tap water containing DBCM and increased risk of bladder cancer or increased risk of adverse pregnancy outcomes. Such studies are confounded by co-exposures to a great variety of identified and unidentified DBPs, including the other THMs discussed in this document. No occupational exposure studies were identified.

To calculate the cancer health-protective level for DBCM, the cancer potency correction for early-in-life exposures to carcinogens was applied. We also incorporate data on water consumption rates by age and applied upper 95th percentile values for drinking water consumption and inhalation rates for various life stages (OEHHA, 2009, 2012, 2015).

Other Cancer Slope Factors

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 (DHS, 1991). For BDCM, an oral or inhalation slope factor of 0.13 $(mg/kg\text{-}day)^{-1}$ has been used as the basis of a No Significant Risk Level (NSRL) under Proposition 65 in California based on a US EPA risk assessment.

Disinfection Benefits versus THM Risk

In interpreting the results of this risk assessment for four THMs, it is important to keep in mind the hazards of microbial pathogens in drinking water. The World Health Organization in its 2011 report *Guidelines for Drinking-Water Quality* discusses the issue as follows:

> "Disinfection is of unquestionable importance in the supply of safe drinking-water. The destruction of pathogenic microorganisms is essential and very commonly involves the use of reactive chemical agents such as chlorine…
> The use of chemical disinfectants in water treatment usually results in the formation of chemical by-products. However, the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection efficacy not be compromised in attempting to control such by-products.

US EPA (2006a) attempted to balance the benefits of chlorination versus risks of exposure to DBPs (DBPs) when it established a drinking water MCL of 80 (ppb) for TTHMs. US EPA stated that "maximizing health protection for sensitive subpopulations requires balancing risks to achieve the recognized benefits of controlling waterborne pathogens while minimizing risk of potential DBP toxicity. Experience shows that waterborne disease from pathogens in drinking water is a major concern for children and other subgroups (e.g., the elderly,

immunocompromised, and pregnant women) because of their greater vulnerabilities." OEHHA agrees that children and other subgroups are sensitive subpopulations for disease from waterborne pathogens. The Drinking Water Program at the California State Water Resources Control Board will be making decisions on the Maximum Contaminant Levels based on balancing the benefits of drinking water disinfection with the risks from disinfection byproducts.

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APPENDIX A. ESTIMATING DERMAL AND INHALATION EXPOSURES VIA TAP WATER USING CALTOX

This appendix describes the multi-route exposure assessment of the four regulated THMs in drinking water using CalTOX modeling. CalTOX 4.0 is a multimedia, multiple pathway exposure model developed for the California Department of Toxic Substances Control by Lawrence Berkeley National Laboratory (available at https://eaei.lbl.gov/tool/caltox). In addition to oral ingestion, exposure to chemical contaminants in tap water can occur via inhalation or dermal contact while performing common household activities, such as bathing, showering, or flushing toilets. OEHHA applies the CalTOX model to assess these multi-route exposures and calculate the relative contribution of each exposure pathway to the total daily exposure to THMs.

Exposure pathways included in CalTOX modeling:

- All inhalation exposures indoor active
- All inhalation exposures indoor resting
- Inhalation exposure in shower/bath
- Use of contaminated water as tap water
- Ingestion of tap water
- Dermal exposure during shower/bath

Table A1 provides OEHHA-derived human exposure parameters for various life stages that are applied during CalTOX exposure modeling of contaminants in drinking water (OEHHA, 2012).

Table A1. OEHHA-derived 95th percentile exposure parameters for various life stages used for CalTOX modeling

Life Stage	Age Range (years)	Drinking Rate (L/kg-day)	Inhalation rate (m^3/kg-hr)	Body Surface Area (m^2/kg)	Reference
Fetus[b]	N/A[a]	0.047	0.015	0.029[b]	OEHHA (2012)
Infant	0-2	0.196	0[c]	0.059	
Child	2-16	0.061	0.031	0.045	
Adult	16-70	0.045	0.012	0.029	

[a]Not applicable

[b]The adult body surface area parameter is used for pregnant women. Fetuses are assumed to be exposed to the same mg/kg-day dose as the pregnant mother.

[c]Infants are expected to be exposed to negligible levels of chemicals in tap water via inhalation (compared to other pathways) because they typically do not shower or flush toilets. These are the dominant inhalation exposure scenarios; therefore the inhalation pathway is excluded for infants.

CalTOX estimates the relative contributions of oral ingestion, inhalation, and dermal exposure to total exposure to contaminants in water based on the input parameters in Table A1 and the exposure pathways selected for inclusion. Liter equivalents for inhalation and dermal exposure are calculated for each life stage using the age-specific drinking water ingestion rate and relative contribution of the oral ingestion value. An example of a CalTOX output is presented in Table A2. For the sake of brevity, only the results using adult exposure parameters for chloroform are included here. Table A3 shows the relative contribution of each exposure pathway for the different life stages.

Table A2. Chloroform CalTOX output, adult exposure scenario

PATHWAYS	**Air (gases & particles)**	**Surface soil**	**Root-zone soil**	**Ground water**	**Surface water**	**Totals**	**%**
INHALATION	7.02E-263	0.00E+00	0.00E+00	6.73E-01	0.00E+00	**6.73E-01**	38.14
INGESTION:							
Water				1.03E+00	0.00E+00	1.03E+00	58.19
Exposed produce	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00
Unexposed produce			0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00
Meat	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00
Milk	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00
Eggs	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00
Fish					0.00E+00	0.00E+00	0.00
Soil		0.00E+00	0.00E+00			0.00E+00	0.00
Total ingestion	0.00 E+00	0.00 E+00	0.00 E+00	1.03 E+00	0.00 E+00	**1.03 E+00**	58.19
DERMAL UPTAKE		0.00E+00	0.00E+00	6.48E-02	0.00E+00	**6.48E-02**	3.67
Dose SUM	**7.02E-263**	**0.00E+00**	**0.00E+00**	**1.76E+00**	**0.00E+00**	**1.76E+00**	100.0

Table A3. CalTOX results for relative contributions of multiple routes of exposure to chloroform in tap water for various life stages

Life Stage	Oral Ingestion (%)	Inhalation (%)	Dermal (%)
Fetus[a] (Pregnancy)	54.2	42.5	3.3
Infant	97.1	0[b]	2.9
Child	42.8	54.1	3.1
Adult	58.2	38.1	3.7

[a]The fetus is assumed to have the same exposure as the pregnant mother.
[b]Infant exposure to endrin in tap water via inhalation is anticipated to be negligible, compared to other pathways, because they typically do not shower or flush toilets. These are the dominant inhalation exposure scenarios; therefore, the inhalation pathway is excluded for infants.

Liter equivalent (L_{eq}) values for inhalation and dermal exposures are calculated using life-stage-specific oral ingestion levels (OEHHA, 2012) and the relative contribution of the oral ingestion

values. These values are presented in Table A4 for chloroform. Chapter 3 shows the values for the four THMs, in Table 3.3

Table A4. Total liter equivalent values for multi-route exposure to chloroform in tap water

Life Stage	Age range (years)	Oral Ingestion (L/kg-day)	Inhalation[a,b] (L_{eq}/kg-day)	Dermal[a] (L_{eq}/kg-day)	Total Exposure (L_{eq}/kg-day)
Fetus (Pregnancy)	N/A[c]	0.047[d]	0.018[d]	0.007[d]	0.072
Infant	0-2	0.196	0.000	0.014	0.210
Child	2-16	0.061	0.039	0.011	0.110
Adult	16-70	0.045	0.015	0.007	0.067
Time-weighted average over lifetime					0.080

[a]Inhalation and dermal estimates are calculated using the life-stage-specific oral ingestion rates (OEHHA, 2012) and relative contribution of the oral ingestion value.
[b]L_{eq} for inhalation assumes 100% absorption in the lung.
[c]Not applicable; a time period of 0.75 year is used to represent the fetus in calculating the time-weighted average total exposure over a lifetime.
[d]The fetus is assumed to be exposed to the same dose as the pregnant mother, thus the liter equivalent values for the fetus are based on exposure parameters for the pregnant woman.

APPENDIX B. PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS

This appendix presents the physiologically based pharmacokinetic (PBPK) models used to model the pharmacokinetics of trihalomethanes. Section B1 provides the PBPK model used by OEHHA to model chloroform pharmacokinetics. Section B2 provides a historical review of pharmacokinetic models used to characterize the THMs, some of which served as a foundation for the models used for chloroform in Section B1. Section B3 provides a list of the computer code that can be used to model chloroform pharmacokinetics.

B1. OEHHA Use of PBPK Model for Chloroform Dose-Response Assessment

PBPK modeling was used to determine which measures of exposure yielded more reliable exposure estimates to establish the dose-response relationship for chloroform for both non-cancer kidney toxicity in the Nagano *et al.* (2006) and Yamamoto *et al.* (2002) studies, and renal cancer data from Nagano *et al.* (2006). These studies are described in Chapter 5.

The PBPK Model

Sasso *et al.* (2013) updated the PBPK model of Corley *et al.* (1990) (described below) with revised rate parameters for renal chloroform metabolism in rats, mice and humans by accounting for microsomal protein (MSP) content and mass of the metabolically active region of the kidney. This served as an improvement over existing models that lacked kidney region-specific data.

Sasso *et al.* revised the model of Corley *et al.* by dividing the renal compartment into cortex and medulla, and assigning 70% of the total kidney volume, 90% of the total blood flow, and 100% of the CYP2E1 activity to the cortex. Metabolic rate constants for the renal cortex were revised using both species-specific data for CYP2E1 content in the cortex and metabolism of chloroform by CYP2E1. Sasso *et al.* utilized updated *in vitro* data from mouse and human kidneys to revise the Corley *et al.* estimates. The kidney cortex metabolic rate was estimated as a fraction of the liver metabolic rate accounting for underlying tissue differences in MSP content and CYP2E1 content in MSP. Model verification indicated sensitivity of the kidney dose metric to the oral exposure profile. For a given average daily dose, if exposure occurred continuously over 24 hours at low levels, the liver metabolic fraction would be high and that of the kidney would be low. Alternatively, if exposure occurred as discrete bolus events a few times a day, liver peak concentrations would be high, resulting in saturated liver metabolism, higher systemic distribution and therefore a higher kidney dose.

OEHHA implemented this model using Berkeley Madonna software (https://berkeley-madonna.myshopify.com/). The parameters used are shown in Table B.1.

Table B.1. PBPK Model Parameters for Chloroform (Sasso *et al.*, 2013, with revisions)

Parameter	Rat	Human
BW, Bodyweight, kg	0.25	70.0
QPC, Ventilation Rate. L/hr/kg$^{0.74}$	14.0	15.0
QCC, Cardiac Output, L/hr/kg$^{0.74}$	14.0	15.0
QLC, Liver Blood Flow, fraction of Cardiac Output	0.25*QC	0.26*QC
QFC, Fat Blood Flow	0.09*QC	0.05*QC
QKC, Kidney Blood Flow	0.14*QC	0.18*QC
QKcortex, Cortex Blood Flow	0.9*QKC	0.9*QKC
QKmedulla, Medulla Blood Flow	0.1*QKC	0.1*QKC
QSC, Slowly Perfused Blood Flow	0.15*QC	0.19*QC
QVrgC, Rapidly Perfused Blood Flow	0.37*QC	0.32*QC
Qpu, Pulmonary Blood Flow	0.93*QC	0.93*QC
Qbr, Bronchial Blood Flow	0.07*QC	0.07*QC
VLC, Volume of Liver, fraction of BW	0.04*BW	0.026*BW
VFC, Volume of Fat	0.07*BW	0.19*BW
VKC Volume of Kidneys	0.0073*BW	0.0044*BW
VKcorr	0.7*VK	0.7VK
VKmed	0.3*VK	0.3*VK
Vvrg, Volume of rapidly perfused	0.253*BW$^{0.61}$-Vlu	0.253*BW$^{0.61}$-Vlu
Vlu, Volume of Lung	0.014*BW	0.014*BW
Vpu, Volume of Pulmonary region of Lung	0.9*Vlu	0.9*Vlu
Vbr, Volume of Bronchial Region of Lung	0.1*Vlu	0.1*Vlu
VSC, Volume of Slowly Perfused Tissues	BW-(Vf+Vl+Vvrg+ Vk+Vs+Vlu)	BW- (Vf+Vl+Vvrg+ Vk+Vs+Vlu)
PB, Blood:Air, Partition Coeff.	17.7	11.34
PL, Liver:Blood, Partition Coeff.	1.0	1.6
PF, Fat:Blood, Partition Coeff.	19.9	31.0
PK, Kidney:Blood, Partition Coeff.	0.62	0.97
PS, Slowly Perfused:Blood, Partition Coeff.	1.0	1.5
Pvrg, Rapidly Perfused:Blood Partition Coeff.	1.0	1.6
Plu, Lung:Blood Partition Coeff.	1.01	1.01
VmaxC, (Liver, mg/kg/hr$^{0.7}$)	5.218	8.956
VmaxkC, (Kidney, mg/kg/hr$^{0.7}$)	0.0417	0.154
Km, Liver, kidney, mg/L	0.12	0.012
KAI, Oral Absorption from Water, /hr	5.0	5.0

An example of the Berkeley Madonna model code is given in Section B3.

Application of PBPK Dosimetry to Model Kidney Toxicity Dose-Response

Sasso *et al.* (2013) used the PBPK model (coded in Advanced Continuous Simulation Language using acslXtreme software) to model non-cancer kidney endpoints. They used the dose metric of internal chloroform metabolite concentration in the kidney cortex (Cintk = Ametk/Vkcor) in units of mg/L-24hr. They used the PBPK models for mice and rats to convert applied doses to Cintk values. Then, they used benchmark dose modeling to characterize the relationship between incidence rate and Cintk and find corresponding BMD_{10} and $BMDL_{10}$ values. They assumed that the Cintk affecting toxic responses in the kidney cortex is approximately equivalent in different species for a given endpoint. For a specific endpoint, they used a human PBPK model to find the human exposure corresponding to the $BMDL_{10}$ based on the internal kidney dose metric (Cintk, mg/L-24hr). Then the human exposure was converted to human equivalent dose (HED, in mg/kg-day). OEHHA used a similar approach for dosimetry calculations in conjunction with benchmark dose-response modeling to derive $BMDL_{05}$s for non-cancer endpoints for chloroform, as presented in Chapter 10 and in Appendix D.

Application of PBPK Dosimetry to Model Kidney Cancer Dose-Response

OEHHA also applied the PBPK approach of Sasso *et al.* (2013) as well as benchmark dose modeling to the Nagano *et al.* (2006) renal cancer data to derive benchmark doses for the cancer endpoint. The cancer study was done in the same laboratory using the same dose protocol as the non-cancer study. The dose response data are shown in Table B.2. The table format is based in part on Table 6 in Sasso *et al.* (2013). The first two rows of Table B.2 give the exposure condition in the experiments for the different dose groups: inhalation only (columns 2-5), *ad lib* drinking water only (column 6), and both inhalation and *ad lib* drinking water (columns 7-9). The third row of the table provides the administered dose uptake estimates in mg/kg-day provided by Sasso. The fourth row gives the internal kidney dose estimates for the different dose groups in mg/L-24hr. The last row gives the corresponding quantal response data for renal tumor incidence observed by Nagano *et al.* (2006).

Table B.2. Administered Chloroform Concentrations in Air and Water, Rat Internal Doses Calculated Using the Sasso *et al.* PBPK Model, and Male Rat Kidney Tumor Incidence Data from Nagano *et al.* (2006)

Concentration in air, 6 hr/day, 5 day/wk, 1wk	**0**	**25**	**50**	**100**	**0**	**25**	**50**	**100**
Concentration in drinking water (ppm) given *ad lib* per schedule irregular pulsed	**0**	**0**	**0**	**0**	**1000**	**1000**	**1000**	**1000**
Uptake, mg/kg-day	0	20	39	78	45	73	93	135
Internal kidney dose, mg/L-24hr	0	42.5	51.8	62.2	65.4	103.9	112.0	124.2
Renal Adenoma or Carcinoma	0/50	0/50	0/50	1/50	0/49	4/50	4/50	18/50

Table B.3 shows the results of the BMD analyses for the dose-response data modeling the internal kidney dose metric vs. renal tumor data. Using all dose groups, there was not an adequate fit for the multistage-cancer model ($p = 0.0024$). As illustrated in Figure B.1a below,

the top dose group fell above the best fit and upper bound curve. Removing the top dose resulted in a good fit of the multistage model, as illustrated in Figure B.1b.

Table B.3. Results of Benchmark Dose Analyses Fitting the Multistage-Cancer Model to the PBPK Internal Dose vs. Tumor Incidence Data from Nagano *et al.* (2006)

Dose Response Data Used	p-value	AIC	BMD_{05}, mg/L-24 hr	$BMDL_{05}$, mg/L-24 hr
All dose groups	0.0024	157.301	67.14	57.76
Top dose group removed	0.7876	72.8447	99.74	77.58

The $BMDL_{05}$ for internal kidney dose in the rat, is 77.58 mg/L-24 hr.

To calculate the human equivalent dose (HED) in mg/kg-day that corresponds to the internal kidney dose of 77.58 mg/L-24 hr, we first characterized the relationship between the values from the human PBPK model of external exposure of 0, 25, 50, and 100 ppm in air and the internal kidney dose (3, 124.2, 177.35, 219.98 mg/L-24hr), based on the assumption that internal dose metric in kidney produces similar responses across species (Sasso *et al.*, 2013). Note that exposure through water was the same in all dose groups in Sasso *et al.* (2013). The polynomial equation fitting the data was $Y(ppm) = -1.8455+0.6290X -0.006656X^2 + 2.6826E-5X^3$, $Chi^2 = 8.1E-28$, X = mg/L-24hr (PSI Plot v. 10 for Windows, Poly Software Intl. Inc., Pearl River, NY). We used this relationship to solve for the human equivalent exposure corresponding to the BMDL05 of 77.58 mg/L-24 hr and obtained a value of 19.42 ppm. Finally, this exposure value was used in the human PBPK model to obtain the HED, which is equivalent to an intake of 30.2 mg/kg-day.

The ratio of the 5% risk level to that lower bound on dose, multiplied by a pharmacodynamics factor (PDF) to adjust for possible pharmacodynamic difference between rats and humans, results in the cancer slope factor (CSF), or cancer potency.

$$CSF = [0.05 \div BMDL_{05}] \times PDF$$

$$PDF = (bw_h/bw_a)^{1/8},$$

where bw_h represents the human bodyweight, assumed to be 70 kilograms, and bw_a represents the rat bodyweight, assumed to be 0.25 kilograms. Thus

$$CSF = [0.05 \div BMDL_{05}] \times (bw_h/bw_a)^{1/8}$$

$$= [0.05 \div 30.2 \text{ mg/kg-d}] \times (70/0.25)^{1/8} = 0.0033 \text{ (mg/kg-day)}^{-1}$$

This is comparable to the value of 0.004 $(mg/kg\text{-}day)^{-1}$ derived applying standard methodology to the Nagano *et al.* (2006) dose response data (see Chapter 10).

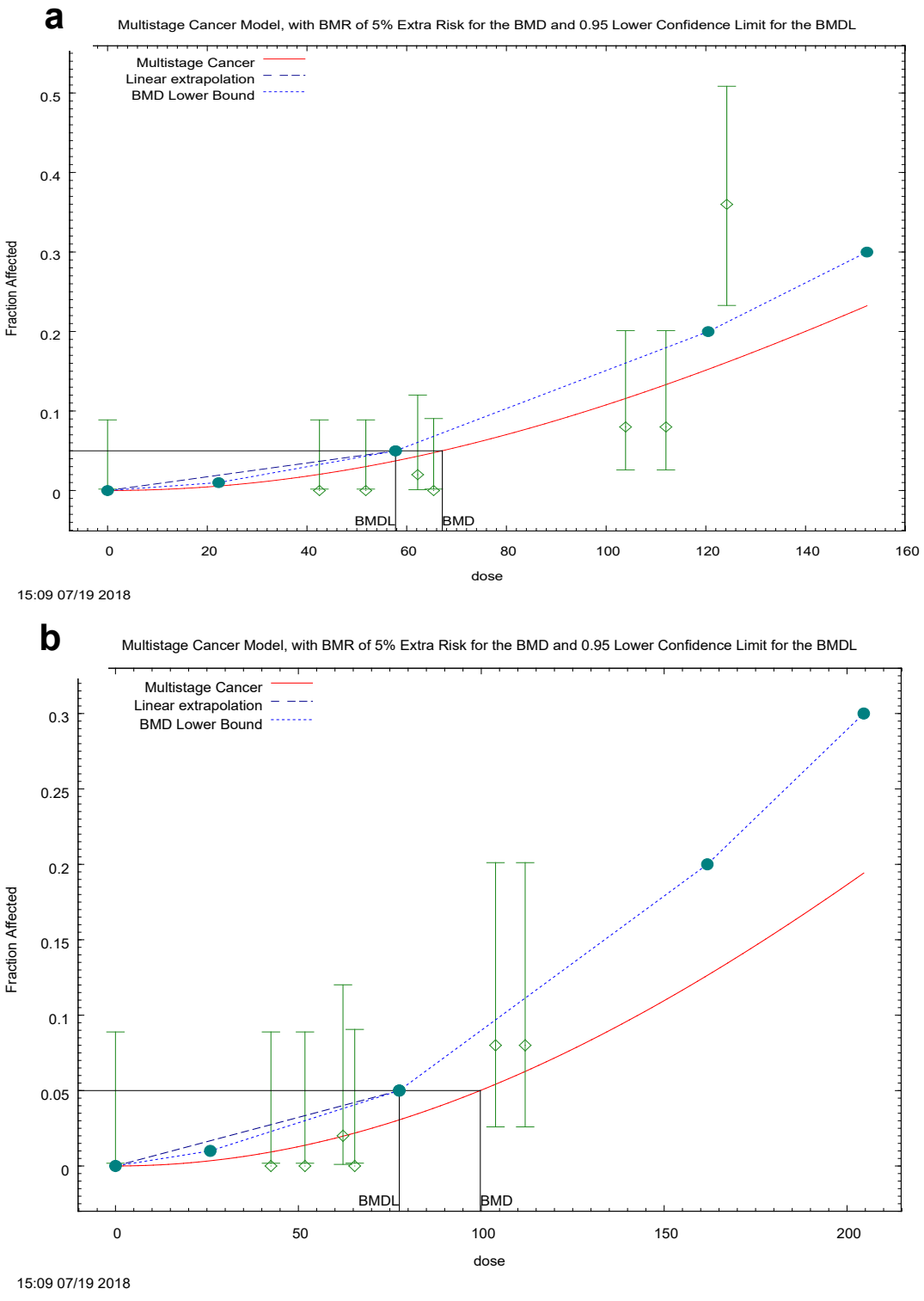

Figure B.1. Fit of the multistage model to the internal kidney dose (rat) vs kidney tumor incidence data observed in Nagano *et al.* (2006). a) Model fit to all data points, b) Model fit with the top dose removed

B2. Physiologically-Based Pharmacokinetic Models: Review of THM Models

Physiologically-based pharmacokinetic (PBPK) models mathematically describe the rate of uptake, distribution, metabolism, and elimination of xenobiotics in humans and experimental animals. These multi-compartment 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.

PBPK models published in the scientific literature that have been developed to study the disposition of THMs are described below and are organized by the exposure route for which they were developed. Table B.4 summarizes the major PBPK models used to study the pharmacokinetics of THMs via the oral and inhalation routes, and Table 7 presents PBPK models developed to describe THM disposition via the dermal route. PBPK models reviewed in this appendix have been developed primarily to study chloroform. Models that have been developed to describe the disposition of other THMs are clearly noted as such.

PBPK Models for Oral and Inhalation Routes

Table B.4. Summary of Major PBPK Models Developed to Describe Disposition of THMs via Oral and Inhalation Routes

Model	Description
For Chloroform	
Feingold and Holaday (1977)	• 5-compartment chloroform PBPK models for humans • One model assumed metabolism is linear, first-order process • Other model assumed specific, non-linear relation between hepatic arterial concentration and metabolized fraction
Corley *et al.* (1990)	• 5-compartment chloroform PBPK models, based on Ramsey and Andersen (1984) model, for mice, rats and humans • Include kidney component of richly perfused tissue compartment, assumed to metabolize chloroform via Michaelis-Menten kinetics • Model incorporated partially recoverable enzymatic self-inhibition • Excluded 1/9 measured enzyme activities in deriving average value for human liver enzyme activity to estimate V_{max} due to "abnormal distribution of [CYPs]" • Model predicted within a factor of two total metabolism, body burden, exhaled parent compound and macromolecular binding for mice and rats exposed via inhalation • Model provided similar or better ability to predict metabolic data for mice, rats and humans from three earlier mass balance studies
Bogen *et al.* (1992b)	• Chloroform PBPK models, based on Ramsey and Andersen (1984) model, for mice, rats and humans • Adjusted V_{max} values so that chloroform metabolism in the models for mice, rats and humans matched that of the studies selected for data extrapolation • Improved models used to estimate metabolized doses in animals and potential effective doses to humans exposed environmentally to chloroform

Model	Description
Gearhart *et al.* (1993)	• Chloroform mouse PBPK model based on Ramsey and Andersen (1984) model • Linear equation incorporated to describe decreases in metabolic activity resulting from decreases in core body temperature due to high doses • Temperature corrections greatly improved overall fit of gas uptake curves at all concentrations • First-order metabolic rate constant also required to adequately represent data at high concentrations
Sasso *et al.* (2013)	• Extension of Corley *et al.* (1990) PBPK model with revised rate parameters for renal chloroform metabolism in rats, mice and humans • Accounted for MSP[a] content and mass of renal metabolically active region • Assumed renal cortex comprises 70% of kidney volume, receives 90% of blood flow and expresses 100% of kidney CYP2E1 • Assumed CYP2E1 activity toward chloroform is equivalent in liver and kidney • Kidney dose metric demonstrated sensitivity to the oral exposure profile
For Bromodichloromethane (or other THMs)	
Lilly *et al.* (1997a)	• 5-compartment PBPK model for inhalation toxicokinetics of BDCM in rats, based on Ramsey and Andersen (1984) model • Metabolism described as a saturable, high-capacity, high-affinity process
Lilly *et al.* (1998)	• 6-compartment PBPK model to study pharmacokinetics of orally ingested BDCM, based on Lilly *et al.* (1997a) model • Incorporated multicompartmental gastrointestinal absorption approach and parameterization using blood and exhaled breath chamber concentration-time data • Assumed 95% metabolism in liver and 5% in kidney
Yoshida *et al.* (1999)	• Used a classical pharmacokinetic, rather than a PBPK, modeling approach to compare the absorption and metabolic elimination of these compounds. • THM compounds were injected into a closed chamber system containing one male Sprague-Dawley rat, and the air concentration of the substances was monitored. • Pharmacokinetics of BDCM and DBCM were evaluated using a model system in which the rat body, chamber and tank were each represented by a single compartment.

[a]Microsomal protein

Feingold and Holaday (1977)

Feingold and Holaday (1977) applied pharmacokinetic models to metabolism and elimination of chloroform in humans. One of their models assumed 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, while their other model assumed a specific, but non-linear relation between the hepatic arterial concentration of chloroform and the metabolized fraction. The first model predicted the percentage of dose metabolized depended on the hepatic fraction value and the exposure duration. The non-linear model predicted that the percentage of chloroform metabolized would not be substantially affected by the duration of exposure.

Corley et al. (1990)

Corley *et al.* (1990) developed a five-compartment PBPK model for chloroform in mice, rats, and humans, which OEHHA found very useful for predicting human risk of exposure to chloroform. The specific tissue volumes, blood flow rates, and PBPK model structure were otherwise similar to those shown in Table B.5 (but scaled for a 28.5 g mouse and 230 g rat), except that partially recoverable enzymatic self-inhibition was incorporated into the Michaelis-Menten model for chloroform metabolism in mice.

The V_{max} for 70 kg humans was estimated by Corley *et al.* (1990) by comparing the *in vivo* metabolic data for mice and rats with corresponding data obtained *in vitro* from liver and kidney microsomes of mice, rats, and humans, given the measured (or assumed) values of K_m used in the analysis. In deriving an average value for human liver enzyme activity to obtain this V_{max} 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 cytochrome P450 isozymes which are inducible by ethanol and barbiturates." No estimate was offered for the extent to which such high-level enzyme activities may be present in the general population.

Table B.5. 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			
$V_{max}C$, mg/hour-kg	22.8	6.8	15.7
K_m, mg/L[a]	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

a The K_m values for mice and rats were determined from gas uptake experiments in those species; the value for humans was taken as the average of the values for mice and rats.

In this model, the metric for tissue-specific effective dose was the daily, time-weighted average amount of chloroform metabolite(s) binding to macromolecules within the target tissue (kidney

or liver), modeled as a first-order accumulation within these tissues. The corresponding rate constants were estimated based on a fit of the model to data on tissue-specific binding measured six hours post-exposure in rats and mice exposed to chloroform by inhalation at three different air concentrations. Values for humans for each tissue were apparently set equal to the average of the corresponding values for mice and rats. To fit the five-compartment PBPK model to data involving exposure by gavage or injection, Corley *et al.* (1990) assumed first-order kinetics for uptake from corn oil and water, and by intraperitoneal injection.

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).

Variants of Corley et al. (1990) PBPK Model

The Corley *et al.* (1990) PBPK model has been adapted by different authors for various areas of investigation. Smith *et al.* (1995) adapted the Corley *et al.* (1990) PBPK 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. Delic *et al.* (2000) added loss and recovery of metabolism to the rodent and human models of Corley *et al.* (1990) to predict metabolic rates of chloroform in rats and humans.

Bogen et al. (1992b)

Bogen *et al.* (1992b) further developed the 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. Bogen *et al.* (1992b) selected values for physiological parameters and K_m and then adjusted the values of V_{max} 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 "V_{max} 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/N rat tissues. It also initially assumed values for V_{max} (7.0 mg/hour) and K_m (0.25 mg/L) determined for F344/N rats, with V_{max} 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 (US EPA, 1988). 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

experimental rat body weight, flowrate parameter values (in units of L/hour) were scaled to the 0.74 power of body weight, and V_{max} was scaled to the 0.70 power of body weight (Ramsey and Andersen, 1984; Gargas *et al.*, 1986a; Paustenbach *et al.*, 1988). Oral absorption kinetics of chloroform were presumed to be similar to those observed by Withey *et al.* (1983). Ingested doses were assumed to be introduced directly into the liver compartment.

Comparing the predictions of the Bogen *et al.* (1992b) model to the data of Mink *et al.* (1986) and one of the data sets (11.9 mg/kg dose) 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 hours); 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 V_{max} estimate of Gargas *et al.* (1988), and the model significantly underestimated the amount of chloroform metabolized. The data of Brown *et al.* (1974) were used to derive an "improved" estimate of V_{max} (37.2 mg/hour for a 1.0 kg rat), which yielded estimated chloroform metabolism in accordance with the best experimental data (73.7 percent of a 60 mg/kg dose 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. Mouse metabolic constants were obtained by setting the mouse K_m equal to that of the rat and scaling the rat V_{max} to the 0.7 power of body weight, as described above. Other physiological parameters for mice were taken from US EPA (1988), and scaled 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, iterative optimization was used to calculate a "corrected" value of V_{max} for mice.

Data on the metabolism and elimination of chloroform in humans via the oral route is largely limited to that of Fry *et al.* (1972), who observed that an average of 49.5 percent of a 500 mg oral dose of ^{13}C-chloroform administered to two humans was metabolized to ^{13}C-carbon dioxide within eight hours post-exposure. The Michaelis constant, K_m, was assumed to be equal to that used for rats. The value of V_{max} for chloroform required for 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 V_{max} 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 dose of chloroform between 250 and 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). Assuming that all unexpired chloroform was actually metabolized in the latter study, these data suggest that as much as 82 percent of an ingested 500 mg dose may be metabolized by humans. Therefore the V_{max} 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.

Table B.6. 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			
V_{max}, mg/hour	4.7	37.2	560
K_m, mg/L	0.25	0.25	0.25

Gearhart et al. (1993)

The PBPK model of Gearhart *et al.* (1993), based on the model of Ramsey and Andersen (1984), incorporated the effects of decreased core body temperature resulting from high chloroform doses on the metabolic components of B6C3F1 mice exposed by applying a linear equation describing the metabolic activity-temperature correlation. Addition of these temperature corrections to the model greatly improved the overall fit of the gas uptake curves at all concentrations. Incorporation of a first-order metabolic rate constant was also required to adequately represent the data at high concentrations.

Models for Bromodichloromethane

Lilly et al. (1997a)

Lilly *et al.* (1997a) developed a PBPK model for inhalation toxicokinetics of BDCM in male rats. Air partition coefficients for blood and key tissues were determined using a vial equilibration technique. BDCM metabolism rates were estimated using both data for bromide ion production during four-hour, constant concentration (50 to 3,200 ppm) inhalation exposures and data from

closed chamber gas uptake experiments. Metabolism was described using a single saturable 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)

Lilly *et al.* (1998) developed a PBPK model to study the pharmacokinetics of orally administered BDCM 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 ion levels in F344/N 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 accurately described BDCM concentrations in kidney and bromide concentrations in plasma without adjustment. However, the model simulations overestimated the measured liver concentrations for both vehicles and were only slightly improved by adjustment for a short period of continued metabolism following tissue excision.

Yoshida et al. (1999)

Yoshida *et al.* (1999) studied absorption and metabolism of chloroform, BDCM, and DBCM in low-level inhalation exposures in rats. They used a classical pharmacokinetic, rather than a PBPK, modeling approach to compare the absorption and metabolic elimination of these compounds. The compounds were injected into a closed chamber system containing one male Sprague-Dawley rat, and the air concentration of the substances was monitored. The pharmacokinetics of BDCM and DBCM were evaluated using a model system in which the rat body, chamber and tank were each represented by a single compartment; in the model for chloroform, the rat body and chamber were each described by one compartment. The rate constants in the metabolic and exhalation elimination process, k_e and k_2, were compared for each compound; k_e was greater than k_2 for BDCM and DBCM. The rate constants of the metabolic elimination process ($V_{max} \div (K_m + C_R) = K$) were calculated. The value of K exceeded k_2 at exposure concentrations less than about 950 ppm chloroform. V_{max} and K_m were estimated to be 86 ppm/hour and 107 ppm, respectively. Fitting Michaelis-Menten kinetics to the rat model, metabolism of the three THMs was found to be linear below 10 ppm in air.

PBPK Models Incorporating a Dermal Component

Table B.7. Summary of Major PBPK Models Developed to Describe Disposition of THMs via the Dermal Route

Model	Description
Blancato and Chiu (1994)	• Addition of dermal absorption component to Corley *et al.* (1990) human PBPK model
Chinery and Gleason (1993)	• Multi-compartment skin component added to Corley *et al.* (1990) human PBPK model and combined with shower exposure model
McKone (1993)	• Combination of dermal uptake models and revised PBPK model based on Corley *et al.* (1990) model to simulate chloroform breath levels following dermal/inhalation exposures while showering • Examined association between exposures and liver metabolized dose, as well as chloroform concentration at which metabolism is nonlinear
Haddad *et al.* (2006)	• 5 human PBPK models describing absorption of THMs through ingestion, inhalation and skin to estimate internal exposures • Incorporation of physiological parameters varying with age, sex and body measures helped predict influence of individual variation on absorbed dose
U.S. EPA (2006b)	• 7-compartment PBPK models based on Gargas *et al.* (1986b, 1990) model • Integration of water use behavior, ventilation and drinking water THM concentrations into PBPK models to estimate internal exposures via oral, inhalation and dermal routes for 6 year-old child and adult male and female • Combination of stochastic and deterministic modeling approaches with measured drinking water THM levels improved estimates of internal doses
Tan *et al.* (2006)	• Combination of PBPK model (based on Corley *et al.*, 2000) and shower exposure model to evaluate residential chloroform exposures via oral, inhalation and dermal routes • Used to estimate exposure distributions consistent with measured chloroform levels in blood and exhaled breath

Blancato and Chiu (1994)

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 for which chloroform exhalation data were available, and reported that the model fit the data well.

Chinery and Gleason (1993)

Chinery and Gleason (1993) have reported a human PBPK model incorporating a dermal absorption component. This model was developed by modifying the Corley *et al.* (1990) PBPK model to include a multi-compartment skin component and a completely mixed shower exposure model. This shower/PBPK model was useful for predicting chloroform concentrations in shower air and exhaled breath of persons exposed dermally and by inhalation while showering in addition to estimating the absorbed dose and tissue distribution.

McKone (1993)

McKone (1993) has also reported a human PBPK model incorporating a dermal absorption component. Dermal uptake models and a revised PBPK model based on the PBPK model of

Corley *et al.* (1990) were developed and combined to simulate chloroform breath levels in persons exposed by dermal or inhalation routes while showering. The model was used to examine the relationship between dermal/inhalation exposure and the liver metabolized dose, as well as to determine the concentration of chloroform at which nonlinear metabolism occurs via dermal/inhalation exposure.

Haddad et al. (2006)

Haddad *et al.* (2006) developed physiologically-based toxicokinetic (PBTK) human models for the THMs (as well as trichloroethylene) to estimate internal exposure to these chemicals for multimedia indoor exposure scenarios. The models enable consideration of the impact of biological variability in estimation of internal doses. Five PBTK models describing absorption through ingestion, inhalation and skin were developed for the contaminants. Incorporation of physiological parameters varying as a function of age, sex, and body measures allowed prediction of the influence of individual variations on absorbed dose and dosimetry of THMs derived from tap water in normal household activities. The simulations estimated that ingestion contributes to less than 50 percent of the total absorbed or metabolized dose for each chemical. The simulations indicate that multimedia exposure is up to twice as high in children as in adults.

US EPA (2006b)

US EPA (2006b) similarly developed a method to estimate internal exposures to THMs via the oral, dermal and inhalation routes from household uses of tap water. The authors integrated several data sets that characterize activity patterns, water use behavior, household volumes and ventilation, and THM concentrations in drinking water. PBPK modeling was used to translate external exposures to internal doses for adult males, females, and a 6-year-old child. The PBPK model used was based on the model by Gargas *et al.* (1986b, 1990) and consisted of seven compartments. Inhalation exposures predominated and children were shown to develop higher internal doses than adults. Combining stochastic and deterministic models and modeling approaches with measured concentrations of THMs in drinking water was shown to provide improved estimates of internal doses for THM risk assessment as well as for evaluation of potential toxicokinetic interactions among various mixtures of chemicals.

Tan et al. (2006)

Tan *et al.* (2006) combined a chloroform PBPK model with an exposure model for showering to estimate the air intake concentrations of chloroform based on measured blood and exhaled breath concentrations of chloroform. The PBPK model was based on the Corley *et al.* (2000) model, which is a modified version of the Corley *et al.* (1990) model consisting of a skin compartment to describe dermal uptake of chloroform. Three previously published studies describing exhaled breath and blood concentrations in people exposed to chloroform under controlled conditions while showering were used to help develop and evaluate the combined shower/PBPK model. This combined-route model (ingestion, inhalation, and dermal absorption) was used to evaluate potential chloroform exposures in residential water use scenarios. The simulation results showed that inhalation and dermal exposure could substantially contribute to total chloroform exposure. Monte Carlo analysis demonstrated that variability in exposure parameters should contribute more to variability in blood concentrations than physiological

variations such as body weight. The residential exposure model was then used for "reverse dosimetry," or estimation of distributions of exposure consistent with concentrations of chloroform measured in human blood and exhaled air.

PBPK Models Joined with a Pharmacodynamic Component

Reitz et al. (1990)

The model of Corley *et al.* (1990) was extended by Reitz *et al.* (1990) by adding a pharmacodynamic (PD) endpoint, 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.

Liao et al. (2007)

Liao *et al.* (2007) developed a PBPK/PD model to estimate chloroform cancer risk based on a presumed cytotoxicity and regenerative cell proliferation MOA. The model links the processes of chloroform metabolism, reparable cell damage, cell death, and regenerative cellular proliferation to support the cancer dose-response assessment. The rodent PD parameters and human age-dependent physiological and metabolism parameters were used to scale up from rodents to humans. The human model was used to predict exposure conditions under which chloroform-mediated cytolethality would be expected to occur in liver and kidney of adults and children. The authors concluded that a child would have less risk than an adult except for liver effects from inhalation, for which a child was estimated to be twice as sensitive. Because of lack of data on chloroform cytotoxicity and cell proliferation and repair rates in human liver and kidneys, as well as uncertainty about other physiological parameters, especially in infants, neither the accuracy nor the validity of this model can be verified as it relates to human cancer risk.

Luke et al. (2010)

Luke *et al.* (2010) developed a combined PBPK/PD model to estimate liver cancer risk from exposure to chloroform, carbon tetrachloride, and N,N-dimethylformamide. The model is based on the proposed MOA for carcinogens characterized by the key events of cytotoxicity and regenerative proliferation. The comparisons of the dose-response model across chemicals suggested to the authors that significant variation among the models relates to chemical-specific parameters such as metabolism and cellular injury rate constants. Optimization of model parameters to tumor data resulted in similar estimates for carbon tetrachloride and N,N-dimethylformamide for the parameters related to cytotoxicity and tumor incidences, but a higher estimate for reduction of death rate of initiated cells for chloroform. This difference among the three chemicals is unexpected if a given level of cytotoxicity will lead to the same level of regenerative proliferation and tumor response, and implies that additional steps beyond cytotoxicity leading to induced cellular proliferation can differ among chemicals that share cytotoxicity as their hypothesized carcinogenic MOA. The authors suggest that focusing on cytotoxicity as the sole mechanism leading to carcinogenicity of chloroform "oversimplifies several possible key steps that may take place *in vivo*," including "other mechanisms, key

events, or MOAs that may exist." The authors did not attempt to extrapolate their model to humans.

Considerations in PBPK Modeling of THMs

Human Variability

Various physiological, dietary and environmental factors can modulate CYP2E1 activity, which may partially account for the large inter-individual differences observed in CYP2E1 activity (see section on Inter-Individual Variability in Metabolism). However, dietary and xenobiotic exposures of individual subjects are typically not well characterized by studies investigating CYP2E1 activity. Given the high degree of CYP2E1 inducibility, this information is clearly critical for understanding the impact on metabolism of chemicals and hence toxicity. Although evidence is suggestive of polymorphisms in the 5' flanking region of the *CYP2E1* gene affecting its expression, particularly in response to inducers, no clear influence of genotype has been demonstrated for CYP2E1 by studies to date. A primary reason for this is inconsistent findings across studies. Taken together, these factors illustrate some of the challenges faced in assessing the influence of genetic polymorphisms on CYP2E1 function *in vivo*. PBPK modeling generally does not adequately account for variations in CYP2E1, or in the enzymes responsible for detoxification in the human population.

Important factors to consider when evaluating *in vivo* CYP2E1 metabolic variability include blood flow to the liver, extra-hepatic metabolism (e.g., in the kidney), potential involvement of compensating pathways for metabolism and clearance, differing interactions between inducers and inhibitors of CYP2E1, the distribution of microsomal protein content per liver mass, and abundance of accessory proteins relative to CYP2E1 enzyme expression (Lipscomb *et al.*, 2003; Lipscomb and Poet, 2008; Neafsey *et al.*, 2009). These sources of *in vivo* variation, many of which are absent from *in vitro* microsomal data used in PBPK models, may have significant influence on enzyme functionality – and consequently internal dose and toxicity – and should be incorporated into analysis of CYP2E1 variability within the human population. This may be best achieved through the use of PBPK models simulating how toxicokinetic factors and CYP2E1 enzyme activity modulate internal dose.

Application of Monte Carlo analysis is a widely adopted approach for estimating variations regarding exposure events and human pharmacokinetics for the purpose of PBPK modeling. For example, Tan *et al.* (2007) performed PBPK modeling of the THMs to estimate doses in humans corresponding to the NHANES III biomonitoring data, and incorporated Monte Carlo analysis to establish an estimate of human variability based on variation in physiological and behavioral parameters. The predicted distributions of THM concentrations in blood were in good agreement with the measured distributions from NHANES III.

Yang *et al.* (2010) used Bayesian hierarchical modeling to estimate uncertainty and variability in human PBPK parameters for multi-route exposures to chloroform using prior information and biomarker data. The PBPK model used was adapted from the model by Corley *et al.* (1990) with the distributed parameter skin model developed by Roy *et al.* (1996) to focus on inhalation-only exposure in a shower stall and dermal-only exposure from hot water baths. Results

showed a variation in blood flow rates under the different exposure scenarios, with a two-fold increase in blood flow rate associated with the hot-bath scenario. Bayesian inference optimized the PBPK model parameters, for the uncertainties in the parameters were reduced.

Valcke and Krishnan (2011) studied the effect of exposure route on the magnitude of human kinetic adjustment factors – i.e., chemical-specific adjustment factors for inter-individual variability in toxicokinetics – for chloroform, bromoform and two other volatile organic drinking water contaminants. A stochastic PBPK modeling approach was used to simulate internal dose metrics in adults, neonates, children, pregnant women and the elderly to calculate route-specific human kinetic adjustment factors per chemical. The multi-route PBPK model by Haddad *et al.* (2006), which facilitates calculation of physiological parameters as a function of body weight, height, age and sex, was modified to generate models relevant to each chemical and subpopulation under investigation. The exposure scenarios included 24-hour inhalation, body weight-adjusted ingestion, and 30-minute dermal contact. Values for human kinetic adjustment factors were highest in neonates for all exposure scenarios (3.6-7.4), and were highest for bromoform. The authors note that the estimates of kinetic variability were dependent on the route of exposure, dose metric and subpopulation considered.

Uncertainty in Selection of Parameters

A common problem in PBPK modeling is choice of model parameters. Smith and Evans (1995) evaluated the uncertainty in fitted estimates of apparent *in vivo* metabolic constants for chloroform. Joint confidence regions for V_{max} and K_m from each experiment, generated using maximum likelihood techniques, were used to evaluate three data sets (Corley *et al.*, 1990; Withey and Collins, 1980). The V_{max} and K_m 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 K_m and a high-affinity low K_m, then the estimated K_m will be dose-dependent. Testai *et al.* (1990) reported evidence for both low- and high-affinity oxidative processes for chloroform. Alternative explanations such as 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 K_m that exhibits some dose-dependency.

Competitive Inhibition and Model Validation

Since co-exposures of THMs and other related compounds are the norm, competitive inhibition of metabolism may influence blood concentrations of the various THMs and their metabolites, and thus toxicity. Da Silva *et al.* (1999) described five-compartment PBPK models for each of the four THMs. The chloroform and BDCM models were based largely on those of 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 were included for metabolism of DBCM or bromoform by the kidney. 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 (V_{max}, K_m, and k_a) were obtained by fitting simulations to the data, the models cannot be said to be validated by independent data.

B3. Chloroform PBPK Computer Code

This section lists computer code that can be used to model chloroform pharmacokinetics using the Berkeley Madonna software program. Using the STIFF integration method, the model simulates a one-week exposure in 188 steps, essentially instantaneously. In Berkeley Madonna, statements in curled parentheses {} are not read by the program. By adding and removing these parentheses we can affect different drinking water, air only, and air plus drinking water exposures. To run the model below, copy the code without this note, open Berkeley Madonna, and choose New Model. Paste the code below default new model code, and backspace over the former to delete the default code. Remove (or add) curled parentheses {} to engage (or disengage) desired exposures: air, DW or both. Choose new graph(s) and select desired variables to follow. Compile the model, run, and check graph(s). Toggle to table(s), scroll to end record values, convert values from mol/L etc. to desired units.

```
METHOD Stiff
STARTTIME = 0
STOPTIME=168
DT = 0.001
{OEHHA air exposure model revised with parameters etc. from Sasso et al. 2013 for inhalation and/or drinking water exposures}
{chloroform moles}
init Af = 0 {fat}
Limit Af >= 0
init Al = 0 {liver}
Limit Al >= 0
init Ak = 0 {kidney}
Limit Ak >= 0
init Akcor = 0 {kidney cortex}
Limit Akcor >= 0
init Akmed = 0 {kidney medulla}
Limit Akmed >= 0
init Am = 0 {muscle}
Limit Am >= 0
init Avrg = 0 {vessel rich group}
Limit Avrg >= 0
init Abr = 0 {bronchi}
Limit Abr >= 0
init Apu = 0 {alveoli}
Limit Apu >= 0
Limit Apu >= 0
init Agi = 0 {stomach_GI tract}
```

Limit Agi >= 0
init AX = 0 {mass exhaled}
Limit AX >= 0
init Ainoral = 0 {mass ingested}
init Ainh = 0 {mass inhaled}
Limit Ainh >= 0
{moles chloroform metabolized by MFO pathway}
init Ametl = 0 {by liver}
init Ametk = 0 {by kidney cortex}
{tissue flows L/hr}
Qtot = Qalv
Qalv = 15.0*BW^0.74
QC = Qf + Ql + Qkcor + Qkmed + Qm + Qvrg
Qf = 0.05*Qtot
Ql = 0.26*Qtot
Qk = 0.18*Qtot
Qkcor =0.9*Qk
Qkmed = 0.1*Qk
Qm = 0.19*Qtot
Qvrg = 0.32*Qtot
Qpu = 0.93*Qtot
Qbr = 0.07*Qtot

{tissue volumes L}
Vf = 0.19*BW
Vl = 0.026*BW
Vk =0.0044*BW
Vkcor = 0.7*Vk
Vkmed = 0.3*Vk
Vm = BW-(Vf+Vl+Vvrg+Vpu+Vbr+Vk)
Vvrg = (0.253*BW^0.61)-Vlu
Vlu = 0.014*BW
Vpu = 0.90*Vlu
Vbr = 0.10*Vlu
BW = 70.0
{blood/air and tissue/blood partition coefficients, chloroform}
Pb = 11.34
Pl = 1.6
Pk = 0.97
Pf = 31.0
Pm = 1.5
Pvrg = 1.6
Ppu = 1.01
Pbr = 1.01

```
{chloroform oxidation metabolic parameters, mol/hr, mol/L}
VmaxC = 7.55E-4
Vmaxk = 1.3E-6*BW^0.7
Vmaxl = VmaxC*BW^0.7
Km = 1.0E-7
{uptake of chloroform from GI tract to liver, /hr}
KAI = 5.0
DW = 2/119.37  {1000ppm*2L/d in mol/day}
{exposure in ppm converted to moles/L}
Cair = 0
{ IF TIME <= 6 THEN 25*(1E-6/25.45) ELSE IF TIME >=24 AND TIME <= 30 THEN  25*(1E-
6/25.45) ELSE IF TIME >= 48 AND TIME <= 54 THEN 25*(1E-6/25.45) ELSE IF TIME >= 72
AND TIME <= 78 THEN 25*(1E-6/25.45) ELSE IF TIME >= 96 AND TIME <= 102 THEN
25*(1E-6/25.45) ELSE 0}
{calculated concentrations of chloroform}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvk = Ak/(Vk*Pk)
Cvkcor = Akcor/(Vkcor*Pk)
Cvkmed = Akmed/(Vkmed*Pk)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Qk*Cvk)/Qpu
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
Limit Cexh >= 0
Cintk = Ametk/Vkcor
RInh = Qalv*Cair
{differential equations for chloroform uptake and metabolism}
d/dt(Agi) = - KAI*Agi +  PULSE(0.25*DW,0,24) + PULSE(0.1*DW,3,24) +
PULSE(0.25*DW,5,24) + PULSE(0.1*DW,8,24) + PULSE(0.25*DW,11,24) +
PULSE(0.05*DW,15,24)
d/dt(Apu) = Qpu*(Cvipu - Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr)
d/dt(Al) = Ql*(Cart - Cvl) - Vmaxl*Cvl/(Km + Cvl) + KAI*Agi
d/dt(Ak) = Qk*(Cart - Cvk)
d/dt(Akcor) = Qkcor*(Cart-Cvkcor) - Vmaxk*Cvkcor/(Km + Cvkcor)
d/dt(Akmed) = Qkmed*(Cart - Cvkmed)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Am) = Qm*(Cart - Cvm)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
```

```
d/dt(AX) = Cexh
d/dt(Ainoral) = KAI*Agi
d/dt(Ainh) = Rinh
{amount of chloroform metabolized by MFO pathway in liver and kidney}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(Ametk) = Vmaxk*Cvkcor/(Km + Cvkcor)
{mass balance}
MassA = Af + Al + Akcor + Akmed + Am + Avrg + Agi + Abr + Apu
Limit MassA >= 0
Amet = Ametl + Ametk
Aremoved = Amet + AX
Ain = Ainoral +  Ainh
MassBalance= Ain - Aremoved
Limit MassBalance >= 0
{Flow Balance}
FlowBalance = Qtot- QC
```

APPENDIX C. EPIDEMIOLOGIC STUDIES OF DISINFECTION BYPRODUCTS AND CANCER

Studies Available

A large number of human epidemiologic studies have examined the association between exposure to disinfection byproducts (DBPs) and cancer incidence or mortality. Many of these have focused on trihalomethanes (THMs), but analyses of these agents are complicated by the coexistence of the other DBPs, including haloacetic acids (HAAs). Studies published prior to 1985 have been critically reviewed elsewhere (Wilkins *et al.*, 1979; NRC, 1980; Cantor, 1982; US EPA, 1985). In its 1985 review of chloroform, the US Environmental Protection Agency (US EPA, 1985) noted that several epidemiologic studies had identified associations between exposure to chlorinated drinking water and rectal, colon, and bladder cancer, but also cited the difficulties in distinguishing the specific effects of any single chemical species because of the high correlation among the different DBPs. IARC's 2013 review of the epidemiological studies notes that many studies identify associations between chlorinated drinking water and urinary bladder cancer. IARC's review also notes that studies have found positive associations between chlorinated drinking water and cancers of the lung, esophagus, kidney, breast and melanoma, although the analyses need to be replicated. US EPA, in promulgating its 2006 Disinfection Byproducts rule (USEPA, 2006) states that new epidemiology and toxicology studies evaluating bladder, colon, and rectal cancers have increased the weight of evidence linking these health effects to DBP exposure. US EPA also considers that a large number of people are exposed to DBPs, and the potential cancer (and reproductive and developmental) risks played a significant role in US EPA's decision to lower DBP exposures.

Since 1985, many new epidemiologic studies have examined the relationship between cancer and drinking water chlorination or concentrations of DBPs in drinking water. OEHHA performed a literature search in January-March 2018 using the PubMed and Google Scholar databases in order to identify these studies. Combinations of the following key words were used in these searches: trihalomethane, haloacetic acid, chlorination, mortality, and cancer. Searches were restricted to those articles published since January 1, 1985. No other restrictions were placed on the searches. The bibliographies of all identified articles and of relevant review articles were also searched. All human epidemiologic studies that provided some estimate of the cancer risks associated with a metric of DBP exposure were identified and these are described in Appendix C Table C1. An overall summary of each study's findings and the quality scores applied to each study are provided in Appendix C Table C2. Studies that were related to the applied literature search criteria but that were not included in Tables C1 and C2 for various reasons are listed in Appendix C Table C3.

The most common outcome assessed in the studies identified was bladder cancer, and a number of case-control studies using retrospective assessments of exposure have identified associations between total THM levels in drinking water or drinking water chlorination and increased odds ratios of incident bladder cancer, especially in men. Most of these studies used exposure modeling and/or extrapolations from more recent THM levels to estimate past exposure. Because the latency of DBP-associated cancer could be many years, and exposures

are likely to change over time in many people (i.e., through the use of new drinking water sources), these methods are likely associated with at least some exposure misclassification. However, because all of these studies appeared to have assessed DBP exposure using the same methods in cancer cases and controls, these types of exposure classification errors would most likely be non-differential and most likely bias results towards the null, not towards the positive findings identified (Greenland, 1998). Clear and consistent associations between DBPs and bladder cancer have not been identified in cohort studies. However, study design factors such as fairly short follow-up periods, small numbers of cases, limited exposure data, or inclusion of only women could have limited the ability of some of these studies to identify true associations.

For cancer types other than bladder cancer, findings have been less consistent across studies or the results were otherwise less supportive of a causal association. For colon or colorectal cancer, seven of the 12 more recent studies (those published since 1985) reported finding no associations between these cancers and various metrics of DBP exposure, including the very recent large case-control study of seemingly good quality by Villanueva *et al.* (2017). Similarly, for kidney, esophageal, ovarian, pancreatic and stomach cancer, the large majority of studies did not find clear evidence for associations. For those that did identify some evidence, most had a number of study quality issues that limited their interpretation and use in causal inference and risk assessment (see Appendix C Table C2).

For breast cancer, five of the seven more recent studies reported some evidence supporting an association, either with drinking water chlorination, TTHM exposure, or some other related exposure metric. However, in several of these, the magnitude of the association was fairly small (e.g., relative risk estimates below 1.2), the findings were not statistically significant, or evidence for a dose-response relationship was not seen or reported. For lung cancer, most studies (five of six) also reported some evidence of an association, although few evaluated potential confounding by smoking or other lung cancer risk factors, and the exposure assessment methods used in most of these studies were limited. In addition, most of the studies examining lung cancer also examined and provided relative risk estimates for a number of other cancer types, raising concerns that some of the positive findings in these studies may be due to chance alone (i.e., multiple comparisons). For rectal cancer, seven of the 12 more recent studies identified some evidence for an association. However, most had a fairly large number of potential weaknesses, and these also limited the interpretation and usefulness of these studies.

Only a few of the studies identified in this review examined the cancer risks associated with individual DBP species. In most studies, the primary exposure metric was drinking water chlorination (as a dichotomous variable, yes or no) or total trihalomethane levels (i.e., the sum of the individual THM chemical species). In the few studies that attempted to separate out the effects of the individual THMs, clear and consistent associations have not been seen for any individual species. Long-term exposure data were not available for any individual chemical species in any study, and most individual DBP species were highly correlated (McGeehin *et al.*, 1993; Salas *et al.*, 2013). Two human epidemiologic studies attempted to examine associations between HAAs and cancer. In these studies, data from the Iowa Women's Health Study were

used to evaluate kidney or ovarian cancer. Neither study found clear or consistent associations for either total HAAs or for any individual HAA species (Inoue-Choi *et al.*, 2015; Jones *et al.*, 2017).

A number of meta-analyses or pooled analyses have been done on drinking water chlorination or DBPs and cancer, mostly bladder cancer (Costet *et al.*, 2011; Morris *et al.*, 1992; Villanueva *et al.*, 2003, 2004, 2006). Most meta-analyses or pooled analyses of bladder cancer have identified statistically significant associations in men but not in women. For example, in a pooled analysis of six case-control studies of bladder cancer from North America and Europe, involving 2,806 cases and 5,254 controls with exposure estimates for at least 70% of the 40 years prior to interview, Villanueva *et al.* (2004) reported summary odds ratios (ORs) in men of 1.00 (reference), 1.10, 1.26, 1.25, and 1.44 (p-trend <0.001) for average THM levels of 0-1, >1-5, >5-25, >25-50, and >50 µg/L, respectively. In men, statistically significant dose-response trends were seen for cumulative THM exposure and for years of drinking chlorinated water. These summary ORs were adjusted for age, smoking, occupation, coffee consumption, and education. Clear associations were not seen in women. Criteria for the inclusion of studies into this meta-analysis were: 1) case-control studies of incident bladder cancer; 2) availability of detailed long-term exposure assessment of THMs; and 3) accessibility to primary data. An earlier meta-analysis by Morris *et al.* (1992) assessed multiple cancer types and identified associations between exposure to drinking water chlorination byproducts and bladder cancer (summary relative risk (RR)=1.21, 95% CI: 1.09-1.34, n=7 studies) and rectal cancer (summary RR=1.38, 95% CI: 1.01-1.87, n=6 studies). In this meta-analysis, Medline was used to identify epidemiologic studies published from 1966 to 1991. According to the authors, “Only those studies that identified morbidity or mortality as well as exposure and potential confounders at the level of the individual (i.e., case-control or cohort studies) were included in the meta-analysis. Studies that considered incidence and exposure at the level of a region or community (i.e., ecological studies) were excluded.” The authors did not mention other specific inclusion criteria. A later evaluation of the Morris *et al.* (1992) meta-analysis showed that the positive result for rectal cancer may have been artificially elevated because of the statistical methods used (Poole and Greenland, 1999). Summary relative risks for other cancers including brain, breast, colon, and lung were not elevated in the Morris *et al.* (1992) meta-analysis. In a 2010 meta-analysis of colon cancer, statistically significant increases in relative risk estimates for elevated exposure to DBPs were reported for colon cancer (summary RR=1.27, 95% CI: 1.08-1.50) and for rectal cancer (summary RR=1.30, 95% CI: 1.06-1.59) (Rahman *et al.*, 2010). However, clear increases were not seen in analyses confined to cohort studies (summary RR=1.11, 95% CI: 0.73-1.70, n=3 studies for colon cancer; summary RR=0.88, 95% CI: 0.57-1.35, n=2 studies for rectal cancer). Overall, the results of these pooled or meta-analyses support an association between DBPs and bladder cancer in men, and provide some suggestive evidence of an association with colon cancer.

Summary

Overall, the consistency of the positive findings across a number of different human epidemiologic studies linking total THM exposure or use of chlorinated drinking water to increased bladder cancer, combined with the presence of dose-response relationships in most

studies and the incorporation of data on several known potential confounding variables such as tobacco smoking, strengthen the likelihood that the positive associations seen in these studies represent true effects. The lack of data on individual THM species limits the usefulness of these findings for quantitative risk assessment of individual chemical compounds. In addition, the lack of long-term exposure data on HAAs and the difficulties in separating out the specific impacts of HAAs from other DBPs limit the usefulness of these epidemiologic data for quantitative risk assessment for HAAs at this time. Some studies have reported associations for cancer types other than bladder cancer, including breast, lung, colon, and rectal cancer. However, these findings are less consistent than those seen for bladder cancer, and various study design issues or other weaknesses limit their interpretation for risk assessment. Regardless, the findings from the studies on these particular cancer types are suggestive, and highlight the potential need for further research on DBPs and these cancer types.

Table C1. Epidemiologic studies of disinfection byproducts and cancer published since 1985

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Min and Min *et al.*, 2016	All cancers combined (mortality)	United States 1994-2011	Retrospective cohort	933 adults over age 17 years who took part in the 1999-2004 National Health and Nutritional Examination Survey	THMs (total and individual)	Blood levels collected in 1999-2004	ORs for the upper tertile vs. the lower tertile (cut-off for the upper tertile given in parentheses): Chloroform (≥20.41 pg/ml): OR=0.80 (95% CI: 0.24-2.66) (p-trend=0.747) BDCM (≥2.71 pg/ml): OR=3.91 (95% CI: 0.98-15.64) (p-trend=0.0869) DBCM (≥1.21 pg/ml): OR=4.97 (95% CI: 1.59-15.50) (p-trend=0.0298) Bromoform (≥1.80 pg/ml): OR=4.94 (95% CI: 1.56-15.61) (p-trend=0.0227) TTHM (27.24 pg/ml): OR=1.58 (95% CI: 0.51-4.85) (p-trend=0.6313)	Age, sex, race, ethnicity, education, income, smoking, alcohol, physical activity, BMI, total cholesterol, diabetes, and hypertension	• Deaths ascertained from the National Death Index through December 2011 • Average follow-up was 8.8 years • Only 19 cases of cancer • Correlation of blood levels to water or intake levels, or to long-term exposure levels are unknown.
Sharma and Goel, 2007	All cancers combined (mortality)	Gangtok, Sikkim, India 2006	Cross-sectional	1810 people age 30 and over who took part in the study house to house survey	Chlorination	Compared areas with and without drinking water chlorination	OR=1.05 (95% CI: 0.42-2.74) comparing chlorinated to non-chlorinated households	Unclear	• Only 23 cancer cases

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Beanne-Freeman *et al.*, 2017	Bladder (incident cases)	New England 2001-04	Case-control	1,213 cases and 1,418 controls ages 30-79 years	THMs (total, chlorinated, brominated)	In person interviews on lifetime residential history, water sources, water intake, showering and bathing, jobs, and other information. Residences linked to utilities and historic TTHM information from utilities as well as other US EPA and state databases. Yearly TTHM levels assigned to each residence and workplace. Showering, bathing, and water intake incorporated in some analyses.	OR for average daily TTHM intake >103.89 µg/day of 1.53 (95% CI: 1.01-2.32) (p-trend=0.16). ORs for lower exposure categories near 1.0. ORs for average TTHM concentrations >45.73 µg/L and cumulative TTHM intakes >1864.16 mg are 1.2-1.4 and not statistically significant. ORs are higher in women ORs for swimming pool use near 1.0 Some evidence of dose-response trends seen when "chlorinated" or "brominated" THMs analyzed separately	Age, sex, race, ethnicity, smoking, state, and high risk occupation	• Cases ascertained from hospital pathology departments and state cancer registries • Controls matched to cases by age, state, and sex ascertained from motor vehicle and Medicare/Medicaid records • Participation rates of 65% in cases and 65% controls • Somewhat inconsistent findings between TTHM and chlorinated or brominated THM analyses
Bove *et al.*, 2007a	Bladder (incident cases)	Western New York 1978-86	Case-control	129 cases and 256 controls; men ages 35-90 years	THMs (total and individual)	Municipal records of THM levels in local water supplies combined with data on water intake; kriging used to interpolate levels between sampling points. Total THM levels based on US EPA's method 551. Current residence and some information on past water sources collected.	ORs of 1.00 (ref), 1.43 (95% CI: 0.78-2.05), 1.93 (95% CI: 0.80-2.98), and 2.34 (95% CI: 1.01-3.66) for TTHM exposures of 0.00-38.04, 38.18-52.58, 52.59-73.82, and 74.10-351.73 µg/day (see notes) Elevated ORs also seen for individual THMs except chlorodibromomethane	Age, smoking, carotene, water intake (see notes), fiber, and alcohol	• White men only • Few details provided on case and control selection • Participation rates unclear • Percent of lifetime using current water source: average ≥89% • Possible discrepancy between exposure units in table heading and footnote • Accuracy of the kriging methods and historical exposures unclear

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Cantor *et al.*, 1987	Bladder (incident cases)	US (ten areas) 1977-78	Case-control	3,805 cases, 5,258 controls; men and women ages 21-84 years	Chlorination	Lifetime residential history linked to historical water utility data	In non-smokers: OR of 3.1 (95% CI: 1.3-7.3) (p-trend=0.01) for ≥60 years exposure to chlorinated surface water and tap water consumption above median levels. Separate results for smokers not provided. p-trend<0.001 The authors state that, "most of the duration effect arose from nonsmokers."	Age, sex, smoking, occupation, population size of residence, and reporting center	• Cases from SEER Registries • Controls randomly selected from random digit dialing and Medicare matched to cases by age, sex, and area • Participation rates of 73% in cases and 83% in controls • Similar odds ratios in male and female non-smokers • Positive results only in non-smokers
Cantor *et al.*, 1998	Bladder (incident cases)	Iowa 1986-89	Case-control	1,123 cases and 1,983 controls; men and women ages 40-85 years	Chlorination; THMs (total)	Lifetime residential history and water intake linked to water records	<u>Chlorination:</u> Men: ORs of 1.0 (ref), 1.0, 1.2, 1.3 and 1.9 for 0, 1-19, 20-39, 40-59, and ≥60 years of exposure (p-trend=0.002) <u>TTHM:</u> Men: ORs of 1.0 (ref), 1.3, 1.1, 1.1, 1.7, and 1.5 for lifetime average of ≤0.7 (ref), 0.8-2.2, 2.3-8.0, 8.1-32.5, 32.6-46.3, ≥46.4 µg/L (p-trend=0.02) Women: no clear associations	Age, study period, education, occupation, and smoking	• Cases ascertained from the Iowa State Health Registry • Controls randomly selected from state driver's licenses and Medicare matched to cases by sex and age • Participation rates of 84.6% in cases and 81.8% in controls • Limited to subjects with ≥70% lifetime residential exposure known • Some evidence of synergy with smoking
Chang *et al.*, 2007	Bladder (mortality)	Taiwan 1996-2005	Case-control	403 cases and 403 controls; men and women ages 50-69 years in 65 municipalities	THMs (total)	2000-2 survey of quarterly TTHM levels in 96 of 361 municipalities of Taiwan. 65 had a single waterworks and clear population. THM levels linked to municipality information at the time of death from death records	ORs of 1.0 (ref), 1.80, and 2.11 for TTHM levels of <13.9, 13.9-21.1, and ≥21.2 ppb (p-trend<0.001)	Age, gender, and urbanization	• Deaths obtained from the Bureau of Vital Statistics • All other deaths used as controls, randomly selected, and matched to bladder cancer cases by gender, year of birth, and year of death, excluding genitourinary disease and some other cancers • May be ecologic. Variability of THM levels within municipalities unknown • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Chevrier *et al.*, 2004	Bladder (incident cases)	France (7 hospitals) 1985-87	Case-control	281 cases and 272 controls; men and women ages 30-80 years	THMs	Residential history from 30 years before cancer diagnosis to five years before interview linked to THM levels estimated based on water source (ground vs. surface) and pre- and post-filtration chlorination	ORs (95% CI) of 1.00 (ref), 1.08 (0.6-2.0), 1.73 (0.7-4.2), and 3.39 (1.2-9.6) for cumulative exposures of 0, 1-150, 151-1500, and >1500 µg/L-years (p-trend=0.08) for all subjects Similar results in men and women although with very small numbers for women	Age, sex, hospital, SES, smoking, coffee consumption, occupation, and water intake	• Cases ascertained from seven hospitals • Controls recruited from the same hospitals were those without cancer, lung disease, or bladder symptoms, matched by age, sex, and area of residence • Participation rates unclear • Limited to subjects with ≥70% of the residential history from 5 to 35 years before interview • Exposure assessment method unclear • Small sample sizes and wide confidence intervals in results for women
Doyle *et al.*, 1997	Bladder (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87	No clear associations, all RRs near 1.0 (n=42 cases)	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Bladder (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women (p>0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
Freedman *et al.*, 1997	Bladder (incident cases)	Washington County, MD 1975-92	Case-control	294 cases and 2,326 controls; men and women ages unknown	Use of municipal (almost all chlorinated) vs. non-municipal water source	Drinking water source in 1975 (from private census data)	Men: ORs of 1.0, 1.1, 1.1, 1.3, 1.5, and 2.2 (95% CI: 0.8-5.1) for 0, 1-10, 11-20, 21-30, 31-40, and >40 years of municipal water use (p-trend=0.07) Women: no clear associations Smokers: OR of 2.8 (95% CI: 1.0-6.9) for >40 years using municipal water Non-smokers: ORs near 1.0	Adjusted for age, smoking, and urbanicity	• Whites only • Cases ascertained from the county cancer registry • Controls randomly selected from the census, matched to cases on age and gender • Participation rates and demographic comparisons unclear or not presented • Drinking water source in 1975 • Nearly all municipal water sources had been chlorinated for >30 years • Water source information collected as part of a private census • Positive results only in smokers

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Isacson *et al.*, 1985	Bladder (incident cases)	Iowa 1969-82	Ecologic	Includes cities and towns with populations 1,000-10,000, and a public water supply with a single major ground water source before 1965	Chlorination	Contaminants measured in finished water supplies of all eligible municipalities in 1979. Information on the treatments used collected from the Iowa Department of Environmental Quality and verified by water plant managers.	Only presents age adjusted risk ratios for nickel and dicloroethane in analyses stratified by chlorination status (yes or no) No clear increase seen for chlorination and bladder cancer in men or women	Age adjusted, sex specific rate ratios	• Numbers of cases obtained from the Iowa Cancer Registry • Only stratified results given • Limited data on other cancer risk factors
Jones *et al.*, 2016	Bladder (incident cases)	Iowa Women's Health Study 1986-2010	Cohort	15,577 women ages 55-69 years at baseline (1986); 130 cases	THMs (total)	Main residential source of drinking water in 1989 and number of years of used. THM levels estimated based on expert assessment, some available measurements, water source, quality, treatment, and disinfectant type.	No association for quartiles of long-term average or for ≥4 years at ≥ ½ the MCL	Age and smoking	• Women only • Cases ascertained for the years 1986-2010 from the State Health Registry and National Death Index • Baseline participation of 42% • Limited to women using their 1989 water supply >10 years • Focus was on nitrates
King and Marrett, 1996	Bladder (incident cases)	Ontario 1992-94	Case-control	696 cases and 1,545 controls; men and women ages 25-74 years	Chlorination; THMs (total)	Residential history for the 40-year period prior to interview and modeled THM levels; models based on 2,494 observations and water source (e.g., surface vs. ground), chlorination level, other treatment procedures.	<u>Chlorination:</u> ORs of 1.0 (ref), 1.04 (95% CI: 0.71-1.53), 1.15 (95% CI: 0.86-1.51), and 1.41 (95% CI: 1.09-1.81) for 0-9, 10-19, 20-34, and ≥35 years of exposure <u>TTHM:</u> ORs of 1.0 (ref), 1.20 (95% CI: 0.88-1.64), 1.08 (95% CI: 0.82-1.42), and 1.44 (95% CI: 1.10-1.88) for 0-583, 584-1,505, 1,506-1,956, and 1,957-6,425 µg/L-years cumulative exposure. OR of 1.11 (95% CI: 1.02-1.21) for each 1,000 µg/L-years increase in exposure	Age, gender, smoking, education, and total calories	• Cases ascertained from the Ontario Cancer Registry • Controls randomly selected from telephone listings matched to cases on age and gender • Participation rates of 73% in cases and 72% in controls • Only included subjects with at least 30 years of exposure data • Population attributable risks of 14-16 percent • Correlation between model predictions and observed TTHM levels was 0.76

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1997	Bladder (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: Women: OR=1.48 (95% CI: 1.01-2.18) Men: OR=1.03 (95% CI: 0.82-1.28)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1998	Bladder (incident cases)	Finland 1991-92	Case-control	732 cases and 914 controls; men and women all ages	Mutagenicity	Residential history and water intake linked to water records; mutagenicity estimated for the years 1950-87 based on TA 100 *Salmonella typhimurium* mutagenicity using data from a previous study of water containing known concentrations of total organic carbon, chlorine level, and ammonia.	Overall: OR=1.16 (95% CI: 0.90-1.47) for each 3,000 net rev/L average increase Men: OR=2.32 (95% CI: 0.99-5.45) for ≥45 years exposure >3,000 net rev/liter. Also positive in categorical analyses by years exposed Men non-smokers: OR=2.59 (95% CI: 1.13-5.94) for each 3,000 net rev/liter average increase ORs in women and in smokers near 1.0	Age, sex, SES, and smoking	• Cases ascertained from the Finnish Cancer Registry • Controls randomly selected from a nationwide population registry • Overall participation rate 68%, only slightly lower in controls • Relevance of the exposure metric is unknown • Positive results mostly in male non-smokers • Results not consistent across sexes
Lynch *et al.*, 1989	Bladder (incident cases)	Iowa 1977-78	Case-control	268 cases and 658 controls; men and women ages 21-84 years	Chlorination	Lifetime residential history and water intake linked to water records for all Iowa towns >1,000 people	ORs of 1.00 (ref), 1.42, 1.70, and 2.14 for 0, 1-25, 26-50, and >50 years of chlorinated water use (p-trend=0.001)	Unadjusted	• Whites only • Cases ascertained from the National Bladder Cancer Study • Controls selected from random digit dialing and Medicare matched to cases on age and sex • Participation rates of 82% in cases and 89% in controls • Demographic comparisons not provided • Only included subjects with exposure data for at least 50% of lifetime • Some evidence of synergy with smoking • Stepwise regression also performed but results not clear • Limited data on other cancer risk factors • Overlap with Cantor *et al.*, 1987

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
McGeehin *et al.*, 1993	Bladder (incident cases)	Colorado 1988-89	Case-control	327 cases and 261 controls; men and women ages 21-84 years	Chlorination; THMs (total)	Residential history from age 20 linked to Colorado water records; THM, chlorine, and nitrate data only based on 1989 levels; water system with major changes in water source or disinfection method classified as "unknown" for the years before change.	<u>Chlorination:</u> ORs of 1.0 (ref), 0.7, 1.4, 1.5, and 1.8 for 0, 1-10, 11-20, 21-30, and >30 years of exposure (p-trend=0.0007) <u>TTHM:</u> ORs of 1.0 (ref), 1.8, 1.1, and 1.8 for 0, 0-200, 201-600, and >600 µg/L-years (cumulative exposure) (p-trend=0.16) Similar results by sex and smoking	Sex, coffee, smoking, water intake, family history, and other urinary conditions	• Whites only • Living cases only • Cases ascertained from the Colorado Central Cancer Registry. • Controls were other cancers (except lung and colon) matched by age and sex to cases randomly selected from same registry • Participation rates of 78.0% in cases and 74.6% in controls • Only limited demographics data given • Similar results when restricted to subjects where >75% of exposure history was known • Inconsistent dose-response for TTHMs

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Villanueva *et al.*, 2007; Cantor *et al.*, 2010; Salas *et al.*, 2013; Villanueva *et al.*, 2009; Michaud *et al.*, 2007; Salas *et al.*, 2014	Bladder (incident cases)	Spain (five areas) 1998-2001	Case-control	1,219 cases and 1,271 controls; men and women ages 20-80 years	THMs (total and individual)	Residential history, water intake, showers and baths, and swimming pool use from age 15 to interview linked to municipal records on THM levels and water source history (e.g., surface vs. ground water)	Average residential TTHM exposure: Men: ORs of 1.00 (ref), 1.53, 2.34, and 2.53 for ≤8, >8.0-26.0, >26.0-49.0, and >49.0 µg/L average residential TTHM exposure (p-trend<0.01) Women: ORs of 1.00 (ref), 0.40, 1.14, and 1.50 for ≤8, >8.0-26.0, >26.0-49.0, and >49.0 µg/L (p-trend<0.61) Duration of chlorinated surface water at residence: Men: similar to women except p-trend=0.20 Women: ORs of 1.00, 2.72, 2.32, and 2.33 for 0-3, >3-25, >25-30, and >30 years use (p-trend=0.62) Individual THMs: inconsistent dose-response relationships, ORs near 1.0, or low power (Salas *et al.*, 2013) OR for swimming pool use: 1.57 (95% CI: 1.18-2.09) ORs by TTHM concentration generally higher when daily water intake is lower (Michaud *et al.*, 2007)	Age, gender, smoking, education, urbanicity, interview quality, and geographic area	• Cases ascertained from 18 hospitals from five areas in Spain • Controls ascertained from the same hospitals with non-cancer outcomes matched to cases by age, gender, and area • Participation rates of 84% in cases and 87% in controls • When water source changed, historical THM levels were based on percentage of surface water used • Similar results when exposures from showering or bathing are incorporated • Evidence of synergy with GST and CYP2E1 polymorphisms (Cantor *et al.*, 2010) • Higher ORs when low quality interviews excluded (Villanueva *et al.*, 2009) • Some evidence of interaction with LINE-1 DNA methylation (p=0.08, Salas *et al.*, 2014) • High p-trends in women

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Bladder (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated TTHM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987..	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=1.4 (95% CI: 0.8-2.4, n=12 exposed cases) Women: SMR=0.4 (95% CI: 0.0-2.0, n=1 exposed case)	Age standardized	• Cancer mortality from the local Department of Public Health for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Bladder (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: Men: SRR=1.86 (95% CI: 1.54-3.50) Women: SRR=3.92 (95% CI: 1.08-4.28)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Chlorination strongly related to surface (vs. private well) water use • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Cantor *et al.*, 1999	Brain (incident cases)	Iowa 1984-87	Case-control	291 cases and 1,983 controls; men and women ages 40-85 years	Chlorination; THMs (total)	Lifetime residential history and water intake linked to municipal water records	Chlorination: Men: ORs of 1.0 (ref), 1.3, 1.7, and 2.5 for 0, 1-19, 20-39, and ≥40 years of exposure (p-trend=0.04) TTHM: Men: ORs of 1.0 (ref), 0.9, 1.0, and 1.4 for lifetime average concentrations of ≤0.7, 0.8-2.2, 2.3-32.5, ≥32.6 µg/L (p-trend=0.04) No clear associations in women	Age, farming, and population size	• Cases ascertained from the Iowa State Health Registry • Proxy interviews in 74.4% of cases • Controls randomly selected from state drivers licenses and Medicare records, matched to cases by age and sex • Participation rates of 74.4% in cases and 79.5-81.8% in controls • Limited to subjects with ≥70% lifetime residential exposure known • Results not consistent across sexes
Koivusalo *et al.*, 1997	Brain (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: All ORs near 1.0 except glioma in women (OR=1.35; 95% CI: 0.94-1.94)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Cancer follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Separate ORs given for men and women for all brain and nervous system combined, brain only, glioma and meningioma • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Brain (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated TTHM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): SMRs in both men and women near or below 1.0	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders
Yang *et al.*, 1998	Brain (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRRs near 1.0 in both men and women	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Chlorination strongly related to surface (vs. private well) water use • Ecologic exposure data • Limited data on other cancer risk factors
Doyle *et al.*, 1997	Breast (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	RR=1.08 (95% CI: 0.85-1.37) for chloroform levels of 14-287 µg/L vs. 1-2 µg/L (n=136 cases in the upper exposure category)	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Breast (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates higher in exposed vs unexposed municipalities (74.6 vs. 65.5 per 100,000; p<0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors • Multiple comparisons may be an issue (15 cancer types in both men and women) • Small increase in relative risk
Font-Ribera *et al.*, 2018	Breast (incident cases)	Spain 2008-13	Case-control	1003 cases and 1458 controls; women ages 20-85 years	THMs (total and individual)	Structured questionnaire and face-to-face interviews. Questions on residential history, water source, bathing, showering, dishwashing. Historic THM levels back to 1940 modeled to create annual average THM levels in each water zone. These were then linked to all addresses age 18 to two years before interview and water source and showering and bathing information.	Chloroform: ORs of 1.0 (ref), 1.25 (95% CI: 0.95-1.65), 1.29 (95% CI: 0.96-1.73), and 1.47 (95% CI: 1.05-2.06) for chloroform values of ≤7.6, >7.6-18.8, >18.8-24.3, and >24.3 µg/L (p-trend=0.026) ORs for TTHMs and brominated THMs near 1.0 For chloroform, TTHM, and brominated THMs, ORs above 1.0 for exposure related to dishwashing, and near 1.0 for exposures from ingestion or showering	Age, area, education, occupation, family history, BMI, energy intake, physical activity, oral contraceptive use, menopause treatment. Smoking also assessed.	• Cases ascertained from cancer and surgical services from 14 hospitals in eight provinces • Controls randomly selected from Primary Health Centers covering “nearly all the population living in the corresponding area…”, frequency matched to cases by 5 year age groups and study area • Low response rates in controls (53%) • Only included subjects with known THM concentrations for at least 70% of years age 18 to two years before interview • Some differences between cases and controls in menopause status, occupational status, family history, menopause treatment, energy intake, and physical activity (mostly adjusted for)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1997	Breast (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	307,967 women of all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	OR for each 3,000 net rev/L increase in mutagenicity: OR=1.11 (95% CI: 1.01-1.22)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Cancer follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Small increase in relative risk
Marcus *et al.*, 1998	Breast (incident cases)	North Carolina 1995	Ecologic	6462 cases, women ages 35-84	THMs (total)	Quarterly THM readings for 1993-94 averaged and assigned to zip codes at the time of diagnosis	OR of 1.1 (95% CI: 0.9-1.2) for TTHMs ≥80 vs. <40 µg/L Similar results in White and Black women	Age, income, education, urban status, and race from Census data	• Black and White women only • Cases ascertained from the North Carolina Cancer Registry • Denominator based on 1990 Census population counts by zip code • Demographics by TTHM levels: some differences in education and percent urban • Includes all state water suppliers serving at least 10,000 customers • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Breast (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=18.4 (95% CI: 1.0-98.6, n=1 exposed case) Women: SMR=1.3 (95% CI: 0.9-1.8)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Small number of cases in men • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Breast (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRR=1.26 (95% CI: 0.89-1.77)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Chlorination strongly related to surface (vs. private well) water use • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Doyle *et al.*, 1997	Colon (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	RRs of 1.00, 1.06, 1.39, and 1.68 for chloroform concentrations of <LOD, 1-2, 3-13, 14-287 µg/L (p-trend <0.01)	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989 • Multiple comparisons may be an issue (RRs for 11 cancer types)
Flaten *et al.*, 1992	Colon (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates higher in exposed vs. unexposed communities for both men (35.9 vs. 28.8 per 100,000; p<0.05) and women (32.6 vs. 27.2 per 100,000; p<0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors • Multiple comparisons may be an issue (15 cancer types in both men and women)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Hildesheim *et al.*, 1998	Colon (incident cases)	Iowa 1986-89	Case-control	560 cases and 1,983 controls; men and women ages 40-85 years	THMs (total); chlorination	Lifetime residential history linked to drinking water records and THM measurements (collected in 1987) from all Iowa water utilities serving at least 1,000 people. Mean THM levels by treatment type and water source measured in 1987 used to estimate historical exposures.	<u>Chlorination:</u> No association (p-trend=0.13) <u>TTHM:</u> No association (p-trends=0.54 to 0.85) Similar results in men and women	Age and sex. Further adjustment for smoking, diet, and other factors did not alter results.	• Cases ascertained from the State Health Registry of Iowa • Controls randomly selected from Iowa driver's license records and Medicare • Participation rates of 85.5% for cases and 80.3% for controls • Limited to subjects with at least 70% exposure history known
Isacson *et al.*, 1985	Colon (incident cases)	Iowa 1969-82	Ecologic	Includes cities and towns with populations 1,000-10,000, and a public water supply with a single major ground water source before 1965	Chlorination	Contaminants measured in finished water supplies of all eligible municipalities in 1979. Information on the treatments used collected from the Iowa Department of Environmental Quality and verified by water plant managers.	Only presents age adjusted risk ratios for nickel and dicloroethane in analyses stratified by chlorination status (yes or no) No clear increases seen by chlorination strata in men or women	Age adjusted, sex specific rate ratios	• Demographic comparisons and exposure assessment not clear • Only stratified results given • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
King *et al.*, 2000	Colon (incident cases)	Ontario 1992-94	Case-control	767 cases and 1,545 controls; men and women ages 30-74 years	Chlorination; THMs (total)	Residential history for the 40-year period 2 years prior to interview and modeled THM levels; models based on information from a water treatment plant survey on water source and treatment characteristics.	Chlorination: Men: ORs of 1.00 (ref), 1.70 (95% CI: 1.07-2.68), 1.33 (95% CI: 0.96-1.86), and 1.53 (95% CI: 1.13-2.09) for 0-9, 10-19, 20-34, and ≥35 years of exposure TTHM: Men: ORs of 1.00 (ref), 1.30 (95% CI: 0.92-11.84), 1.11 (95% CI: 0.78-11.57), and 1.74 (95% CI: 1.25-2.43) for cumulative exposures of 0-583, 584-1,505, 1,506-1,956, and 1,957-6,425 µg/L-years of cumulative exposure. OR of 1.17 (95% CI: 1.06-1.29) for each 1,000 µg/L-years increase in exposure Women: no clear associations	Age, sex, education, BMI, total calories, cholesterol, calcium, alcohol, and coffee	• Cases ascertained from the Ontario Cancer Registry • Controls randomly selected from residential telephone listings • Participation rates 73% in cases and 72% in controls • Cases less educated than controls, but this is adjusted for • Only included subjects with at least 30 years of exposure data • Results not consistent across sexes • No information on smoking • Somewhat inconsistent dose-response pattern
Koivusalo *et al.*, 1997	Colon (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: ORs near 1.0 in men and women	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Kuo *et al.*, 2010b; Kuo *et al.*, 2011; Kuo *et al.*, 2009	Colon (mortality)	Taiwan 1998-2007	Case-control	2,180 cases and 2,180 controls; men and women ages 50-69 years in 53 municipalities	THMs (total)	2000-2 survey of quarterly THM levels in 96 of 361 municipalities of Taiwan. 53 had a single waterworks, a clear population, and water sources did not change in last decade. THM levels linked to municipality information at the time of death from death records.	OR=1.14 (95% CI: 1.01-1.28) comparing TTHM levels above and below the median (4.9 ppb)	Age, gender, marital status, and urbanization	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • All other non-gastrointestinal deaths used as controls, matched to rectal cancer cases by gender, year of birth, and year of death; deaths from certain cancers also excluded (e.g., bladder, lung) • May be ecologic. Variability of THM levels within municipalities unknown • Limited data on other cancer risk factors • Some evidence of additive effects with low water calcium and low water magnesium levels • Small increase in relative risk • Clear associations not seen in Kuo *et al.* 2009 although municipalities and years are slightly different
Rahman *et al.*, 2014	Colon (incident cases)	New South Wales 2001-06	Ecologic	Number of subjects is not clear; men and women ages ≥35 years	THMs (total and individual)	Yearly mean THM concentrations in municipal water supplies for each local government area for the years 1995-2001	Bromoform: Incidence rate ratios in men of 1.035 (95% CI: 1.017-1.053) for each interquartile increase in exposure (2 µg/L) ORs for other THMs near 1.0	Area level data on SES, alcohol, smoking, water source, and year of diagnosis	• Cancer incidence data from the New South Wales Central Cancer Registry • Indirect standardization on age and gender • Limited data on other cancer risk factors • Small increase in relative risk
Villanueva *et al.*, 2017	Colorectal (incident cases)	Spain and Italy 2008-13	Case-control	2,047 cases and 3,718 controls; men and women ages 20-85 years	THMs (total and individual)	Residential histories for age 18 to two years before interview and data on showering and bathing linked to municipal data on THM levels for 2004-10 (Spain) and varying lengths (Italy)	Total THMs: no association Chloroform: OR of 0.31 (95% CI: 0.24-0.41) comparing the highest to lowest quartile (p-trend <0.001) Similar results in men and women	Age, sex, area, education, non-steroidal anti-inflammatory drugs, smoking, physical activity, and family history. Adjustment for diet had little impact on results	• Case ascertainment is unclear • Controls were hospital controls or from randomly selected family practitioners in the same catchment areas as the participating hospitals providing the cases, matched to cases by age, sex, and area • Participation rates of 68-93% in cases and 53-95% in controls • Demographics mostly similar between cases and controls • Some evidence of synergy seen with CYP2E1 polymorphisms

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Colon (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): ORs in both men and women near 1.0	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders
Yang *et al.*, 1998	Colon (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRRs in both men and women near 1.0	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorinated and non-chlorinated areas similar in terms of population density, percentage white and blue collar workers, and percent involved in agriculture jobs • Chlorination strongly related to surface water (vs. private well water) use • Ecologic exposure data • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Young *et al.*, 1987	Colon (incident cases)	Wisconsin (exact years unknown)	Case-control	347 cases and 1,250 controls; men and women ages 35-90 years	Chlorination; THMs (total)	Lifetime residential history and water intake linked to modeled THM concentrations; TTHM estimates based on models using water characteristics and treatment variables (e.g., source, temperature, lime:alum dose…) from 81 Wisconsin water sources (approximately 47% of the state's water supply).	Chlorination: Some ORs > 1.2 depending on source of controls and latency but no clear or consistent patterns TTHMs: ORs near 1.0	Age, sex, and population size	• Whites only • Cases ascertained from the Wisconsin Cancer Reporting System • Controls included other cancers (other than gastrointestinal or urinary) and population (motor vehicle registration) controls • Overall participation rates <50% • Ecologic exposure data • Limited data on other cancer risk factors
Koivusalo *et al.*, 1997	Esophageal (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs for each 3,000 net rev/L increase in mutagenicity: Women: OR=1.90 (95% CI: 1.02-3.52) Men: OR=0.92 (95% CI: 0.51-1.66)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Tsai *et al.*, 2013	Esophageal (mortality)	Taiwan 2006-10	Case-control	881 cases and 881 controls; men and women ages 50-69 years in 53 municipalities	THMs (total)	2000-2 survey of quarterly THM levels in 96 of 361 municipalities of Taiwan. 53 had a single waterworks, a clear population, and water sources did not change in last decade. THM levels linked to municipality information at the time of death from death records.	OR=1.02 (95% CI: 0.84-1.23) comparing TTHM levels above and below the median (4.9 ppb) Some evidence of additivity or synergy with low calcium or low magnesium. For example, OR of 1.78 (95% CI: 1.19-2.68) in subjects with TTHM ≥4.9 µg/L and water magnesium concentrations <7.7 mg/L compared to those below (TTHM) and above (magnesium) these levels	Age, gender, marital status, and urbanization	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • All other non-cancer and non-gastrointestinal deaths used as controls, matched to cases by gender, year of birth, and year of death • May be ecologic. Variability in THM levels within municipalities unknown • Limited data on other cancer risk factors • Positive results only seen in strata of low magnesium or low calcium water concentrations
Yang *et al.*, 1998	Esophageal (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRRs in both men and women near or below 1.0	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Hematopoietic: lymphatic or other hematopoietic (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women ($p>0.05$)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
Infante-Rivard *et al.*, 2001; Infante-Rivard *et al.*, 2002	Hematopoietic: acute lymphoblastic leukemia (incident cases)	Quebec 1980-93	Case-control	491 cases and 491 controls ages 0-9 years	THMs (total and individual)	Telephone interview used to collect child's residential history and water sources. THM levels collected from municipalities, from the Ministry of Environment (after 1985), and a survey of 227 homes. Only 112 of 305 municipalities sent "usable" exposure data. Levels were assigned to subjects residences, and average and cumulative exposure estimated.	<u>Chloroform:</u> OR for cumulative total chloroform ≥95th percentile is 1.63 (95% CI: 0.84-3.19) (>102 µg/L). OR of 0.91 (95% CI: 0.59-1.41) for >75th percentile (44 µg/L) vs. ≤24th percentile (12.9 µg/L). <u>TTHMs:</u> OR of 1.54 (95% CI: 0.78-3.03) for cumulative total TTHM >95th percentile (level not clear) vs. ≤95th percentile post-natal exposure. ORs near 1.0 for average TTHM exposures, for prenatal exposures, and for other individual THMs.	Maternal age and education. Rates of maternal smoking similar in cases and controls.	• Cases recruited from centers designed to treat children with cancer ("population based ascertainment"), excluding children from less populated areas (approximately 10%) • Controls ascertained from family allowance files, which appear to be fairly complete, randomly selected and matched on age, sex, and region • Participation rates of 96.3% in cases and 83.8% in controls • Some evidence of synergy with CYP2E1 and GSTT1 (Infante-Rivard *et al.*, 2002) • Some elevated ORs but not statistically significant

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Kasim *et al.*, 2006	Hematopoietic: leukemia (incident cases)	Canada (eight provinces) 1994-97	Case-control	686 cases and 3,420 controls; men and women ages 20-74 years	THMs (total and bromo-dichloro-methane)	Lifetime residential history for the 40 years prior to interview linked to multiple sources of THM survey data from municipalities	TTHM: No associations for all leukemia types combined. OR of 1.70 (95% CI: 1.00-3.03) for >31 years >20 µg/L vs. no exposure for CML. Dose-response pattern seen but not statistically significant (p-trend=0.11). OR of 0.47 (95% CI: 0.32-0.68) for >31 years >20 µg/L vs. no exposure for chronic lymphocytic leukemia BDCM: Elevated OR for CML (1.63, 95% CI: 1.00-3.10, p-trend=0.12) for >24 years >5 µg/L. No association for all leukemias and other subtypes	Age, gender, occupation, benzene, and radiation	• Cases from the Canadian National Enhanced Cancer Surveillance System • Controls from random sampling of health insurance plans, property assessment database, and from random digit dialing • Participation rates of 53.5% in cases and 63% in controls • Smoking and SES variables similar between cases and controls • Limited to subjects with at least 30 years of exposure data. • Adult leukemia • Elevated ORs for CML
Koivusalo *et al.*, 1997	Hematopoietic (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs for each 3,000 net rev/L increase in mutagenicity: NHL: Women: OR=1.40 (95% CI: 0.98-1.98) Men: OR=1.03 (95% CI: 0.75-1.41) ORs in men and women mostly near 1.0 for Hodgkin's lymphoma and leukemia	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Hodgkin's lymphoma in men: "the statistical analysis did not converge" • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Hematopoietic (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): SMRs in both men and women near or below 1.0 except lymphatic leukemia in women, OR=3.2 (95% CI: 0.8-8.8)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • SMRs given for NHL and lymphatic leukemia • Results not consistent across sexes • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Doyle *et al.*, 1997	Kidney (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	All RRs near 1.0	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Kidney (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women (p>0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
Jones *et al.*, 2017	Kidney (incident cases)	Iowa Women's Health Study 1986-2010	Prospective cohort	125 kidney cancer cases among 15,577 women ages 55-69 at baseline	THMs (total and individual); HAA5	Main residential source of drinking water in 1989 and number of years of used. THM levels estimated based on expert assessment, some available measurements, water source, quality, treatment, and disinfectant type.	ORs near or below 1.0 for the following: • TTHM >14.30 µg/L • ≥36 years with TTHM >40 µg/L • HAA5 >6.43 µg/L • ≥16 years with HHA5 >30 µg/L	Age, education, hypertension, obesity, physical activity, smoking, parity, estrogen use, and family history of cancer	• Women only • Cases ascertained for the years 1986-2010 from the State Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited to women using their 1989 water supply >10 years • Focus was on nitrates

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1997	Kidney (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs for each 3,000 net rev/L increase in mutagenicity: ORs near 1.0 in men and women	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up was for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors
Koivusalo *et al.*, 1998	Kidney (incident cases)	Finland 1991-92	Case-control	703 cases and 914 controls; men and women all ages	Mutagenicity	Residential history and water intake linked to water records; mutagenicity estimated based on TA 100 *Salmonella typhimurium* mutagenicity using data from a previous study of water containing known concentrations of total organic carbon, chlorine level, and ammonia.	ORs for each 3,000 net rev/L increase in mutagenicity: Men: OR=1.47 (95% CI: 1.07-2.02). Also positive in categorical analyses by years exposed Women: no clear associations	Age, sex, SES, and smoking	• Cases ascertained from the Finnish Cancer Registry • Controls randomly selected from a nationwide population registry matched to cases by age and sex • Overall participation rate 68%, only slightly lower in controls • Relevance of the exposure metric is unknown • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Liao *et al.*, 2012	Kidney (mortality)	Taiwan 1998-2007	Case-control	500 cases and 500 controls; men and women ages 50-69 years in 53 municipalities	THMs (total)	2000-2 survey of quarterly THM levels in 96 of 361 municipalities of Taiwan. 53 had a single waterworks, a clear population, and water sources did not change in last decade. THM levels linked to municipality information at the time of death from death records.	OR=0.98 (95% CI: 0.77-1.25) comparing TTHM levels above and below the median (4.9 ppb)	Age, gender, marital status, and urbanization	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • All other non-genitourinary deaths used as controls, matched to kidney cancer cases by gender, year of birth, and year of death; deaths from certain cancers also excluded (e.g., colon, lung) • May be ecologic. Variability in THM levels within municipalities unknown • Limited data on other cancer risk factors • Some evidence of additivity or synergy with water softness although not statistically significant
Vinceti *et al.*, 2004	Kidney (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): ORs in both men and women near or below 1.0	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders
Yang *et al.*, 1998	Kidney (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: Men: SRR=2.51 (95% CI: 1.27-4.94) Women: SRR=2.20 (95% CI: 1.84-5.78)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Doyle *et al.*, 1997	Lung (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	RRs of 1.00, 1.24, 1.81, and 1.59 for chloroform concentrations of <LOD, 1-2, 3-13, 14-287 µg/L (p-trend=0.025)	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989
Flaten *et al.*, 1992	Lung (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Higher rate in chlorinating vs. non-chlorinating municipalities but only in women (11.5 vs. 9.5 per 100,000; p<0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • Includes trachea and bronchus cancers • No information on residential history • Limited data on other cancer risk factors • Multiple comparisons may be an issue (15 cancer types in both men and women)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Isacson *et al.*, 1985	Lung (incident cases)	Iowa 1969-82	Ecologic	Includes cities and towns with populations 1,000-10,000, and a public water supply with a single major ground water source before 1965	Chlorination	Contaminants measured in finished water supplies of all eligible municipalities in 1979. Information on the treatments used collected from the Iowa Department of Environmental Quality and verified by water plant managers.	Only presents age adjusted risk ratios for nickel and dicloroethane in analyses stratified by chlorination status (yes or no). Slightly higher risk ratios in chlorination group in men (e.g. risk ratio=1.22 vs. 1.15 in chlorination vs. non-chlorination groups of men with elevated water nickel levels), but statistical significance is unknown Risk ratios across strata appear similar in women	Age adjusted, sex specific rate ratios	• Demographic comparisons and exposure assessment not clear • Only stratified results given • Limited data on other cancer risk factors
Koivusalo *et al.*, 1997	Lung (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: Women: OR=0.95 (95% CI: 0.75-1.22) Men: OR=1.21 (95% CI: 1.07-1.36)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Results not consistent across sexes

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Lung (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=1.3 (95% CI: 1.0-1.6) Women: SMR=1.0 (95% CI: 0.6-1.7)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Results not consistent across sexes • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Lung (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: Men: SRR=1.60 (95% CI: 1.39-1.85) Women: SRR=1.95 (95% CI: 1.45-2.59)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface water (vs. private well water) use • Chlorinated and non-chlorinated areas similar in terms of population density, percentage white and blue collar workers, and percent involved in agriculture jobs • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Doyle *et al.*, 1997	Ovarian (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	RR=0.91 (95% CI: 0.36-2.30) for 14-287 µg/L vs. 1-2 µg/L (n=8 cases in the upper exposure category)	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, BMI, and several reproductive/developmental factors	• Women only • Cases identified from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989
Flaten *et al.*, 1992	Ovarian (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women (p>0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Inoue-Choi *et al.*, 2015	Ovarian (incident cases)	Iowa Women's Health Study 1986-2010	Cohort	28,555 women ages 55-69 years at baseline (1986); 315 cases	THMs (individual and total); HAAs (individual and total)	Main residential source of drinking water in 1989 and number of years of used. THM and HAA levels estimated based on expert assessment, some available measurements, water source, quality, treatment, and disinfectant type.	Some individual HRs above 1.4 and statistically significant, but not in the highest exposure categories and no clear dose-response pattern (i.e., highest ORs in the middle exposure category and p-trends >0.05).	Age, BMI, family history, number of live births, oral contraception use, estrogen use, oophorectomy, and other factors	• Women only • Cases ascertained from the Iowa State Health Registry and National Death Index • Baseline participation of 42% • Limited to women with >11 years using residential water source in 1989 • Only assessed the residential drinking water source used in 1989 • Focus was on nitrates
Koivusalo *et al.*, 1997	Ovarian (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	307,967 women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: OR=1.15 (95% CI: 0.95-1.39)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Ovarian (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): SMR=1.6 (95% CI: 0.8-2.9)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Ovarian (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRR=1.02 (95% CI: 0.47-2.23)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Chiu *et al.*, 2010	Pancreas (mortality)	Taiwan 1998-2007	Case-control	1056 cases and 1056 controls, men and women age 50-69 years in 53 municipalities	THMs (total)	2000-2 survey of quarterly THM levels in 96 of 361 municipalities of Taiwan. 53 had a single waterworks, a clear population, and water sources did not change in last decade. THM levels linked to municipality information at the time of death from death records.	OR of 1.01 (95% CI: 0.85-1.21) for TTHM >4.9 vs. <4.9 µg/L	Age, gender, marital status, and urbanization	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Controls were deaths from other causes (non-gastro-intestinal and not bladder, lung, or kidney cancer) matched by sex, year of birth and year of death • May be ecologic. Variability in THM levels within municipalities unknown • Limited data on other cancer risk factors • Some evidence of synergy with lower water magnesium concentrations. • No synergy seem with water calcium concentrations
Do *et al.*, 2005	Pancreas (incident cases)	Canada (six provinces) 1994-97	Case-control	486 cases, and 3,596 controls; men and women ages 30-75 years	THMs (total and BDCM and chloroform)	Lifetime residential history for the 30 years ending 3 years prior to interview linked to multiple sources of THM survey data from municipalities.	No clear associations Similar results by sex and for various latency periods	Age, sex, province, BMI, weight change, smoking, coffee, beer, liquor, fat intake, and total calories	• Cases from the National Enhanced Cancer Surveillance System of Canada • Controls from random sampling of health insurance plans, property assessment database, and from random digit dialing matched to cases on age and sex • Participation rates of 70% in cases and 65% in controls

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Pancreas (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women (p>0.05)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
Ijsselmuiden *et al.*, 1992	Pancreas (incident cases)	Washington County, MD 1975-89	Case-control	101 cases and 206 controls; men and women ages ≥35 years	Chlorination	Drinking water source in 1975 (from private census data)	OR=2.18 (95% CI: 1.20-3.95) for chlorinated vs. non-chlorinated water use	Age and smoking	• Whites only • Cases from the Washington County cancer registry (only one hospital) • Controls selected from the 1975 census, but limited information provided on control selection • Participation rates not provided • Average time at 1975 residence was 11.7 years • Age and employment differences between cases and controls • No information on alcohol use

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1997	Pancreas (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: ORs in men and women near 1.0	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors
Quist *et al.*, 2018	Pancreas (incident cases)	Iowa Women's Health Study 1986-2011	Prospec tive cohort	189 pancreas cancer cases among 15,577 women ages 55-69 at baseline	THMs (total)	Main residential source of drinking water in 1989 and number of years of used. THM levels estimated based on expert assessment, some available measurements, water source, quality, treatment, and disinfectant type.	ORs near or below 1.0 for the following: • Average TTHM >14.30 µg/L • ≥36 years with TTHM >40 µg/L	Smoking, body mass index, diabetes, estrogen use, menopause, occupation, rural vs. city, water nitrate, and medications	• Women only • Cases ascertained for the years 1986-2011 from the State Health Registry of Iowa • Baseline participation of 42% • Only women using public water supplies >10 years • Focus was on nitrates • No information on alcohol use

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Pancreas (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=0.9 (95% CI: 0.4-1.9) Women: SMR=1.6 (95% CI: 0.8-2.8)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Results not consistent across sexes • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Pancreas (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: Men: SRR=1.49 (95% CI: 0.93-2.40) Women: SRR=1.22 (95% CI: 0.73-2.05)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface water (vs. private well water) use • Chlorinated and non-chlorinated areas similar in terms of population density, percentage white and blue collar workers, and percent involved in agriculture jobs • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Prostate (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates higher in chlorinating vs. non-chlorinating municipalities (87.5 vs. 79.1 per 100,000; $p<0.05$)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors • Multiple comparisons may be an issue (15 cancer types in both men and women) • Small increase in relative risk
Koivusalo *et al.*, 1997	Prostate (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	313,464 men all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: OR=0.97 (95% CI: 0.83-1.13)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Prostate (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): SMR=1.4 (95% CI: 0.8-2.2)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Prostate (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRR=1.18 (95% CI: 0.78-1.78)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors
Bove *et al.*, 2007b	Rectal (incident cases)	Western New York 1978-86	Case-control	128 cases and 253 controls; men ages 35-90 years	THMs (total and individual)	Municipal records of THM levels in local water supplies combined with data on residences and water intake; kriging used to interpolate levels between sampling points.	<u>Bromoform:</u> ORs of 1.00 (ref), 1.42, 1.63, and 2.32 for 0.00-0.64, 0.65-0.97, 0.98-1.68, and 1.69-15.43 µg/day (p-trend=0.002) <u>Other THMs:</u> Marginal increases in ORs for chlorodibromomethane and bromodichloromethane	Alcohol, carotene, and total calories; smoking also assessed	• White men only • Cases ascertained from all major hospitals in the three county study area • Next door neighbor controls • Participation rates of 71% in cases and 57% in controls • Accuracy of the kriging methods and historical exposures unclear

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Doyle *et al.*, 1997	Rectal (incident cases)	Iowa Women's Health Study 1986-93	Cohort	28,237 women ages 55-69 years at baseline (1985)	THMs (chloroform)	Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87.	For rectum and anus cancer: RR=1.07 95% CI: 0.60-1.93) for 14-287 µg/L vs. 1-2 µg/L	Age, education, smoking, physical activity, diet, total energy intake, waist-to-hip circumference, and BMI	• Women only • Cases ascertained from the Health Registry of Iowa and the National Death Index • Baseline participation of 42% • Limited follow-up: through 1993 • Only assessed the residential drinking water source used in 1989
Flaten *et al.*, 1992	Rectal (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates higher in chlorinating vs. non-chlorinating municipalities for both men (25.0 vs. 20.2 per 100,000, $p<0.05$) and women (15.8 vs 12.1 per 100,000, $p<0.05$)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors • Multiple comparisons may be an issue (15 cancer types in both men and women)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Hildesheim *et al.*, 1998	Rectal (incident cases)	Iowa 1986-88	Case-control	537 cases and 1,983 controls; men and women ages 40-85 years	THMs (total); chlorination	Lifetime residential history linked to drinking water records and THM measurements (collected in 1987) from all Iowa water utilities serving at least 1,000 people. Mean THM levels by treatment type and water source measured in 1987 used to estimate historical exposures.	Chlorination: ORs of 1.0 (ref), 0.88, 1.11, 1.41, and 2.13 for 0, 1-19, 20-39, 40-59, and ≥60 years of exposure (p-trend=0.0002) TTHM: ORs of 1.0 (ref), 1.05, 1.24, 1.23, 1.66, and 1.66 for lifetime average exposure of ≤0.7, 0.8-2.2, 2.3-8.0, 8.1-32.5, 32.6-46.3, and ≥46.4 µg/L (p-trend=0.01) Similar results in men and women	Age and sex. Further adjustment for smoking, diet, and other factors did not alter results.	• Cases ascertained from the State Health Registry of Iowa • Controls randomly selected from Iowa driver's license records and Medicare • Participation rates of 82.0% for cases and 81.5% for controls • Limited to subjects with at least 70% exposure history known
Isacson *et al.*, 1985	Rectal (incident cases)	Iowa 1969-82	Ecologic	Includes cities and towns with populations 1,000-10,000, and a public water supply with a single major ground water source before 1965	Chlorination	Contaminants measured in finished water supplies of all eligible municipalities in 1979. Information on the treatments used collected from the Iowa Department of Environmental Quality and verified by water plant managers.	Only presents age adjusted risk ratios for nickel and dicloroethane in analyses stratified by chlorination status (yes or no). Risk ratios are higher for chlorination strata for female rectal cancer but estimates of variance not given. No clear associations for male rectal cancer.	Age adjusted, sex specific rate ratios	• Demographic comparisons and exposure assessment not clear • Only stratified results given • Limited data on other cancer risk factors
King *et al.*, 2000	Rectal (incident cases)	Ontario 1992-94	Case-control	661 cases and 1,545 controls; men and women ages 30-74 years	Chlorination; THMs (total)	Residential history for the 40-year period 2 years prior to interview and modeled THM levels; models based on information from a water treatment plant survey on water source and treatment characteristics.	No clear associations in men or women	Age, sex, education, BMI, medical history, total calories, cholesterol, calcium, and coffee	• Cases ascertained from the Ontario Cancer Registry • Controls randomly selected from residential telephone listings matched to cases on age and gender • Participation rates of 73% in cases and 72% in controls • Cases less educated than controls, but this is adjusted for • Only included subjects with at least 30 years of exposure data • No information on smoking

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo *et al.*, 1997	Rectal (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity: Women: OR=1.38 (95% CI: 1.03-1.85) Men: OR=0.85 (95% CI: 0.66-1.09)	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors • Multiple comparisons may be an issue (ORs given for 26 cancer types and in both men and women) • Results not consistent across sexes
Kuo *et al.*, 2010a	Rectal (mortality)	Taiwan 1998-2007	Case-control	1,106 cases and 1,106 controls; men and women ages 50-69 years in 53 municipalities	THMs (total)	2000-2 survey of quarterly THM levels in 96 of 361 municipalities of Taiwan. 53 had a single waterworks, a clear population, and water sources did not change in last decade. THM levels linked to municipality information at the time of death from death records.	No clear association overall comparing those above and below the median TTHM level (4.9 ppb)	Age, gender, marital status, and urbanization	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • All other non-gastrointestinal deaths used as controls, matched to rectal cancer cases by gender, year of birth, and year of death; deaths from certain cancers also excluded (e.g., bladder, lung) • May be ecologic; variability in TTHM levels within municipalities unknown • Limited data on other cancer risk factors • OR in those with THM levels ≥4.9 and water magnesium <5.9 mg/L is 1.43 (95% CI: 1.00-2.04)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Rectal (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=0.2 (95% CI: 0.1-0.8, n=1 exposed case) Women: SMR=1.4 (95% CI: 0.6-2.8, n=7 cases)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Results not consistent across sexes • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Rectal (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: Men: SRR=1.42 (95% CI: 1.23-2.25) Women: SRR=1.42 (95% CI: 1.13-1.98)	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors • Multiple comparisons may be an issue (SRRs given for 11 and 14 cancer types in men and women, respectively)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Flaten *et al.*, 1992	Stomach (incident cases)	Norway	Ecologic	Men and women of all ages in 96 municipalities	Chlorination	1982-83 national survey of drinking water. Includes municipalities where water source and treatment method was unchanged from 1965 to 1983 and >60% of the municipality was served by a sampled water source. Considered exposed if >60% of municipality received chlorinated water and unexposed if water chlorination never used.	Rates similar in chlorinating and non-chlorinating municipalities in both men and women ($p>0.05$)	Age adjusted, sex specific rates given	• Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
Koivusalo *et al.*, 1997	Stomach (incident cases)	Finland (56 towns) 1970-93	Retro-spective cohort	621,431 men and women all ages	Mutagenicity	Includes people who in 1970 were living in the same town where born and the town had a water pipe connection. Residential history after 1970 obtained from Statistics Finland. Residential history and water intake linked to water records for the years 1955 and 1960. Mutagenicity estimated based on total organic carbon, chlorine level, and ammonia concentration.	ORs for each 3,000 net rev/L increase in mutagenicity: ORs in men and women both near 1.0	Age, urbanization, and SES	• Cases ascertained from the Finnish Cancer Registry • Follow-up for the years 1970-93 • Demographic comparisons not presented • Relevance of the exposure metric unknown • Limited data on other cancer risk factors

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Vinceti *et al.*, 2004	Stomach (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retro-spective cohort	5,144 subjects in the exposed city; men and women over age 20 years	THMs (total)	Residents of Guastalla from 1966-1986. This municipality had elevated THM levels (e.g., 39.7-70.7 µg/L) from 1965 to 1987.	SMRs comparing residents of Guastalla (exposed) to residents of Reggio Emilia (lower TTHM levels): Men: SMR=1.7 (95% CI: 1.2-2.5) Women: SMR=1.2 (95% CI: 0.7-1.9)	Age standardized	• Cancer mortality for Guastalla was compared to that in a region with similar ethnic and lifestyle factors (Reggio Emilia) for the years 1987 to 1999 • Limited exposure data • Data on occupations and education were available but no direct subject interviews and no or limited data on other potential confounders • Results not consistent across sexes • Multiple comparisons may be an issue (SMRs given for 15 cancer types in both men and women)
Yang *et al.*, 1998	Stomach (mortality)	Taiwan 1982-91	Ecologic	Residents of 28 municipalities in Taiwan	Chlorination	Municipality at the time of death. Municipalities in which greater than 90% (exposed) or less than 5% of the population (unexposed) was served by chlorinated water, matched by urbanization index and sociodemographic characteristics.	Comparing exposed and unexposed municipalities: SRRs near 1.0 in both men and women	Mortality rate ratios by sex, standardized by age	• Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health • Chlorination strongly related to surface (vs. private well) water use • Chlorinated and non-chlorinated areas similar in population density, percentage white and blue collar workers, and percent involved in agriculture • Ecologic exposure data • Limited data on other cancer risk factors

Abbreviations: BDCM, bromodichloromethane; BMI, body mass index; CI, confidence interval; CML, chronic myeloid leukemia; CYP2E1, cytochrome P450; DBCM, dibromochloromethane; GST, glutathione-S-transferase; HAA, haloacetic acids; HAA5, sum of monochloro-, dichloro-, trichloro-, monobromo-, and dibromoacetic acids; HR, hazard ratio; LOD, limit of detection; OR, odds ratio; NA, not applicable; NHL, Non-Hodgkin lymphoma; ppb, parts per billion; Ref, reference; RR, relative risk estimate; rev, revertants (units of mutagenicity); SEER, Surveillance, Epidemiology, and End Results; SES, socioeconomic status; SMR, standardized mortality ratio; SRR, standardized rate ratio; TTM, trihalomethanes; TTHMs, total trihalomethanes; US EPA, US Environmental Protection Agency

Table C2. Association and quality score criteria* for epidemiologic studies of disinfection byproducts and cancer

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Beane Freeman et al. (2017)	BL	CC	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+		+	+	0	None	TTHM	>103.9 µg/d	4+	B	-0
Bove et al. (2007a)	BL	CC	+	+	+	+	-	-	u	+	0	+	+	+	+	-	-	+		+	-	0	Recruitment unclear; kriging unclear; white males only	TTHM/IND	≥74.1	4+	M	-6
Cantor et al. (1987)	BL	CC	+	+	+	-	+	+	+	u	0	+	+	+	+	-	0	+		+	+	0	Subgroup: non-smokers	Chlorination	≥60 years	3+	NS	-2
Cantor et al. (1998)	BL	CC	+	+	+	+	+	+	+	+	0	+	+	+	+	-	0	+		+	+	0	Subgroup: men	Chlorin/TTHM	≥46.4	4+	M	-1
Chang et al. (2007)	BL	CC	+	+	+	+	+	+	+	u	0	+	u	-	-	-	0	-		+	+	0	Cross-sectional; municipality; confounding	TTHM	≥21.2	4+	C	-6
Chevrier et al. (2004)	BL	CC	+	+	+	+	+	+	u	+	0	+	+	+	+	-	-	+		+	+	0	TTHM assessment unclear; few women cases	TTHM	>1500 µg/L-yr	4+	M	-3
Doyle et al. (1997)	BL	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+		+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	BL	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-		+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-6
Freedman et al. (1997)	BL	CC	+	-	+	-	+	+	u	u	0	+	+	+	-	-	0	+		+	-	0	Subgroup: male smokers; water source 1975; Whites only	Chlorination	Yes/no	2+	MS	-5
Isacson et al. (1985)	BL	E	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-		+	+	-	Demographics and exposure unclear; stratified results; confounding	Chlorination	Yes/no	-	B	-7
Jones et al. (2016)	BL	CO	-	-	-	-	+	+	-	u	0	+	+	+	+	-	0	+		+	+	0	Women only; focused on nitrates; low participation	TTHM	≥40	-	W	-3
King and Marrett (1996)	BL	CC	+	+	+	+	+	+	+	+	0	+	+	+	+	-	0	+		+	+	0	None	Chlorin/TTHM	≥35 yrs/1957 µg/L-yrs	4+	C	-1

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Koivusalo et al. (1997)	BL	CO	+	+	+	-	+	+	+	u	0	+	+	+	u	-	-	-		-	+	0	Subgroup: women; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	3+	W	-6
Koivusalo et al. (1998)	BL	CC	+	+	+	-	+	+	+	+	0	+	+	+	+	-	-	+		+	+	0	Subgroup: male non-smokers; mutagenicity	Mutagenesis	NA	3+	MN	-2
Lynch et al. (1989)	BL	CC	+	+	+	+	+	+	+	u	0	+	+	+	+	-	0	-		+	-	0	Unadjusted; stepwise regression unclear; Whites only; confounding	Chlorination	>50 years	4+	C	-4
McGeehin et al. (1993)	BL	CC	+	+	+	+	+	+	+	u	0	+	+	+	+	-	0	+		+	-	-	Whites only; living cases only	Chlorin/ TTHM	>30 yrs/>600 µg/L-yrs	4+	B	-4
Villanueva et al. (2007b)	BL	CC	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+		+	+	0	Results in women somewhat weaker	TTHM/IND	>49/NA	4+	B	-0
Vinceti et al. (2004)	BL	CO	+	-	-	+	+	+	+	+	0	+	+	+	u	-	0	-		-	+	-	Subgroup: men; no interviews; confounding; 1 female case	TTHM	70.7	1+	M	-5
Yang et al. (1998)	BL	E	+	+	-	+	+	+	+	+	0	+	-	-	-	-	0	-		-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	3+	B	-6
Cantor et al. (1999)	BR	CC	+	+	+	-	+	+	+	+	0	+	+	+	+	-	0			+	+	-	Subgroup: men; 74% proxy interviews	Chlorin/ TTHM	≥40 yrs/≥32.6	3+	M	-2
Koivusalo et al. (1997)	BR	CO	+	-	+	-	+	+	+	u	0	+	+	+	u	-	-			-	+	0	Glioma; subgroup: women; mutagenicity; exposure 1955-60	Mutagenesis	NA	2+	W	-5
Vinceti et al. (2004)	BR	CO	-	-	-	-	+	+	+	+	0	+	+	+	u	-	0			+	+	0	No interviews	TTHM	70.7	-	B	-2
Yang et al. (1998)	BR	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0			+	+	0	Cross-sectional; ecologic	Chlorination	Yes/no	-	B	-4
Doyle et al. (1997)	BT	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+		+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	BT	E	-	+	-	+	+	+	+	u	0	+	-	-	-	-	0	-		-	+	0	Ecologic, no residential history; confounding; low RR	Chlorination	Yes/no	2+	W	-7

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Font-Ribera et al. (2018)	BT	CC	+	+	+	+	+	+	-	+	0	+	+	+	+	+	0	+		+	+	0	Low participation	Chloroform	>24.3	4+	W	-1
Koivusalo et al. (1997)	BT	CO	-	+	+	+	+	+	+	u	0	+	+	+	u	-	-	-		-	+	0	Mutagenicity; exposure 1955-60; confounding; low RR	Mutagenesis	NA	3+	W	-6
Marcus et al. (1998)	BT	E	-	-	-	-	+	+	+	-	0	+	-	-	-	-	0	-		+	-	0	Cross-sectional; ecologic; White and Black women only	TTHM	≥80	-	W	-7
Vinceti et al. (2004)	BT	CO	+	-	-	+	+	+	+	+	0	+	+	+	u	-	0	-		-	+	0	No interviews; confounding; 1 male case	TTHM	70.7	2+	W	-4
Yang et al. (1998)	BT	E	+	-	-	+	+	+	+	+	0	+	-	-	-	-	0	-		-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	2+	W	-6
Doyle et al. (1997)	CO	CO	+	+	+	+	+	+	-	+	0	+	+	-	-	-	0	+	+	-	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	4+	W	-5
Flaten (1992)	CO	E	+	+	-	+	+	+	+	u	0	+	-	-	-	-	0	-	-	-	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	3+	B	-8
Hildesheim et al. (1998)	CO	CC	-	-	-	-	+	+	+	u	0	+	+	+	+	-	0	+	+	+	+	0	None	Chlorin/ TTHM	≥60 yrs/≥46.4	-	B	-2
Isacson et al. (1985)	CO	E	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	-	Demographics and exposure unclear; stratified results; confounding	Chlorination	Yes/no	-	B	-8
King et al. (2000)	CO	CC	+	+	u	-	+	+	+	+	0	+	+	+	+	-	0	-	+	+	+	0	Subgroup: men; inconsistent dose-response; no smoking data	Chlorin/ TTHM	≥35 yrs/≥1957 µg/L-yrs	2+	M	-2
Koivusalo et al. (1997)	CO	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	B	-6
Kuo et al. (2010b)	CO	CC	-	+	-	+	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	0	Cross-sectional; municipality; confounding; low RR	TTHM	≥4.9	2+	C	-6

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Rahman et al. (2014)	CO	E	-	+	+	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Subgroup: men; ecologic; low RR; confounding	Bromoform	Per 2 µg/L	2+	M	-7
Villanueva et al. (2017)	CO	CC	-	-	-	-	u	u	-	+	0	+	+	+	+	+	0	+	+	+	+	0	Low participation in some areas	Chloroform	>23.4	-	B	-3
Vinceti et al. (2004)	CO	CO	-	-	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	+	+	0	No interviews; confounding	TTHM	70.7	-	B	-4
Yang et al. (1998)	CO	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0	-	-	+	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	-	B	-6
Young et al. (1987)	CO	CC	-	-	-	-	+	+	-	u	0	+	+	+	+	-	0	-	-	+	-	0	Whites only; low participation; confounding	Chlorin/ TTHM	Yes/no/> 40	-	C	-6
Koivusalo et al. (1997)	ES	E	+	+	+	-	+	+	+	u	0	+	+	+	u	-	-	-		-	+	0	Subgroup: women; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	3+	W	-6
Tsai et al. (2013)	ES	CC	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-		+	+	0	Cross-sectional; municipality; confounding; Ca/Mg synergy	TTHM	≥4.9	-	C	-6
Yang et al. (1998)	ES	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0	-		+	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	-	B	-5
Doyle et al. (1997)	K	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+	+	+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	K	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
Jones et al. (2017)	K	CO	-	-	-	-	+	+	-	u	0	+	+	+	+	-	0	+	+	+	+	0	Women only; focused on nitrates; low participation	TTHM/ HAA5	≥14.3/≥30	-	W	-3
Koivusalo et al. (1997)	K	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	B	-6
Koivusalo et al. (1998)	K	CC	+	+	+	-	+	+	+	+	0	+	+	+	+	-	-	+	-	+	+	0	Subgroup: men; mutagenicity; confounding	Mutagenesis	NA	3+	M	-3

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Liao et al. (2012)	K	CC	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	0	Cross-sectional; municipality; confounding; synergy softness	TTHM	≥4.9	-	C	-6
Vinceti et al. (2004)	K	CO	-	-	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	+	+	0	No interviews; confounding	TTHM	70.7	-	B	-4
Yang et al. (1998)	K	E	+	+	-	+	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	3+	B	-7
Doyle et al. (1997)	L	CO	+	+	+	+	+	+	-	+	0	+	+	-	-	-	0	+	+	-	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	4+	W	-5
Flaten (1992)	L	E	+	+	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	-	+	0	Subgroup: women; ecologic, no residential history; confounding	Chlorination	Yes/no	2+	W	-8
Isacson et al. (1985)	L	E	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	-	Demographics and exposure unclear; stratified results; confounding	Chlorination	Yes/no	-	B	-8
Koivusalo et al. (1997)	L	CO	+	+	+	-	+	+	+	u	0	+	+	+	u	-	-	-	-	-	+	0	Subgroup: men; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	3+	M	-7
Vinceti et al. (2004)	L	CO	+	+	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	Subgroup: men; no interviews; confounding	TTHM	70.7	2+	M	-5
Yang et al. (1998)	L	E	+	+	-	+	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	3+	B	-7
Flaten (1992)	L H	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
Infante-Rivard et al. (2001)	L H	CC	+	-	-	+	+	+	+	+	0	+	+	+	+	-	-	+	+	+	+	0	ALL; limited usable exposure data	TTHM/ Chorofo rm	Unc/>10 2	2+	C	-2
Kasim et al. (2006)	L H	CC	+	+	+	+	+	+	-	+	0	+	+	+	+	-	0	+	+	+	+	0	CML; BDCM; low participation	TTHM/ BDCM	>20/>5	4+	C	-2
Koivusalo et al. (1997)	L H	CO	+	-	+	-	+	+	+	u	0	+	+	+	u	-	-	-	+	-	+	0	NHL; subgroup: women; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	2+	W	-6

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Vinceti et al. (2004)	L H	CO	+	-	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	Lymphatic leukemia; subgroup: women; no interviews; confounding	TTHM	70.7	1+	W	-5
Doyle et al. (1997)	O	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+	+	+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	O	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	W	-7
Inoue-Choi et al. (2015)	O	CO	+	-	-	+	+	+	-	u	0	+	+	+	+	-	0	+	+	+	+	0	Women only; water 1989, follow-up >11 years; low participation	TTHM/ HAA5	≥14.5/≥8.17	2+	W	-3
Koivusalo et al. (1997)	O	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	W	-6
Vinceti et al. (2004)	O	CO	+	-	-	+	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	No interviews; confounding	TTHM	70.7	2+	W	-5
Yang et al. (1998)	O	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0	-	-	+	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	-	W	-6
Chiu et al. (2010)	P N	CC	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	0	Cross-sectional; municipality; confounding	TTHM	≥4.9	-	C	-7
Do et al. (2005)	P N	CC	-	-	-	-	+	+	+	+	0	+	+	+	+	-	0	+	+	+	+	0	None	TTHM/IND	>50	-	B	-1
Flaten (1992)	P N	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
IJsselmuiden et al. (1992)	P N	CC	+	+	-	+	+	-	u	-	0	+	+	u	u	-	0	+	-	+	-	0	Whites only; confounding	Chlorination	Yes/no	3+	C	-8
Koivusalo et al. (1997)	P N	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	B	-6
Quist et al. (2018)	P N	CO	-	-	-	-	+	+	-	u	0	+	+	+	+	-	0	+	-	+	+	0	Women only; low participation; confounding	TTHM	>14.3	-	W	-4
Vinceti et al. (2004)	P N	CO	+	-	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	Subgroup: women; no interviews; confounding	TTHM	70.7	1+	W	-5

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Yang et al. (1998)	PN	E	+	-	-	+	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	2+	B	-7
Flaten (1992)	PR	E	-	+	-	+	+	+	+	u	0	+	-	-	-	-	0			-	+	0	Ecologic, no residential history; low RR	Chlorination	Yes/no	2+	M	-6
Koivusalo et al. (1997)	PR	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-			+	+	0	Mutagenicity; exposure 1955-60	Mutagenesis	NA	-	M	-4
Vinceti et al. (2004)	PR	CO	+	-	-	+	+	+	+	+	0	+	+	+	u	-	0			-	+	0	No interviewers	TTHM	70.7	2+	M	-3
Yang et al. (1998)	PR	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0			+	+	0	Cross-sectional; ecologic	Chlorination	Yes/no	-	M	-4
Bove et al. (2007b)	R	CC	+	+	+	+	+	-	-	+	0	+	+	u	u	-	-	+	+	+	-	0	White males only; neighbor controls; kriging; bromoform; low participation	Bromoform	≥1.69 µg/d	4+	M	-7
Doyle et al. (1997)	R	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+	+	+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	R	E	+	+	-	+	+	+	+	u	0	+	-	-	-	-	0	-	-	-	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	3+	B	-8
Hildesheim et al. (1998)	R	CC	+	+	+	+	+	+	+	u	0	+	+	+	+	-	0	+	+	+	+	0	None	Chlorin/TTHM	≥60 yrs/≥46.4	4+	B	-2
Isacson et al. (1985)	R	E	+	u	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	-	+	-	Subgroup: women; demo. and exposure unclear; stratified results; confounding	Chlorination	Yes/no	1+	W	-9
King et al. (2000)	R	CC	-	-	-	-	+	+	+	+	0	+	+	+	+	-	0	-	+	+	+	0	No smoking data	Chlorin/TTHM	≥35 yrs/≥1957 µg/L-yrs	-	B	-2
Koivusalo et al. (1997)	R	CO	+	+	+	-	+	+	+	u	0	+	+	+	u	-	-	-	-	-	+	0	Subgroup: women; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	3+	W	-7

Study (first author, year)	Cancer	Design	Strength of the association				Study quality															Notes	Summary				
							Selection					Outcome	Exposure					Confounding		Multiple comparisons: no	Generalizable	Other		Chemical	Level	Overall association	Who	Potential major flaws
			RR >1.2	Statistically sig.	Dose-response	No subgroup only	Cases	Controls	Participation	Similar demo	Other		Individual	Past	Long-term	Other sources	Other	#1	#2									
Kuo et al. (2010a)	R	CC	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	0	Cross-sectional; municipality; confounding; synergy Mg	TTHM	≥4 .9	-	C	-7
Vinceti et al. (2004)	R	CO	+	-	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	Subgroup: women; no interviews; confounding	TTHM	70.7	1+	W	-5
Yang et al. (1998)	R	E	+	+	-	+	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	3+	B	-7
Flaten (1992)	ST	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
Koivusalo et al. (1997)	ST	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	B	-6
Vinceti et al. (2004)	ST	CO	+	+	-	-	+	+	+	+	0	+	+	+	u	-	0	-	-	-	+	0	Subgroup: men; no interviews; confounding	TTHM	70.7	2+	M	-5
Yang et al. (1998)	ST	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0	-	-	+	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	-	B	-6

Table C2 Notes: Association and quality score criteria

The scores presented above are based on two major aspects of causal inference. The first is the strength of the association. This was assessed using four criteria: the magnitude of the association, whether or not the result was statistically significant, the presence of a dose-response pattern, and whether or not the association was seen in only a specific subgroup (e.g., women only). The second aspect of causal inference is the quality of the study. This is based on factors that were identified as being common sources of potential bias and confounding in the studies reviewed here. These include the potential for selection bias ("Selection"), outcome misclassification ("Outcome"), exposure misclassification ("Exposure"), confounding, multiple comparisons issues, and generalizability. Findings that generally weaken the evidence that a strong or true association exists, and study characteristics that were evidence of low study quality, were given a score of "-." Criteria for which adequate data were not provided to assess the criteria were given a score of "u" (for "unknown"). Findings or study characteristics supporting a true association or that were evidence of good study quality were given a score of "+." Studies were given a "0" if there were no other weaknesses not covered by the other criteria evaluated (described below), and left blank if not appropriate for the cancer type assessed or the study design used. Further details on each criterion and on the other data provided in the table are presented below.

Cancers Evaluated:
This gives the cancer type evaluated. Abbreviations in this column are:
BL, bladder cancer
BR, brain
BT, breast
CO, colon (also includes results for colorectal cancers combined)
ES, esophageal
K, kidney
L, lung
LH, lympho-hematopoietic
O, ovarian
PN, pancreatic
PR, prostate
R, rectal
ST, stomach

Design:
This column describes the study design. Abbreviations in this column are:
E, ecologic
CC, case control
CO, cohort

RR >1.2:

This criterion is used as an indicator of the magnitude of the association, specifically whether or not the relative risk estimate (RR) is greater or less than 1.2. Relative risk estimates include odds ratios, rate ratios, risk ratios, and mortality ratios. This criterion is similar to the first Bradford-Hill criterion ("strength"). The Bradford-Hill criteria are a set of criteria commonly used to evaluate the strength of epidemiologic evidence for evaluating causal inference (Bradford-Hill, 1965). This particular criterion described here is based on the idea that relative risk estimates further away from 1.0 are more likely to represent true effects than those closer to 1.0. The basis of this is that relative risks further away from 1.0 are less likely to be solely due to small degrees of bias and confounding than relative risks closer to 1.0 (e.g., RR=1.1). Axelson (1978) presents an excellent explanation and example of this concept. OEHHA acknowledges that there are many exceptions to this general rule, which is why a large number of other association and quality score criteria are also incorporated into this review. OEHHA also acknowledges that there is no widely accepted boundary that defines whether a relative risk is "close" to 1.0. Here, a relative risk estimate of 1.2 is chosen. This was based on a review of the studies identified and OEHHA's judgement relating to the sample sizes and statistical power seen in these studies. This cut-off point may be considered somewhat arbitrary. As such, this criterion was used only as a general guide and was not the sole criterion used to evaluate causal inference.

Statistically sig.:
This is statistical significance. Scoring this was based on whether the lower 95% confidence interval was above 1.0 or whether the two-sided p-value was below 0.05. Studies were given a "+" if the relevant result was statistically significant and a "-" if it was not. It should be noted that a study may identify a true effect, but the result may not be statistically significant. This can occur if the study is too small and has insufficient statistical power. Because of this, statistical significance was not the only criterion used to evaluate the strength of the associations identified. The magnitude of the association was also considered. Many studies gave results for multiple exposure categories (e.g., separate relative risks for low, medium, and high levels of the DBP). This review focused on the statistical significance of the result provided for the highest exposure category.

Dose-response:
This criterion was based on the principle that in many true causal relationships, greater levels of exposure will lead to greater levels of effect. Again, OEHHA acknowledges that there are many exceptions to this principle. However, no convincing information was identified to suggest that this general principle would not apply to DBPs and cancer. Studies were given a "+" if the relative risk estimates generally increased as the exposure level increased, and were given a "-" if this type of monotonic dose-response pattern was not seen. Statistical significance was not a part of this criterion (it was evaluated in the criterion above).

No subgroup only:
This criterion is related to the concept that as a study evaluates a greater and greater number of individual subgroups, the probability that a statistically significant result will occur by chance alone will also increase. (Note: the multiple comparisons criterion described below was used to evaluate studies that assessed multiple cancer types rather than multiple subgroups.) Here, studies were given a "-" if associations were only seen in an "unexplained" subgroup or were not

consistently seen in other studies. For example, studies were given a "-" for this criterion if an association was only seen in men but not women, without an obvious explanation or biological justification for why this might be the case, or without similar findings seen in other studies. For bladder cancer, two studies found associations in non-smokers but not in smokers, while another one found an association in smokers but not in non-smokers. Since there was no clear pattern across these studies and no obvious reason why non-smokers might be more susceptible to DBP-related bladder cancer, these studies were given a "-" for this criterion. Studies were given a "+" if findings were seen in a broad and generalizable group of subjects (e.g., all subjects including men and women, smokers and non-smokers combined) or if findings regarding a particular subgroup were mostly consistent across different studies. For bladder cancer, a number of studies found associations in males but not in females. Since this pattern was somewhat consistent across studies, these particular studies were given a "+" for this criterion. It is acknowledged that an association limited to only a particular unexplained or inconsistent subgroup may still represent a true causal effect. Given this, a "-" in this category was not used as the sole criterion for evaluating each study. Studies finding no association overall and no associations in any particular subgroup were given a "-" for this criterion. This was done because adding a "+" here would interfere with the "Overall Association" score described below.

Cases:
Studies were give a "+" if cancer cases were ascertained from a government or otherwise established cancer or health registry, or were ascertained from a clearly defined set of hospitals or clinics.

Controls:
Studies were given a "+" if the selection process used to ascertain controls (i.e., comparison subjects) was clear and the control subjects appeared to be randomly selected from a population from which cases were ascertained. Prospective or retrospective studies were given a "+" if all non-cancer subjects were included in the analyses. Poor follow-up rates in prospective cohort studies were evaluated under the "Participation" criterion.

Participation:
Case-control studies were given a "+" if the participation rates for both cases and controls were >60% and there was not a >20% difference between them. Participation rates not meeting these criteria raise concerns about generalizability and could introduce significant bias. Ecologic studies were given a "+" here since participation in these types of studies is generally not voluntary. Prospective studies were given a "+" if baseline ascertainment rates or follow-up rates were >60%. Studies were given a "-" if they did not meet these criteria and a "u" if participation or follow-up rates were not provided or were not clear.

Similar demo:
This criterion evaluates whether the groups being compared are similar with regard to major sociodemographic factors. Studies were given a "+" if there were no major differences in sociodemographic factors (e.g., smoking, education, ages, genders, socioeconomic status (SES)) between the groups being compared (e.g., between cases and controls or between

people with high and low levels of DBP exposure). Studies were given a "-" if major differences were identified or a "u" if information needed to evaluate this was not provided.

Other (selection):
Studies with any other potential major source of selection bias were given a "-," otherwise studies were given a "0."

Outcome:
Studies were given a "+" if there was no obvious source of misclassification of cancer status. Histologic confirmation of cancer cases has been used to evaluate study quality in other reviews. However, it was not used in the scoring here since it seemed an unlikely source of major bias in the studies reviewed.

Individual:
Studies were given a "+" if exposure was evaluated in each individual subject, that is, if the exposure data were not ecologic. Studies with ecologic exposure data were given a "-." Items that were unclear were given a "u."

Past:
This criterion is similar to the Bradford-Hill criteria of "temporality." This is the concept that if an exposure truly causes an outcome, the exposure must occur before the outcome. It also incorporates the concept of latency. For a number of environmental carcinogens like arsenic or asbestos, the cancers that are caused by these agents are usually not diagnosed until many years after the exposure occurred. Given this, studies that only evaluate recent exposures (those close to the time of cancer diagnosis) may miss the relevant exposure period. Here, studies in which exposure was assessed only at the time of cancer diagnosis or death ("cross-sectional" studies) were given a "-". Studies that evaluated an exposure at least two years before cancer diagnosis were given a "+."

Long-term:
As mentioned above, the most relevant exposure period for DBP-related cancer (if it exists) may have occurred at some point over a substantial number of years in the past. Studies that evaluated participants' exposures for at least a period of 5 years or more were given a "+." Studies that evaluated exposure at only a single residence, without knowledge of how long study participants lived there were given a "-."

Other sources:
Exposure to some DBPs may also occur from swimming, washing dishes, or showering and bathing. Studies that assessed these other sources, in addition to exposure through drinking water consumption, were given a "+." If only drinking water DBP concentrations and water consumption were assessed, studies were given a "-" for this criterion.

Other (exposure):
Studies with some other source of potential exposure misclassification or other potential weakness related to exposure assessment were given a "-." Otherwise, studies were given a "0." The most common reasons that studies received a "-" for this criterion were that they used modeled exposure data without providing good information on the methods used or accuracy of

the modeling, or they used a somewhat vague and difficult to interpret exposure metric (e.g., mutagenicity).

Confounding:

The likelihood of important confounding is primarily related to the following three factors:

1. The magnitude of the association between the potential confounder and the exposure variable of interest (DBPs);
2. The magnitude of the association between the potential confounder and the outcome of interest (cancer);
3. The prevalence of the confounder among the study population.

Potential confounding factors that are only weakly associated with the exposure or only weakly associated with the outcome, or that are not prevalent in the study population can cause major confounding, but this is relatively unlikely (Axelson, 1978). These three factors, along with information from the American Cancer Society (https://www.cancer.org/cancer), the National Cancer Institute (https://www.cancer.gov), relevant review articles, and other sources, were used to select the most likely potential confounders for each cancer type. The following potential confounders were selected:

Bladder cancer: #1 smoking (certain occupational exposures were considered although almost all studies were population based and these exposures were likely not highly prevalent)
Brain: none
Breast: #1 smoking (reproductive and developmental history were also considered but seemed unlikely to be related to DBP exposure)
Colon cancer: #1 smoking; #2 diet or related variable
Esophageal: #1 smoking; #2 diet or related variable
Kidney: #1 smoking; #2 obesity or hypertension
Lympho-hematopoietic: #1 smoking; #2 socioeconomic status
Ovarian: #1 smoking; #2 obesity
Pancreatic: #1 smoking; #2 alcohol use
Prostate: none
Rectal: #1 smoking; #2 diet or related variable
Stomach: #1 smoking; #2 diet or related variable

A study received a “+” if these factors were adjusted for in the statistical analysis or if the authors stated that their adjustment did not markedly alter study results. Otherwise the study received a “-” or “u.”

Multiple comparisons:

A number of studies assessed many different cancer types and presented results for each. This large number of comparisons raises concerns that statistically significant associations may be identified solely because of chance. Studies that found evidence of an association (defined here as a relative risk estimate >1.2 or a statistically significant finding) and that assessed more than 10 different cancer types were given a “-.” Otherwise they were given a “+.”

Generalizable:
Several studies only evaluated certain racial subgroups (e.g., whites only). The rationale usually given was that sample sizes in other groups were too small. However, this rationale doesn't account for the fact that data in small subgroups could still be used in meta-analyses or that efforts could have been made for oversampling some groups. Because some important subgroups of people may be missing in these studies, they were given a score of "-" for this criterion.

Other:
Studies were given a "-" if any other potential weakness was identified that was not covered by the preceding criteria. Otherwise studies were given a "0."

Notes:
This column provides brief notes on the major participant subgroups or chemical subclasses where associations were seen, or on some of the major potential weaknesses.

Chemical:
This column lists the primary chemical(s) assessed, with a focus on those chemicals where associations were seen.

Level:
This column lists the lower cutoff point for the highest exposure category assessed. This is listed as "yes/no" if the exposure variable was assessed as a dichotomous variable. Units are in µg/L unless otherwise noted. Cumulative exposure is usually given in units of µg/L-years. The purpose of this column was to identify any studies in which the exposures may have been too low to expect to see an association (if one truly exists).

Overall association:
This column is the sum of the "+"'s in the four "Association" columns (RR>1.2, statistical significance, dose-response, no subgroups only columns). In general, one could consider a score of 0 to be evidence of no association, a score of 1-2 as weak evidence for an association, and a score of 3-4 to be fairly strong evidence for an association. Importantly though, exceptions are possible and this column was only used as a guide.

Who:
In studies with Overall Association scores of 1 or more, this column lists the sexes (or other subgroup) in which the association was identified. In studies with Overall Association scores of 0, it indicates the sexes that were assessed. The codes are:
B, men and women assessed separately ("both")
C, men and women combined
M, men only
MN, male non-smokers
MS, male smokers
NS, non-smokers
W, women only

Potential major flaws:
This column is the sum of all the "-"and "u" designations in the Quality section and is presented to provide a general overall assessment of the quality of each study. Because the scores presented here do not incorporate information on the relative importance or relative likelihood of each weakness they cannot be used to directly compare one study to another. Because of this, these scores were only used as a general guide, and were not used as the sole determinant in the evaluations of causal inference.

Table C3. Epidemiologic studies not used in the review of disinfection byproduct exposure and cancer

Article Not Used	Reason
Fernández-Navarro P, Villanueva CM, García-Pérez J, Boldo E, Goñi-Irigoyen F, Ulibarrena E, Rantakokko P, García-Esquinas E, Pérez-Gómez B, Pollán M, Aragonés N. Chemical quality of tap water in Madrid: multicase control cancer study in Spain (MCC-Spain). Environ Sci Pollut Res Int. 2017 Feb;24(5):4755-4764.	Exposure assessment study
Bogen, K.T., Slone, T., Gold, L.S., Manley, N., and Revzan, K.. New perspectives on the cancer risks of trichloroethylene, its metabolites, and chlorination by-products. United States: N. p., 1994.	Risk assessment
Marienfeld CJ, Collins M, Wright H, Reddy R, Shoop G, Rust P. Cancer mortality and the method of chlorination of public drinking water: St. Louis City and St. Louis County, Missouri. J Environ Pathol Toxicol Oncol. 1986 Sep-Dec;7(1-2):141-57.	Rates in same community over time
Goel S. Impact of chlorination on the incidence of cancers and miscarriages in two different campus communities in India. J Environ Sci Eng. 2008 Jul;50(3):175-8.	Unclear exposure assessment
Abbas S, Hashmi I, Rehman MS, Qazi IA, Awan MA, Nasir H. Monitoring of chlorination disinfection by-products and their associated health risks in drinking water of Pakistan. J Water Health. 2015 Mar;13(1):270-84.	Risk assessment
Benson NU, Akintokun OA, Adedapo AE. Disinfection Byproducts in Drinking Water and Evaluation of Potential Health Risks of Long-Term Exposure in Nigeria. J Environ Public Health. 2017;2017:7535797. doi: 10.1155/2017/7535797. Epub 2017 Aug 16.	Risk assessment
Bull RJ, Charles Gerba & R. Rhodes Trussell (2009) Evaluation of the health risks associated with disinfection, Critical Reviews in Environmental Control, 20:2, 77-113,	Review
Bull RJ, Birnbaum LS, Cantor KP, Rose JB, Butterworth BE, Pegram R, Tuomisto J. Water chlorination: essential process or cancer hazard? Fundam Appl Toxicol. 1995 Dec;28(2):155-66.	Review
Bull RJ, Meier JR, Robinson M, Ringhand HP, Laurie RD, Stober JA. Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. Fundam Appl Toxicol. 1985 Dec;5(6 Pt 1):1065-74.	In vitro and mouse study
Castaño-Vinyals G, Cantor KP, Villanueva CM, Tardon A, Garcia-Closas R, Serra C, Carrato A, Malats N, Rothman N, Silverman D, Kogevinas M. Socioeconomic status and exposure to disinfection by-products in drinking water in Spain. Environ Health. 2011 Mar 16;10:18.	Exposure factors
Cech I, Holguin AH, Littell AS, Henry JP, *et al.* (1987). Health significance of chlorination byproducts in drinking water: the Houston experience. Int J Epidemiol 16(2): 198-207.	Rates in same communities over time
Chen K, Yu W, Ma X, Yao K, Jiang Q. The association between drinking water source and colorectal cancer incidence in Jiashan County of China: a prospective cohort study. Eur J Public Health. 2005 Dec;15(6):652-6.	Drinking water source
Chen K, Yu WP, Ma XY, Yao KY, Zheng S, Jiang QT. [Association of drinking water source and colorectal cancer incidence: a prospect cohort study]. Ai Zheng. 2004 May;23(5):550-4.	Drinking water source
Chen K, Zhou L, Shen G, Yu H. [An epidemiological study on the incidence rates of colorectal cancer through different drinking water sources]. Zhonghua Liu Xing Bing Xue Za Zhi. 2000 Aug;21(4):249-52.	Drinking water source
Chernichenko IA, Balenko NV, Litvichenko ON. [Carcinogenic hazard of chloroform and other drinking water chlorination by-products]. Gig Sanit. 2009 May-Jun;(3):28-33.	Review
Chowdhury S, Rodriguez MJ, Sadiq R. Disinfection byproducts in Canadian provinces: associated cancer risks and medical expenses. J Hazard Mater. 2011 Mar 15;187(1-3):574-84.	Risk assessment
Dunnick JK, Melnick RL. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. J Natl Cancer Inst. 1993 May 19;85(10):817-22.	Animal study
Florentin A, Alexis Hautemanière, Philippe Hartemann, Health effects of disinfection by-products in chlorinated swimming pools, International Journal of Hygiene and Environmental Health, Volume 214, Issue 6, 2011, Pages 461-469,	Review
Goebell, P.J., Villanueva, C.M., Rettenmeier, A.W. *et al.* World J Urol (2004) 21: 424.	Review

Article Not Used	Reason
Hang C, Zhang B, Gong T, Xian Q. Occurrence and health risk assessment of halogenated disinfection byproducts in indoor swimming pool water. Sci Total Environ. 2016 Feb 1;543(Pt A):425-31.	Risk assessment
Holme JA, Steffensen IL, Brunborg G, Becher G, Alexander J. [Chlorination of drinking water--possible cancer risk from a by-product]. Tidsskr Nor Laegeforen. 1999 Jun 30;119(17):2528-30.	Review
Hsu CH, Jeng WL, Chang RM, Chien LC, Han BC. Estimation of potential lifetime cancer risks for trihalomethanes from consuming chlorinated drinking water in Taiwan. Environ Res. 2001 Feb;85(2):77-82.	Risk assessment
Inoue-Choi M, Weyer PJ, Jones RR, Booth BJ, Cantor KP, Robien K, Ward MH. Atrazine in public water supplies and risk of ovarian cancer among postmenopausal women in the Iowa Women's Health Study. Occup Environ Med. 2016 Sep;73(9):582-7.	Atrazine
Kogevinas M, Villanueva CM, Font-Ribera L, Liviac D, Bustamante M, Espinoza F, Nieuwenhuijsen MJ, Espinosa A, Fernandez P, DeMarini DM, Grimalt JO, Grummt T, Marcos R. Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environ Health Perspect. 2010 Nov;118(11):1531 7.	Genotoxicity
Koivusalo , M. & Vartiainen , T. (2011). Drinking Water Chlorination By-Products And Cancer. Reviews on Environmental Health, 12(2), pp. 81-90.	Review
Kuo HW, Chiang TF, Lo II, Lai JS, Chan CC, Wang JD. Estimates of cancer risk from chloroform exposure during showering in Taiwan. Sci Total Environ. 1998 Jul 11;218(1):1-7.	Risk assessment
Kusamran WR, Tanthasri N, Meesiripan N, Tepsuwan A. Mutagenicity of the drinking water supply in Bangkok. Asian Pac J Cancer Prev. 2003 Jan-Mar;4(1):31-8.	No cancer data
Lee H-K, Yir-Yarn YehEmail authorWei-Ming Chen. Cancer risk analysis and assessment of trihalomethanes in drinking water. Stochastic Environmental Research and Risk Assessment. November 2006, Volume 21, Issue 1, pp 1–13	Risk assessment
Lee J, Kim ES, Roh BS, Eom SW, Zoh KD. Occurrence of disinfection by products in tap water distribution systems and their associated health risk. Environ Monit Assess. 2013 Sep;185(9):7675-91.	Risk assessment
Legay C, Rodriguez MJ, Sadiq R, Sérodes JB, Levallois P, Proulx F. Spatial variations of human health risk associated with exposure to chlorination by-products occurring in drinking water. J Environ Manage. 2011 Mar;92(3):892 901.	Risk assessment
Lévesque B, Ayotte P, Tardif R, Charest-Tardif G, Dewailly E, Prud'Homme D, Gingras G, Allaire S, Lavoie R. Evaluation of the health risk associated with exposure to chloroform in indoor swimming pools. J Toxicol Environ Health A. 2000 Oct 27;61(4):225-43.	Exposure and risk assessment
Lévesque B, Ayotte P, Tardif R, Ferron L, Gingras S, Schlouch E, Gingras G, Levallois P, Dewailly E. Cancer risk associated with household exposure to chloroform. J Toxicol Environ Health A. 2002 Apr 12;65(7):489-502.	Risk, exposure assessment
Li XF, Mitch WA. Drinking Water Disinfection Byproducts (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities. Environ Sci Technol. 2018 Feb 20;52(4):1681-1689.	Review
Liu S, Zhu Z, Fan C, Qiu Y, Zhao J. Seasonal variation effects on the formation of trihalomethane during chlorination of water from Yangtze River and associated cancer risk assessment. J Environ Sci (China). 2011;23(9):1503-11.	Exposure and risk assessment
Makris KC, Andrianou XD, Charisiadis P, Burch JB, Seth RK, Ioannou A, Picolos M, Christophi CA, Chatterjee S. Association between exposures to brominated trihalomethanes, hepatic injury and type II diabetes mellitus. Environ Int. 2016 Jul-Aug;92-93:486-93.	Not cancer
McDonald TA, Komulainen H. Carcinogenicity of the chlorination disinfection by-product MX. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2005;23(2):163-214.	MX toxicity
McElroy JA, Gangnon RE, Newcomb PA, Kanarek MS, Anderson HA, Brook JV, Trentham-Dietz A, Remington PL. Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in Wisconsin. J Expo Sci Environ Epidemiol. 2007 Mar;17(2):207-14.	Atrazine

Article Not Used	Reason
Melnick RL, Dunnick JK, Sandler DP, Elwell MR, Barrett JC. Trihalomethanes and Other Environmental Factors That Contribute to Colorectal Cancer. Environ Health Perspect. 1994 Jun;102(6-7):586-8.	Review
Melnick RL, Gary A. Boorman Vicki Dellarco. Water Chlorination, 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), and Potential Cancer Risk JNCI: Journal of the National Cancer Institute, Volume 89, Issue 12, 18 June 1997, Pages 832–833,	Review
Messing RB, Gust LD, Petersen DW. Carcinogenicity of chloroform. Science. 1994 Jun 10;264(5165):1518-9.	Comment
Nieuwenhuijsen MJ, Grellier J, Smith R, Iszatt N, Bennett J, Best N, Toledano M. The epidemiology and possible mechanisms of disinfection by-products in drinking water. Philos Trans A Math Phys Eng Sci. 2009 Oct 13;367(1904):4043-76.	Review
Nieuwenhuijsen MJ, Smith R, Golfinopoulos S, Best N, Bennett J, Aggazzotti G, Righi E, Fantuzzi G, Bucchini L, Cordier S, Villanueva CM, Moreno V, La Vecchia C, Bosetti C, Vartiainen T, Rautiu R, Toledano M, Iszatt N, Grazuleviciene R, Kogevinas M. Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE. J Water Health. 2009 Jun;7(2):185-207.	Study design description
Pan S, An W, Li H, Su M, Zhang J, Yang M. Cancer risk assessment on trihalomethanes and haloacetic acids in drinking water of China using disability-adjusted life years. J Hazard Mater. 2014 Sep 15;280:288-94.	Risk assessment
Regli S, Chen J, Messner M, Elovitz MS, Letkiewicz FJ, Pegram RA, Pepping TJ, Richardson SD, Wright JM. Estimating Potential Increased Bladder Cancer Risk Due to Increased Bromide Concentrations in Sources of Disinfected Drinking Waters. Environ Sci Technol. 2015 Nov 17;49(22):13094-102.	Risk assessment
Salas LA, Bustamante M, Gonzalez JR, Gracia-Lavedan E, Moreno V, Kogevinas M, Villanueva CM. DNA methylation levels and long-term trihalomethane exposure in drinking water: an epigenome-wide association study. Epigenetics. 2015;10(7):650-61.	DNA methylation, not cancer
Salas LA, Font-Ribera L, Bustamante M, Sumoy L, Grimalt JO, Bonnin S, Aguilar M, Mattlin H, Hummel M, Ferrer A, Kogevinas M, Villanueva CM. Gene expression changes in blood RNA after swimming in a chlorinated pool. J Environ Sci (China). 2017 Aug;58:250-261.	Gene expression, not cancer
Tao X, Zhu H, Matanoski GM. Mutagenic drinking water and risk of male esophageal cancer: a population-based case-control study. Am J Epidemiol. 1999 Sep 1;150(5):443-52.	No data on chemicals (Ames test)
Terrell ML, Rosenblatt KA, Wirth J, Cameron LL, Marcus M. Breast cancer among women in Michigan following exposure to brominated flame retardants. Occup Environ Med. 2016 Aug;73(8):564-7.	No water DBP data
Tokmak B, Capar G, Dilek FB, Yetis U. Trihalomethanes and associated potential cancer risks in the water supply in Ankara, Turkey. Environ Res. 2004 Nov;96(3):345-52.	Risk assessment
Trihalomethanes and colorectal cancer. Environ Health Perspect. 1994 Feb;102(2):151-2.	Review
Villanueva CM, Cantor KP, Grimalt JO, Castaño-Vinyals G, Malats N, Silverman D, Tardon A, Garcia-Closas R, Serra C, Carrato A, Rothman N, Real FX, Dosemeci M, Kogevinas M. Assessment of lifetime exposure to trihalomethanes through different routes. Occup Environ Med. 2006 Apr;63(4):273-7.	Exposure assessment study
Villanueva CM, Cordier S, Font-Ribera L, Salas LA, Levallois P. Overview of Disinfection By-products and Associated Health Effects. Curr Environ Health Rep. 2015 Mar;2(1):107-15.	Review
Villanueva CM, Kogevinas M, Grimalt JO. [Chlorination of drinking water in Spain and bladder cancer]. Gac Sanit. 2001 Jan-Feb;15(1):48-53.	Review
Zierler S, Danley RA and Feingold L (1986). Type of disinfectant in drinking water and patterns of mortality in Massachusetts. Environ Health Perspect 69: 275-279.	Compared chlorination to chloramine

Appendix C References

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Bove GE, Rogerson PA, Vena JE (2007b). Case control study of the geographic variability of exposure to disinfectant byproducts and risk for rectal cancer. Int J Health Geogr 6:18.

Bradford-Hill A (1965). The Environment and Disease: Association or Causation? Proceedings of the Royal Society of Medicine 58 (5) 295-300.

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Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, Lubin J, *et al.* (1999). Drinking water source and chlorination byproducts in Iowa. III. Risk of brain cancer. Am J Epidemiol 150(6):552-60.

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APPENDIX D. BENCHMARK DOSE ANALYSIS RESULTS FOR NON-CANCER ENDPOINTS

Chloroform

Sasso *et al.* (2013) provided BMD analyses of five kidney toxicity endpoints for chloroform in male and female rats and male mice from Yamamoto *et al.* (2002) and Nagano *et al.* (2006) (see Sasso *et al.*, Table 7), studies that used inhalation or inhalation plus drinking water as the medium for exposure. The endpoints included in the Sasso analysis were: nuclear enlargement of proximal tubules; atypical tubule hyperplasia; dilation of the tubular lumen; cytoplasmic basophilia; and proliferation. Sasso et al (2013) used $BMDL_{10}$ values with a human PBPK model to estimate human equivalent doses (HEDs) ranging from 33.5 to 45.8 mg/kg-day for continuous dosage and 4.24 to 6.48 mg/kg-day for pulsed bolus dosage simulating 24 hour drinking water consumption.

Since the HEDs calculated by Sasso et al (2013) were based on $BMDL_{10}$ values and OEHHA's BMD analyses use $BMDL_{05}$, OEHHA reanalyzed the data to obtain $BMDL_{05}$ values and revised HEDs based on these $BMDL_{05}$ values. OEHHA analyzed the four kidney toxicity endpoints presented in Sasso *et al.*, Table 6: nuclear enlargement and dilation of tubular lumen (from Yamamoto *et al.* (2002) and Nagano *et al.* (2006)), and atypical tubular hyperplasia and cytoplasmic basophilia (from Nagano *et al.* (2006)). Good agreement was obtained with the $BMDL_{10}$ values of Sasso *et al.* (2013). The $BMDL_{05}$ values for each of these endpoints were 42.46, 36.32, 30.25, and 73.71 mg/L-24 hr., respectively. HED values estimated from $BMDL_{05}/BMDL_{10}$ ratios and continuous exposure for each of these endpoints were 32.62, 29.90, 24.15, and 36.96, respectively. For pulsed exposure revised HEDs were 4.11, 4.23, 3.04, and 5.22, respectively.

Table D1. Evaluation of human equivalent doses from Sasso *et al.*, (2013) based on OEHHA-derived $BMDL_{05}$ values

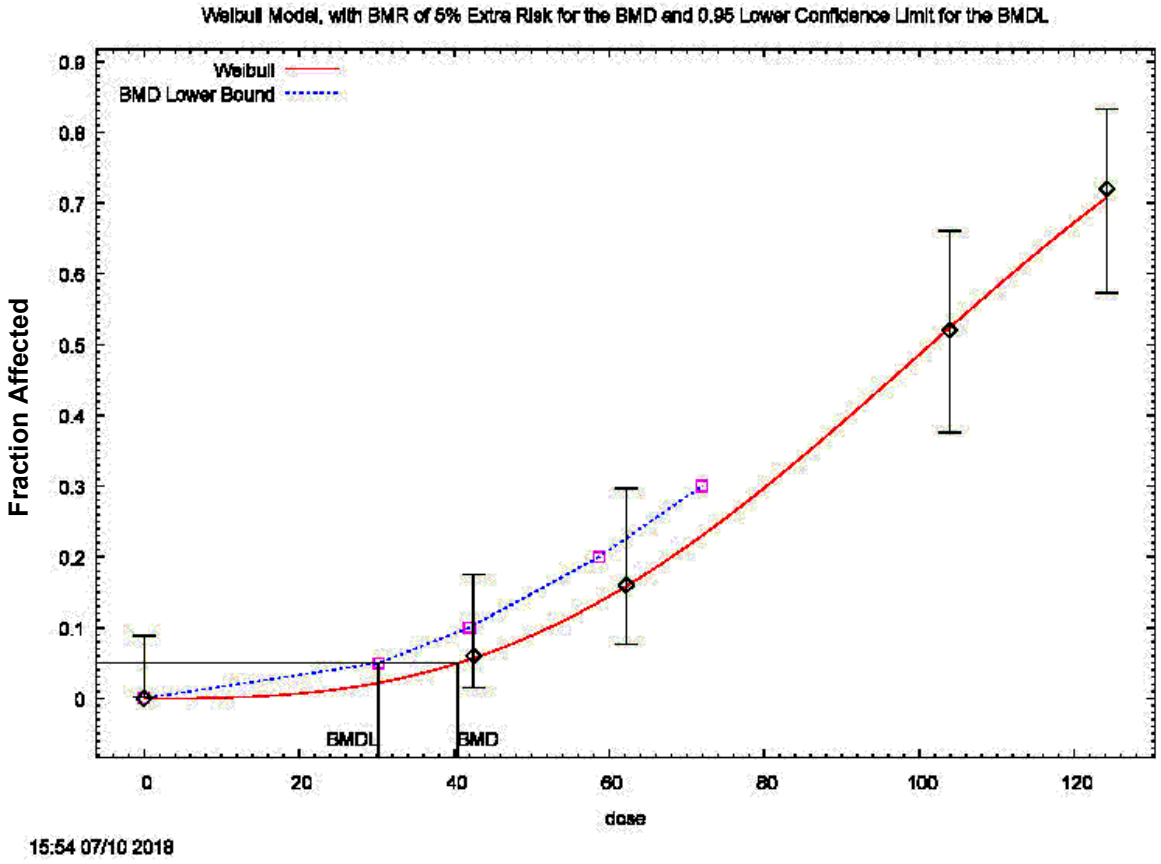
Kidney Toxicity Endpoints (Sasso *et al.* Table 6)	Model Fit stats	Internal Dose mg/L 24 hr; Quantal response	BMD/ BMDL10 Sasso *et al.*,2013	BMD/ BMDL10 OEHHA	BMD/ BMDL05 OEHHA	HEDd Sasso *et al.*, 2013	HEDc Sasso *et al.*, 2013	HEDa/b, OEHHA
Nuclear Enlargement of Proximal Tubules		0,27.8,42.5, 45.0,51.8, 60.2,62.2; 0/100,0/50, 0/50,5/50, 6/50,32/50, 33/50						
Loglogistic	X^2 = 7.72, P = 0.1726				45.7 43.1			
Logprobit	X^2 = 8.82, P = 0.128				45.65 43.32			
Weibull	X^2 = 6.93, P = 0.2261		48.99 46.61	48.82 46.39	45.44 42.46	35.64	4.49	32.62 4.11
Dilation of the Tubular Lumen		0, 28.7, 42.5, 45.0, 51.8, 60.2; 0/100,0/50, 3/50, 9/50,11/50, 7/50						
Loglogistic	X^2 = 3.13 P = 0.5369		43.60 40.60	43.93 40.70	39.66 35.55			
Logprobit	X^2 = 2.94, P = 0.5685		43.60 40.60	43.76 40.73	40.13 36.32	33.53	4.74	29.90 4.23

Kidney Toxicity Endpoints (Sasso *et al.* Table 6)	**Model Fit stats**	**Internal Dose mg/L 24 hr; Quantal response**	**BMD/ BMDL10 Sasso *et al.*,2013**	**BMD/ BMDL10 OEHHA**	**BMD/ BMDL05 OEHHA**	**HEDd Sasso *et al.*, 2013**	**HEDc Sasso *et al.*, 2013**	**HEDa/b, OEHHA**
Weibull	X^2 = 3.09, P = 0.5424			43.91 40.4	39.18 34.84			
Cytoplasmic Basophilia		0, 42.5, 62.2, 103.9, 124.2;0/50, 3/50,8/50, 26/50, 36/50						
Loglogistic	X^2 = 0.44, P = 0.932		49.71 40.45	52.4 43.4	43.4 33.1			
Logprobit	X^2 = 0.80, P = 0.8488			51.91 43.82	43.40 35.05			
Weibull	X^2 = 0.03, P = 0.9985			52.03 41.94	40.40 30.25	33.48	4.23	24.15 3.04
Tubular Hyperplasia		0, 42.5, 65.4,103.9, 112.0, 124.2; 1/50,0/50, 2/49, 4/50, 7/50,15/50						
Loglogistic	X^2 = 2.01 P = 0.5710			109.2 93.98	101.2 77.0			
Logprobit	X^2 = 2.04, P = 0.5633			109.28 93.44	102.35 77.28			
Weibull	X^2 = 1.99, P = 0.5742		106.03 96.43	109.08 93.96	100.58 76.51			
Multistage	X^2 = 8.42, P = 0.0774			90.31 77.19	63.02 50.97			

Kidney Toxicity Endpoints (Sasso *et al.* Table 6)	**Model Fit stats**	**Internal Dose mg/L 24 hr; Quantal response**	**BMD/ BMDL10 Sasso *et al.*,2013**	**BMD/ BMDL10 OEHHA**	**BMD/ BMDL05 OEHHA**	**HEDd Sasso *et al.*, 2013**	**HEDc Sasso *et al.*, 2013**	**HEDa/b, OEHHA**
Gamma	X^2 = 2.76, P = 0.5983			106.19 91.36	96.36 73.71	45.81	6.48	36.96 5.22

Note: d and a based on continuous dosage; b and c use pulsed dosage simulating water consumption. HEDa/b estimates based on BMDL05/BMDL10 ratios.

Figure D1. BMDS model output for cytoplasmic basophilia from Nagano *et al.* (2006) using PBPK simulated doses from Sasso *et al.* (2013)




```
==========================================================================
Weibull Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
Input Data File: C:/Users/jbrown1/Desktop/CHCl3_CytoBaso_MRat_Weib_05.(d)
  Gnuplot Plotting File:  C:/Users/jbrown1/Desktop/CHCl3_CytoBaso_MRat_Weib_05.plt
Tue Jul 10 15:54:43 2018
==========================================================================
BMDS_Model_Run
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = Effect
Independent variable = Dose
Power parameter is restricted as power >= 1.000000

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0192308
Slope = 2.06384e-006
Power = 2.75678

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Slope	Power
Slope	1	-1
Power	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	1.38592e-006	2.77064e-006	-4.04444e-006	6.81628e-006
Power	2.8439	0.431283	1.9986	3.6892

NA - Indicates that this parameter has hit a bound by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-97.5969	5			
Fitted model	-97.6127	2	0.0316917	3	0.9985
Reduced model	-150.983	1	106.773	4	<.0001

AIC: 199.225

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50.000	0.000
42.5000	0.0575	2.877	3.000	50.000	0.075
62.2000	0.1606	8.028	8.000	50.000	-0.011
103.9000	0.5291	26.453	26.000	50.000	-0.128
124.2000	0.7137	35.687	36.000	50.000	0.098

Chi^2 = 0.03 d.f. = 3 P-value = 0.9985

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 40.3977
BMDL = 30.2474

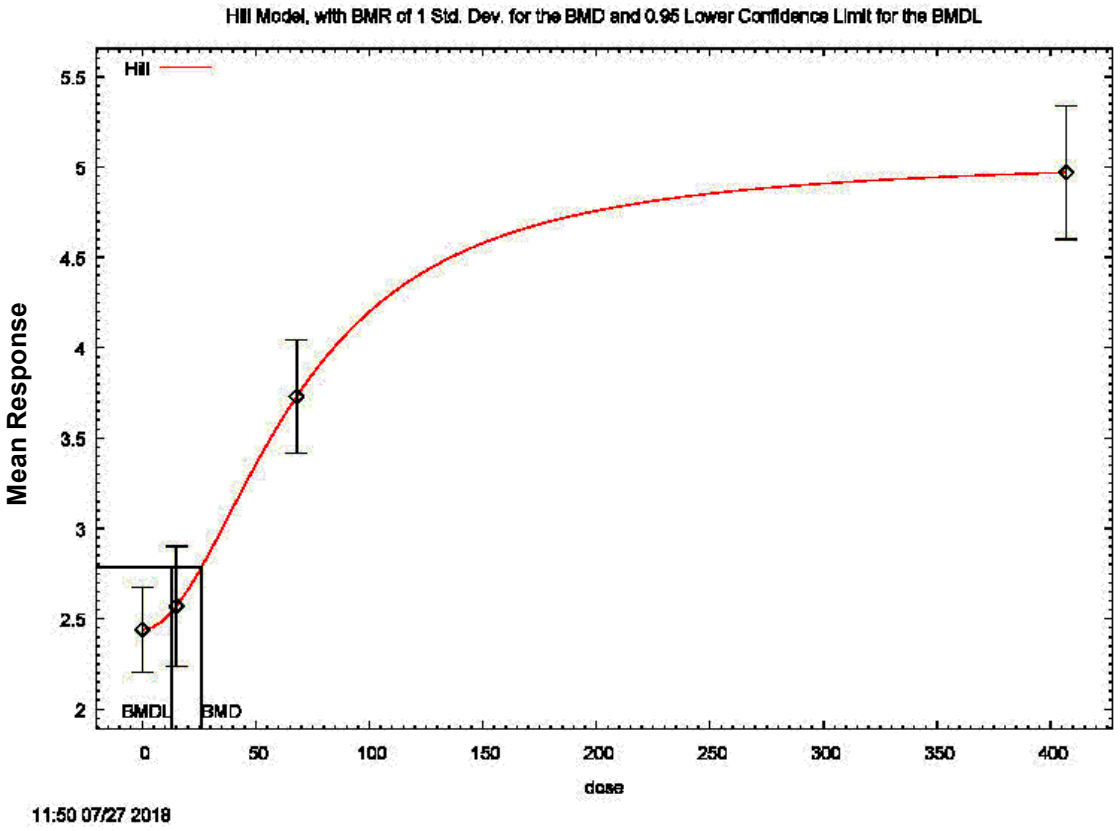
Bromoform

OEHHA applied BMD models for continuous data to the observed increased relative liver weight in bromoform-exposed male and female rats at 24 months of exposure reported in the dietary study by Tobe *et al.* (1982). The best fit model was the Hill model for continuous data (Table D2). This model yielded a $BMDL_{1SD}$ (lower 95% confidence limit of the dose corresponding to a 1 standard deviation increase in relative liver weight) for these data in female rats of 12.9 mg/kg-day. The model yielded a $BMDL_{1SD}$ of 36.38 mg/kg-day for male rats, indicating that females were more sensitive as measured by this indicator of hepatotoxicity.

Table D2. BMD analyses of bromoform induced chronic non-cancer effects, Tobe *et al.* (1982) with revised OEHHA doses

Data Set Model	P for fit	AIC	BMD_1SD	$BMDL_1SD$
Relative liver Weight				
Male Rats				
Linear	0.1524	14.03	280.5	193.6
Polynomial	0.4259	12.90	71.5	39.7
Polynomial – restricted to positive coefficients	0.1524	14.03	280.5	193.6
Hill	NA (perfect fit)	14.3	82.91	32.45
Relative Liver Weight				
Female Rats				
Linear	<0.0001	-8.05	82.7	65.9
Polynomial	0.2354	-27.75	16.6	12.7
Polynomial; restricted to positive coefficients	<0.001	-8.06	82.7	65.9
Hill	NA (perfect fit)	-27.16	26.05	12.90

Figure D2. BMD model output for relative liver weight – female rats in Tobe *et al*. (1982)



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Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/asalmon/Documents/BMDS/BMDS2704/Data/Bromoform rel liver wt Tobe Hil.dax.(d)
Gnuplot Plotting File: C:/Users/asalmon/Documents/BMDS/BMDS2704/Data/Bromoform rel liver wt Tobe Hil.dax.plt
Fri Jul 27 11:50:02 2018
==========
BMDS Model Run
~~~~~~~~~~

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
~~~~~~~~~~

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.135762
rho = 0 Specified
intercept = 2.44
v = 2.53
n = 0.954015
k = 69.1422

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	alpha	intercept	v	n	k
alpha	1	-1.9e-008	-5.9e-008	9.6e-010	-1.3e-007
intercept	-1.9e-008	1	-0.66	0.54	0.067
v	-5.9e-008	-0.66	1	-0.77	0.48
n	9.6e-010	0.54	-0.77	1	-0.4
k	-1.3e-007	0.067	0.48	-0.4	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.119306	0.0293711	0.0617397	0.176872
intercept	2.44	0.0997104	2.24457	2.63543
v	2.61122	0.25519	2.11105	3.11138
n	1.93526	0.878107	0.214199	3.65632
k	68.8454	9.77849	49.6799	88.0109

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	12	2.44	2.44	0.37	0.345	7.9e-008
15	7	2.57	2.57	0.36	0.345	-5.01e-008
68	7	3.73	3.73	0.34	0.345	-4.4e-007
407	7	4.97	4.97	0.4	0.345	4.25e-007

Degrees of freedom for Test A3 vs fitted <= 0

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)

Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	18.580042	5	-27.160084
A2	18.688939	8	-21.377878
A3	18.580042	5	-27.160084
fitted	18.580042	5	-27.160084
R	-18.540048	2	41.080097

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	74.458	6	<.0001
Test 2	0.217794	3	0.9747
Test 3	0.217794	3	0.9747
Test 4	1.84741e-013	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 26.0474
BMDL = 12.9062
BMDU = 63.0206

Bromodichloromethane

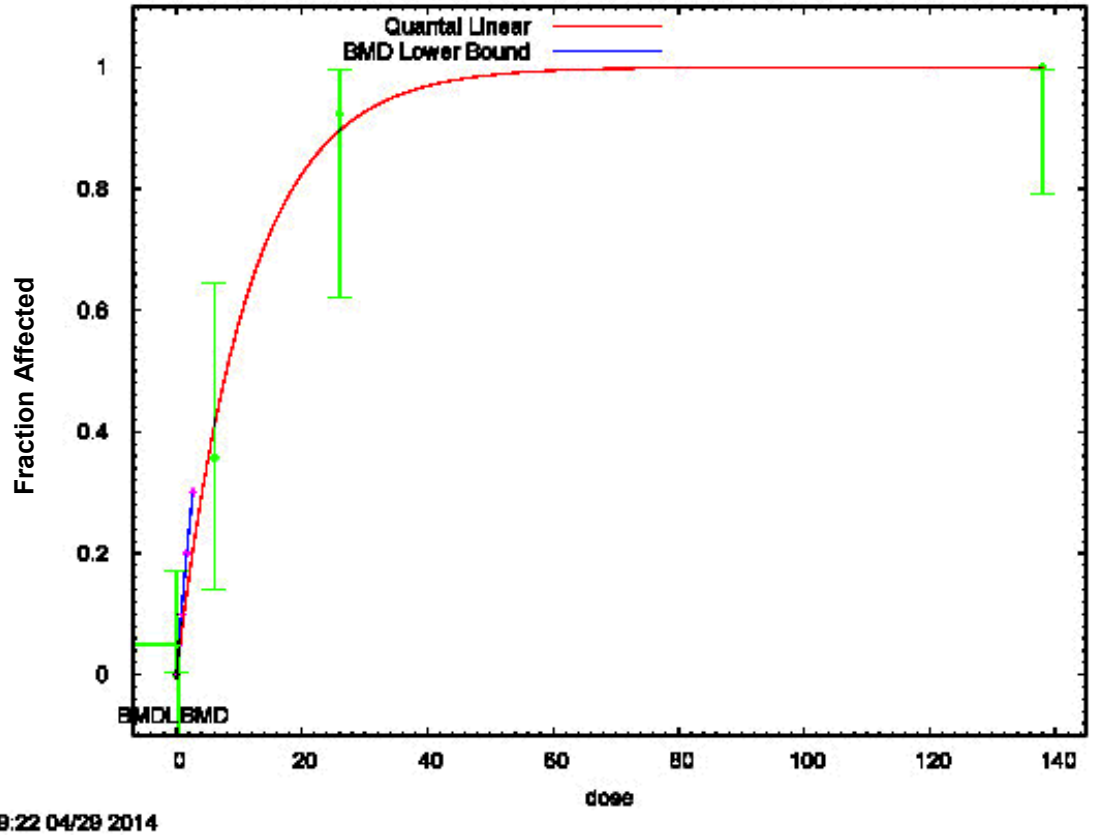
Results of the chronic studies conducted in male and female Wistar rats by Aida *et al.*, (1992b) were selected for quantification of non-carcinogenic effects. Dose-response relationships were assessed for hepatic fatty degeneration and granuloma in male and female rats using the BMD approach.

Table D3. Model output summary for BDCM – hepatic lesions in wistar rats from Aida *et al.* (1992b) Study

Model	AIC	p-value	Benchmark Dose (mg/kg-day)	
			BMD_{05}	$BMDL_{05}$
Hepatic Fatty Degeneration in Males				
Gamma	29.3	1	1.15	0.386
Logistic	34.5	0.14	2.64	1.53
LogLogistic	29.4	0.98	2.04	0.478
LogProbit	29.3	1	2.13	0.974
Multistage (Mst2)	29.3	1	0.765	0.386
Multistage (Mst3)	31.3	1	0.728	0.386
Probit	34.1	0.15	2.58	1.57
Weibull	29.3	1	1	0.386
Quantal-Linear[a]	27.6	0.97	0.589	0.378

Figure D3. Quantal-Linear model output for BDCM – hepatic fatty degeneration in male wistar rats from Aida *et al.* (1992b) Study

Quantal Linear Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
================================================================
Quantal Linear Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
Input Data File: K:/BMDS240/Data/qln_080113BDCMmaleHepaticFattyDegeneration_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/qln_080113BDCMmaleHepaticFattyDegeneration_Opt.pltTue Apr 29
 ================================================================
 BMDS_Model_Run
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The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-slope*dose)]

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
Background = 0.0384615
Slope = 0.0217775
Power = 1 Specified

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.0870329	0.0242606	0.039483	0.134583

NA - Indicates that this parameter has hit a boundimplied b y some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-12.6501	4			
Fitted model	-12.7786	1	0.257082	3	0.9679
Reduced model	-48.4917	1	71.6833	3	<.0001

AIC: 27.5572

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	24	0.000
6.0000	0.4068	5.695	5.000	14	-0.378
26.0000	0.8959	11.647	12.000	13	0.320
138.0000	1.0000	19.000	19.000	19	0.011

Chi^2 = 0.25 d.f. = 3 P-value = 0.9699
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 0.589355
BMDL = 0.378474

Dibromochloromethane

We performed benchmark dose analyses on the data for ground glass cytoplasmic change in the liver in male rats and fatty change in the liver in female rats. Several models fit the data well for ground glass cytoplasmic change in the livers of male rats, including the gamma, multistage, and Weibull models, producing the same $BMDL_{05}$. The BMD_{05} ranged from 6.03 to 8.45 for the three models and the $BMDL_{05}$ was 3.4 mg/kg-day.

Table D4a. Model output summary for DBCM – ground glass cytoplasmic change in males

Model	AIC	p-value	Scaled Residual for dose group	Benchmark Dose (mg/kg-day)	
				BMD_{05}	$BMDL_{05}$
Gamma*	181.247	NA	0	8.45406	3.39565
Logistic	179.563	0.5743	-0.279	8.8612	7.13954
LogLogistic*	181.247	NA	0	10.6433	2.28891
LogProbit*	181.247	NA	0	12.7034	8.87914
Multistage*	181.247	NA	0	6.0342	3.39565
Probit	179.492	0.6203	-0.228	8.35481	6.8439
Weibull*	181.247	NA	0	7.66244	3.39565
Quantal-Linear[a]	179.461	0.6443	0.084	4.48554	3.35935

[a]This model yields the lowest AIC and highest p-value. However, the AIC values are similar for all models.
*These 5 models yielding p-values of "NA" provide perfect curve fits to the. Except for the LogProbit, they all yield similar BMDL values, with Gamma, Multistage and Weibull providing identical BMDL values (but different BMD values).

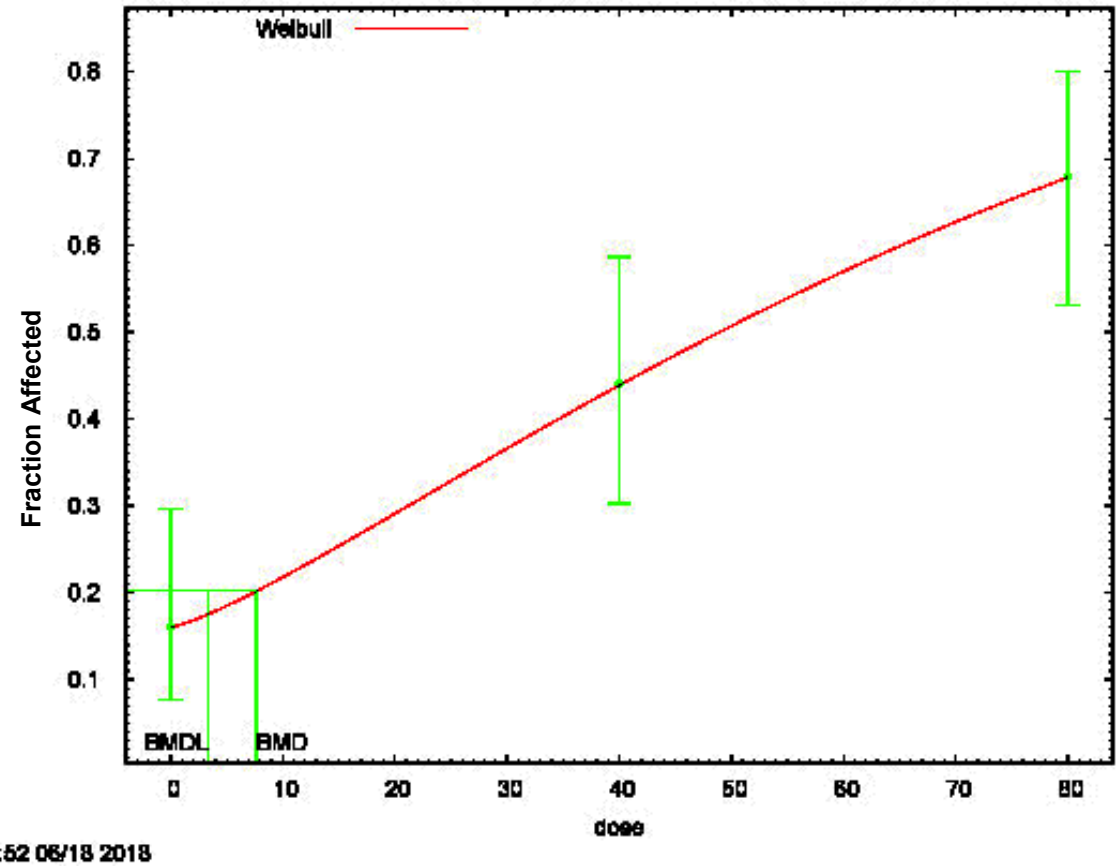
Table D4b. Model output summary for DBCM – fatty change in females

Model	AIC	P-value	Scaled Residual for dose group	Benchmark Dose (mg/kg-day)	
				BMD_{05}	$BMDL_{05}$
Gamma	127.145	0.6914	-0.135	29.536	23.0008
Logistic	140.4	0.0014	1.367	5.25377	4.07609
LogLogistic[a]	126.853	0.9854	0	35.5836	26.1672
LogProbit	128.853	NA	0	35.1254	25.9056
Multistage	135.842	0.0105	0.58	10.932	6.4103
Probit	139.044	0.0019	1.279	4.8105	3.8707
Weibull	128.853	NA	0.001	28.0778	17.5395
Quantal-Linear	150.242	0	0.547	2.28151	1.76636

[a]This model gives the best fit of the data.

Figure D4. Weibull Model Output for DBCM – Ground Glass Cytoplasmic Change in Males

Weibull Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
==========================================================================
Weibull Model using Weibull Model (Version: 2.16;  Date: 2/28/2013)
Input Data File: C:/Users/ndangleben/Desktop/BMDS March
2018/wei_NTP1985MaleCytoplasmicChange_Opt.(d)
Gnuplot Plotting File:  C:/Users/ndangleben/Desktop/BMDS March
2018/wei_NTP1985MaleCytoplasmicChange_Opt.plt
Mon Jun 18 08:52:39 2018
==========================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = Effect
Independent variable = Dose
Power parameter is restricted as power >= 1.000000
```

Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.173077
Slope = 0.00415766
Power = 1.23415

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	-0.27	0.23
Slope	-0.27	1	-1
Power	0.23	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.16	0.0518462	0.0583835	0.261617
Slope	0.00401464	0.00941531	-0.014439	0.0224683
Power	1.25108	0.556936	0.159506	2.34265

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-87.6235	3			
Fitted model	-87.6235	3	2.26436e-010	0	NA
Reduced model	-102.353	1	29.4589	2	<.0001

AIC: 181.247

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1600	8.000	8.000	50	-0.000
40.0000	0.4400	22.000	22.000	50	0.000
80.0000	0.6800	34.000	34.000	50	-0.000

Chi^2 = 0.00 d.f. = 0 P-value = NA

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 7.66244
BMDL = 3.39565

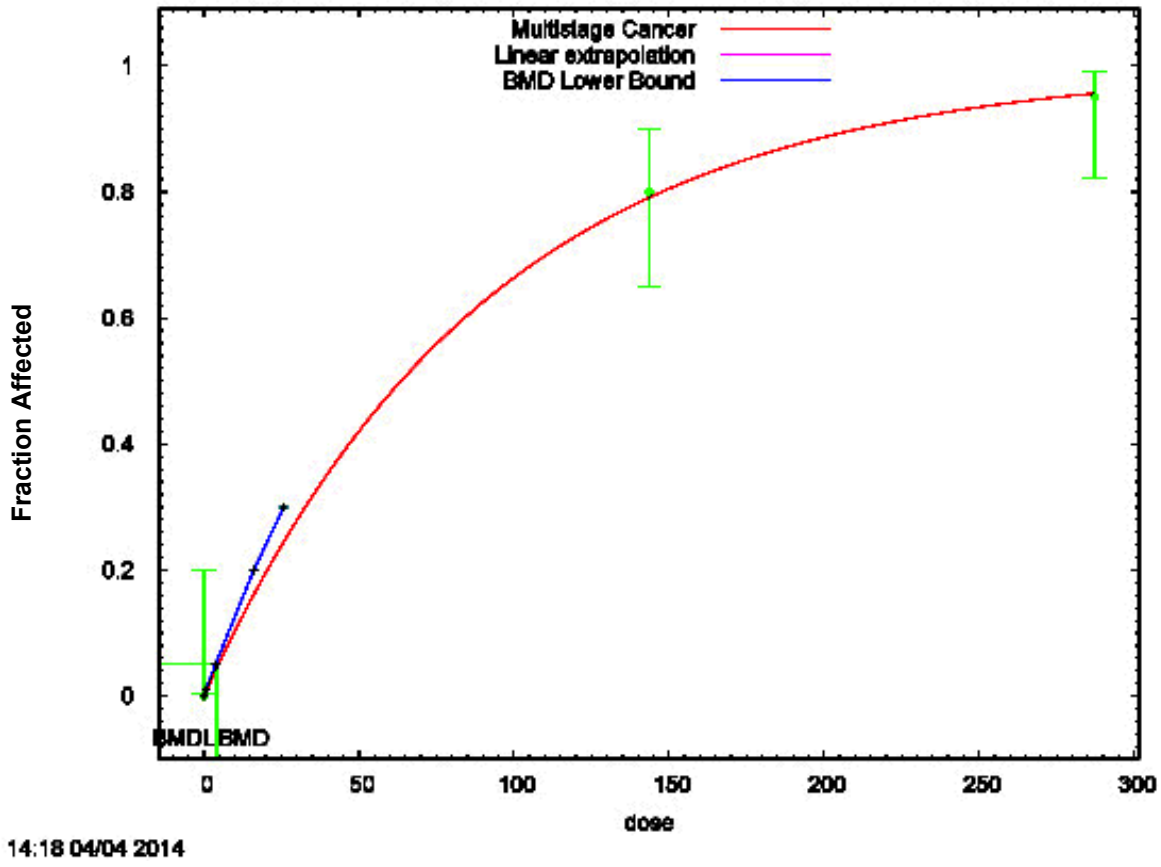
APPENDIX E. BENCHMARK DOSE ANALYSIS RESULTS FOR CANCER ENDPOINTS

This appendix provides the BMD modeling outputs for each THM. The Multistage-Cancer model, which is optimized for cancer data, was run with default parameters and a benchmark response of 5 percent. Outputs were checked for goodness of fit p-value ≥ 0.05, scaled residual ≤ the absolute value of 2, and visual inspection of the dose-response curve prior to selection for use in the PHG calculation.

Chloroform

Figure E1. Multistage-cancer model output for chloroform – hepatocellular carcinoma in female B6C3F1 mice from NCI (1976) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



==========
Multistage Cancer Model. (Version: 1.10; Date: 02/28/2013)
Input Data File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_NCI1976_Female_Mice_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_NCI1976_Female_Mice_Opt.plt
Fri Apr 04 15:18:17 2014
==========

BMDS_Model_Run
~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]
The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0325342
Beta(1) = 0.0105146
Beta(2) = 0

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.0109258	*	*	*
Beta(2)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-30.5094	3			
Fitted model	-30.5327	1	0.0466268	2	0.977
Reduced model	-64.0593	1	67.0999	2	<.0001

AIC: 63.0654
~~~~~~

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	0.000
143.6300	0.7918	35.631	36.000	45	0.135
287.2600	0.9567	39.223	39.000	41	-0.171

Chi^2 = 0.05 d.f. = 2 P-value = 0.9765

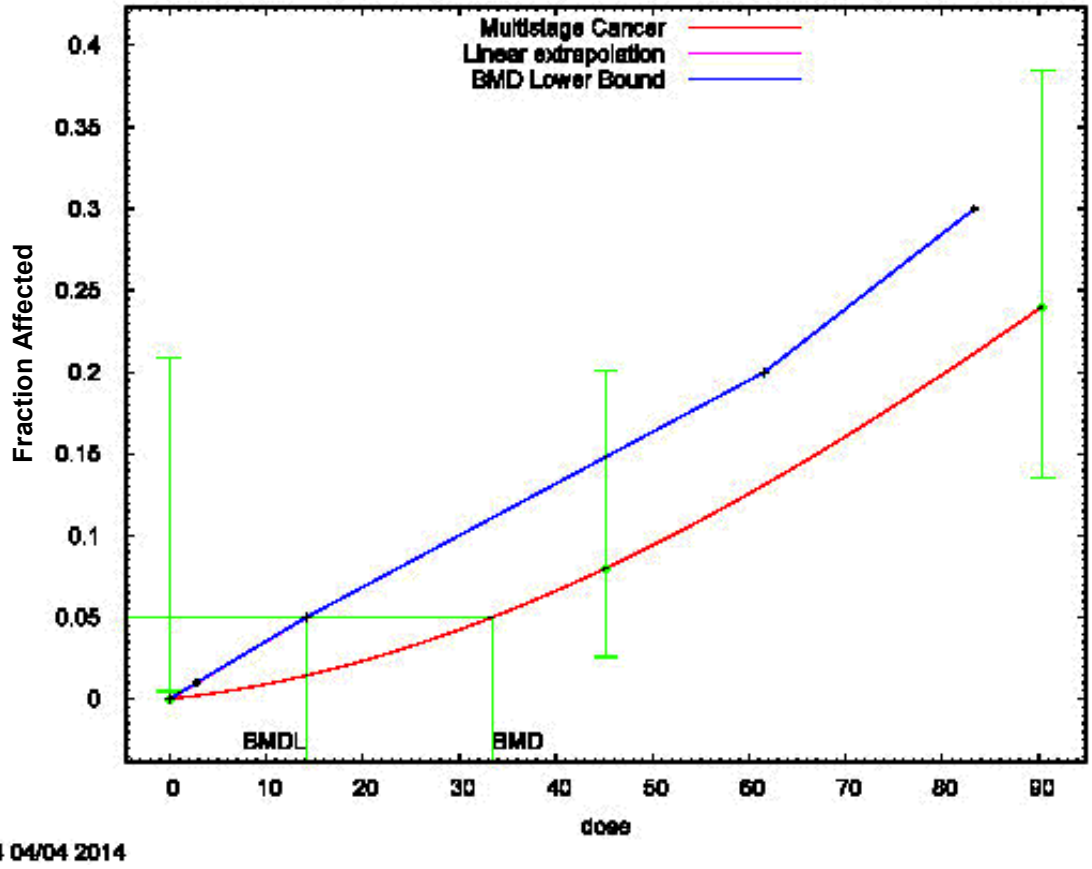
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 4.6947
BMDL = 3.70591
BMDU = 11.3762

Taken together, (3.70591, 11.3762) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.013492

Figure E2. Multistage-cancer model output for chloroform – renal tubular cell adenoma and carcinoma in male Osborne-Mendel rats from NCI (1976) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
==========================================================================
Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
Input Data File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_NCI1976_Male_Rats_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_NCI1976_Male_Rats_Opt.plt
Fri Apr 04 15:34:21 2014
==========================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]
The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
```

Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 1.14709e-016
Beta(1) = 0.00065468
Beta(2) = 2.63731e-005

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)	Beta(2)
Beta(1)	1	-0.97
Beta(2)	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00065468	*	*	*
Beta(2)	2.63731e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-41.4925	3			
Fitted model	-41.4925	2	1.42109e-013	1	1
Reduced model	-46.9772	1	10.9695	2	0.00415

AIC: 86.9849

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	19	0.000
45.1700	0.0800	4.000	4.000	50	-0.000
90.3500	0.2400	12.000	12.000	50	-0.000

Chi^2 = 0.00 d.f. = 1 P-value = 1.0000

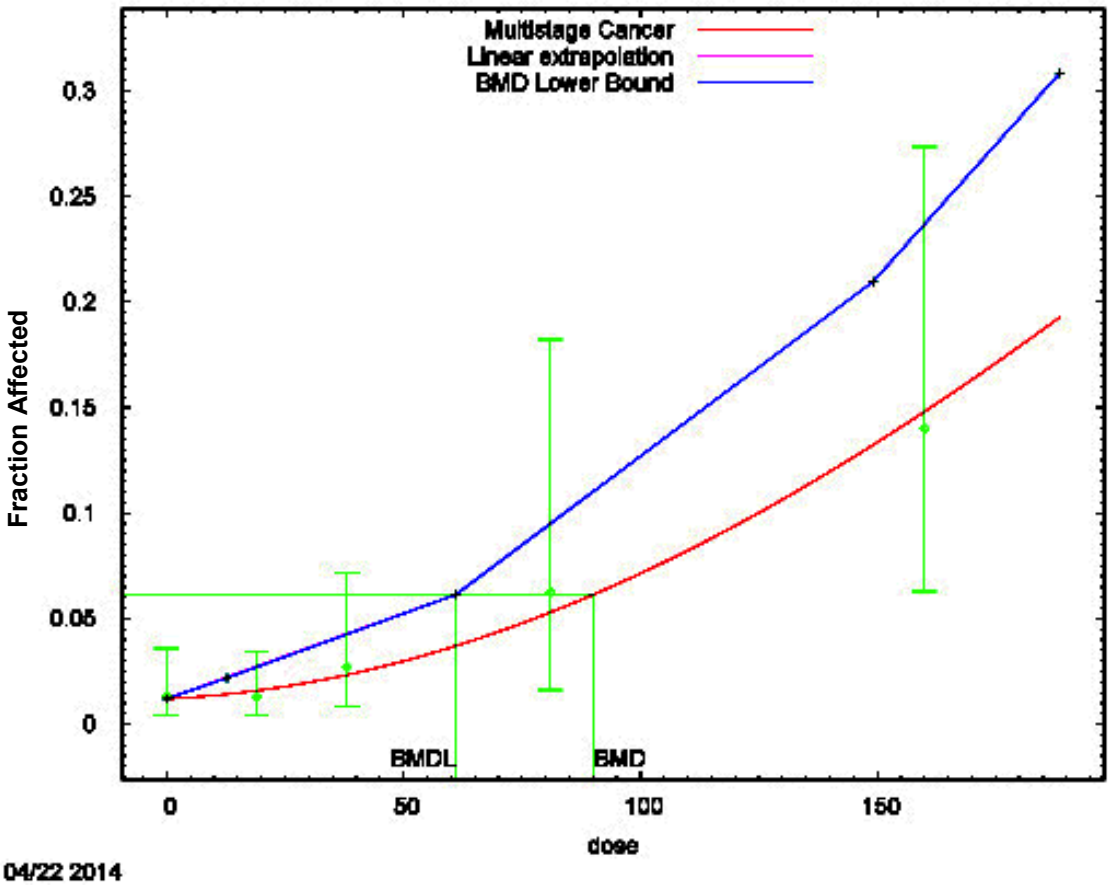
Benchmark Dose Computation
Specified effect = 0.05
Risk Type= Extra risk
Confidence level = 0.95
BMD = 33.4026

BMDL = 14.1487
BMDU = 51.6299

Taken together, (14.1487, 51.6299) is a 90 % two-sided confidenceinterval for the BMD
Multistage Cancer Slope Factor = 0.00353388

Figure E3. Multistage-Cancer Model Output for Chloroform – Renal Tubular Cell Adenoma and Adenocarcinoma in Male Osborne-Mendel Rats from Jorgenson *et al.* (1985) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the




```
 ================================================================
Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
Input Data File:
K:/BMDS240/Data/msc_ChloroformCancerJorgenson1985MaleRatsRenalTumors_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/msc_ChloroformCancerJorgenson1985MaleRatsRenalTumors_Opt.plt
Tue Apr 22 10:44:29 2014
================================================================
BMDS_Model_Run
```

~~~~~~

The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.00936056
Beta(1) = 0.000390669
Beta(2) = 3.10775e-006

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.69	0.55
Beta(1)	-0.69	1	-0.93
Beta(2)	0.55	-0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	95.0% Wald Confidence Interval Upper Conf. Limit
Background	0.0120191	*	*	*
Beta(1)	0.000109536	*	*	*
Beta(2)	5.096e-006	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-92.5298	5			
Fitted model	-92.7492	3	0.438715	2	0.803
Reduced model	-102.366	1	19.6718	4	0.0005797

AIC: 191.498

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0120	3.618	4.000	301	0.202
19.0000	0.0159	4.972	4.000	313	-0.439
38.0000	0.0233	3.454	4.000	148	0.297
~~~~~~

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
81.0000	0.0529	2.541	3.000	48	0.296
160.0000	0.1479	7.396	7.000	50	-0.158

Chi^2 = 0.43 d.f. = 2 P-value = 0.8046

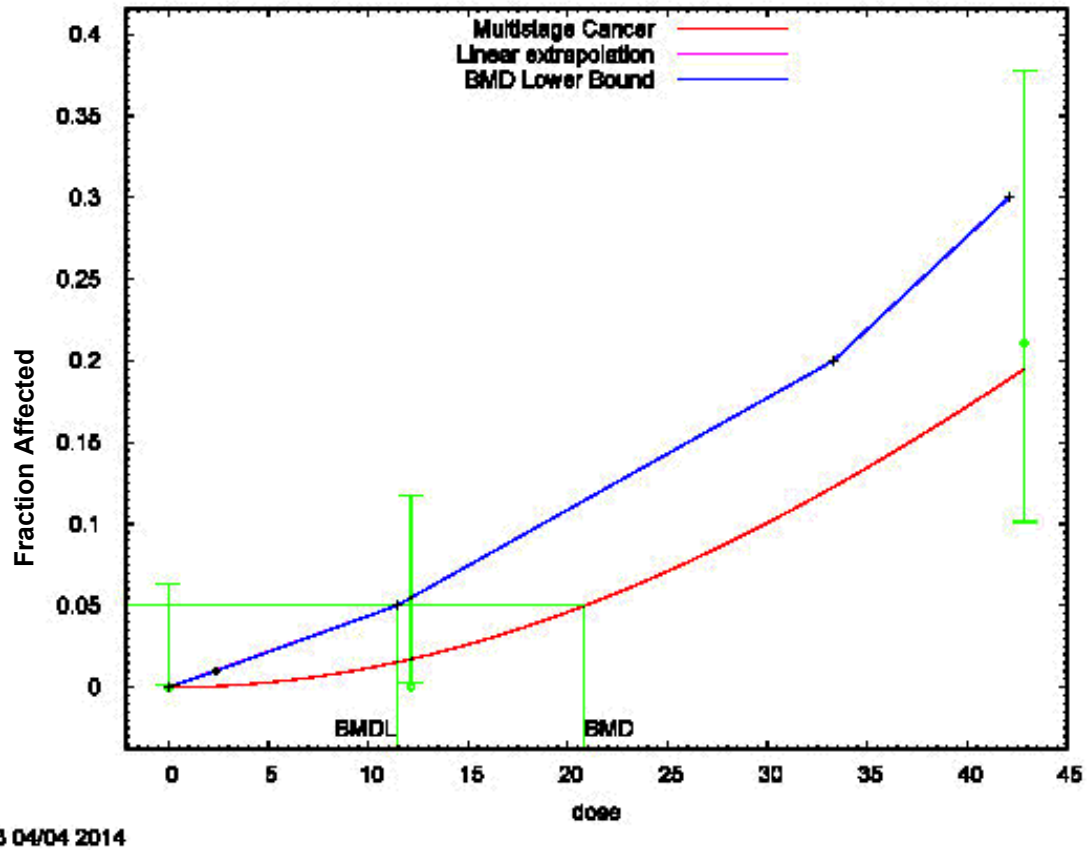
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95

BMD = 90.1532
BMDL = 61.0585
BMDU = 131.452

Taken together, (61.0585, 131.452) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.000818887

Figure E4. Multistage-cancer model output for chloroform – renal adenoma and carcinoma in male ICI mice from Roe *et al.* (1979) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



==========

Multistage Cancer Model. (Version: 1.10; Date: 02/28/2013)
Input Data File: K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_Roe_Opt.(d)

Gnuplot Plotting File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_Chloroform_Roe_Opt.plt
Fri Apr 04 15:53:17 2014
==========
BMDS_Model_Run
~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0.000133362

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000118265	*	*	*

* - Indicates that this value is not calculated.

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-19.5568	3			
Fitted model	-20.2293	1	1.34502	2	0.5104
~~~~~~~~~~

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Reduced model	-31.0662	1	23.0187	2	<.0001

AIC: 42.4587

Goodness of Fit

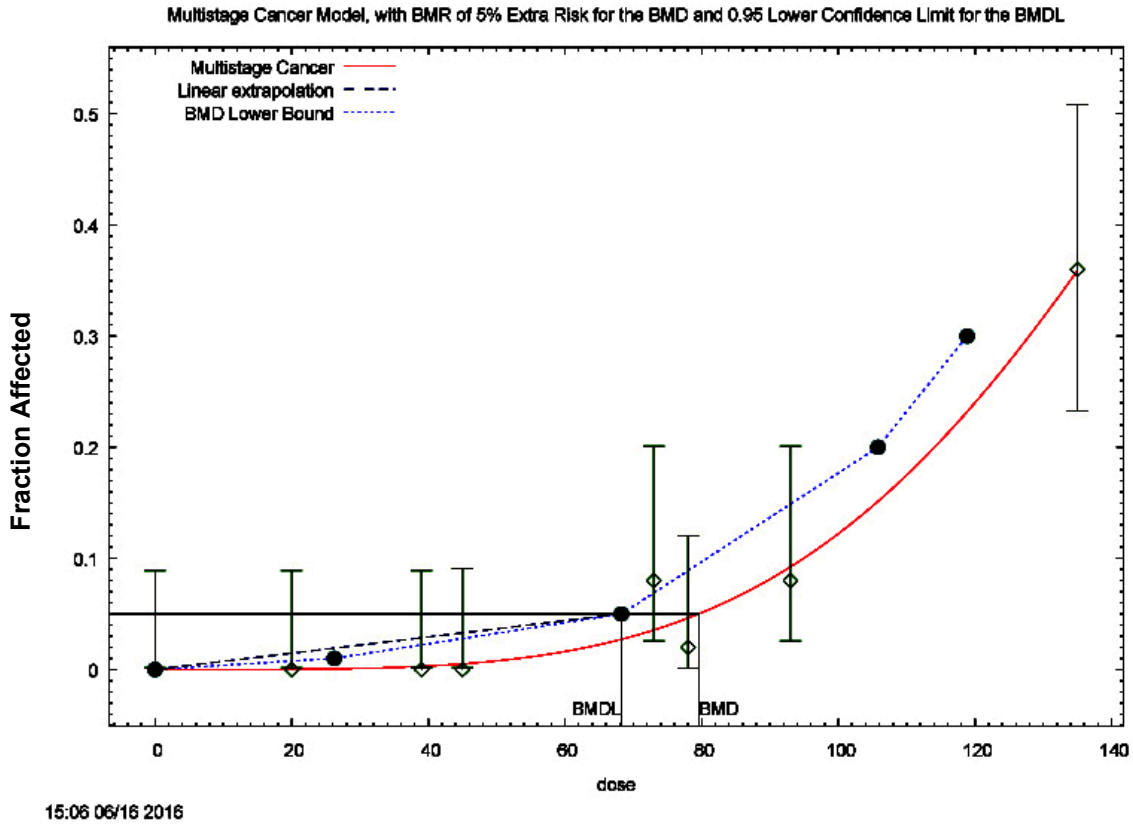
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	72	0.000
12.1400	0.0173	0.639	0.000	37	-0.807
42.8600	0.1953	7.420	8.000	38	0.237

Chi^2 = 0.71 d.f. = 2 P-value = 0.7023

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 20.8258
BMDL = 11.4337
BMDU = 28.7479
Taken together, (11.4337, 28.7479) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00437303

Figure E5. Multistage-cancer model output for chloroform – renal adenoma and carcinoma in male F344 rats from Nagano *et al.* (2006) Study



```
 ================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: C:/BMDS260/Data/msc_Dax_Setting.(d)
Gnuplot Plotting File:  C:/BMDS260/Data/msc_Dax_Setting.plt
Thu Jun 16 15:06:15 2016
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4-beta5*dose^5-beta6*dose^6-
beta7*dose^7)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 8
Total number of records with missing values = 0
Total number of parameters in model = 8
Total number of specified parameters = 0
Degree of polynomial = 7

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background =  0
Beta(1) = 4.11235e-005
Beta(2) = 3.69597e-006
Beta(3) =  0
Beta(4) =  0
Beta(5) = 8.36028e-012
Beta(6) =  0
Beta(7) =  0

Asymptotic Correlation Matrix of Parameter Estimates

 ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)    -Beta(3)    -Beta(6)    -
Beta(7)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )
              Beta(4)         Beta(5)
 Beta(4)      1               -0.98
 Beta(5)      -0.98           1

Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Beta(1)	0	NA		
Beta(2)	0	NA		
Beta(3)	0	NA		
Beta(4)	1.19625e-009	1.17284e-009	-1.10247e-009	3.49498e-009
Beta(5)	1.02975e-012	9.84773e-012	-1.82714e-011	2.0331e-011
Beta(6)	0	NA		
Beta(7)	0	NA		

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-65.4498	8			
Fitted model	-67.4672	2	4.03471	6	0.672
Reduced model	-98.7795	1	66.6594	7	<.0001

AIC: 138.934

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50.000	0.000
20.0000	0.0002	0.010	0.000	50.000	-0.099
39.0000	0.0029	0.143	0.000	50.000	-0.378
45.0000	0.0051	0.249	0.000	50.000	-0.500
73.0000	0.0355	1.773	4.000	50.000	1.703
78.0000	0.0462	2.308	1.000	50.000	-0.881
93.0000	0.0921	4.606	4.000	50.000	-0.296
135.0000	0.3582	17.911	18.000	50.000	0.026

Chi^2 = 4.17 d.f. = 6 P-value = 0.6539
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 79.5911
BMDL = 68.2513
BMDU = 91.5095

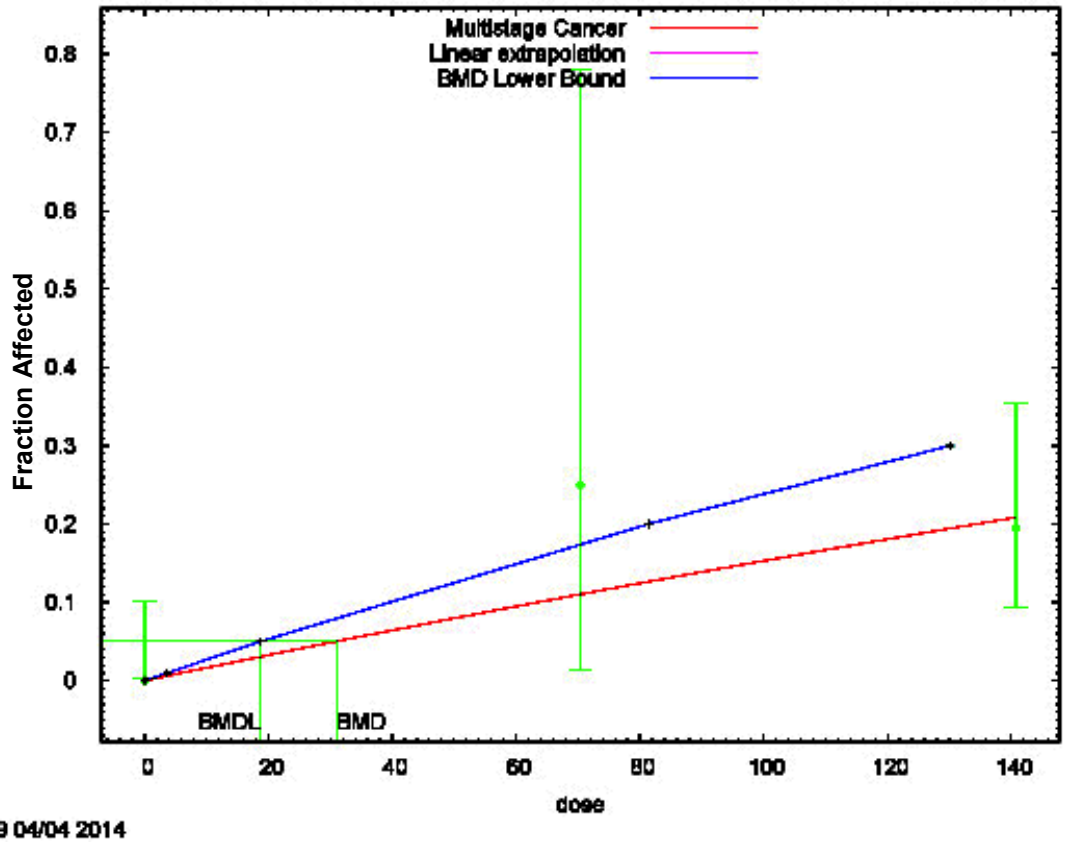
Taken together, (68.2513, 91.5095) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.000732587

Bromoform

Figure E6. Multistage-cancer model output for bromoform – large intestine adenomatous polyps and adenocarcinoma in female F344/N rats from NTP (1989a) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
==========================================================================
Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
Input Data File: K:/BMDS240/Data/msc_April2014BMDS_Cancer_Bromoform_Opt.(d)
Gnuplot Plotting File:  K:/BMDS240/Data/msc_April2014BMDS_Cancer_Bromoform_Opt.plt
Fri Apr 04 15:59:51 2014
==========================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
            -beta1*dose^1-beta2*dose^2)]
The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Total number of observations = 3
Total number of records with missing values = 0
```

Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0579685
Beta(1) = 0.00154165
Beta(2) = 0

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00165641	*	*	*
Beta(2)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-22.4855	3			
Fitted model	-22.8137	1	0.656355	2	0.7202
Reduced model	-29.0442	1	13.1174	2	0.001418

AIC: 47.6274

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	43	0.000
70.4000	0.1101	0.440	1.000	4	0.894
140.8000	0.2080	8.529	8.000	41	-0.204

Chi^2 = 0.84 d.f. = 2 P-value = 0.6567
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 30.9666
BMDL = 18.711
BMDU = 82.7333

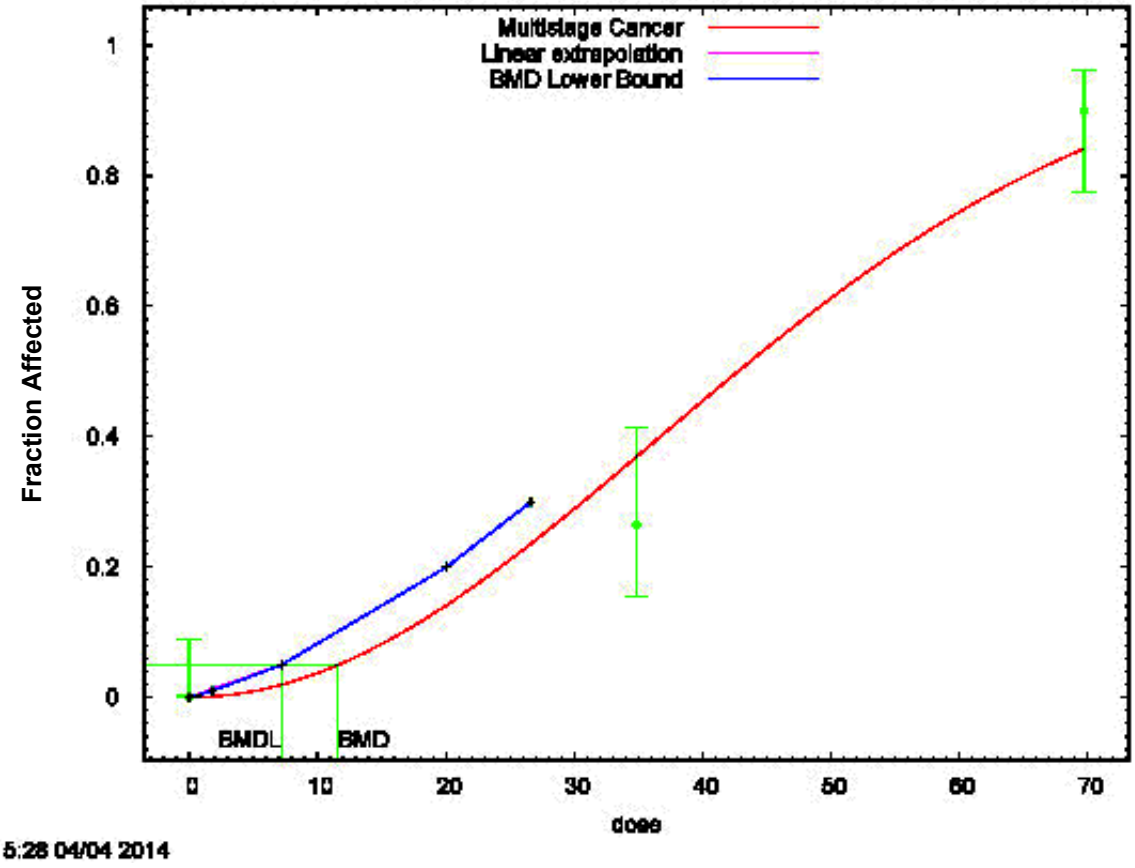
Taken together, (18.711 , 82.7333) is a 90 % two-sided confidence

interval for the BMD

Multistage Cancer Slope Factor = 0.00267222
Bromodichloromethane

Figure E7. Multistage-cancer model output for BDCM – large intestine adenoma and adenocarcinoma in male F344/N rats from NTP (1987) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
 ================================================================
Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
Input Data File: K:/BMDS240/Data/msc_April2014BMDS_Cancer_BDCM_Male_Rats_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_BDCM_Male_Rats_Opt.plt
Fri Apr 04 16:28:54 2014
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive
```

Dependent variable = Effect
Independent variable = Dose
Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0.00049062

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	95.0% Wald Confidence Interval Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000379432	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-44.6023	3			
Fitted model	-46.5129	1	3.8212	2	0.148
Reduced model	-99.5941	1	109.984	2	<.0001

AIC: 95.0258

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
34.8600	0.3694	18.101	13.000	49	-1.510
69.7200	0.8419	42.094	45.000	50	1.126

Chi^2 = 3.55 d.f. = 2 P-value = 0.1696

Benchmark Dose Computation

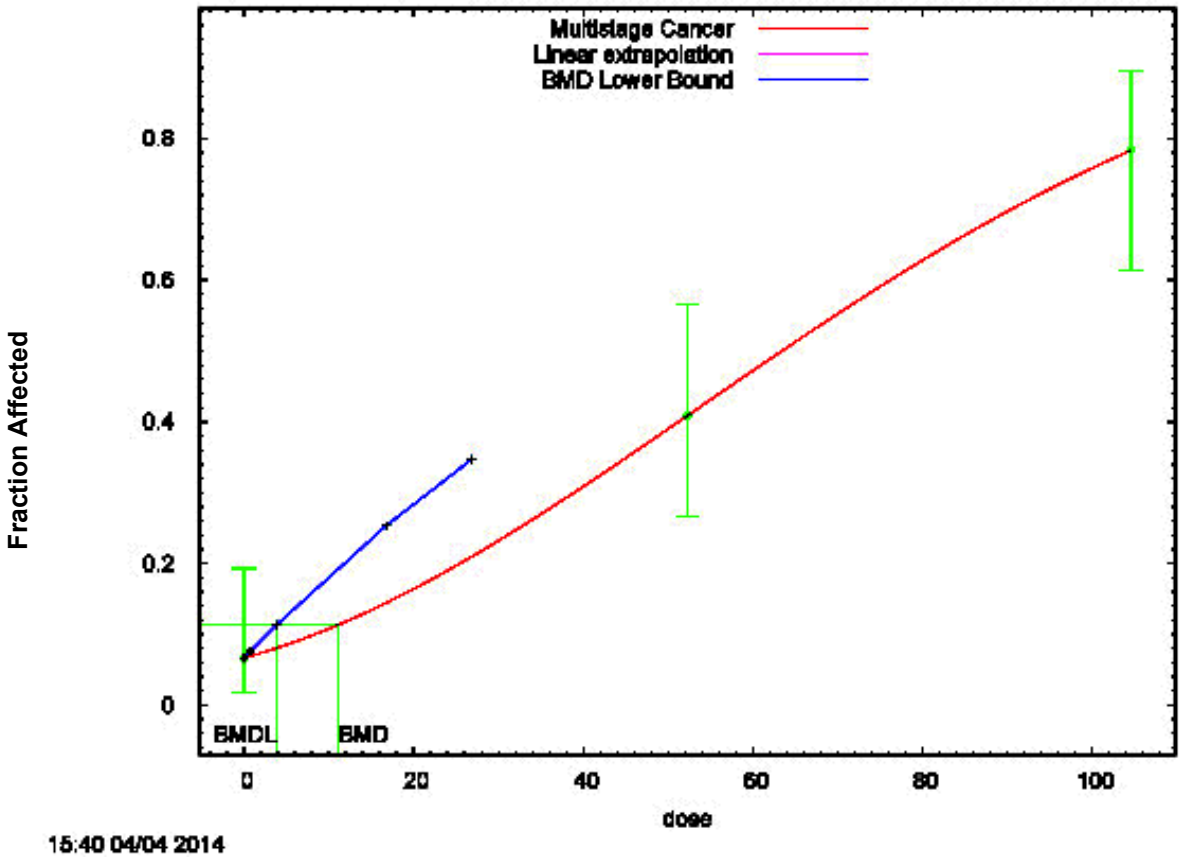
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 11.6269
BMDL = 7.21873
BMDU = 13.1501

Taken together, (7.21873, 13.1501) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00692642

Figure E8. Multistage-cancer model output for BDCM – hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice from NTP (1987) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



==========
Multistage Cancer Model. (Version: 1.10; Date: 02/28/2013)
Input Data File: K:/BMDS240/Data/msc_April2014BMDS_Cancer_BDCM_Female_Mice_Opt.(d)
Gnuplot Plotting File:
K:/BMDS240/Data/msc_April2014BMDS_Cancer_BDCM_Female_Mice_Opt.plt
Fri Apr 04 16:40:49 2014
==========
BMDS_Model_Run

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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0666667
Beta(1) = 0.00349892
Beta(2) = 0.000100262

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.47	0.28
Beta(1)	-0.47	1	-0.94
Beta(2)	0.28	-0.94	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0666667	*	*	*
Beta(1)	0.00349892	*	*	*
Beta(2)	0.000100262	*	*	*

* - Indicates that this value is not calculated.
Error in computing chi-square; returning 2

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-60.1058	3			
Fitted model	-60.1058	3	0	0	NA
Reduced model	-84.6346	1	49.0576	2	<.0001

AIC: 126.212

Goodness of Fit
~~~~~~

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0667	3.000	3.000	45	0.000
52.2900	0.4091	18.000	18.000	44	-0.000
104.5800	0.7838	29.000	29.000	37	0.000

Chi^2 = 0.00 d.f. = 0 P-value = NA

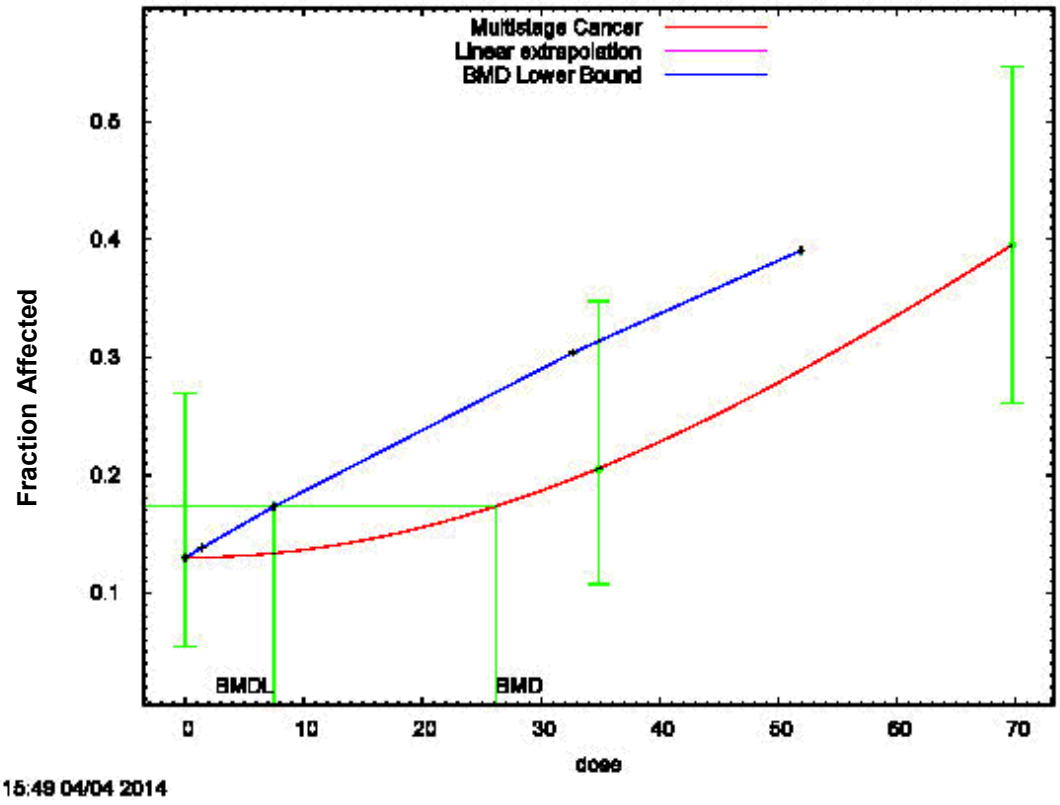
Benchmark Dose Computation
Specified effect = 0.05
Risk Type Extra risk
Confidence level = 0.95
BMD = 11.1178
BMDL = 3.85761
BMDU = 21.9401
Taken together, (3.85761, 21.9401) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0129614

Dibromochloromethane

Figure E9. Multistage-cancer model output for DBCM – hepatocellular adenoma and carcinoma in female B6C3F1 mice from NTP (1985) Study

Multistage Cancer Model, with BMR of 5% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL




```
================================================================
Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
Input Data File: K:/BMDS240/Data/msc_April2014BMDS_Cancer_DBCM_Opt.(d)
Gnuplot Plotting File:  K:/BMDS240/Data/msc_April2014BMDS_Cancer_DBCM_Opt.plt
Fri Apr 04 16:49:09 2014
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
            -beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
```

Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.129415
Beta(1) = 0
Beta(2) = 7.49877e-005

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.66
Beta(2)	-0.66	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.129759	*	*	*
Beta(1)	0	*	*	*
Beta(2)	7.47633e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-74.8279	3			
Fitted model	-74.8283	2	0.00081218	1	0.9773
Reduced model	-79.5794	1	9.50298	2	0.008639

AIC: 153.657

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1298	5.969	6.000	46	0.014
34.8800	0.2054	10.066	10.000	49	-0.023
69.7600	0.3952	18.968	19.000	48	0.009

Chi^2 = 0.00 d.f. = 1 P-value = 0.9773

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk

Confidence level = 0.95
BMD = 26.193
BMDL = 7.51301
BMDU = 39.1437

Taken together, (7.51301, 39.1437) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00665513

APPENDIX F. DETERMINATION OF MULTIROUTE EXPOSURES

Human exposure to chemical contaminants in tap water can occur via oral ingestion, as well as inhalation or dermal contact while performing common household activities, such as bathing, showering, and flushing toilets. This appendix describes the multi-route exposure assessment of chemicals in drinking water using equations extracted from CalTOX.[8] CalTOX is a multimedia total exposure model with built-in physicochemical property values for over 200 chemicals and mathematical equations to calculate total human exposure to contaminants in the environment (air, soil, and water).

For PHG development, exposures to chemicals in tap water over a lifetime (70 years) are considered. Exposure estimates differ across life stages (fetus, infant, child, and adult) due to physiological and activity pattern changes. CalTOX equations are used to calculate how much each route (oral, inhalation, and dermal) contributes to total daily exposure to a contaminant in tap water. The relative contributions of the different routes are then used to estimate a daily drinking water intake equivalent (DWI, in L_{eq}/kg-day) of multiroute exposure to tap water for each life stage. The lifetime daily multiroute intake rate of tap water in L_{eq}/kg-day is the time-weighted average of these life-stage specific tap water intake rates.[9] The liter equivalent (L_{eq}/kg-day) value represents the equivalent of how much water a person would have to drink to account for exposures via ingestion, inhalation and dermal uptake. Table 1 shows the descriptions and values of parameters applied in the exposure equations. Tables 2 and 3 show life stage-specific exposure parameter values.

[8] A multimedia total exposure model developed for the Department of Toxic Substances Control, California Environmental Protection Agency (Cal/EPA), by the Lawrence Berkeley National Laboratory (2002, Version 4.0 Beta).

[9] A 0.75-yr exposure duration for the fetus is used to derive the time-weighted average for the lifetime daily exposure rate (e.g., 0.75/70*0.047+2/70*0.196+14/70*0.061+54/70*0.045=0.053 L/kg-day for exposure via oral ingestion) in calculating the non-cancer health protective concentration. A 0.25-yr duration (3rd trimester) is applied as the life-stage-specific exposure of the fetus in calculating the age sensitivity factor (ASF)-adjusted life-stage-specific exposures to tap water.

Table F1. Descriptions and values of model defaults, chemical-specific and exposure-specific parameters

Symbol	Parameter	Value	Unit	Source
Inputs and Calculated Outputs				
$Intake_{oral}$	chemical intake via oral ingestion of tap water	-	mg/kg-day	calculated
$Intake_{inh}$	chemical intake via inhalation	-	mg/kg-day	calculated
$Uptake_{dermal}$	chemical uptake via dermal contacts	-	mg/kg-day	calculated
C_{tap_water}	chemical concentration in tap water	100[a]	mg/L	input
C_{air}	chemical concentration in indoor air	-	mg/m^3	calculated
C_{bath_air}	chemical concentration in bathroom air	-	mg/m^3	calculated
Exposure Parameters				
Ifl	fluid (water) intake, normalized to body weight	0.045 to 0.196[b]	L/kg-day	OEHHA, 2012
BR_a	active breathing rate, normalized to body weight	0.012 to 0.045[b]	m^3/kg-hr	OEHHA, 2012
BR_r	resting breathing rate, normalized to body weight	0.012 to 0.045[b]	m^3/kg-hr	OEHHA, 2012
SA_b	surface area, normalized to body weight	0.029 to 0.059[b]	m^2/kg	OEHHA, 2012
ET_{ai}	exposure time, active indoors	5.71 to 8[c]	hr/day	model default
ET_{ri}	exposure time, resting indoors	8 to 11[c]	hr/day	model default
ET_{sb}	exposure time, in shower or bath	0.27[c]	hr	model default
δ_{skin}	skin thickness	0.0025	cm	model default
f_s	fraction of skin in contact of water during showering or bathing	0.80	unitless	model default
CF	conversion factor for dermal uptake calculation	10	L/cm-m^2	calculated
Physicochemical and Other Parameters				
W_{house}	Water use in the house	40	L/hr	model default
VR_{house}	Room ventilation rate, house	750	m^3/hr	model default
W_{shower}	Water use in the shower	8	L/min	model default
VR_{bath}	Room ventilation rate, bathroom	1	m^3/min	model default
D_{water}	Diffusion coefficient in pure water	chemical specific	m^2/day	literature
D_{air}	Diffusion coefficient in pure air	chemical specific	m^2/day	literature
Z_{water}	fugacity capacity of pure water	volatiles=1/H semivolatiles=1 (H: Henry's Law constant)	mole/Pa-m^3	literature
R_{gas}	gas constant	8.31	Pa-m^3/mol-K	literature
t_{lag}	diffusion lag time in skin	chemical specific	hr	calculated
K_m	skin-water partition coefficient	chemical specific	unitless	literature
K_p^w	steady-state skin permeability coefficient	chemical specific	cm/hr	literature
MW	molecular weight	chemical specific	g/mole	literature
K_{ow}	octanol/water partition coefficient	chemical specific	unitless	literature

[a] As long as the chemical concentration in tap water is low (well below the saturation concentration in water), the input value of C_{tap_water} does not affect the calculation of relative contributions from the multiroute exposures and 100 ppm is an arbitrarily assigned low value.
[b] See Table A.2 for life-stage specific values.
[c] See Table A.3 for life-stage specific values.

Table F2. OEHHA calculated exposure parameters (OEHHA, 2012[10])

Life Stage	Water Intake Rate[a] (L/kg-day)	Breathing Rate[b] (m^3/kg-hr)	Surface Area[c] (m^2/kg)
Infant (0<2 yrs)	0.196	0.045	0.059
Child (2<16 yrs)	0.061	0.031	0.045
Adult (16-70 yrs)	0.045	0.012	0.029
Fetus[d]	0.047	0.015	0.029

[a] 95th percentile water intake rates (L/kg-day) are obtained from Table 8.1 of OEHHA (2012) risk assessment guidelines.
[b] 95th percentile breathing rates (L/kg-day) are obtained from Table 3.1 of OEHHA (2012) risk assessment guidelines and converted to m^3/kg-hr. The same life stage-specific breathing rate is used for BR_a and BR_r.
[c] 95th percentile values for total body surface area over body weight (m^2/kg) are obtained from Table 6.5 of OEHHA (2012) risk assessment guidelines.
[d] In utero exposure dose of the fetus is assumed to be the same as that of the pregnant mothers. Therefore the breathing rate and water intake rate for pregnant women are applied in the exposure estimates for fetuses (OEHHA, 2012). Pregnant women are assumed to have the same total body surface area over body weight as adults. Therefore, the total body surface area per body weight for adults is applied in the fetal dermal exposure estimation.

Table F3. CalTOX model default exposure durations

Life Stage	CalTOX Exposure Factors Set[a]	Exposure Time, Active Indoors (hr/day)	Exposure Time, Resting Indoors (hr/day)	Exposure Time, Shower or Bath (hr/day)
Infant (0<2 yrs)	Female 0-1	5.71	11.01	0.27
Child (2<16 yrs)	Female 7-9	5.71	11.01	0.27
Adult (16-70 yrs)	Female 19+	8.00	8.00	0.27
Fetus	Female 19+	8.00	8.00	0.27

[a] These Exposure Factors Sets provide the best estimates of the multi-route exposure for the corresponding life stages. Between the age groups within a particular life stage, the differences in relative contribution of a particular route are negligible, predominantly well below 1%. Within the same age group, the male and female inputs provide almost the same model outputs. Therefore, for internal consistency, use of the female Exposure Factor Sets is recommended for all life stages.

A. Oral Intake: Ingestion of Tap Water

Oral intake through ingestion of tap water can be calculated as follows:[11]

$$\mathbf{Intake_{oral} = C_{tap\_water} \times Ifl}$$

B. Inhalation Intake: Inhalation of Indoor Air in Active State, Resting State, and Shower/Bath

[10] OEHHA (2012). Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document for Exposure Assessment and Stochastic Analysis. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, California.
[11] Abbreviations and symbols used in equations are defined in Table A.1.

Chemicals in tap water can be transferred to indoor air during domestic activities such as showering, bathing, and toilet flushing. The total inhalation intake ($Intake_{inh}$) for a chemical in indoor air is obtained by summing the inhalation intakes in the active state, resting state, and in the shower/bath for each life-stage, as shown in the following equation:

$$\mathbf{Intake_{inh} = C_{air} \times (BR_a \times ET_{ai} + BR_r \times ET_{ri} - BR_a \times ET_{sb}) + C_{bath\_air} \times BR_a \times ET_{sb}}$$

The chemical concentration in indoor air and bathroom air are derived from the two equations below:

$$\mathbf{C_{air} = \frac{3 \times 10^6 \times 0.7 \times \left(\frac{W_{house}}{VR_{house}}\right) \times C_{tap\_water}}{\frac{2.5}{(D_{water}/86400)^{2/3}} + \frac{R_{gas} \times 298 \times Z_{water}}{(D_{air}/86400)^{2/3}}}}$$

and

$$\mathbf{C_{bath\_air} = \frac{3 \times 10^6 \times 0.6 \times \left(\frac{W_{shower}}{VR_{bath}}\right) \times C_{tap\_water}}{\frac{2.5}{(D_{water}/86400)^{2/3}} + \frac{R_{gas} \times 298 \times Z_{water}}{(D_{air}/86400)^{2/3}}}}$$

C. Dermal Uptake: Dermal Exposure to Tap Water during Shower/Bath

Dermal uptake of a chemical is dependent on exposure time and chemical-specific parameters, including diffusion through the skin. As a result, the dermal uptake of chemicals in tap water while showering or bathing are derived from one of the following equations:

1. When exposure time < diffusion lag time in skin[12] (t_{lag}):

   a. Exposure time << diffusion lag time, i.e. $\frac{t_{lag} \times 2}{ET_{sb}} > 3$:

$$\mathbf{Uptake_{dermal} = C_{tap\_water} \times \left(\frac{\delta_{skin} \times K_m}{2}\right) \times f_s \times CF \times SA_b \times \frac{ET_{sb}}{2 \times tlag} \times \frac{1\ event}{day}}$$

   b. For $1 \leq \frac{t_{lag} \times 2}{ET_{sb}} \leq 3$:

$$\mathbf{Uptake_{dermal} = C_{tap\_water} \times \left(\frac{\delta_{skin} \times K_m}{2}\right) \times f_s \times CF \times SA_b \times \frac{1\ event}{day}}$$

[12] Diffusion lag time in the skin is the amount of time it takes a chemical to permeate through the skin until it reaches a steady state of diffusion.

2. When exposure time > diffusion lag time, i.e. $\frac{t_{lag} \times 2}{ET_{sb}} < 1$:

$$\mathbf{Uptake_{dermal}} = \mathbf{C_{tap\_water}} \times \left[\frac{\delta_{skin} \times K_m}{2} + \left(\frac{\mathbf{ET_{sb}}}{2} - t_{lag}\right) \times \mathbf{K_p^w}\right] \times \mathbf{f_s} \times \mathbf{CF} \times \mathbf{SA_b} \times \frac{\mathbf{1\ event}}{\mathbf{day}}$$

where the chemical-specific t_{lag} is obtained from:

$$t_{lag} = \frac{\delta_{skin} \times K_m}{6 \times K_p^w}$$

For chemicals with no steady-state skin-permeability coefficient (K_p^w) and skin/water partition coefficient (K_m) available in the literature, these values are derived from the following equations, using chemical molecular weight (MW) and octanol/water partition coefficient (K_{ow}):

1. K_p^w is calculated using one of the equations below:

   a. Chemicals with MW < 280 g/mole:

$$K_p^w = \frac{1}{(MW)^{0.6}} \times \frac{2.4 \times 10^{-6} + 3 \times 10^{-5} \times (K_{ow})^{0.8}}{\delta_{skin}}$$

   b. Chemicals with MW ≥ 280 g/mole:

$$K_p^w = 0.0019 \times (K_{ow})^{0.71} \times 10^{(-0.0061 \times MW)}$$

   c. Chemicals with calculated $K_p^w > 1$:

$$K_p^w = 1$$

2. K_m is calculated using this equation:

$$K_m = 0.64 + 0.25 \times (K_{ow})^{0.8}$$

D. Relative Contributions from Each Route of Exposure

Finally, the relative contributions of chemical exposure to tap water via multiple routes are derived from the $Intake_{oral}$, $Intake_{inh}$, and $Uptake_{dermal}$ as follows:

Relative Contribution from Oral Ingestion (%)

$$= \frac{\mathbf{Intake_{oral}}}{\mathbf{Intake_{oral}} + \mathbf{Intake_{inh}} + \mathbf{Uptake_{dermal}}} \times 100\%$$

Relative Contribution from Inhalation[13] (%)

$$= \frac{\mathbf{Intake_{inh}}}{\mathbf{Intake_{oral} + Intake_{inh} + Uptake_{dermal}}} \times \mathbf{100\%}$$

Relative Contribution from Dermal Uptake (%)

$$= \frac{\mathbf{Uptake_{dermal}}}{\mathbf{Intake_{oral} + Intake_{inh} + Uptake_{dermal}}} \times \mathbf{100\%}$$

[13] Infant exposure to chemicals in tap water via inhalation are anticipated to be negligible, compared to the other exposure pathways, because they typically do not shower or flush toilets. Thus, the relative contribution from inhalation is zero for infants.