

Public Health Goals

Haloacetic Acids in Drinking Water:

Monochloroacetic Acid

Dichloroacetic Acid

Trichloroacetic Acid

Monobromoacetic Acid

Dibromoacetic Acid

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Public Health Goals for Chemicals in California Drinking Water
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PREFACE

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 occurrence of the respective chemical in California drinking water supplies and the availability of new scientific data. This document presents PHGs for five haloacetic acids (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid) that are created through the disinfection of water.

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

PREFACE	III
SUMMARY	3
1. INTRODUCTION	7
PURPOSE	7
PHYSICAL AND CHEMICAL PROPERTIES	8
2. PRODUCTION, USE, AND ENVIRONMENTAL OCCURRENCE	11
PRODUCTION AND USE	11
ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE	12
3. PHARMACOKINETICS	23
ABSORPTION	23
DISTRIBUTION.....	26
METABOLISM	29
EXCRETION	33
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS	34
4. HUMAN EPIDEMIOLOGY STUDIES ON DISINFECTION BYPRODUCTS	39
REPRODUCTIVE AND DEVELOPMENTAL EFFECTS	39
CANCER EFFECTS.....	46
5. TOXICOLOGICAL PROFILE: MONOCHLOROACETIC ACID	50
ACUTE TOXICITY	50
SUBCHRONIC TOXICITY	52
GENETIC TOXICITY	55
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY IN ANIMALS.....	58
IMMUNOTOXICITY IN ANIMALS.....	59
NEUROTOXICITY IN ANIMALS	59
CHRONIC TOXICITY	60
CARCINOGENICITY	61
6. TOXICOLOGICAL PROFILE: DICHLOROACETIC ACID	63
ACUTE TOXICITY	63
SUBCHRONIC TOXICITY	71
GENETIC TOXICITY.....	81
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY	87
IMMUNOTOXICITY	92
NEUROTOXICITY	93
CHRONIC TOXICITY	98
CARCINOGENICITY	104
7. TOXICOLOGICAL PROFILE: TRICHLOROACETIC ACID	118
ACUTE TOXICITY	118
SHORT-TERM TOXICITY IN ANIMALS	119
SUBCHRONIC TOXICITY	124
GENETIC TOXICITY.....	127
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY	134

IMMUNOTOXICITY	137
NEUROTOXICITY	137
CHRONIC TOXICITY	138
CARCINOGENICITY	142
8. TOXICOLOGICAL PROFILE: MONOBROMOACETIC ACID.....	153
ACUTE TOXICITY	153
SUBCHRONIC TOXICITY	153
GENETIC TOXICITY	157
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY	160
IMMUNOTOXICITY	162
NEUROTOXICITY	162
CARCINOGENICITY	162
UNPUBLISHED STUDIES	162
IN VITRO STUDIES AND MECHANISTIC CONSIDERATIONS	163
9. TOXICOLOGICAL PROFILE: DIBROMOACETIC ACID	167
ACUTE TOXICITY	167
SUBCHRONIC TOXICITY	169
GENETIC TOXICITY	172
DEVELOPMENTAL AND REPRODUCTIVE TOXICITY	174
IMMUNOTOXICITY	182
NEUROTOXICITY	184
CHRONIC TOXICITY	185
CARCINOGENICITY	186
10. DOSE-RESPONSE ASESMENT	189
NONCANCER DOSE-RESPONSE ANALYSES AND ACCEPTABLE DAILY DOSE CALCULATIONS	189
MONOCHLOROACETIC ACID	193
DICHLOROACETIC ACID	195
TRICHLOROACETIC ACID.....	200
MONOBROMOACETIC ACID.....	202
DIBROMOACETIC ACID.....	204
CANCER DOSE-RESPONSE ANALYSES AND CANCER POTENCY DERIVATION	208
DICHLOROACETIC ACID	211
TRICHLOROACETIC ACID.....	214
DIBROMOACETIC ACID.....	217
11. HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS.....	220
NONCANCER HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS	220
MONOCHLOROACETIC ACID	221
DICHLOROACETIC ACID	222
TRICHLOROACETIC ACID.....	222
MONOBROMOACETIC ACID.....	222
DIBROMOACETIC ACID	222
CANCER HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS.....	222
DICHLOROACETIC ACID	223
TRICHLOROACETIC ACID.....	224
DIBROMOACETIC ACID.....	224

12. RISK CHARACTERIZATION	225
MECHANISTIC EVIDENCE AND OTHER CONSIDERATIONS.....	225
MONOCHLOROACETIC ACID	227
DICHLOROACETIC ACID	227
TRICHLOROACETIC ACID.....	228
MONOBROMOACETIC ACID.....	228
DIBROMOACETIC ACID.....	229
DISINFECTION BENEFITS VERSUS HAA RISK	230
OTHER REGULATORY STANDARDS.....	230
REFERENCES	232
APPENDIX A. DETERMINATION OF MULTIROUTE EXPOSURES	278
APPENDIX B. EPIDEMIOLOGIC STUDIES OF REPRODUCTIVE OUTCOMES	284
APPENDIX C. EPIDEMIOLOGIC STUDIES OF CANCER OUTCOMES	347
APPENDIX D. BENCHMARK DOSE ANALYSIS RESULTS FOR NONCANCER ENDPOINTS	411
MONOCHLOROACETIC ACID	411
DICHLOROACETIC ACID	419
TRICHLOROACETIC ACID.....	436
DIBROMOACETIC ACID.....	438
APPENDIX E. BENCHMARK DOSE ANALYSIS RESULTS FOR CANCER ENDPOINTS	450
DICHLOROACETIC ACID	450
TRICHLOROACETIC ACID.....	461
DIBROMOACETIC ACID.....	467
APPENDIX F. AN EVALUATION OF CONFOUNDING IN EPIDEMIOLOGIC STUDIES OF DISINFECTION BYPRODUCT EXPOSURE AND ADVERSE REPRODUCTIVE OUTCOMES	481

List of Commonly Used Abbreviations

ADD – acceptable daily dose

ALT – alanine aminotransferase

ASF – age sensitivity factor

AST – aspartate aminotransferase

AUC – area under the curve

BMD – Benchmark Dose

BMDL – 95% lower confidence limit on the benchmark dose

BMR – Benchmark Response

CAS # – Chemical Abstracts Service number

CNS – central nervous system

CSF – cancer slope factor

CYP – cytochrome p450 enzyme

DBA – dibromoacetic acid

DCA – dichloroacetic acid

DBP – disinfection byproduct

DWI – daily water intake

GD – gestation day

HAA – haloacetic acid

HAA5 – five regulated haloacetic acids: MCA, DCA, TCA, MBA, DBA

IARC - International Agency for Research on Cancer

i.p. – intraperitoneal

i.v. – intravenous

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

LD₅₀ – lethal dose to 50% of test animals

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

LOAEL – Lowest Observed Adverse Effect Level

MBA – monobromoacetic acid

MCA – monochloroacetic acid

MCL – Maximum Contaminant Level

µg/L – micrograms per liter

µg/ml – micrograms per milliliter

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
mmol/L– millimoles per liter
MOA – mode of action
MTD – maximum tolerated dose
nmol/ml – nanomoles per milliliter
NCI – National Cancer Institute
NA – not applicable
ND – not determined
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
RoC – Report on Carcinogens
SWRCB – State Water Resources Control Board
TCA – trichloroacetic acid
THM – trihalomethane
UF – uncertainty factor
UF_A – interspecies uncertainty factor
UF_H – intraspecies uncertainty factor
US EPA – United States Environmental Protection Agency
VOC – volatile organic compounds
WHO – World Health Organization

SUMMARY

Haloacetic Acid Public Health Goals

This document presents a health risk assessment and a Public Health Goal (PHG) for each of the five regulated haloacetic acids (HAAs) found in drinking water as a result of disinfection methods: monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), monobromoacetic acid (MBA), and dibromoacetic acid (DBA). The HAAs are one of the major categories of disinfection byproducts (DBPs) formed in the chlorination disinfection process. The five PHGs represent concentrations of HAAs in drinking water that do not pose significant health risks, including risks of cancer. The table below provides the PHGs, based on cancer or noncancer effects, depending on the specific HAA compound, and health-protective concentrations for the noncancer effects of carcinogenic HAAs. 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 ($\mu\text{g/L}$).

Table S1. PHGs and Noncancer Health-Protective Concentrations (HPCs)

Chemical Name	PHG (ppb)	PHG Effect	HPC (ppb)	HPC Effect
Monochloroacetic acid	53	Systemic toxicity	-	-
Dichloroacetic acid	0.2	Liver cancer	115	Liver toxicity
Trichloroacetic acid	0.1	Liver cancer	128	Liver toxicity
Monobromoacetic acid	25	Muscular degeneration	-	-
Dibromoacetic acid	0.03	Liver and lung cancer	5	Male reproductive toxicity

ppb, parts per billion

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 (SWRCB). Each MCL must be set as close to the corresponding PHG as is economically and technologically 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 California and federal MCLs of 60 ppb for total HAAs represent the highest allowable annual average sum of the concentrations of MCA, DCA, TCA, MBA, and DBA. A single PHG for the five HAAs combined was not developed due to the potencies and the distinct critical effects of the individual chemicals.

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 toxic chemicals, known as disinfection byproducts (DBPs), in drinking water. These DBPs include the HAAs.

Up to 13 different types of HAAs have been identified in disinfected drinking water (NTP, 2017) and in 1996, US EPA began to regulate the five HAAs (MCA, DCA, TCA, MBA, and DBA) assessed in this document. In California drinking water, average concentrations of the regulated HAAs range from 0.6 to 4.2 ppb.

In considering exposures to the HAAs, 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 World Health Organization’s International Agency for Research on Cancer (IARC) in 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 PHGs and Health-Protective Concentrations

As shown in Table S1, PHGs for the HAAs are based on cancer for DCA, TCA and DBA, and noncancer endpoints for MCA and MBA. The PHGs based on cancer are set at a level where the cancer risk is one per one million persons exposed over a 70-year lifetime. The PHGs and noncancer health-protective concentrations take into consideration 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 noncancer health-protective concentrations for the five HAAs follows.

Monochloroacetic Acid

Cancer effects: Chronic oral toxicity/carcinogenicity studies (DeAngelo et al., 1997; NTP, 1992) did not show evidence of carcinogenic activity associated with MCA exposure in mice and rats. US EPA (2003a) described MCA as “not classifiable as to its human carcinogenicity” and the National Toxicology Program (2018) concluded in its Report on Carcinogens (RoC) Monograph on Haloacetic Acids that evidence of

carcinogenicity from animals studies was not sufficient and did not support identifying MCA as reasonably anticipated to be a human carcinogen.

Noncancer effects: In animal experiments, the most sensitive effects as a result of subchronic and chronic exposure to MCA is systemic toxicity and cardiac effects. In order to calculate the PHG for noncancer health effects, OEHHA performed a dose-response assessment of the rat systemic toxicity results reported in DeAngelo et al. (1997). OEHHA derived a point of departure (POD) of 3.5 mg/kg-day based on a NOAEL for decreased body weight and changes in relative liver weight. After applying uncertainty factors, the acceptable daily dose is 0.0035 mg/kg-day, resulting in a health-protective concentration for noncancer effects and PHG of 53 ppb.

Dichloroacetic Acid

Cancer effects: In 1996, DCA was listed as a carcinogen under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (also known as Proposition 65) based on US EPA's classification of the chemical as a likely carcinogen. IARC classified DCA as possibly carcinogenic to humans (Group 2B) based on inadequate evidence for carcinogenicity in humans but sufficient evidence of carcinogenicity in animals (IARC, 2014). In the NTP (2018) Monograph on Haloacetic Acids, DCA was found to be reasonably anticipated to be a human carcinogen based on sufficient evidence in animals and supportive mechanistic data. Liver tumors were the most sensitive endpoint observed in multiple studies in mice (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Daniel et al., 1992; Anna et al., 1994; Ferreira-Gonzalez et al., 1995; DeAngelo et al., 1999; Bull et al., 2002; Wood et al., 2015; Wehmas et al., 2017). To determine the health-protective concentration for cancer, that is, the concentration of DCA in drinking water that is associated with a one-in-one-million risk of cancer for people exposed over a lifetime, OEHHA first derived a human cancer potency for DCA of $0.041 \text{ (mg/kg-day)}^{-1}$ based on the combined incidence of liver adenomas and carcinomas in male mice in a study by DeAngelo et al. (1999). Using this cancer potency value, a PHG of 0.2 ppb was derived.

Noncancer effects: Liver and reproductive toxicity were the most sensitive endpoints observed in animal bioassays for DCA. OEHHA performed a dose-response analysis on hepatic toxicity data from a chronic mouse study by DeAngelo et al. (1991). A NOAEL of 7.6 mg/kg-day for increased relative liver weight was estimated and used as the POD. After applying uncertainty factors, the acceptable daily dose is 0.0076 mg/kg-day resulting in a health-protective concentration for noncancer effects of 115 ppb.

Trichloroacetic Acid

Cancer effects: IARC (2014) classified TCA as possibly carcinogenic to humans (Group 2B). US EPA concluded that "there is suggestive evidence of carcinogenic potential for TCA based on significantly increased incidences of liver tumors" in male and female mice (US EPA, 2011, 2013). In 2013, TCA was listed as a carcinogen under Proposition 65. The most sensitive and consistent endpoint in animal cancer bioassays for TCA is

hepatocellular adenoma or carcinoma in male mice. OEHHA derived a human cancer potency of $0.071 \text{ (mg/kg-day)}^{-1}$, resulting in a PHG of 0.1 ppb. In contrast, NTP found that “evidence of carcinogenicity from studies in experimental animals is not sufficient to meet the RoC criteria for listing...trichloroacetic acid (TCA)” in regards to its carcinogenic potential to humans (NTP, 2018).

Noncancer effects: The most sensitive endpoints seen in animal toxicity studies are liver effects. OEHHA performed a dose-response assessment on mouse hepatotoxicity observed in a study by DeAngelo et al. (2008) to calculate a health-protective concentration. A POD of 8.5 mg/kg-day was derived from benchmark dose modeling of hepatocellular necrosis data. After applying uncertainty factors, the acceptable daily dose is 0.0085 mg/kg-day, resulting in a health-protective concentration for noncancer effects of 128 ppb.

Monobromoacetic Acid

Cancer effects: OEHHA did not locate any carcinogenicity studies for MBA.

Noncancer effects: There is a limited database for subchronic toxicity studies and no chronic toxicity studies for MBA. In order to calculate the PHG for noncancer health effects, OEHHA performed a dose-response assessment of the toxicity observed in pigs reported in Dalgaard-Mikkelsen et al. (1955). OEHHA derived a POD of 5 mg/kg-day based on a NOAEL from this multigenerational study, in which the first generation animals dosed at 5 mg/kg-day did not show toxic effects such as muscular degeneration and pulmonary edema observed in the second generation. The MCA dose for the second generation was increased by design relative to the first generation but the exact daily dose could not be calculated. After applying uncertainty factors, the acceptable daily dose is 0.0017 mg/kg-day and the resulting PHG is 25 ppb.

Dibromoacetic Acid

Cancer effects: NTP (2018) concluded DBA was reasonably anticipated to be a human carcinogen based on sufficient evidence from studies in experimental animals and supporting mechanistic data. Using the linear multi-stage model, OEHHA derived a cancer potency of $0.25 \text{ (mg/kg-day)}^{-1}$ based on a significant increase in hepatic tumors and alveolar/bronchiolar tumors (NTP, 2007a). The PHG is 0.03 ppb.

Noncancer effects: The most sensitive endpoints were changes in organ weights and reproductive toxicity. OEHHA performed a dose-response assessment of testicular lesions and decreased incidence of morphologically normal sperm in a study with male rabbits by Veeramachaneni et al. (2007). A lowest-observed-adverse-effect level (LOAEL) of 1 mg/kg-day was used as a POD and after applying uncertainty factors, an acceptable daily dose of 0.0003 mg/kg-day was derived. The resulting health-protective concentration for noncancer effects is 5 ppb.

1. INTRODUCTION

Purpose

The purpose of this document is to estimate health-protective concentrations for the five regulated haloacetic acids (HAA5) found in drinking water as a result of disinfection methods – monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), monobromoacetic acid (MBA), and dibromoacetic acid (DBA) – and to develop a public health goal (PHG) for each individual HAA5. The PHGs and estimated public health-protective concentrations are based on a comprehensive analysis of the toxicology of each compound. PHGs are based solely on the 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 equivalent water intake relative to bodyweight of the young.

Disinfection is a critically important process for the 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, 2017). Other waterborne diseases may result in diarrhea, and are likely to have serious consequences in infants and the elderly.

In the US, over 200 million people are served by public water systems that apply a disinfectant such as chlorine, chlorine dioxide, ozone, or chloramine to water to protect against infectious diseases caused by microorganisms. Chlorine disinfection is widely accepted as one of the major public health advances of the 20th century, greatly decreasing the incidence of water-borne diseases. However, in addition to killing bacteria, the disinfectants react with natural organic and inorganic matter in the water to form disinfection byproducts (DBPs) such as HAAs (Richardson et al., 2008). This poses a health concern because many DBPs are toxic (Boorman, 1999; Richardson et al., 2007; Richardson et al., 2008; Woo et al., 2002). The type and concentrations of DBP chemicals produced depend on the treatment method, climate, upstream vegetation, light, temperature, and other factors (Urbansky, 2000).

In a normal water chlorination process, the chlorine concentration is limited to 1 to 10 mg/L. This generates relatively nonvolatile degradation products, such as HAAs, in concentrations commonly ranging from about 1 to 100 µg/L, and volatile haloforms, such as the trihalomethanes (THMs), in concentrations commonly ranging from about 10 to 100 µg/L (Richardson et al., 2007). Unlike THMs, HAAs have been shown to be biodegradable in drinking water (Bayless and Andrews, 2008).

Many recent research studies have associated the presence of certain DBPs in drinking water with risks to human health. A review of human studies IARC summarized the suggestive associations of bladder, colorectal, and other cancers with the consumption of chlorinated drinking water (WHO, 2004b). US EPA (2006) in the Stage 2 Disinfectant

and Disinfection Byproducts Rule (DBPR) acknowledged that the human bladder cancer studies appear to provide the strongest evidence of increased cancer risk from exposure to DBPs. Though the large number of positive epidemiological studies raises concern about the carcinogenicity of DBPs as a whole, the studies do not provide a definitive link between any specific individual DBP and cancer (ATSDR, 1997; IARC, 1999; US EPA, 2001, 2006; Villanueva et al., 2003a). In addition, adverse noncancer effects such as liver toxicity, developmental and reproductive toxicity, and kidney toxicity have been linked to DBPs (Davis, 1986; Mori et al., 2004; Villanueva et al., 2015).

Although the cumulative risk of the individual DBPs, such as THMs and HAAs, or DBP mixtures in drinking water has not yet been adequately assessed (Simmons et al., 2008), these DBPs are regulated under the Stage 1 and Stage 2 DBPRs of the federal Safe Drinking Water Act (US EPA, 1998a, 2005b). These rules have been developed to balance the benefits and risks posed by drinking water disinfection. HAA5 is a group of five contaminants regulated under the DBPRs. HAAs represent the second largest DBP group on a weight basis, after THMs. The remaining two regulated DBPs are bromate and chlorate. Since 1998, under the Stage 1 DBPR, monitoring of HAA5 has been required in the US with a HAA5 MCL of 60 µg/L or 60 ppb. The Stage 2 DBPR of 2006 instituted minimum reporting level requirements of 2 µg/L for MCA and 1 µg/L for the other four HAA5 (US EPA, 2006).

Depending on the bromide level in the source water as well as the amount of chlorinated disinfectants added, varying amounts of chlorinated, brominated, and mixed bromochlorinated HAAs are produced (WHO, 2004a). Nine HAAs, known as HAA9, have been identified in drinking water, including the HAA5 plus bromochloroacetic acid (BCA), bromodichloroacetic acid (BDCA), dibromochloroacetic acid (DBCA), and tribromoacetic acid (TBA). The concentrations of BCA, BDCA, DBCA, and TBA found in drinking water are generally lower than those of the chlorinated HAA5, but are increased in drinking waters with higher bromide levels. The brominated and mixed halogenated HAAs tend to be more toxic than the chlorinated analogs, but fewer studies have been carried out on them (WHO, 2004a). This document focuses on the HAA5 that are regulated by the US EPA and the State Water Resources Control Board (SWRCB).

Physical and Chemical Properties

The HAAs are analogs of acetic acid with chlorine or bromine replacing one, two or three of the hydrogen atoms of the methyl group. The physical and chemical properties for mono-, di- and trichloroacetic acid, and mono- and dibromoacetic acid are summarized in Table 1 (Bowden et al., 1998a, 1998b; NCBI, 2018a, 2018b, 2018c, 2018d, 2018e; NTP, 2018; Sander, 2015; Sigma-Aldrich, 2018).

At the drinking water pH range of 6.8-8.5 and in biological tissues, more than 99.99% of the HAA5 exist as the dissociated carboxylate anions. Whenever any of the HAA5 acids is used as the test article in vivo, it becomes the corresponding acetate after it leaves the stomach (NTP, 2007a, 2009).

The pure HAA5 are solids or liquids at room temperature and are soluble in water (WHO, 2004a, 2004b). Unlike the volatile THMs, these halogenated organic chemicals have relatively low vapor pressure and are not expected to volatilize from drinking water or contaminated environmental media to any appreciable extent. The mono-substituted acids like MCA and MBA with higher pKa values are weaker acids than the tri-substituted acids like TCA and TBA with lower pKa values (Bowden et al., 1998a, 1998b). The Henry's Law constants of the HAA5 are higher than that of acetic acid, 50 mol/m³·Pa (Sander, 2015), indicating lower volatility. Almost all the HAA5 present in air will partition into droplets within clouds and be removed by rain.

Table 1.1 Physical and Chemical Properties of the HAA5

	Monochloroacetic acid	Dichloroacetic acid	Trichloroacetic acid	Monobromoacetic acid	Dibromoacetic acid
Formula	CH ₂ ClCOOH	CHCl ₂ COOH	CCl ₃ COOH	CH ₂ BrCOOH	CHBr ₂ COOH
CAS No.	79-11-8	79-43-6	76-03-9	79-08-3	631-64-1
Molecular weight (g/mole)	94.497	128.942	163.387	138.948	217.844
Physical state at ambient temperature	colorless to white crystalline (hygroscopic)	colorless to yellowish liquid	colorless to off-white crystalline	colorless crystalline	beige crystalline (hygroscopic)
Melting point (°C)	52-64	9-13.5	54-58	47-51	32-48
Boiling point (°C)	189	194	195.5-198	208	128-130
Density (g/cm ³)	1.328-1.58 (20 °C)	1.563 (25 °C)	1.62 (25 °C)	1.93 (25 °C)	2.38 (25 °C)
Solubility in water (g/L)	858-3170 (25 °C)	1,000 (20 °C) miscible	44 (25 °C) 82 (20 °C) very high in some sources (miscible)	94 (25 °C)	2110 (25 °C)
Other solubility	ethanol, methanol, diethyl ether, benzene, acetone, chloroform	ethanol, diethyl ether, acetone	ethanol, diethyl ether, methanol, acetone, benzene	ethanol, methanol, ether, acetone, benzene	ethanol, diethyl ether
Vapor pressure (mm Hg)	0.065	0.179	0.06	0.119	0.023
Henry's Law constant (mol/m ³ Pa)	8.8×10 ² – 1.1×10 ³	3.9×10 ² – 1.2×10 ³	7.3×10 ²	1.5×10 ³	2.2×10 ³ – 2.3×10 ³
Log K _{ow}	0.22-0.34	0.92-0.94	1.33-1.7	0.41	0.7
Acidity, pKa	2.87-2.97	1.26-1.41	0.51-0.66	2.89-2.96	1.39-1.48

References: (Bowden et al., 1998a, 1998b; Sander, 2015; NCBI, 2018a, 2018b, 2018c, 2018d, 2018e; NTP, 2018; Sigma-Aldrich, 2018)

2. PRODUCTION, USE, AND ENVIRONMENTAL OCCURRENCE

Production and Use

Monochloroacetic Acid

MCA is an industrial chemical, used mainly as a building block for carboxylation reactions in organic synthesis. MCA is commercially manufactured via chlorination of acetic acid or hydrolysis of trichloroethylene. MCA was used as a post-emergence contact herbicide (Bhat et al., 1991; Daniel et al., 1991; Bryant et al., 1992; NTP, 1992; ECETOC, 1999; WHO, 2004c, 2004d, 2009). As of 2018, there are no registered pesticide products containing MCA in California. It was once used as a food stabilizer and preservative for beverages because of its fungicidal and bactericidal properties (Fuhrman et al., 1955; Reimann et al., 1996b).

Medicinal applications of MCA include use as a wart remover (Steele et al., 1988; Rogers, 1995), despite reports of serious complications and fatality (Kulling et al., 1992; Pirson et al., 2003; Chapman et al., 2006; Tan Baser et al., 2008). In comparison, TCA and DCA have been reported to be safe for topical wart treatments (Pirson et al., 2003; Tan Baser et al., 2008).

Dichloroacetic Acid

DCA is commercially manufactured by chlorination of acetic acid, chlorination of MCA, reduction of TCA, dechlorination of ethyl trichloroacetate, and other techniques (WHO, 2004b, 2005; Koenig et al., 2012). DCA is used in industrial synthesis of glyoxylic acid and in production of polyethylene terephthalate (NTP, 2018).

DCA has been used as a fungicide and as a pesticide inert ingredient; but as of 2018, it has not been used for these purposes in California.

DCA has been investigated and used clinically for treatment of a variety of metabolic disorders in humans such as diabetes mellitus, lactic acidosis, and primary mitochondrial disorders (Stacpoole et al., 1998a; Stacpoole et al., 1998b; Stacpoole et al., 2008a; Stacpoole et al., 2008b; Stacpoole, 2011). DCA is also used as a medical disinfectant and a cauterizing agent to treat calluses, corns, papular xanthomata, and other skin conditions (Stacpoole et al., 1998a; WHO, 2004b; Levine, 2008). DCA has been proposed as an anticancer drug (IARC, 2014).

Trichloroacetic Acid

TCA is commercially manufactured by chlorination of acetic acid (WHO, 2004b). Beginning in the 1940s, TCA and sodium and calcium salts of TCA were commonly used as herbicides to remove grasses from dicotyledonous fields (Bailey and White, 1965). However, all registered products have now been voluntarily cancelled (US EPA, 1994a).

TCA is used as a peeling agent for wrinkled or sun-damaged skin and tattoos (Piggot and Norris, 1988; Bhunya and Jena, 1996). TCA is also used for treating acne, removal of genital warts, and as a cauterizing agent to treat calluses, corns, papular xanthomata, and other skin conditions (WHO, 2004c).

Monobromoacetic Acid

MBA is commercially manufactured by bromination of acetic acid (Yoffe et al., 2013). MBA has been used in organic synthesis, commercial printing, in the electronic industry, and in hospitals (WHO, 2004a).

MBA can be used as an abscission agent (decreasing the force needed for picking) in citrus fruits, although it is not currently registered for use as a pesticide in the US. MBA has also been used as a food additive to inhibit alcoholic fermentation or other metabolic processes of molds, yeasts, and bacteria and as a preservative for beverages (Morrison 1946; Reimann et al., 1996a). MBA, like other monohaloacetates, is phytotoxic due to its electrophile reactivity with nucleophilic amino, hydroxyl, and thiol groups via carboxymethylation (Frank et al., 1995). MBA was evaluated as a potential pancreatic cancer treatment due to its ability to modify the induction of new blood vessel formation in tumors by angiogenin (Shapiro et al., 1988).

Dibromoacetic Acid

DBA is primarily found in the environment as a byproduct of water chlorination. DBA does not have known industrial uses, and only small quantities are produced for research purposes (WHO, 2004a; IARC, 2013).

Environmental Occurrence and Human Exposure

HAAs have been detected in tap water and food as well as in the environment, including air, water, soil, and plants (IARC, 1995; Amy et al., 2000; WHO, 2004a, 2004b, 2004c, 2004d, 2005). HAAs can be produced from anthropogenic as well as natural sources (Ellis et al., 2001; Laturnus et al., 2005; Scott et al., 2005; Cape et al., 2006). The majority of human exposure to HAAs occurs through disinfected water. In disinfection, gaseous chlorine or bleach react with water to form hypochlorous acid (in the presence of bromine, hypobromous acid), which interacts with organic matter present in water to form a wide variety of DBPs.

Anthropogenic sources, such as waste incineration, biomass burning, forest fires, and anthropogenic emissions of chlorine, as well as photochemical oxidation of volatile organochlorine compounds such as trichloroethylene (TCE), and perchloroethylene (PCE) contribute to the formation of HAAs in the air, followed by deposition in the form of snow, rain or fog water.

HAAs are present in the environment even in the absence of anthropogenic sources, albeit at much lower levels. Naturally occurring organohalogens have been identified as the main precursors for halogenated acetic acids in the marine and terrestrial environments (Eurochlor, 2002; McCulloch, 2002; Laturnus et al., 2005; Cape et al., 2006). Soil is a natural source of DCA and TCA (Hoekstra et al., 1999a, 1999b; Fahimi et al., 2003; Hoekstra, 2003). The THMs as well as chloroacetic acids are produced by natural chlorination of organic matter by soil, marine and salt lake microorganisms, which share similar metabolism pathways.

The presence of HAAs, especially DCA and TCA, in environmental water is well documented. There are very low concentrations in remote lakes, glacier ice, and in some precipitation,

moderate concentrations in surface water, snow, fog, rain, marine water, and relatively high concentrations in treated water and water associated with industrial activities (Scott et al., 2002; Scott et al., 2005). Samples of precipitation, soils and conifer needles collected from various countries indicate that concentrations of HAAs are greater in the industrialized northern hemisphere than in the less industrialized southern hemisphere (Scott et al., 2000; Scott et al., 2002; Scott et al., 2005).

In contrast to the mostly non-biodegradable THMs, HAA5 have been shown to undergo significant biological degradation in drinking water, soil, and enriched bacterial cultures (Bayless and Andrews, 2008). Levels of DCA and TCA in drinking water declined with time in the distribution system (Chen and Weisel, 1998). McRae et al. (2004) found that aerobic biodegradation is a potential loss or removal mechanism for HAA5 in surface waters and in drinking water distribution systems. In general, brominated HAA5 are better biodegraded than the corresponding chlorinated species, and mono-halogenated compounds are removed to a greater extent than the di-halogenated species, with the tri-halogenated being biologically removed to the least extent (Bayless and Andrews, 2008).

Air

Direct emission of all HAA5 has been reported from combustion processes including heating, wood burning, municipal waste incineration, and forest fires (Mowrer and Nordin, 1987; Schöler et al., 2003). In air, the HAAs are most likely dissolved in atmospheric water vapor due to their high hydrophilicity, or attached to particles (Frank et al., 1989a; Frank et al., 1989b; Frank et al., 1990; Schöler et al., 2003; WHO, 2004a, 2004b, 2004c, 2004d, 2005). HAA5 can also form in air from the breakdown of solvents, degreasers, pesticides and other organic compounds (Wilson and Mabury, 2000; Eurochlor, 2002; Fahimi et al., 2003).

Mean values for MCA, DCA and TCA in samples of urban and rural air in Ontario, Canada were 1.5-2.5 nanograms per cubic meter (ng/m^3), 0.66-1.1 ng/m^3 and 0.13-0.22 ng/m^3 , respectively (Martin et al., 2003). In the air discharged from municipal waste incinerators, MCA concentrations were 3.2-7.8 $\mu\text{g}/\text{m}^3$ (Mowrer and Nordin, 1987). No information was identified by OEHHA on concentrations of HAA5 in indoor air in the US. HAA5 from disinfected water is not expected to contribute to the indoor or outdoor air burden because of the low Henry's Law constants.

MCA in the atmosphere is derived from the photodechlorination reactions of many volatile chlorinated hydrocarbons such as methyl chloroform, TCE, and PCE (Frank et al., 1994), and from photolysis reactions of chloroacetamide herbicides including metolachlor, alachlor, and butachlor (Wilson and Mabury, 2000). In addition to these sources, MCA may be emitted via combustion of chlorohydrocarbons (Mowrer and Nordin, 1987).

DCA has also been found in the atmosphere from the photodechlorination reactions of chlorinated hydrocarbons such as TCE, PCE, and methyl chloroform (Frank et al., 1994). DCA has also been identified as a metabolite of dichlorvos, dichloroethylene, chloral hydrate, 1-chloroethene, 1,2-dichloroethane, 1,1-dichloroethane, 1,1,2-trichloroethane, and 1,1,2,2-

tetrachloroethane (Schultz et al., 1971; Yllner, 1971a, 1971b; Hales et al., 1987; Delinsky et al., 2005a; Delinsky et al., 2005b).

TCA is an air contaminant due to the photodechlorination of chlorinated hydrocarbons such as TCE, PCE and methyl chloroform (Frank et al., 1994). It is a biological metabolite of TCE, PCE, methyl chloroform and chloral hydrate (Nolan et al., 1984; IARC, 2014; Cichocki et al., 2016). TCA can be formed both naturally and anthropogenically; the fluxes of TCA between air, biota, soil, and groundwater have been reviewed by Schöler et al. (2003).

The review of Schöler et al. (2003) reported TCA in air from less than 0.03 ng/m³ to 0.32 ng/m³ with a mean of 0.1 ng/m³ in urban areas, and from 0.29 to 2.6 ng/m³ with a mean of 1 ng/m³ in forested areas. The National Air Toxics Information Clearinghouse (NATICH) 1993 data on ambient TCA concentrations in seven US states reported a mean 8-hour time-weighted average for TCA of 73 ng/m³ with a range of 7 to 119 ng/m³, and a mean 24-hour time-weighted average of 58 ng/m³ (range 1.7 to 110 ng/m³) (US EPA, 1994a).

MCA is listed by US EPA as being a Hazardous Air Pollutant under the Clean Air Act (Cal. Code Regs. tit. 17, § 93001; C.F.R. tit. 42, § 7412). MCA is on the California Air Resources Board's list of Toxic Air Contaminants (TACs) (CARB, 2019).

Soil

HAA5 can reach soil by dry and wet deposition, and can be formed directly through both biotic and abiotic processes (Haiber et al., 1996; Hoekstra et al., 1999a, 1999b; Fahimi et al., 2003; Peters, 2003). Concentrations of individual HAAs in soil appear to vary geographically and across the vertical profile in a given location (Scott et al., 2005).

Schöler et al. (2003) reviewed the potential for TCA fluxes between soil, groundwater and plant compartments. With its high water solubility and low volatility, TCA formed in the air is adsorbed onto aerosol particles and precipitated during rainfall. In addition, TCA can be formed from PCE in plants, with high concentrations detected in needles, leaves and in forest soil especially in mountain regions (Ahlers et al., 2003; Cape et al., 2006). The concentrations in vegetation samples are 10 to 20 times higher than the soil concentrations (Peters, 2003). TCA generally declined with soil depth while MCA and DCA showed no trend (Scott et al., 2005).

Water

HAA5 occur at very low concentrations in natural waters, including surface waters and precipitation. In unpolluted ground water, HAA5 are generally below detection limits. In contrast, disinfection of water dramatically increases HAA5 levels, and the overall concentration as well as the HAA5 speciation depend on multiple factors, including disinfection method, residual disinfectant concentration, organic carbon content of water, bromine levels, temperature, pH, time in the distribution system, etc. Chloramination is thought to reduce HAA5 levels in comparison with chlorination, while higher bromine content increases the DBA and MBA fractions. In certain applications requiring higher residual disinfectant, such as in swimming pool water, very high concentrations of individual HAA5 have been reported in some studies (Cardador and Gallego, 2011; Simard et al., 2013; Hang et al., 2016). This may be of concern due to human exposure from ingested pool water.

HAA5 were included in Stage 1 and Stage 2 DBPRs, with the goal of reducing the observed levels below the proposed MCL of 60 µg/L. Prior to and during development of these rules, several large-scale studies were conducted to determine HAA5 baseline levels in public water systems (US EPA, 1998b; Weinberg et al., 2002; Obolensky et al., 2003; US EPA, 2005b), and following implementation, wide-scale monitoring of HAA5 levels was routinely conducted. Thus, the available reports documenting HAA5 levels in US drinking water range from peer-reviewed articles to water system reports and number in the thousands. Just a small selection is presented in Tables 2.1 and 2.2, which also includes several representative reports on HAA5 levels in other sources, such as fog water or swimming pool water.

Table 2.1 Concentrations of combined haloacetic acids (HAA5) in drinking water

Reference	HAA5	Mean concentration [Range], µg/L	Location	Year	Type of water
Krasner et al. (1989)	HAA5	19 ^a [13-21]	US	1988-9	Drinking water
ICR data set, as cited in Obolensky et al. (2003)	HAA5	23, 18 ^a	US	1997-8	Drinking water
Weinberg et al. (2002)	HAA5	[9-46]	EPA region 9	2000-2	Drinking water in distribution system at average detention time
Weinberg et al. (2002)	HAA5	[17-55]	EPA region 6	2000-2	Drinking water in distribution system at average detention time
Weinberg et al. (2002)	HAA5	[18-27]	EPA region 5	2000-2	Drinking water in distribution system at average detention time
Weinberg et al. (2002)	HAA5	[21-79]	EPA region 4	2000-2	Drinking water in distribution system at average detention time

Reference	HAA5	Mean concentration [Range], µg/L	Location	Year	Type of water
Weinberg et al. (2002)	HAA5	[7.8-72]	EPA region 3	2000-2	Drinking water in distribution system at average detention time
Weinberg et al. (2002)	HAA5	[5-134]	EPA regions 5&7	2000-2	Drinking water in distribution system at average detention time
US EPA (2005b)	HAA5	29, 24 ^a , 52 ^b [0-116]	US	1999-2000	Surface - drinking
US EPA (2005b)	HAA5	8.4, 2.2 ^a , 22 ^b [0-71]	US	1999-2000	Ground – drinking
NRWA data set, as cited in US EPA (2005b)	HAA5	45, 34 ^a , 84 ^b [0-262]	US	1999-2000	Surface - drinking, small systems
AWAA data set, as cited in US EPA (1998b)	HAA5	28.1, 25 ^a [nd-91]	US		Drinking water
AWWSCo study, as cited in U.S. EPA (1998b)	HAA5	41.3, 37.0 ^a , 72.3 ^b [4.7-134]			Drinking water
Singer et al. (1995) as cited in US EPA (1998b)	HAA5	77, 81 ^a [36-106]	North Carolina		Drinking water
US EPA (1998b)	HAA5	69, 56 ^a [01.-284]	Missouri	1997	Drinking water
Peters et al. (1991)	HAA5	[0.5-15]	Netherlands		Surface – drinking
Peters et al. (1991)	HAA5	nd	Netherlands		Ground – drinking
Cowman and Singer (1996)	HAA5	15.3-20.6	US		Finished drinking
Nieminski et al. (1993)	HAA5	2.1-42.1	Utah	1990-1	Drinking water in plant effluent
Jacangelo et al. (1989)	HAA5	[8.8-64]	US		Drinking water
Simard et al. (2013)	HAA9	[12-113]	Canada		Drinking water
Simard et al. (2013)	HAA9	[348-510]	Canada		Indoor swimming pools
Simard et al. (2013)	HAA9	[634-983]	Canada		Outdoor swimming pools

^a median

^b 90th percentile

nd, not detected

HAA5 comprise MCA, DCA, TCA, MBA and DBA; HAA9 comprise HAA5 as well as bromochloroacetic acid, chlorodibromoacetic acid, bromodichloroacetic acid and tribromoacetic acid.

Table 2.2 Concentrations of individual haloacetic acids (HAAs) in drinking water

Reference	HAAs	Mean concentration [Range], µg/L	Location	Year	Type of water
Williams et al. (1997)	DCA	5-19 [0.3-120]	Canada	1999	Drinking water from distribution system
Williams et al. (1997)	TCA	4-57 [0.1-473]	Canada	1999	Drinking water from distribution system
Williams et al. (1997)	MCA	[0.3-9.7]	Canada	1999	Drinking water from distribution system
Williams et al. (1997)	MBA	[<0.01-9.2]	Canada	1999	Drinking water from distribution system
Williams et al. (1997)	DBA	[<0.01-1.9]	Canada	1999	Drinking water from distribution system
Cancho et al. (1999)	DCA	0.9 (nd-2.0)	Spain	1997-8	Drinking water
Cancho et al. (1999)	TCA	1.5 [0.3-2.5]	Spain	1997-8	Drinking water
Cancho et al. (1999)	DBA	3.7 [2.1-5.7]	Spain	1997-8	Drinking water
Nissinen et al. (2002)	MCA	[nd-4.7]	Finland	1994-5	Drinking water (drinking water plant effluent)
Nissinen et al. (2002)	DCA	[nd-42]	Finland	1994-5	Drinking water (drinking water plant effluent)
Nissinen et al. (2002)	TCA	[nd-210]	Finland	1994-5	Drinking water (drinking water plant effluent)
Nissinen et al. (2002)	MBA	[nd-1.1]	Finland	1994-5	Drinking water (drinking water plant effluent)
Nissinen et al. (2002)	DBA	[nd-27]	Finland	1994-5	Drinking water (drinking water plant effluent)
Ding et al. (1999)	DCA	0.1 [0.1-0.2]	California (Santa Ana River)	1994-6	Surface water (study control)
Ding et al. (1999)	TCA	0.2 [nd-0.5]	California (Santa Ana River)	1994-6	Surface water (study control)
Ding et al. (1999)	DBA	nd	California (Santa Ana River)	1994-6	Surface water (study control)
Ding et al. (1999)	DCA	nd	California (Santa Ana River)	1994-6	Ground water (study control)
Ding et al. (1999)	TCA	0.2	California (Santa Ana River)	1994-6	Ground water (study control)

Reference	HAAs	Mean concentration [Range], µg/L	Location	Year	Type of water
Ding et al. (1999)	DBA	nd	California (Santa Ana River)	1994-6	Ground water (study control)
Palacios et al. (2000)	DCA	1.1, 0.2 ^a [nd-3.9]	European Union	1980-2000	Treated surface water
Palacios et al. (2000)	TCA	0.25, 0.08 ^a [nd-1]	European Union	1980-2000	Treated surface water
Palacios et al. (2000)	MBA	0.06, 0.06 ^a [nd-0.11]	European Union	1980-2000	Treated surface water
Palacios et al. (2000)	DBA	7.0, 1.15 ^a [nd-30]	European Union	1980-2000	Treated surface water
Palacios et al. (2000)	MCA	nd	European Union	1980-2000	Treated ground water
Palacios et al. (2000)	DCA	0.83, 1.0 ^a [nd-1.5]	European Union	1980-2000	Treated ground water
Palacios et al. (2000)	TCA	nd	European Union	1980-2000	Treated ground water
Palacios et al. (2000)	DBA	3.0, 2.0 ^a [nd-7.0]	European Union	1980-2000	Treated ground water
Richardson et al. (2003)	DBA	[nd-38.7]	Israel	1999	Treated drinking
Hang et al. (2016)	MCA	10.1-41.1 [nd-475]	China	2014	Indoor swimming pool water
Hang et al. (2016)	DCA	12-434 [nd-2435]	China	2014	Indoor swimming pool water
Hang et al. (2016)	TCA	16.0-156 [nd-636]	China	2014	Indoor swimming pool water
Hang et al. (2016)	MBA	1.9-16.1 [nd-103]	China	2014	Indoor swimming pool water
Hang et al. (2016)	DBA	nd	China	2014	Indoor swimming pool water
Cardador and Gallego (2011)	MCA	20 [8.5-33.5]	Spain	2010	Indoor swimming pool water
Cardador and Gallego (2011)	DCA	71-83 [67-125]	Spain	2010	Indoor swimming pool water
Cardador and Gallego (2011)	TCA	105-117 [67-166]	Spain	2010	Indoor swimming pool water
Cardador and Gallego (2011)	MCA	25.5 [19.5-33.5]	Spain	2010	Outdoor swimming pool water
Cardador and Gallego (2011)	DCA	148-154 [99-166]	Spain	2010	Outdoor swimming pool water
Cardador and Gallego (2011)	TCA	118-122 [109-170]	Spain	2010	Outdoor swimming pool water
Reckhow and Singer (1990)	TCA	[15-64]	US	1983-4	Drinking water
Reckhow and Singer (1990)	DCA	[8-79]	US	1983-4	Drinking water
Berg et al. (2000)	MCA	1.8 [0.06-7.2]	Switzerland	1996-7	Rain and snow
Berg et al. (2000)	DCA	1.0 [0.03-7.2]	Switzerland	1996-7	Rain and snow

Reference	HAAs	Mean concentration [Range], µg/L	Location	Year	Type of water
Berg et al. (2000)	TCA	0.24 [nd-2.1]	Switzerland	1996-7	Rain and snow
Berg et al. (2000)	MCA	0.1 [nd-0.32]	Switzerland	1996-7	Rivers
Berg et al. (2000)	DCA	0.05 [nd-0.24]	Switzerland	1996-7	Rivers
Berg et al. (2000)	TCA	0.11 [nd-0.7]	Switzerland	1996-7	Rivers
Berg et al. (2000)	MCA	0.07 [0.02-0.2]	Switzerland	1996-7	Drinking water
Berg et al. (2000)	DCA	0.09 [nd-0.2]	Switzerland	1996-7	Drinking water
Berg et al. (2000)	TCA	0.08 [nd-0.2]	Switzerland	1996-7	Drinking water
Römpp et al. (2001)	MCA	0.54 ^a [0.28-11]	Germany	1998-9	Fog water
Römpp et al. (2001)	DCA	0.60 ^a [0.12-5.0]	Germany	1998-9	Fog water
Römpp et al. (2001)	TCA	0.28 ^a [0.02-2.0]	Germany	1998-9	Fog water
Römpp et al. (2001)	MBA	0.30 ^a [0.02-1.0]	Germany	1998-9	Fog water
Römpp et al. (2001)	DBA	0.14 ^a [0.02-0.45]	Germany	1998-9	Fog water

^a median

^b 90th percentile

nd, not detected

HAA5 comprise MCA, DCA, TCA, MBA and DBA; HAA9 comprise HAA5 as well as bromochloroacetic acid, chlorodibromoacetic acid, bromodichloroacetic acid and tribromoacetic acid.

Analysis of HAA occurrence data supports several general conclusions:

- HAAs occur at higher levels in disinfected water;
- MCA and MBA occur at lower levels compared to other HAAs;
- DBA can be high in bromine-rich water (Richardson and Thruston Jr, 2003).

OEHHA analyzed HAA5 occurrence in California drinking water reported by SWRCB for 2014-2015. The median, average, and 95th percentile for individual HAA5 levels are presented in Table 2.3. Detection limits for purposes of reporting are included in the first column. MCA and MBA were typically present at lower concentrations and in mixture with other HAAs (Table 2.3). In at least 5 percent of all samples, DBA was the only reported HAA5 (data not shown).

Table 2.3 Concentration of HAA5 in California Drinking Water (2014-2015)

Chemical (detection limit, ppb)	Concentrations, ppb			
	Median	Mean	95 th percentile	Maximum
MCA (2)	1.9 ^a	1.1	3.2	28.0
DCA (1)	2.5	4.2	16.7	63.7
TCA (1)	1.7	3.6	15.1	19.0
MBA (1)	1	0.6	1.7	29.8
DBA (1)	1.9	2.3	6.9	55.7

^a More than half of the samples were nondetects.

HAAs appear enriched in treatment plant effluents compared to upstream water (Krasner et al., 1989; Ding et al., 1999).

In several studies, boiling water was shown to change HAA concentrations, resulting in a decrease in TCA and an increase in DCA (Wu et al., 2001; Krasner and Wright, 2005; Levesque et al., 2006). Effects of boiling water on DCA was specific to the disinfection method, suggesting that interactions with the residual disinfectant may play a role (Krasner and Wright, 2005).

Food

HAAs, particularly MCA and MBA, have been previously used as cleansing or preserving agents in the beverage industry, due to their fungicidal and bactericidal properties. These and all other food additive uses were disallowed by the US Food and Drug Administration (US FDA) due to either a determination that they presented a potential risk to public health and/or the inadequacy of the scientific data to support safe use in human foods. The US FDA permits an MCA migration level of up to 10 ppb in food-packaging adhesives, but notes that approved protocols do not detect MCA at this level. European Union regulations do not permit the addition of HAAs to provide stabilization or preservation (Willetts et al., 1991; Reimann et al., 1996a; Reimann et al., 1996b).

MCA, DCA, and TCA have been detected in vegetables, fruits, and grains, and can be taken up into food from the cooking water (Raymer et al., 2001; US EPA, 2003a). Reimann et al. (1996b) examined the concentrations of chlorinated acetic acids in vegetables, fruits, grain, bread and beer from several countries, and found MCA, DCA, and TCA at levels up to about 20 µg/kg in solid foods and 15 µg/L in beer.

MCA is a minor breakdown product of chloroacetanilide herbicides, such as alachlor and acetochlor; however, estimated MCA exposure from pesticide-treated foodstuffs would be very small. In general, it is anticipated that MCA exposure from foods is insignificant compared to drinking water exposure.

HAAs may also be present in bottled water, depending on the treatment methodology and source of water used by bottlers. In the US, bottled water is under the jurisdiction of US FDA, but standards are typically regulated in accordance with and incorporated by reference to the US EPA drinking water standards, which is the case for the HAAs.

Human Exposure

Humans can be exposed to DBPs in drinking water through multiple routes including ingestion, inhalation, and dermal exposures (US EPA, 1998a; Nieuwenhuijsen et al., 2000a; Nieuwenhuijsen et al., 2000b; Nieuwenhuijsen et al., 2009a). Due to low HAA volatilization from water and low skin permeability, ingestion is the main route of exposure from tap water (US EPA, 1994a, 1994b; Xu et al., 2002).

While showering, bathing and dishwashing can result in inhalation and dermal exposure, the resulting doses are estimated to be very small. One shower stall exposure study investigated aerosol formation, characteristics and decay during and after showering, and estimated that at 250 ppb combined HAA in water, the inhalation dose of HAA from shower water would constitute less than 1% of the corresponding oral dose (Xu and Weisel, 2003). While the investigated HAA concentration was at the high end of the observed range (e.g., as found in swimming pools), due to the linearity of the Henry's law constant, lower tap water concentrations would also result in very low inhalation exposures. Thus, inhalation is not considered an important HAA exposure route and is not quantified in this risk assessment.

Similarly, dermal absorption of DCA and TCA from chlorinated water is considered minor compared to ingestion (Kim and Weisel, 1998), due to low skin permeability (Xu et al., 2002). US EPA estimated that the dermal dose from bathing or showering for an average adult constitutes an insignificant fraction of the daily ingestion dose for the HAAs (US EPA, 2011). Therefore, dermal exposure from HAAs in tap water is not quantified in this risk assessment.

Estimating exposure to HAAs is complicated by the variability in water ingestion across a population. Urinary levels of TCA and DCA were compared to exposure estimates calculated from in-home tap water concentrations and responses to a water usage questionnaire (Kim et al., 1999; Weisel et al., 1999). Urinary TCA excretion rates were correlated with ingestion exposure, and the correlation was stronger in individuals who consumed beverages primarily within their home where the concentration measurements were made. No correlation was observed between an average 48-hour exposure estimate and the urinary DCA excretion rate, presumably because of its high metabolism and short biological half-life. In contrast, the slow kinetics of TCA elimination suggested a long half-life (Kim et al., 1999), indicating the potential usefulness of urinary TCA as a biomarker of DBP exposure.

Kuklennyik et al. (2002) determined TCA in 76% of urine samples from 402 US adults. The 90th percentile concentration was 23 µg/L (22 µg TCA/g creatinine), and the geometric mean and median concentrations were 2.9 µg/L (2.6 µg/g creatinine) and 3.3 µg/L (3.2 µg/g creatinine), respectively (Calafat et al., 2003). Urban residents had higher mean TCA levels (men, 5.3 µg/L, 3.8 µg/g creatinine; women, 2.9 µg/L, 2.8 µg/g creatinine) than did rural residents (men, 2.2 µg/L, 1.7 µg/g creatinine; women, 2.6 µg/L, 2.7 µg/g creatinine). The higher urban levels (and more frequent detections) may reflect use of chlorinated public water supplies, versus water from private wells, which typically is not chlorinated.

Froese et al. (2002) evaluated urinary excretion of TCA as an exposure biomarker in Australians who normally consume domestic tap water and concluded that while urine TCA possessed

several qualifiers as a biomarker to DPB exposure in drinking water, it was not completely specific, suggesting other oral sources in addition to drinking water.

3. PHARMACOKINETICS

Absorption

Monochloroacetic Acid

MCA was rapidly absorbed after gavage of adult male Sprague-Dawley rats with a carbon-14 radiolabeled MCA ($[^{14}\text{C}]\text{MCA}$) solution without pH adjustment at either a subtoxic dose of 10 mg/kg or a lethal dose for 20% of the rats (LD_{20}) of 225 mg/kg (Saghir and Rozman, 2003). At 10 mg/kg, MCA disappearance from the rat stomach was fast (less than 10% remaining in the stomach after 45 min) and followed apparent first-order kinetics. The peak concentration of $[^{14}\text{C}]\text{MCA}$ in the plasma was reached at two hours. Absorption of the high dose from the stomach was saturated at between 0.25 and 8 hours, following apparent zero-order kinetics. An accidental oral exposure to MCA led to the rapid onset of toxic effects (Rogers, 1995) indicating fast oral absorption of MCA.

Hayes et al. (1973) subcutaneously injected 53 or 162 mg/kg radiolabeled $[^{14}\text{C}]\text{MCA}$ into rats and measured distribution between internal organs over 17 hours. The absorption and distribution of MCA were similar at the two doses.

Saghir and Rozman (2003) administered 125 mg/kg radiolabeled $[^{14}\text{C}]\text{MCA}$ (250 milligrams per milliliter [mg/ml] in acetone) without pH adjustment on the shaved back of adult male Sprague-Dawley rats and measured radioactivity in plasma, tissues, and excreta at several post-administration times. More than 95% of the dose penetrated into the skin within 0.25 hour, and was slowly released. Plasma levels peaked at 0.36% of dose at 0.75 hour and remained about this level for up to four hours. Peak tissue concentrations were reached between two and four hours. At 0.75 hour, 9% of the absorbed dose had been eliminated through bile, all of which was subsequently reabsorbed. In a case of human skin contamination, MCA appeared to be rapidly absorbed. Several accidental occupational exposures to highly concentrated MCA in water ($\geq 80\%$ by volume) splashed onto the skin have resulted in systemic toxicity within minutes to hours of exposure (Dancer et al., 1965; Kulling et al., 1992), as has crystalline MCA applied medicinally to the skin by itself (Pirson et al., 2003; Chapman et al., 2006) or as a mixture with DCA and TCA (Baser et al., 2008). However, at lower concentrations relevant to the drinking water exposures, the steady state skin permeability coefficient (K_p) of MCA is estimated to be very low (Xu et al., 2002), and the resulting dermal absorption (e.g., while showering) would be negligible relative to the oral dose, as discussed in the Human Exposure section of this document.

Dichloroacetic Acid

Radiolabeled DCA was readily absorbed after oral dosing of rats and mice, with only 1% to 2% of the label found in the feces, indicating almost complete gastrointestinal absorption (Larson and Bull, 1992a; Lin et al., 1993; James et al., 1997; James et al., 1998). James et al. (1998) gavaged young adult Sprague-Dawley rats with 50 mg/kg radiolabeled sodium dichloroacetate (SDCA; 42.4 mg/kg DCA), which initially distributed to muscle and liver and subsequently to other organs. Following a single oral 100 mg/kg dose of DCA to adult Sprague-Dawley rats, peak hepatic concentration of DCA occurred at three hours (Evans, 1982). DCA appeared in

the blood of male Fischer 344 rats within minutes after oral dosing with 500 micromoles of neutralized DCA per kilogram of bodyweight ($\mu\text{mole/kg}$) equivalent to 64.5 mg/kg, with maximum blood concentration at about one hour (Schultz et al., 1999).

Comparing ratios of the area under the curve (AUC) for blood after oral and i.v. doses of DCA, Schultz et al. (1999) found that 80% of the oral dose was bioavailable. Saghir and Schultz (2002) studied disposition of DCA after single and multiple oral and i.v. doses (0.05 to 20 mg/kg) to male Fischer 344 rats. After oral dosing of animals, DCA was rapidly absorbed. Absorption and bioavailability were compared between naïve and GSTZ1-depleted rats, which were pretreated with DCA for 7 days. After i.v. administration, plasma concentrations rapidly declined in naïve rats, while plasma decline was slower in GSTZ1-depleted rats. After oral administration, DCA was rapidly absorbed in both naïve and GSTZ1-depleted rats. At low doses, DCA was rapidly metabolized and plasma concentrations declined after the initial peak in concentration. At high doses, a secondary peak was present after the initial absorption, however it was less evident in the GSTZ1-depleted rats. Thus, the apparent oral bioavailability of DCA was both dose-dependent and pre-treatment-related: 0 to 13% in naïve rats, and 14 to 100% in GSTZ1-depleted rats.

Following oral exposure, DCA was rapidly absorbed in humans (Stacpoole et al., 1998a; Stacpoole et al., 1998b). Stacpoole et al. (1998a) reported peak plasma concentrations occurring within 15 to 30 minutes after oral dosing. After an i.v. dose of SDCA (10 or 20 mg/kg), the plasma half-life of DCA in humans was 0.43 hours on average (Lukas et al., 1980). The estimated low permeability constant K_p of DCA across human skin (Xu et al., 2002) likely results in low dermal absorption and a negligible fraction of dermal exposure relative to ingestion in exposure scenarios such as swimming (Kim and Weisel, 1998).

Oral systemic bioavailability of DCA was determined by comparing the area under the plasma time curve in male and female humans simultaneously administered DCA orally at 2 mg/kg-day and by i.v. injection at 0.3 mg/kg-day of radiolabeled [^{13}C]DCA (Schultz and Shangraw, 2006). It was estimated that on average 54% of the oral dose was absorbed in males (range 28-100%) and 59% in females (range 40-90%). There was no significant difference in bioavailability between men and women in this experiment; however, pre-treatment for 14 days with the very low dose of 20 $\mu\text{g/kg-day}$ DCA prior to the oral or i.v. administration of DCA significantly increased bioavailability and delayed elimination of DCA in women only (Schultz and Shangraw, 2006). While these effects may be relevant to GSTZeta polymorphism and DCA-dependent inhibition, the underlying mechanisms have not been directly examined in humans.

Trichloroacetic Acid

Following oral administration, TCA was shown to be rapidly absorbed into the bloodstream in mongrel dogs (Hobara et al., 1988), in male Fischer 344 rats (Schultz et al., 1999), and male B6C3F1 mice (Styles et al., 1991). TCA appeared in the blood of male Fischer 344 rats within minutes after oral dosing with 500 $\mu\text{mole/kg}$ of neutralized TCA, with maximum blood concentration reached around one hour (Schultz et al., 1999).

Styles et al. (1991) studied the absorption and distribution of 500 mg/kg radiolabeled [¹⁴C]TCA, as free acid in water or corn oil or as sodium trichloroacetate (STCA) in water, in male B6C3F1 mice. Plasma TCA levels peaked within one hour and fell to below 20% of peak levels at 24 hours. In the liver, TCA levels also peaked at one hour and declined more slowly to less than half of peak levels at 24 hours. Little of the radioactivity was covalently bound to plasma or liver proteins. The three TCA formulations had similar rates of absorption and elimination.

Schultz et al. (1999) studied the disposition of TCA in male Fischer 344 rats. Blood samples were collected at several time points up to 48 hours after i.v. or gavage administration of 500 μmole/kg (81.7 mg/kg) of neutralized TCA. TCA blood levels peaked one hour after oral administration, and declined to roughly 30% of peak levels at 24 hours. Oral bioavailability was close to 100%. There appeared to be significant non-covalent binding of TCA to plasma proteins after dosing by both routes (approximately 50% in bound fraction), as also observed by Templin et al. (1993) and Yu et al. (2000) (23-60% in bound fraction).

TCA is rapidly absorbed into the bloodstream via the oral route in humans (Kim and Weisel, 1998). TCA also appeared in the bloodstream quickly after dermal exposure, but was judged to be absorbed at much lower levels, compared to ingestion (Kim and Weisel, 1998; Weisel et al., 1999). Percutaneous absorption of TCA in vitro was low, with a K_p of 1.9 × 10⁻³ centimeters per hour (cm/hour) (Xu et al., 2002), and this value was within the range, 1-8×10⁻³ cm/hour, of the K_p estimate from the Kim and Weisel (1998) study. Based on these K_p estimates, dermal exposure was determined to contribute less than 1% of a dose of TCA received by the ingestion of 1.4 L/day of water.

Monobromoacetic Acid

Only one animal study on MBA was located. MBA was administered to male Fischer 344 rats either orally or by i.v. injection in a mixture of 25 μmole/kg each of MBA, DCA, chlorodibromoacetic acid (CDBA), and tribromoacetic acid (TBA) (Saghir and Schultz, 2005). MBA could not be detected in the rat plasma samples collected three minutes after i.v. injection and one minute after oral dosing, indicating possibly very rapid elimination. The three other compounds were rapidly absorbed as shown by their detection in plasma within one minute after the oral dosing. The K_p of MBA through human skin was estimated to be about 1.4×10⁻³ cm/hour (Xu et al., 2002).

Dibromoacetic Acid

DBA was rapidly absorbed by male and female Fischer 344/N rats and B6C3F1 mice orally as shown by measurable blood levels within five minutes of gavage dosing with a pH 5 aqueous solution (NTP, 2007a). Schultz et al. (1999) also found DBA in the blood of male Fischer 344 rats within minutes after oral dosing with 500 μmole/kg of neutralized DBA. The maximum blood concentration of DBA in male Fischer 344/N rats was reached within one hour after gavage. DBA oral bioavailability was estimated to be about 30% (Schultz et al., 1999). Absorption through human skin is greatest for DBA among the HAAs with an estimated K_p of DBA 2.6×10⁻³ cm/hour (Xu et al., 2002).

Distribution

The HAAs are rapidly distributed with body water, as expected for small hydrophilic compounds. This is followed by some specific tissue uptake and retention, which appears to vary among the HAAs. Most of the available studies involved MCA. Plasma binding was only investigated in detail for TCA, in part due to its relative resistance to metabolism.

Monochloroacetic Acid

Saghir and Rozman (2003) gavaged adult male Sprague-Dawley rats with single 10 or 225 mg/kg doses of radiolabeled [¹⁴C]MCA solution without pH adjustment, and radioactivity levels were determined in plasma and organs at 0.25, 0.45, 2, 4, 8, 16 and 32 hours. At 10 mg/kg, the highest radioactivity levels were found in kidney, liver, thymus, brown and white fat, spleen, testis, and heart, with the peak plasma concentration at 2 hours. At 225 mg/kg, absorption was much slower (with most of the dose remaining in the stomach for several hours) and the plasma concentration was highest at the earliest time point (0.25 h). The highest radioactivity levels after the 225 mg/kg dose were found in thymus, kidney, liver, brown and white fat, and brain.

Hayes et al. (1973) subcutaneously (s.c.) injected 53 or 162 mg/kg radiolabeled [¹⁴C]MCA to rats and measured distribution over 17 hours. MCA was distributed throughout the body within 90 minutes. The highest concentrations after two hours were found in the kidney and liver, relative to plasma concentrations.

Kaphalia et al. (1992) gavaged male Sprague-Dawley rats with 0.1 millimole per kilogram of bodyweight (mmol/kg), or 9.45 mg/kg, radiolabeled [¹⁴C]MCA and studied distribution of MCA at several time points up to 48 hours. MCA was widely distributed within four hours, with the greatest concentrations in the intestinal tract, followed by kidney, liver, spleen, testis, lung, brain and heart. In a second experiment, male Sprague-Dawley rats were gavaged with a 10-fold higher dose of [¹⁴C]MCA and tissues were assayed after 24 hours. At this dose, the kidney and liver again had the highest MCA levels, but concentrations were just 2.5 to 3.6 times higher than those found at the lower dose. The relative distribution patterns were the same. In a third experiment, Kaphalia et al. (1992) gavaged male Sprague-Dawley rats daily for three days with 1.0 mmol/kg (94.5 mg/kg) [¹⁴C]MCA and assayed tissues 24 hours after the last dose. Compared to a single dose, repetitive dosing resulted in higher residual concentrations in the kidney, intestine, lung, spleen, heart, brain and testis. Only in the liver were concentrations similar to that of single-dosed animals.

Bhat et al. (1990) injected male Sprague-Dawley rats intravenously with a tracer dose of radiolabeled [¹⁴C]MCA (68 µg/kg) and conducted whole body autoradiographs at up to 48 hours. At five minutes, MCA was distributed widely, with the highest concentrations in the kidney cortex, stomach walls, liver and myocardium. MCA was also present in the duodenal cavity, indicating biliary excretion, and had started to accumulate in the brain, esophagus, trachea, and in ganglionic fibers. Bhat et al. (1990) concluded that MCA and its metabolites distribute first into hydrophilic tissues, then into lipophilic tissues.

Saghir et al. (2001) injected 10 or 75 mg/kg radiolabeled [¹⁴C]MCA into the tail vein of adult male Sprague-Dawley rats and assayed tissues at various time points. At 10 mg/kg, the highest concentrations occurred five minutes after administration with about the same concentrations in plasma, heart, and brown fat, and lower levels in brain and thymus. Distribution of MCA into tissues at 75 mg/kg appeared much slower than at 10 mg/kg as shown by a 22- to 23- times higher area under curve of total ¹⁴C and intact MCA in plasma, instead of the expected 7- to 8-fold, based on the dose ratio. This was probably due to saturation of metabolism at the higher dose rather than a difference in rate of distribution per se. A higher percent of radioactivity was found in the liver and kidney at 10 mg/kg than at 75 mg/kg. The areas under the curve for liver and kidney were about 11 times higher after the high dose than after the lower dose. Radioactivity in bile was associated with one metabolite more polar than MCA. A very large fraction of the dose was found in the gastrointestinal tract, almost all of which was reabsorbed.

Kulling et al. (1992) monitored the plasma concentration of MCA in a man who was dermally exposed to an 80% solution of MCA in an occupational accident. At four, six, eight, and 12 hours after exposure, MCA levels were 33, 15, 7.8, and 0.22 mg/L, respectively, indicating that MCA is rapidly cleared from the blood, with an approximate half-life of 2 hours.

Dichloroacetic Acid

Lin et al. (1993) gavaged male Fischer 344 rats with 28.2 or 282 mg/kg of 2-[¹⁴C]DCA (DCA with the chlorinated carbon radiolabeled) or 28.2 mg/kg of 1-[¹⁴C]DCA (DCA with the non-chlorinated carbon radiolabeled). At 48 hours after administration, 21% to 36% of the ¹⁴C was recovered from tissues. The tracer from the 28.2 mg/kg dose of 2-[¹⁴C]DCA was found at highest levels in the liver and muscle, followed by skin, blood, and intestines. The percentage of the dose expired as carbon dioxide (CO₂) was 34.4% at 28.2 mg/kg and 25.0% at 282 mg/kg, while the percentage in the urine increased from 12.7% to 35.2%. This increase in the urinary excretion was mostly attributable to unmetabolized DCA, which was less than 1% at the lower dose and over 20% at the higher dose. The rest of the tissues combined contained 1-2% of the dose.

During short-term oral administration of DCA to humans at therapeutic doses, DCA was rapidly and virtually completely absorbed and was minimally bound to plasma proteins (Ammini and Stacpoole, 2003; Jia et al., 2006). Lukas et al. (1980) intravenously administered SDCA at 10 mg/kg to two normal adult humans. The plasma half-life of SDCA was 0.33 to 0.36 hours, the volume of distribution was 308 to 366 ml/kg, and the plasma clearance rate was 10.9 to 11.8 ml/min-kg. Another two normal human adults given i.v. doses of 20 mg/kg SDCA showed plasma half-lives of 0.41 to 0.61 hours, a volume of distribution of 186 to 195 ml/kg, and a plasma clearance of 3.5 to 5.6 ml/min-kg.

Trichloroacetic Acid

TCA is well absorbed by the gastrointestinal tract, followed by rapid urinary elimination, mostly unmetabolized, with small amounts excreted through feces and exhaled air (US EPA, 2011). Larson and Bull (1992a) gave male Fischer 344 rats and B6C3F1 mice 5 to 100 mg/kg radiolabeled [¹⁴C]TCA, as a single oral dose in water. Urine, feces and expired air were collected for up to 48 hours. About 57-72% of the radioactivity appeared in urine, 4-8% in

expired air as CO₂ and 2-4% in feces. Unmetabolized TCA accounted for 81-90% of the urinary ¹⁴C and 48-65% of the dose. Urinary metabolites comprised 1-3% of the dose as DCA and combined glyoxylate, glycolate, and oxalate at 5-11%.

Xu et al. (1995) gavaged male B6C3F1 mice with 100 mg/kg radiolabeled [¹⁴C]TCA. Twenty-four hours later, about 55% of the label was in urine, 5% in expired air as CO₂, 5% in feces, and the remainder in the carcass. Urinary radioactivity was identified as 44.5% TCA, 0.2% DCA, 0.03% MCA, 0.06% glyoxylate, 0.11% glycolate, 1.5% oxalate, and 10.2% unidentified compounds.

Following oral or i.v. administration to rats, TCA appeared to bind to plasma proteins and to distribute to the liver to be metabolized (Templin et al., 1993; Schultz et al., 1999; Yu et al., 2000). In the liver, TCA was reduced through dehalogenation to DCA and small amounts of DCA were further dehalogenated to MCA, then to thiodiglycolate, glyoxylate, oxalate, glycolate, glycine, and CO₂ (Larson and Bull, 1992a; Ketcha et al., 1996; Bull, 2000; Merdink et al., 2000). DCA is considered to be a reactive intermediate but not a major metabolite of TCA, and MCA a minor metabolite of DCA (Yan et al., 1997; Stacpoole et al., 1998a; US EPA, 2003a).

TCA binding to plasma proteins was evaluated in humans, rats, and mice (Lumpkin et al., 2003; US EPA, 2011). TCA plasma protein binding was much higher in humans (more than 80%), and was constant over concentrations varying by nearly four orders of magnitude. Binding in mice was lower and not constant, ranging from 47% at the lowest concentration to 19% at higher concentrations. Binding in the rat was in between, with 67% at low concentrations and 39% at high concentrations.

Templin et al. (1993) assayed plasma and liver TCA levels in male B6C3F1 mice given TCA orally at 0.03, 0.12, and 0.61 mmol/kg. Peak TCA levels were higher in blood than in liver at all doses. The AUC was also higher in plasma than in liver. The plasma half-life of TCA was about six hours for all doses. TCA binding to plasma proteins (and other constituents) was 50-57% at plasma concentrations below 306 nanomoles per milliliter (nmol/ml) and 41-23% at TCA concentrations from 306 to 1,224 nmol/ml (with higher binding at lower plasma concentrations).

Yu et al. (2000) intravenously administered radiolabeled TCA at 6.1, 61, or 306 μmole/kg to male Fischer 344 rats. Tissue TCA levels peaked within minutes and declined with time in a parallel manner, although the liver levels tended to level off more than plasma levels. The investigators suggested that decreased binding of radioactivity in rat plasma at higher doses represented saturation of binding sites.

Monobromoacetic Acid

Saghir and Schultz (2005) gave MBA to male Fischer 344 rats either orally or intravenously in a mixture of 25 μmole/kg each of MBA, DCA, DBA, and TBA. MBA could not be detected in rat plasma three minutes after i.v. dosing and one minute after oral dosing; the three other compounds were detectable under these conditions. Thus MBA appears to be very rapidly distributed and eliminated.

Dibromoacetic Acid

Schultz et al. (1999) found that following i.v. injection in rats, DBA did not bind significantly to plasma proteins or accumulate in blood cells. The extent of tissue distribution was not clear in this study, but DBA did not appear to be lipophilic at physiological pH, indicating a low tendency to accumulate in body fat.

Holmes et al. (2001) gave five gavage doses of 250 mg/kg-day DBA to male Sprague-Dawley rats. Thirty minutes after the last dose, DBA was measured in the testicular interstitial fluid with a peak level of 79 micrograms per milliliter ($\mu\text{g/ml}$). The DBA half-life was estimated to be about 1.5 hours.

Christian et al. (2001) provided DBA in drinking water at 0, 125, 250, 500, or 1,000 mg/L to 50 Sprague-Dawley rats/sex/group for 14 days pre-mating through gestation and lactation, for a total exposure period of 63 to 70 days. DBA was measurable in parental and fetal plasma, placental tissue, amniotic fluid, and maternal milk, indicating that DBA crossed the placenta and was taken up by the fetus.

Metabolism

Interactions among the DBPs may alter their metabolism and toxicity (Austin and Bull, 1997). MCA, DCA or TCA can change chloroform metabolism and toxicity in a sex-specific manner (Davis, 1992; Davis and Berndt, 1992). Results of animal studies suggested in vivo competitive interactions between tri- and di-substituted HAAs (Saghir and Schultz, 2005). Specifically, at high oral and i.v. doses, DCA was shown to inhibit its own metabolism and that of other di-substituted HAAs via glutathione S-transferase zeta (GSTZ1) (Curry et al., 1991; James et al., 1997; Saghir and Schultz, 2005).

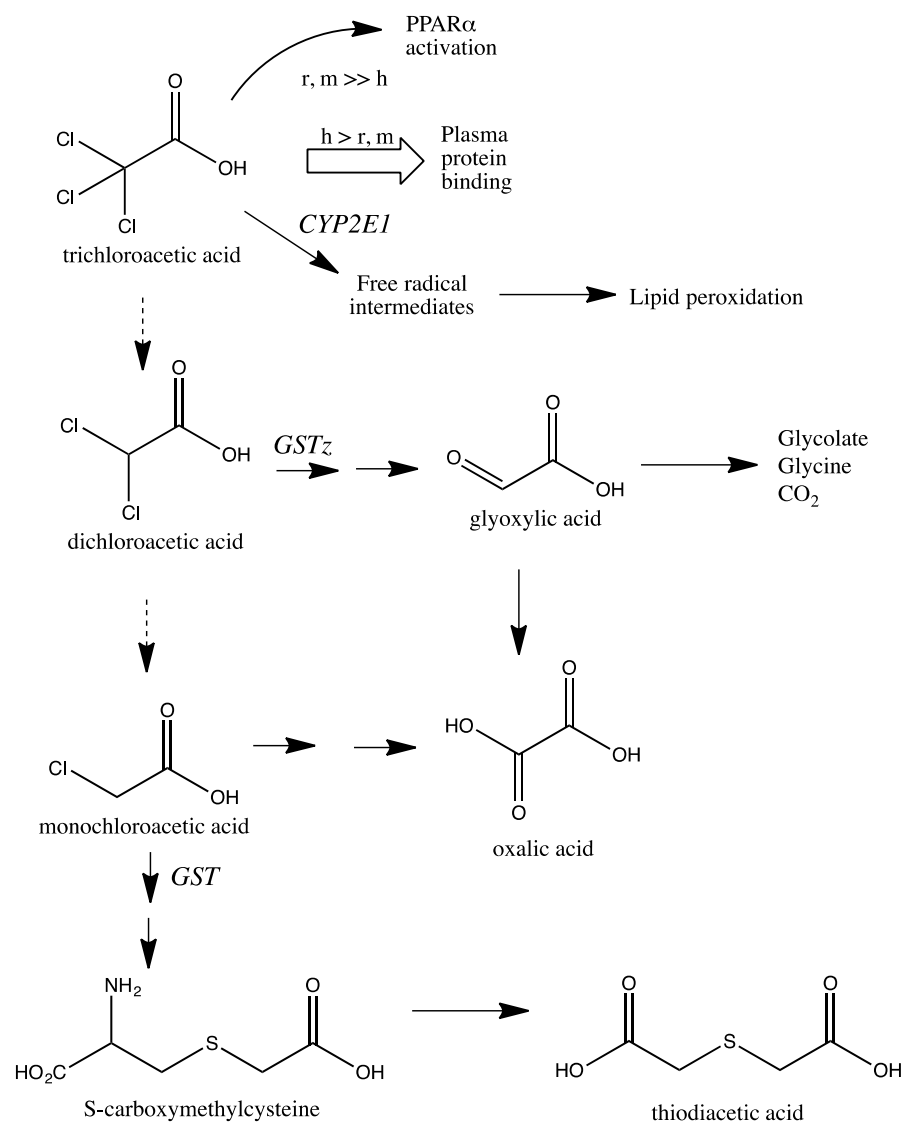
Chloroacetic Acids (MCA, DCA, TCA)

Haloacetic acids are electrophilic compounds that can react with thiol groups (Dickens, 1933; Plewa et al., 2010; Stalter et al., 2016) and undergo dehalogenation through the action of cytochrome P450 enzymes and GSTZ1. Brominated acetic acids are generally more reactive than their chlorinated counterparts, and tri-substituted acetic acids (such as TCA) have lower reactivity than di- and mono-substituted acetic acids (Stalter et al., 2016). Generally speaking, TCA is classified as possessing “low metabolism with moderate renal clearance,” while the rest of the HAA5 group demonstrate properties of “high metabolism with low renal clearance” (NTP, 2018). Tri- and di-substituted haloacetic acids were found to inhibit GSTZ1 and consequently to decrease the GSTZ1-dependent metabolism of reactive HAAs (Anderson et al., 1999). Additional toxicokinetic data on HAAs were obtained from the studies of chlorinated ethylenes, which are thought to be metabolized to TCA and DCA, among other intermediates. Although HAA metabolism appears to be qualitatively similar among mice, rats and humans, there are species-specific differences in GSTZ1 activity toward HAA metabolism and inhibition, which may explain the higher metabolic stability of DCA in humans (NTP, 2018).

The major proposed pathways and metabolites of the tri-, di- and mono-chlorinated acetic acids are summarized in Fig. 3.1.

Figure 3.1 Metabolic pathways of chloro-substituted acetic acids. Summarized from (Xu et al., 1995; Stacpoole et al., 1998b; NTP, 2018)

Solid arrows indicate main flow of metabolism, dashed arrows indicate attenuated directions. m, mouse; r, rat; h, human.



Monochloroacetic Acid

MCA is metabolically labile. Yllner (1971b) proposed a glutathione conjugation-mediated pathway of MCA metabolism, which produces two major urinary metabolites, S-carboxymethylcysteine and thiodiacetic acid (Figure 3.1). In this study, the urine of albino mice was collected over 3 days after a single intraperitoneal (i.p.) dose of 70-100 mg of radiolabeled [¹⁴C]MCA (Yllner, 1971b). Additional minor metabolites were identified as glycolic acid and oxalic acid and were proposed to form in an independent minor pathway (Figure 3.1).

Dichloroacetic Acid

Like MCA, DCA is metabolically labile and is rapidly converted to a wide range of metabolites, including glyoxylic acid, oxalic acid, glycolate, glycine and CO₂ (Larson and Bull, 1992a; James et al., 1997; Yan et al., 1997; Stacpoole et al., 1998a; Tong et al., 1998a; Bull, 2000; Ammini and Stacpoole, 2003). Less than 2% of unmodified DCA was excreted in urine following treatment of male F344 rats and B6C3F1 mice with a single oral dose of 5-100 mg/kg, while at least 50% of unmodified TCA from equivalent dosing was excreted within 48 hours in rats and 24 hours in mice (Larson and Bull, 1992a). DCA appears to be a metabolite in the TCA pathway detected in the urine (mice and rats) and in the rat plasma (Larson and Bull, 1992a, 1992b). DCA was not detected in the plasma of TCA-treated B6C3F1 mice, likely due to rapid elimination (Merdink et al., 1998). In addition to direct metabolic conversion, DCA can be formed by dechlorination from TCA by gut microflora under anaerobic conditions (Moghaddam et al., 1996; George et al., 2000). Physiological dechlorination of DCA (either directly administered or as a metabolite in the TCA pathway) to MCA appears to be a minor pathway (Shroads et al., 2008).

The primary route of DCA metabolism is through glutathione conjugation in the liver, and GSTZ1 has been demonstrated to catalyze the initial step in glutathione-dependent oxygenation of DCA to glyoxylic acid (Tong et al., 1998a, 1998b; Board and Anders, 2005). Mice with a homozygous knockout for the *GSTz1-1* gene lost their ability to metabolize DCA, suggesting a lack of alternative pathways (Ammini et al., 2003). In male Fischer 344 rats, GSTZ1 depletion slowed down DCA plasma clearance and changed its kinetics (Saghir and Schultz, 2002). GSTZ1 appears to be inhibited by DCA through multiple mechanisms, resulting in the well-documented effect of DCA pretreatment to dramatically slow down DCA elimination from plasma in laboratory animals and humans (Anderson et al., 1999; Cornett et al., 1999; Wempe et al., 1999; Saghir and Schultz, 2002; Ammini et al., 2003; Jia et al., 2006; Shroads et al., 2008). This effect was particularly pronounced in young mice (10 weeks old) compared to older mice (60 weeks old) (Schultz et al., 2002). In contrast, following a six-month long twice-daily 12.5 mg/kg DCA dosing regimen, DCA clearance was more dramatically delayed in adult human patients (aged 14.0-33.9 years) in comparison to child patients (aged 2.2 – 7.1 years), with elimination half-life increased about 10-fold in adult patients, and only about 2-fold in child patients (Stacpoole et al., 2008b). This study concluded that older individuals or those with impaired liver metabolism may be at higher risk for DCA neurotoxicity observed in DCA clinical trials (Shroads et al., 2008; Stacpoole et al., 2008b). Similarly, polymorphisms in human *GSTz1-1* genes may result in differences in susceptibility to DCA toxicity among subpopulations (Tzeng et al., 2000).

Trichloroacetic Acid

TCA is a metabolite of trichloroethylene, tetrachloroethylene and chloral hydrate, and studies on these compounds have provided additional insights into TCA metabolism (Davidson and Beliles, 1991; Delinsky et al., 2005a; US EPA, 2011). TCA does not appear to undergo significant metabolism: for single dose studies, 75% of the human dose, 57-95% of the mouse dose and 59-84% of rat dose of labeled TCA were excreted unchanged in urine (Paykoc and Powell, 1945; Larson and Bull, 1992a; Xu et al., 1995; Yu et al., 2000). For comparison, 2% or less of the radiolabeled [¹⁴C]DCA at any dose were excreted unchanged in urine in either rats or mice in the same study (Larson and Bull, 1992a) that found 57-72% (higher with increasing dose) of the original single oral radiolabeled [¹⁴C]TCA dose unmetabolized in urine.

One reason for the relative metabolic stability of TCA in human could be due to its binding to plasma proteins, which was higher in humans than in rats and mice (Templin et al., 1993; Templin et al., 1995; Toxopeus and Frazier, 1998; Schultz et al., 1999; Yu et al., 2000; Toxopeus and Frazier, 2002; Lumpkin et al., 2003; US EPA, 2011).

Non-extractable radioactivity was present in the plasma and the liver of F344 rats at 24 hours post-treatment, attributed to adducts of macromolecules with transient reactive metabolites of radiolabeled [¹⁴C]TCA, but no metabolites were detected in plasma or urine (Yu et al., 2000). In contrast, Larson and Bull (1992a) observed small but significant levels of [¹⁴C]DCA in the urine and plasma of [¹⁴C]TCA-treated F344 rats and B6C3F1 mice, and proposed reductive dechlorination of TCA to DCA as the primary metabolic event for TCA (Figure 3.1).

Cytochromes P450, including CYP2E1, were proposed to be responsible for the metabolism of TCA (as well as to play a role in the metabolism of its precursors, such as trichloroethylene) generating free radical intermediates, which would contribute to increased lipid peroxidation (Davidson and Beliles, 1991; Larson and Bull, 1992b, 1992a; Austin et al., 1995; Xu et al., 1995; US EPA, 2011). Alternatively, TCA-dependent increase in lipid peroxidation was proposed to result from receptor-driven peroxisome proliferation (Austin et al., 1995), and TCA was found to directly activate mouse peroxisome proliferator-activated receptor alpha (PPAR α) within the concentration range observed in mouse plasma in carcinogenicity studies (Bull, 2000), as summarized in the TCA Toxicity/Mechanism section of this document.

Bromoacetic Acids (MBA, DBA)

The metabolism studies of bromo-substituted acetic acids, particularly of MBA, are few, and it is currently thought that these compounds share metabolic pathways with their chloro-substituted analogs.

Monobromoacetic Acid

In a study of male Fischer 344 rats dosed with a mixture of molar-equivalent concentrations of MBA, DCA, DCBA, and TBA (25 μ mol/kg of each HAA), MBA underwent fast metabolism and was not detected in most plasma samples collected 3 min after i.v. dosing and 1 min after oral dosing (Saghir and Schultz, 2005). Furthermore, reduction of GSTZ1 activity due to previous DCA exposure did not stabilize MBA levels in this study.

Dibromoacetic Acid

Tong et al. (1998b) investigated biotransformation of dihaloacetic acids by GSTZ1 and concluded that DBA is metabolized in a GSTZ1-dependent pathway, which was similar to that of DCA, leading to eventual formation of glyoxylic acid. Several other studies reached a similar conclusion of GSTZ1-mediated conversion of DBA into glyoxylate (Stacpoole et al., 1998a; Tong et al., 1998a; Schultz et al., 1999). Based on the much faster elimination rate of DBA in comparison to DCA in male F344/N rats, Schultz et al. (1999) suggested a greater first-pass liver metabolism of DBA, and Tong et al. (1998b) found a greater in vitro rate of glyoxylate formation from DBA compared to DCA using purified GSTZ1. Similar to DCA, DBA was found to inhibit liver GSTZ1 (Wempe et al., 1999).

Excretion

HAAs and their metabolites are primarily eliminated from the body via urine. In agreement with its metabolic stability, TCA is primarily recovered unchanged in the urine, while the more metabolically labile DCA and MCA produce a wider spectrum of metabolites (Yllner, 1971c; Lukas et al., 1980; Larson and Bull, 1992a; Kim and Weisel, 1998; Schultz et al., 1999).

Oral administration of labeled MCA to female mice (~2 mg) resulted in 82-88% of the dose eliminated in the urine (Yllner, 1971c). Excretion in the feces was insignificant, and about 8% was expired as CO₂. In several rat studies, urinary excretion of MCA and metabolites was at 32-72% of the oral dose, depending on the strain and the administered dose (Berardi et al., 1987; Kaphalia et al., 1992; Saghir et al., 2001; Saghir and Rozman, 2003). S-carboxymethylcysteine and thiodiacetic acid are major MCA metabolites in the urine (Yllner, 1971c). A higher MCA dose resulted in higher urinary elimination and a larger fraction of unchanged MCA in the urine (Saghir et al., 2001). The major route of MCA elimination after dermal administration was also via urine (Saghir and Rozman, 2003). The total body clearance rate was twice as high for oral administration compared to the i.v. or dermal routes, implicating hepatic first pass metabolism as a main contributor to elimination when administered orally (Saghir and Rozman, 2003).

Plasma clearance of DCA is much slower in dogs than in rats and humans (Lukas et al., 1980). Following an intravenous (i.v.) injection of 100 mg/kg SDCA, the half-life of DCA in male Sprague-Dawley rats was about 2.1 to 4.4 hours, while in dogs DCA had an estimated half-life of 17.1 to 24.6 hours.

In F344 rats and B6C3F1 mice, most of the orally administered DCA dose is excreted via urine, 1-2% is found in the feces and 17-46% can be excreted as CO₂ (Larson and Bull, 1992a, b; Lin et al., 1993; James et al., 1998). DCA is extensively metabolized to glyoxylate, oxalate, CO₂ and other metabolites, and only a small fraction of the parent compound (1-5%) has been found in the urine in animal and human studies (Larson and Bull, 1992a, 1992b; Lin et al., 1993; Kim and Weisel, 1998; Kim et al., 1999). After a single oral dose of 50 mg/kg, the plasma half-life of DCA was about 0.5 to 2 hours in human subjects (Lukas et al., 1980).

Following oral administration of TCA to male B6C3F1 mice, the half-life of TCA in blood was 5.4-6.4 hours (Templin et al., 1993). TCA half-life in F344 male rats was 8 hours (Schultz et al., 1999). Urinary excretion of TCA was 48-84% of administered dose in F344 rats and 48-55% of administered dose in B6C3F1 mice (Larson and Bull, 1992a; Yu et al., 2000). Up to 3% of administered TCA dose was recovered in the feces of F344 rats or B6C3F1 mice, and 6.4-7.8% (rats) or 3.6% (mice) of the TCA dose were metabolized to CO₂ (Larson and Bull, 1992a).

In humans, the half-life of TCA absorbed from drinking water ranged from 2.1 to 6.3 days (Froese et al., 2002; Bader et al., 2004). In a toxicokinetic study of a single i.v. dose of TCA (28-60 mg/kg) administered to human volunteers, plasma half-life was 30 hours immediately following the injection, but elimination was much slower at later times (Paykoc and Powell, 1945). In this study, approximately 75% of unmetabolized TCA was recovered in urine at the end of day 10. In another human study, Froese et al. (2002) estimated 17-67% of the absorbed dose of TCA was excreted unchanged in the urine although this study reported high uncertainty of human exposure estimates, high variation in measured TCA concentrations in tap water at sampling points over the study period, and several other limitations.

It would be problematic to directly compare the mouse and human half-lives of TCA, due to the dramatic differences in the experimental design. As such, additional factors to account for differences in elimination kinetics between mice and humans were not considered due to the uncertainty in the available toxicokinetic information for TCA. Therefore, default species dose conversion methods were applied for the dose-response analysis.

Limited evidence is available on excretion of MBA and DBA. Both MBA and DBA undergo rapid metabolism, and the metabolites would be expected to undergo fast renal elimination (Schultz et al., 1999; WHO, 2004a; Saghir and Schultz, 2005; NTP, 2007a).

Physiologically Based Pharmacokinetic Models

HAA5 physiologically based pharmacokinetic (PBPK) models were developed to track TCA or DCA formed as P450-mediated metabolites of TCE, PCE or chloral hydrate (Abbas et al., 1996; Abbas and Fisher, 1997; Qiu et al., 2009). TCA is the product of the oxidative branch of PCE metabolism, while DCA would be either formed from dechlorination of TCA or possibly, through GSH (glutathione) conjugation of PCE, which is its second metabolic branch.

A harmonized PBPK model for PCE toxicokinetics was developed in mice, rats and humans (Chiu et al., 2009; Chiu and Ginsberg, 2011). This model includes twelve compartments in the main PCE module and two distinct blocks for PCE metabolism: oxidative metabolism in the liver, lung and kidney, and conjugative metabolism in the liver. In this model, TCA is the final product of the oxidative metabolism. The PBPK module for TCA includes three separate compartments for plasma, body and liver, as well as two elimination routes (through urine, starting in plasma, and 'other,' starting in the liver). The Chiu and Ginsberg (2011) PCE model was calibrated using PCE inhalation and gavage studies that primarily measured TCA in the blood or urine as the marker of exposure. The oxidative block was also independently calibrated using TCA oral and i.v. studies (Chiu et al., 2009). Although this model considers DCA formation through the

conjugation metabolism of PCE, it targets DCA for direct excretion in the urine without possibility of distribution to other tissues.

In a subsequent report, the TCA PBPK module was further developed as an independent model to incorporate TCA exposure from drinking water in mice using three additional drinking water TCA studies in male mice for validation (Chiu, 2011). The oral absorption of TCA was optimized and three diurnal exposure patterns were considered that would mimic drinking behavior in mice. The model was optimized using the Bayesian approach. In this model, the fractional absorption of TCA (bioavailability) was study-specific and also depended on the dose, gradually decreasing with increasing doses. The resulting model was able to successfully predict the reported blood and liver TCA concentrations after 5 or 14 days of exposure to 0.5-2.5 mg/L TCA in drinking water (Chiu, 2011). Due to the absence of human oral studies of TCA toxicokinetics, human oral TCA bioavailability is unknown, and the existing human PBPK sub-module of the harmonized PCE model (Chiu and Ginsberg, 2011) cannot be validated as an independent oral PBPK model for TCA. Further studies, specifically on human bioavailability of TCA, would be required for the development of the human TCA PBPK model, and currently, no such model has been described in the literature.

While earlier DCA models accounted for its metabolism by GSTZ1, next generation models incorporated the inhibitory effect of DCA on its own metabolism (Barton et al., 1999; Keys et al., 2004; Li et al., 2008). These included (i) a mouse model to evaluate the relationship between liver cancer incidence and hepatic dosimetry for oral DCA (Barton et al., 1999), (ii) rat and mouse models to evaluate the impact of reduced liver metabolism by suicide inhibition and regeneration of GSTZ1 on DCA kinetics (Keys et al., 2004), and (iii) a human model to describe DCA biotransformation and kinetics at either low environmental doses or a high therapeutic dose in humans given DCA orally or by an i.v. infusion (Li et al., 2008).

Barton et al. (1999) developed a three-compartment mouse PBPK model for DCA that included liver, body and gastrointestinal (GI) tract compartments. Absorption is modeled from the GI tract into the liver compartment. Urinary excretion from the body compartment and metabolism in the liver are elimination routes. The model accommodates i.v. and oral doses. The body/blood and liver/blood partition coefficients are assumed to be unity (value of 1). The model was derived using original pharmacokinetic (PK) data from i.v. and oral gavage dosing experiments. It is not clear from the report whether DCA solutions were neutralized prior to dosing. The model parameters, such as absorption constant and maximum rate of DCA metabolism were optimized using single dose data (20 mg/kg or 100 mg/kg) from naïve and pretreated mice that received drinking water containing DCA at 2 g/L for 2 weeks. The Michaelis-Menten affinity constant (K_m) for DCA metabolism in the liver was assigned 0.5 mg/L and kept constant in all exposure scenarios. At the outcome of the model optimization, the maximum rate of DCA metabolism (V_{max}) varied dramatically between the naïve and pre-treated mice, which would be consistent with self-inhibition of a DCA-metabolizing enzyme, such as GSTZ-1. Interestingly, the PK data for the lower dose (20 mg/kg) in pre-treated animals could not be fitted to the model, and was discarded from further consideration (Barton et al., 1999).

Therefore, the model may not adequately predict internal doses at the low-end range of a chronic DCA treatment.

Keys et al. (2004) developed a DCA PBPK model in mouse and rat, with further focus on the suicide inhibition of DCA metabolism. In this four-compartment model (including liver, rapidly perfused, slowly perfused and kidney compartments), DCA metabolism/elimination in the liver is modeled with two independent fluxes as the first-order non-inhibitable metabolism and the rate-controlled inhibitable metabolism. Unlike the Barton et al. (1999) model described above, there is no elimination route from the kidney, due to presumably low urinary elimination of unmetabolized DCA. DCA ingested from drinking water is assumed to directly enter the liver compartment. Physiological and some metabolic parameters are from previously published studies. Specifically, the Michaelis-Menten affinity constants (9.0 mg/L for rat, 10.6 mg/L for mouse) are from a previously published in vitro study (Tong et al., 1998b). In contrast, the initial maximum rate of inhibitable metabolism and the inhibition rate served as optimization parameters in calibrating the model using experiments with a single dose or multiple daily doses. The resulting initial rate of maximal inhibitable metabolism (V_{max0C}) was greater in mouse (190 mg/h·kg^{0.75}) compared to rat (77.5 mg/h·kg^{0.75}). Only a limited number of validation experiments are described in the report, and the model appears to adequately track the observed DCA concentrations, with some exceptions. For example, the model appears to overpredict the blood concentrations in mice on the fifteenth day of exposure to 2 g/L DCA in drinking water (Figure 8 in Keys et al. (2004)).

As a limitation, the Keys et al. (2004) mouse model does not account for the reported effect of age on the suicide inhibition of DCA metabolism. In young mice (8 week old) pre-treated with 2 g/L DCA in drinking water for 2 weeks, the rate of DCA elimination from blood following a single i.v. dose (20 mg/kg) was dramatically lower compared to naïve animals (Schultz et al., 2002). In contrast, 56-week-old mice demonstrated no difference in inhibition rates between DCA pre-treated and naïve animals. As presented in the original report, only some of the data from Schultz et al. (2002) were used for the model validation, and the issue of age sensitivity of suicide inhibition was not discussed (Keys et al., 2004). Interestingly, in humans, adult subjects appear to demonstrate about a five-fold stronger DCA metabolic inhibition compared to the young subjects (Stacpoole et al., 2008b), as described above in the *Metabolism* section. This difference between mouse and human metabolic rates for DCA may be critical for interspecies extrapolation of toxicity values. However, neither the Keys et al. (2004) mouse model, nor the human PBPK model described below account for age-related differences in DCA metabolism.

Due to pharmacological uses for DCA, considerable human pharmacokinetic data are available for this compound, including serum concentration measurements in clinical trials and in patients prescribed DCA. Li and coworkers developed a human PBPK model for DCA using the Keys et al. (2004) mouse/rat model as a starting point (Li et al., 2008). The model additionally incorporates DCA binding to plasma proteins and first-order elimination from the kidney compartment. Oral absorption is modeled with two sequential GI compartments (there are assignments to specific GI compartments in the model). Physiological and chemical-specific parameters are from previously published reports, and when human values are not available,

mouse values are used instead. Sex-specific parameter values are given, as well as sex-averaged values for use with datasets in which the subject's sex is not specified. The model has a duplicate structure to separately describe radiolabeled [¹²C]DCA and [¹³C]DCA in order to incorporate the available isotope data (Schultz and Shangraw, 2006) into the model calibration protocol. The two absorption constants, the maximal rate of metabolism and the enzyme inhibition constant served as optimization parameters to calibrate the model to available pharmacokinetic data. The average optimized value for the maximal rate of DCA metabolism was 109.9 mg/h·kg^{0.75}. Twenty-three PK data sets were used for model validation, and generally, predicted values agreed well with the reported plasma concentrations.

Schultz and colleagues developed a rat PBPK model for DBA, which demonstrated an overall similarity with DCA toxicokinetics including little direct urinary excretion and primarily hepatic DBA metabolism with suicide inhibition of the metabolizing enzyme GSTZ1 (Schultz et al., 1999; Matthews et al., 2010). The DBA model distinguishes the liver tissue (and its capillary space, as a separate block), kidney tissue (plus capillary space) and kidney tubule, and other aggregated tissues (plus capillary space). Oral absorption is modeled through a separate stomach compartment. Two similarly sub-divided blocks are incorporated for the DBA metabolites glyoxylate and oxalate. GSTZ1-mediated DBA metabolism has a complex reaction structure, with four reaction steps leading to glyoxylate and a GST-independent DBA → glyoxylate conversion step. Physiological parameters are as previously published, and for some chemical-specific parameters (such as partition coefficients) DCA values are used. In all, 12 parameters were optimized in model calibration, including various reaction constants. Interestingly, the proposed reaction scheme does not include a Michaelis-Menten type reaction, and instead, DBA metabolism would occur as sequential irreversible reactions. At the validation step, the model appears to predict well the observed DBA blood concentration. However, it is possible that a simpler model (with fewer optimized parameters) would similarly describe the observed data.

No PBPK models have been reported for MCA and MBA.

Use of HAA PBPK Models in Risk Assessment

The use of PBPK models in risk assessment has been proposed to reduce uncertainties associated with interspecies and high-dose-to-low-dose extrapolations. PBPK models can also provide additional insights into the mechanism of action to inform the weight of evidence (WoE) component of the risk assessment process. Because of the availability of human and mouse models for both TCA and DCA, PBPK-assisted approaches were considered for use in risk assessment and PHG derivation for TCA and DCA.

The main limitation of the TCA PBPK model (as the sub-block of the Chiu and Ginsberg (2011) PCE model) is the lack of calibration or validation with human oral studies. As optimization of the mouse TCA PBPK model demonstrated (Chiu, 2011), oral absorption in mice decreased with dose, and adjusting for absorption appeared critical for successful model calibration. Since there are no available pharmacokinetic data on orally administered TCA in humans, one simply cannot know how accurate the human PBPK model would be in predicting plasma

concentrations from oral exposures. Additionally, the mechanism of action of TCA is unknown and the involvement of minor metabolites, such as those resulting from oxidative P450 metabolism, cannot be ruled out. The current TCA models do not account for TCA metabolism or pharmacokinetics of possible metabolites. With all these considerations, OEHHA decided against using the existing TCA PBPK models as part of the dose-response analysis of candidate critical studies.

The DCA PBPK models also have limitations. Unlike TCA, DCA undergoes extensive metabolism. Since it is not known whether the parent compound (DCA) or some of its metabolites (such as glyoxylate) would be responsible for toxic action, the available PBPK models, which do not include dedicated modules for any metabolites, may not fully account for toxicokinetic differences underlying the mechanisms of DCA toxicity. Additionally, the extent of suicide inhibition of DCA-metabolizing GSTZ1 appears to be age-dependent and species-specific. Since neither human nor mouse PBPK models account for age-dependent differences in metabolic self-inhibition, these unaccounted for differences may further amplify with dose extrapolation between models, leading to increased uncertainty. Based on these considerations, OEHHA decided against using PBPK models for DCA risk assessment, at least not at the current state of knowledge. Further PBPK studies are required to clarify DCA metabolism and interspecies differences.

4. HUMAN EPIDEMIOLOGY STUDIES ON DISINFECTION BYPRODUCTS

Reproductive and Developmental Effects

A large number of human epidemiologic studies have examined the association between exposure to DBPs and reproductive outcomes. Most of these have focused on THMs, although a smaller number have specifically examined haloacetic acids (HAAs). OEHHA searched PubMed and Embase for all human epidemiologic studies related to DBP exposure and reproductive outcomes.

The search included any epidemiologic study on DBP exposure and a reproductive outcome that presented some metric of an association (e.g., relative risk estimate, mean difference, etc.). This comprised case-control, cohort, cross-sectional, and ecologic studies. Case reports were not reviewed because they generally do not include unexposed or lesser exposed comparison groups. No language restrictions were used in the search, and studies published up to 4/26/2018 were included.

This review included epidemiologic studies that have exposure information through drinking water, work-related activities (e.g., laboratory work), showering or bathing, and/or swimming. Studies that only provided results for tap water intake or time spent showering, bathing, or swimming, without some measurement or estimate of DBP exposure levels or chlorination status, were not included in this review. In vitro studies, studies of cord blood DNA methylation, micronuclei, or chromosomal aberrations were also not reviewed. A few conference abstracts involving studies of DBP exposures and reproductive outcomes were identified, however, none of these provided sufficiently detailed information that allowed evaluation of the study's strengths and weaknesses and therefore were also not included.

The bibliographies of the studies meeting the inclusion criteria described above and of relevant review articles and reports were also searched. A diagram of the literature search results is included in Appendix B. Details of the designs and results of the studies meeting these criteria are provided in Appendix B, Tables B1-B9.

Previous Reviews

Several comprehensive reviews have been published on DBPs and reproductive outcomes, with most concluding that either no or only weak or suggestive associations exist. These reviews have not focused specifically on HAAs, but rather on THMs or on DBPs in general. With regard to overall DBP exposure, an extensive review by Graves et al. (2001) concluded that, "The weight of the evidence demonstrated that no association with DBP exists for over a dozen outcomes including low and very low birth weight, preterm delivery, some specific congenital abnormalities, and neonatal death." However, the authors did note that there was evidence to suggest a positive association with some measures of growth retardation such as small for gestational age (SGA) and congenital anomalies of the urinary tract. In a 2009 review, Nieuwenhuijsen et al. (2009a) concluded that there was "some evidence" for an association between DBPs, specifically THMs, and SGA/intrauterine growth retardation, and to a lesser extent, preterm birth, but found the evidence for low birth weight, stillbirth, congenital anomalies,

and semen quality to be inconsistent or inconclusive. A more recent review by Villanueva et al. (2015) noted the difficulties in assessing DBP exposures in human studies and concluded, "...there is no clear evidence linking exposure to DBPs and reproductive outcomes, with the exception of a slight association with fetal growth related outcomes and sporadic associations with some categories of congenital anomalies." In a 2010 meta-analysis of human epidemiologic studies, Grellier et al. (2010) found no associations between total trihalomethane (TTHM) exposure during pregnancy and low birth weight or preterm birth. A statistically significant association was identified for SGA although the effect size was small (summary odds ratio (OR) = 1.01 per each 10 µg/L increase in TTHM exposure; 95% confidence interval (CI): 1.00-1.02; n = 6 studies). The authors found no evidence of publication bias based on Egger's test, but noted that because of the relatively small number of studies (six or fewer in each analysis), the "robustness of this test was limited." A 2009 meta-analysis of DBPs and congenital malformations reported statistically significant associations for "any congenital anomaly" (summary relative risk (RR) estimate = 1.17; 95% CI: 1.02-1.34) and for ventricular septal defects (summary RR = 1.59; 95% CI: 1.21-2.07) although the number of studies in both analyses was small (five and three, respectively) (Nieuwenhuijsen et al., 2009b). Statistically significant associations were not seen for other organ sites including major cardiac defects (summary RR = 1.16; 95% CI: 0.98-1.37; n = 8 studies) and urinary tract defects (summary RR = 1.33; 95% CI: 0.92-1.92; n = 4 studies).

Review of Potential Strengths and Weaknesses

The lack of consistent or convincing associations in the epidemiologic studies of DBP exposures and reproductive outcomes to date could indicate that true associations do not exist. However, this inconsistency or lack of convincing evidence could also be related to various biases, confounding, or other weaknesses in the design, implementation, or analysis of these studies.

One critical aspect is exposure assessment, and a number of study design issues may have introduced errors into this process. In most studies, the primary metric of exposure was the estimated DBP levels in the study subjects' residential drinking water. While some studies collected and incorporated additional data on whether the residential tap water was actually consumed, how much was consumed, and data on other sources or determinants of exposure (e.g., swimming, bathing, showering, water filter use, bottled water use, or water use away from the home), most studies did not. Very few studies assessed water temperature, shower size, or ventilation, factors that can also impact exposure. A number of studies assessed exposure based on the maternal residence at the time of birth and assumed this was the exposure throughout pregnancy regardless of whether a woman may have changed residences or water sources during pregnancy. The large majority of studies also relied on exposure information collected as part of ongoing regulatory requirements or other routine surveillance, usually performed by the drinking water providers themselves. In most cases this testing occurred relatively infrequently (e.g., annually or quarterly) and at only a limited number of sites in any given water distribution system. Given this limited testing, spatial or temporal (e.g., seasonal) variation in DBP levels within these systems could be another source of misclassified exposure. A number of studies, especially those involving very large cohorts, relied solely on the addresses of the subjects that were listed on either birth certificates or in other medical records.

In these studies, these addresses could potentially be incorrect or some subjects may not have lived at the same address throughout their entire pregnancy.

Each of the issues discussed above may have resulted in inaccurate or misclassified exposure information (Villanueva et al., 2007a). Since all of the studies reviewed appear to have assessed DBP exposures using the same methods in people with and without the adverse reproductive outcomes being studied, these errors would most likely bias study results towards the null and therefore could lead to true associations being missed.

Many studies involved personal interviews with the study subjects. Personal interviews may allow researchers to collect more thorough and valid information on the actual amount and source of water consumed and used. However, the study participants' ability to accurately recall water use and other exposure determinants may differ among those with and without the adverse pregnancy event under study, especially if collected after the adverse event occurred. This type of differential recall could artificially inflate measures of association (Hertz-Picciotto, 1991; Rockenbauer et al., 2001).

Widely accepted biomarkers of DBP exposure for use in epidemiologic studies have not been developed. The use of THM levels in blood for epidemiologic studies is limited by their very short half-life (Savitz, 2012). A few studies used urinary TCA as a biomarker of DBP exposure, although questions or issues regarding the degree to which this marker correlates with or represents other DBP agents, the limited sensitivity of current laboratory methods, and the impact on TCA levels caused by other chemical exposures limits the usefulness of this particular biomarker for human studies (Savitz, 2012).

Another potentially critical issue is confounding (discussed in greater detail in Appendix F). Failure to account or adjust for factors known to cause adverse reproductive outcomes, including smoking, second hand smoke, and alcohol consumption could cause erroneous results. This is especially true if these factors are also strongly related to DBP exposure. As detailed in Appendix Tables B1-B9, most of the studies reviewed attempted to control or adjust for at least a few of these potential confounding factors. In many studies, the source of the information on these factors was from birth certificates or other medical records, and the accuracy or thoroughness of these sources was not evaluated. As noted in Tables B1-B9, in a few studies statistical adjustments for potential confounders led to large differences between the adjusted and unadjusted results. This is usually indicative of marked confounding in the unadjusted results. It does not necessarily invalidate study findings but does raise concerns about residual or remaining confounding in the adjusted results. In other studies, adjusted and unadjusted relative risk estimates were similar, providing at least some evidence that major confounding by the adjustment factors was not a significant problem. Overall, only a few studies provided detailed information on all potentially important confounding variables, and most importantly on whether these variables were related to both the primary exposure (i.e., DBP exposure) and primary outcome (i.e., adverse reproductive outcome) of interest. OEHHA reviewed the potential impact of confounding due to smoking and alcohol consumption, and found that while these two factors were associated with several adverse reproductive outcomes, their association with DBP exposure varied from study to study. In general, this evaluation

suggested that confounding by smoking was unlikely to cause relative risk estimates greater than 1.10, although greater confounding in some studies could not be ruled out. The impact of most other potential confounders is likely to be smaller than this, although again, greater impacts could not be ruled out.

Another concern is the issue of correlated exposures. DBPs can include a large number of individual chemical species, and in many water sources these species are highly correlated with each other. For example, in a case-control study in Nova Scotia and Ontario, Canada, (King et al., 2005) reported that the correlation between total HAA and THM tap water concentrations was 0.81 (p-value not provided). In a large cohort study of DBP exposure and ovarian cancer in Iowa, Inoue-Choi et al. (2015) reported similarly high correlations between HAA and THM water levels (Table 4.1). Overall, these high correlations make it difficult to separate out the effects of any given individual chemical, and therefore it is difficult to make any firm conclusions regarding their individual toxicities.

Table 4.1 Spearman correlation coefficients (r) between water DBP concentrations in the Iowa Women’s Health Study (Inoue-Choi et al., 2015).

	TTHM	Chloroform	BCA	BDCM	DCA	HAA5
Chloroform	0.98	-				
BCA	0.82	0.76	-			
BDCM	0.97	0.95	0.82	-		
DCA	0.79	0.81	0.67	0.72	-	
HAA5	0.89	0.91	0.75	0.84	0.93	-
TCA	0.92	0.90	0.81	0.93	0.71	0.87

BCA, bromochloroacetic acid; BDCM, bromodichloromethane; DCA, dichloroacetic acid; HAA5, haloacetic acid5, sum of monochloroacetic acid, trichloroacetic acid, dichloroacetic acid, monobromoacetic acid, and dibromoacetic acid; TCA, trichloroacetic acid; TTHM, total trihalomethanes

Two other issues to be considered in the interpretation of the results in Tables B1-B9 are the problem of multiple comparisons and the possibility of selection bias. Several studies tested for associations between a large number of different outcomes and a large number of different exposure variables. For example, Hwang et al. (2008) examined associations between THM levels at the municipal level and 14 different congenital anomalies. Similarly, the prospective cohort study by (Grazuleviciene et al., 2013) reported relative risk estimates for three different outcomes (congenital heart abnormalities, congenital musculoskeletal abnormalities, and congenital urogenital abnormalities), four different exposures (TTHMs, chloroform, BDCM, and DBCM), and four different time periods (the first, second, and third months and the third trimester of pregnancy). Testing for multiple associations like this is appropriate in some situations, but does raise the likelihood that statistically significant findings will occur simply due to chance (Rothman, 1990). On the other hand, if a particular outcome is consistently linked to the exposure of interest in multiple studies, whether or not there are multiple associations evaluated in the studies, then it increases the likelihood that the association between the exposure and that specific outcome is real.

A large number of studies in Tables B1-B9 used population-based methods for selecting study subjects, and had good response or participation rates. One particular area of concern however relates to the selection of subjects for the studies of semen quality. In most of these studies, subjects were recruited from among patients seeking care in fertility clinics. Although it was not known whether the male or their partner were the source of the fertility issue, pre-existing fertility problems unrelated to DBP exposure in the male study subjects could potentially mask any true adverse impacts of DBP exposure in these men.

Summary of Study Results

Haloacetic Acids

For outcomes related to fetal growth, including low birthweight, SGA, and intrauterine growth retardation (IUGR), four studies found no associations and five reported weak or modest evidence for a positive association (Table B1). The case-control study by Levallois et al. (2012) reported an OR of 1.4 (95% CI: 1.1-1.9) comparing HAA5 concentrations >60 µg/L to concentrations <60 µg/L. The test for a linear dose-response trend based on the Wald X² test gave a statistically significant result (p = 0.03), although a U-shaped dose-response pattern was seen in the categorical analysis (ORs of 1.00 (reference), 1.2 (95% CI: 0.9-1.6), 1.0 (95% CI: 0.7-1.3), and 1.4 (95% CI: 1.0-1.8) for HAA5 concentrations of <12.72, 12.72-21.35, 21.36-39.59, and >39.59 µg/L, respectively). Some evidence of an association was also seen for TCA and DCA water concentrations in this study. In Hinckley et al. (2005), statistically significant associations and dose-response trends were identified for specific HAA species (i.e., DCA, TCA, and DBA) for either IUGR or low birthweight, although ORs in the highest categories of exposure were somewhat low (e.g., below 1.5). Overall, the epidemiologic data on HAAs and reproductive outcomes related to fetal growth are mixed, with a few studies reporting modest evidence of an association but without overall consistent findings for any given outcome or any individual HAA chemical species.

The epidemiologic findings on HAAs and other reproductive outcomes such as congenital malformations, preterm birth, and sperm quality are also mostly mixed (Table B2). In a case-control study, Wright et al. (2017) identified a fairly strong association between HAA5 water concentrations and Tetralogy of Fallot, but this outcome has not been examined in any other HAA study. Four studies examined HAAs and preterm birth with none identifying clear evidence of an association. Four studies examined sperm quality. Although two of these found some evidence of an association, lack of clear dose-response relationships (Zeng et al., 2014a) or co-existing THM exposures (Zeng et al., 2016) limit their interpretation. Some suggestive evidence on time-to-pregnancy (MacLehose et al., 2008) and stillbirth (King et al., 2005) has been reported but to date have not been replicated.

Trihalomethanes

The epidemiologic data on THM exposure and low birthweight or birthweight as a continuous variable are also mixed, with approximately equal numbers of studies finding associations and finding no associations (Table B3). Using information on quarterly water monitoring of THM concentrations, combined with personal interview data on water consumption, showering, and

bathing, the prospective cohort study by Grazuleviciene et al. (2011) reported ORs of 1.00 (reference), 1.77 (95% CI: 0.95-3.30), and 2.13 (95% CI: 1.17-3.87) for estimated TTHM uptakes of 0.0025-0.0386, 0.0386-0.3545, and 0.3545-2.4040 mg/day. They also reported an OR of 1.08 (95% CI: 1.01-1.16) for each 0.1 µg/day increase in TTHM uptake. Data on swimming pool attendance were also collected but it does not appear this information was used in the exposure assessment. The authors reported that only 7% of participants attended swimming pools. They also reported that showering and bathing were the main contributors of estimated TTHM uptakes and made up 92% of the total estimated internal dose. Uptake via oral ingestion contributed only 8%. The authors also noted that variability in the frequency and duration of showering and bathing was the main determinant of the variability in the estimated TTHM internal doses although the ranges for the TTHM internal doses from these two sources were not provided.

Several other studies identified at least some evidence of an association with birthweight or low birthweight, although most did not involve personal subject interviews to confirm water sources, estimate water consumption, or collect information on other water use habits. These findings support those of Grazuleviciene et al. described above. However, two other fairly recent high quality prospective cohort studies (Villanueva et al., 2011; Kogevinas et al., 2016) with personal information on exposure, large sample sizes, and data on multiple potential confounders found no evidence of an association between THM exposures from water and birthweight or low birthweight.

Twelve of the 16 studies (75%) reporting data for THM exposures and SGA found some evidence of an association. However, all had at least one study design weakness (e.g., a lack of personal data on water sources and water use habits), limited data on potential confounding, or inconsistent or weaker findings (e.g., dose-response patterns that were not consistent with other studies, ORs only slightly above 1.0, or findings that were not statistically significant). Relative risk estimates (like ORs) that are only slightly above 1.0 may represent true associations. However, all else being equal, they are also more likely to be solely due to relatively smaller amounts of bias or confounding than relative risk estimates that are much further away from 1.0 (Axelson, 1978; Schlesselman, 1978). Only two studies found fairly clear evidence that THM exposures were not associated with SGA, including the high quality prospective cohort study by Villanueva et al. (2011). For IUGR or fetal growth retardation (FGR), two studies reported weak evidence for an association and two studies found no evidence of an association. For preterm birth, usually defined as birth before gestational week 37, the very large majority of studies found no evidence for an association (Table B4).

Four of the eight epidemiologic studies involving some form of pregnancy loss (e.g., spontaneous abortion (SAB), stillbirth, or neonatal death) found at least some evidence for an association with THM exposures (Table B5). Two of these positive studies assessed stillbirths, one assessed SABs, and for one the outcome was defined simply as “pregnancy loss.” The prospective cohort study by Waller et al. (1998) reported an OR of 2.0 (95% CI: 1.1-3.6) for spontaneous abortions for THM water concentrations ≥ 75 vs. < 75 µg/L among those drinking at least 5 glasses of cold tap water per day. The case-control study by Savitz et al. (1995) also

assessed THM exposures and SAB although results were inconsistent across various analyses. Several studies of pregnancy loss reported results that were difficult to interpret (e.g., only small increases in relative risk, unclear dose-response patterns, or inconsistent results across different exposure metrics). As a whole, the current epidemiologic literature provides some suggestive evidence for a relationship between THM exposure and pregnancy loss, although further confirmatory studies are needed.

As shown in Table B6, several studies have reported statistically significant associations between THM exposures and a number of specific congenital abnormalities including neural tube defects, central nervous system defects, cardiac abnormalities, and cleft lip or palate. However, for each of these outcomes, a number of studies reported finding no associations. For example, four studies reported relative risk estimates of 1.8 or higher for neural tube defects while three others reported relative risk estimates close to or below 1.0. Similarly, for cardiac defects, while five studies reported relative risk estimates that were statistically significant or greater than 1.5, five other studies reported estimates below 1.0. Clear differences in study quality or differences in exposure levels or other scenarios that might explain these inconsistent results are not obvious. Overall, the inconsistency in the results across studies for any given organ anomaly prevents any firm conclusions from being made regarding a causal link between THM exposure and congenital malformations.

Several studies have assessed the relationship between THM exposures and sperm quality (Table B7). In a cross-sectional study by Zeng et al. (2016), the OR for having a low sperm concentration (<20 million/ml) was 6.35 (95% CI: 1.83-22.06) in subjects with both an elevated blood TTHM concentration (above the median of 54.70 ng/L) and an elevated urinary TCA concentration (above the median of 8.42 µg/L) compared to those with both metrics below these levels. Similar findings were also seen for low sperm counts (<40 million). The ORs in those with only an elevated TTHM level was 2.97 (95% CI: 0.81-10.87), suggesting that THMs were at least partially responsible for some of this association. Other studies have found less clear results. For example, sperm count and concentration were not associated with THM exposures in the US prospective cohort study by Luben et al. (2007). Overall, although several positive associations have been reported between THM exposures and sperm quality, the specific outcomes for these positive associations (e.g., count vs. morphology vs. motion) are not consistent from study to study. Given the inconsistencies across studies, firm conclusions regarding the relationship between THM exposure and sperm quality cannot be made at this time.

Studies of THM exposure and other reproductive outcomes including menstrual cycle length, time-to-pregnancy, and premature rupture of membranes are described in Table B8. None of these studies provides convincing evidence of adverse effects on these reproductive outcomes related to THM exposure. Studies assessing DBP exposures other than HAAs and THMs are shown in Table B9. The majority of these simply assessed whether a particular water treatment process (e.g., chlorination) was used in the water systems being studied. As such, these studies did not account for the possibility that DBP concentrations may vary within a water system, and their results could not be used to evaluate whether a particular chemical species

might be responsible for the majority of any association identified. In almost all of these studies, no personal data were collected to help confirm that the subjects actually drank the water or to help estimate how much they drank. Overall, consistent evidence was not seen between drinking water chlorination and preterm birth or low birthweight outcomes. Two of the studies listed in Table B9 investigated chloroform exposures in the workplace. While one of these reported a potential association with spontaneous abortions (OR = 2.3, 95% CI: 0.9-5.9) in female laboratory workers, the presence of co-existing chemical exposures in these workers or the reliance on self-reported exposure data limits the interpretation of this finding (Wennborg et al., 2000).

Conclusion

In conclusion, although a large number of human epidemiologic studies have been performed on various DBP exposures and a large number of different adverse reproductive outcomes, consistent and convincing associations have not been seen for most of them. Exceptions might include SGA, and possibly birth weight and low birthweight where a number of studies provided at least some evidence linking THM exposures with these outcomes. Importantly though, most of these studies had at least one design feature or weakness that limits their usefulness in defining clear dose-response relationships. For example, only a minority of the studies collected personal data on water consumption or other water use habits like showering or bathing. Of those that did, weaknesses included dose-response patterns that were not consistent from study to study and low statistical power (e.g., elevated ORs that were not statistically significant). In some studies dose-response patterns appeared to be monotonic or close to monotonic, in others they were U-shaped (relative risk estimates were greater in the lower and higher exposure categories than in the middle exposure categories), and in others they were λ -shaped (relative risk estimates were greater in the middle exposure categories than in the lower or higher exposure categories). Issues of confounding (Appendix F), correlated exposures, and multiple comparisons also limited OEHHA's ability to use these data to quantify risks associated with any given individual DBP species.

Cancer Effects

A large number of human epidemiologic studies have examined the association between exposure to DBPs and cancer incidence or mortality. Many of these have focused on THMs, but analyses of these agents are complicated by the coexistence of the other DBPs, including HAAs. Studies published prior to 1985 have been critically reviewed elsewhere (Wilkins et al., 1979; NRC, 1980; US EPA, 1985; Cantor et al., 2010). In its 1985 review of chloroform, 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. The IARC (2013) review of the epidemiologic 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 (US EPA, 2006), stated 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 considered 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. A diagram of the literature search results for cancer is included in Appendix C. 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 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 Table C3.

Summary of Study Results

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, 1996). 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. (2016). 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 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 (Morris et al., 1992; Villanueva et al., 2003a; Villanueva et al., 2004; Villanueva et al., 2006; Costet et al., 2011). 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) 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 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.

Conclusion

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 DBP 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.

5. TOXICOLOGICAL PROFILE: MONOCHLOROACETIC ACID

Acute Toxicity

Due to its strong acidic properties ($pK_a = 2.87-2.97$, Table 1.1), acute exposure to concentrated monochloroacetic acid (MCA) results in corrosive damage at the site of contact (skin, airways or GI tract). Such irritant acute effects of concentrated MCA are not expected to occur when individuals are exposed to environmentally relevant concentrations of MCA.

Effects in Humans

The acute health effects of MCA discussed in this section are primarily obtained from reports of accidental dermal or oral exposures to concentrated aqueous or solid MCA.

Concentrated or molten MCA is highly corrosive to tissues and can cause serious complications and even death following dermal or ingestion exposure. Exposures to MCA have resulted in neurological symptoms including convulsions and loss of consciousness as well as cardiovascular irregularities including tachycardia, hypotension, and abnormal electrocardiograph (Millischer et al., 1987; Kusch et al., 1990; Chapman et al., 2006) as cited in (WHO, 2004d). Severe cardiotoxic effects have also been reported in fatal poisoning cases (Kulling et al., 1992; Rogers, 1995; Pirson et al., 2003; Nayak et al., 2007).

Hospitalization has been recommended for individuals with as little as 10% of the skin exposed to technical grade formulations (Kusch et al., 1990; Kulling et al., 1992)). The estimated lethal oral dose of MCA is 50 to 500 mg/kg for an adult, or about one to six teaspoons for a 70 kg person (Pohanish, 2002; NIH, 2005). The one-minute irritation threshold for acute inhalation exposure is 5.7 mg/m³ or 1.48 ppm (Maksimov and Dubinina (1974) and Rodionova and Ivanov (1979), as cited in NAS (2009)). No data were found on effects of acute inhalation of MCA in industrial or accidental exposures.

Localized effects occur because highly concentrated MCA can hydrolyze protein and result in inflammation and tissue destruction (Chapman et al., 2006). It is an irritant to the skin, mucous membranes, eyes, and lungs (O'Neil et al., 2001). Ingestion of MCA may cause gastrointestinal irritation, ulcerations or burns, perforation, necrosis, and subsequent peritonitis (Pohanish, 2002).

Effects in Animals

In LD₅₀ experiments, un-neutralized MCA was more toxic than neutralized MCA (Maksimov and Dubinina (1974) as cited in NAS (2009)), which in turn was more toxic than neutralized dichloroacetic or trichloroacetic acid (Woodard et al., 1941). An overview of LD₅₀ studies for different species and routes of exposure is presented in Table 5.1.

Table 5.1 Summary of LD₅₀ studies of MCA

LD ₅₀	Species	Route	Reference
<i>Studies of neutralized MCA</i>			
76 mg/kg	Rat	Oral gavage	Woodard et al. (1941)
70-100 mg/kg	Rat	Intravenous injection	Saghir et al. (2001)
80 mg/kg	Guinea pig	Oral gavage	Woodard et al. (1941)
165 mg/kg	Mouse	Oral gavage	Morrison (1946) as cited in NAS (2009)
255 mg/kg	Mouse	Oral gavage	Woodard et al. (1941)
269 mg/kg	Mouse	Intraperitoneal injection	Le Poidevin (1965)
580 mg/kg	Rat	Oral gavage	Maksimov and Dubinina (1974) as cited in NAS (2009)
<i>Studies of un-neutralized MCA</i>			
5 mg/kg	Rat	Subcutaneous injection	As cited in Lewis (2004) ^a
55 mg/kg	Rat	Oral gavage	Maksimov and Dubinina (1974) as cited in NAS (2009)
55 mg/kg	Rat	Intravenous injection	As cited in Lewis (2004) ^b
98-130 mg/kg	Rat	Oral gavage	(NTP, 1992)
180 mg/m ³	Rat	Inhalation	As cited in Lewis (2004) ^b
120 mg/kg (LD ₂₀)	Rat	Dermal	Saghir and Rozman (2003)
225 mg/kg (LD ₂₀)	Rat	Oral gavage	Saghir and Rozman (2003)
226 mg/kg	Mouse	Oral gavage	NTP (1992)
250 mg/kg	Mouse	Subcutaneous injection	WHO (2004d)
<i>Studies of unknown treatment of MCA</i>			
60 mg/kg	Rat	Subcutaneous injection	Hayes et al. (1973)
260 mg/kg	Mouse	Drinking water	Berardi et al. (1987)

^a Abstracts of papers for the Eleventh Annual Meeting of the Society of Toxicology, Williamsburg, Virginia March 5–9, 1972. *Toxicol Appl Pharmacol* 22: 303 as cited in Lewis (2004).

^b *Gigiena Truda i Professional'nye Zabolevaniya*. Labor Hygiene and Occupational Diseases V.18 (9) p.32 (1974) as cited in Lewis (2004).

A 2009 report by the National Academy of Sciences (NAS) provides a detailed review of studies in mice and rats that reported neurological effects from acute exposures to MCA at doses close to the LD₅₀, noting mobility problems, tremors, convulsions, and seizures among observed effects (NAS, 2009). The mechanism of action of these neurotoxic effects is presumed to involve damage to the blood-brain barrier and inhibition of reactions in the Krebs cycle. MCA blocks the Krebs cycle by interfering with ATP formation and gluconeogenesis (NTP, 1992). The reaction of MCA with sulfhydryl groups in enzymes can cause severe tissue damage in energy-rich organs like the brain, liver, and kidney (Hayes et al., 1973; Bryant et al., 1992; NTP, 1992). Reduction in cholinesterase levels may also explain some of the neurotoxic properties associated with MCA exposure (NTP, 1992).

Selected acute or short-term oral studies of MCA are listed in Table 5.2.

Table 5.2 Summary of acute or short-term oral studies of MCA

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male Swiss-Webster mice (8-10 mice for acute toxicity studies, 6 for blood-brain barrier damage studies)	0, 80, 118, 174, 257, 380 mg/kg un-neutralized MCA once orally	Neurological dysfunction	NOAEL: 174 mg/kg-day	Berardi et al. (1987) as cited in US EPA (1994a)
Male Swiss-Webster mice (8-10 mice for acute toxicity studies, 6 for blood-brain barrier damage studies)	0, 380 mg/kg once orally	Blood brain barrier damage	LOAEL: 380 mg/kg-day	Berardi et al. (1987) as cited in US EPA (1994a)
Male/Female Sprague-Dawley rats (3-8/sex/dose)	0 or 188 mg/kg (males); 0 or 94 mg/kg (females) neutralized MCA by oral gavage	Decreased bile production and glomerular filtration rate in females	LOAEL: 94 mg/kg-day	Davis and Berndt (1992)
Male/Female B6C3F ₁ mice (5/sex/dose)	0, 11, 21, 43, 86, 171 mg/kg-day (males); 0, 21, 43, 86, 171, 343 mg/kg-day (females) un-neutralized MCA by oral gavage for 16 days	Lacrimation in females	NOAEL: 43 mg/kg-day	NTP (1992)
Male/Female F344/N rats (5/sex/dose)	0, 5, 11, 21, 43, 86 mg/kg-day un-neutralized MCA by oral gavage for 16 days	Nasal discharge	LOAEL: 5 mg/kg-day	NTP (1992)

Subchronic Toxicity

Effects in Humans

No adverse effects in three human volunteers were reported after daily oral exposure to approximately 2.1 mg/kg-day of MCA in water for 60 days (Morrison and Leake (1941) as cited in NAS (2009)).

Effects in Animals

Subchronic exposure of laboratory animals to neutralized or un-neutralized MCA by oral gavage or in drinking water caused a wide array of changes in serum biochemistry, which were indicative of organ toxicity. Organ weight changes and inflammatory processes were also consistently observed. Key organs affected are the heart, liver, and kidney, as summarized in Table 5.3. The lowest subchronic LOAEL of 15 mg/kg-day is identified for significant changes in clinical chemistry indicative of adverse liver and heart effects.

In finding the appropriate dose range for chronic studies (NTP, 1992), groups of 20 rats of both sexes were exposed by oral gavage to doses of 0, 21, 43, 64, 86, or 107 mg/kg-day for 13 weeks. All male and female rats died at the doses greater than 86 mg/kg-day. All rats except one male died at the 64 mg/kg-day dose. Acute or subacute cardiomyopathy was considered the cause of death in these animals. MCA-related cardiomyopathy was observed in all but the lowest dose group. At doses of 43, 86, and 107 mg/kg-day, male and female rats had increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, which is indicative of hepatocellular or cardiac muscle damage. Cholinesterase levels were significantly decreased at doses higher than 21 mg/kg-day, a sign of neurotoxicity.

Groups of 20 mice in both sexes were exposed by oral gavage to doses of 0, 18, 36, 71.5, 107, and 143 mg/kg-day for 13 weeks (NTP, 1992). All males and two females at the high dose died. Cholinesterase was significantly decreased in females at ≥ 107 mg/kg-day.

Male and female Sprague-Dawley rats (10 animals/sex/dose) were exposed by oral gavage to 0, 15, 30, 60 or 120 mg/kg-day neutralized MCA for 90 days (Daniel et al., 1991). Mortality was observed in the highest dose in males and females. There were significant relative liver and kidney weight increases in males and females at 60 mg/kg-day. There was a significant increase in ALT levels in males at the lowest dose. Increased incidence of heart inflammation was found in males and females with a significant trend; however, there was a high background rate in the male control group and no statistical significance in pairwise analysis for both sexes. Chronic kidney nephropathy and spleen pigment was significantly increased in males at 60 mg/kg-day.

Male Sprague-Dawley rats were exposed to 0 or 18.5 mg/kg-day of neutralized MCA in drinking water for 90 days (Bhat et al., 1991). MCA treated rats exhibited increased absolute liver weights. Collagen deposition and portal vein dilation was observed in the liver of MCA treated rats with severity ranging from minimal to moderate.

Table 5.3 Summary of subchronic studies of MCA

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL ^a	Reference
Male/Female Sprague-Dawley rats (10/sex/dose)	0, 15, 30, 60, 120 mg/kg-day neutralized MCA by oral gavage for 90 days	Heart inflammation ^b F: 1/10, 1/10, 3/10, 3/10, 4/7 M: 4/10, 5/9, 6/10, 7/9, 2/2 Increased relative liver and kidney weight (males and females); increased spleen pigment and chronic nephropathy (males) Increased ALT (males) Mortality at highest dose (males and females)	LOAEL: 15 mg/kg-day (increased ALT in males)	Daniel et al. (1991)
Male Sprague-Dawley rats (5/dose)	0 or 18.5 mg/kg-day neutralized MCA in drinking water for 90 days	Hepatic collagen deposition (4/5 animals) and portal vein dilation (4/5 animals); increased inflammation of liver and lungs	LOAEL ^c : 18.5 mg/kg-day	Bhat et al. (1991)
Male/Female F344/N rats (20/sex/dose)	0, 21, 43, 64, 86, 107 mg/kg-day un-neutralized MCA by oral gavage for 13 weeks	Increased relative liver and kidney weight (males); decreased relative heart weight (females); decreased plasma cholinesterase (males) Cardiomyopathy (males, females); Increased ALT and AST; decreased plasma cholinesterase (females) Mortality due to acute or subacute cardiomyopathy in all animals but one male at ≥64 mg/kg-day	LOAEL: 21 mg/kg-day (cardiomyopathy, increased relative liver and kidney weight in males, decreased relative heart weight in females. decreased plasma cholinesterase)	NTP (1992)

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL ^a	Reference
Male/Female B6C3F1 mice (20/sex/dose)	0, 18, 36, 71.5, 107, 143 mg/kg-day un-neutralized MCA by oral gavage for 13 weeks	Decreased plasma cholinesterase (females) Mortality in all males and two females at highest dose; hepatocellular vacuolization in males and one female that died	NOAEL: 71.5 mg/kg-day (decreased plasma cholinesterase)	NTP (1992)

^a Only the lowest NOAEL and/or LOAEL for each study are presented. NOAELs and LOAELs are not identified for single-dose studies.

^b No pairwise significance compared to controls but significant trend in both sexes.

^c This LOAEL was identified from a single-dose study.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable.

Genetic Toxicity

The evidence of genetic toxicity of MCA is mixed, with several reports showing some genotoxicity, and other reports finding none. The data are presented below in Table 5.4. Within each table, studies are organized by experimental system (e.g., human studies before animal studies, primary cells before cell culture), and then by type of genotoxic effect.

No human in vivo studies were located. Several available MCA genotoxicity studies in human lymphocytes and human culture cells were positive for genotoxicity effects. The Comet assays, which measure DNA damage, were positive in human and animal cell lines. The results of DNA alkaline unwinding assays were negative (Change et al., 1992). The bacterial assays with various strains of *S. typhimurium* were largely, although not entirely, negative; the discrepancy could be due to cytotoxicity (Rannug et al., 1976; Giller et al., 1997; Stalter et al., 2016). In in vitro genotoxicity studies, MCA was not specifically neutralized prior to application; however, exposures were performed in buffered media, which would effectively neutralize MCA at the concentrations examined.

Table 5.4 Genetic toxicity studies of MCA

Assay	Results Without S9	Results With S9	Concentration	Reference
Whole animal: DNA alkaline unwinding assay (single-strand breaks), liver, spleen, and stomach and duodenal epithelial tissues from male B6C3F1 mice	-	NA	Liver, spleen: 1 and 5 mmol/kg (94.5 and 472 mg/kg), stomach and duodenum cells: 10 mmol/kg (945 mg/kg), by oral gavage for 4 h	Chang et al. (1992)

Assay	Results Without S9	Results With S9	Concentration	Reference
DNA alkaline unwinding assay (single-strand breaks), hepatic tissue from male F344 rats	-	NA	1 mmol/kg (94.5 mg/kg), by oral gavage for 4 h	Chang et al. (1992)
Primary human cells: Chromosome aberrations assay in human lymphocytes	+	ND	1-1470 µM	Escobar-Hoyos et al. (2013)
SCGE (Comet) assay (genomic DNA damage) in human lymphocytes	+	ND	1– 2940 µM	Escobar-Hoyos et al. (2013)
Primary mammalian cells: DNA alkaline unwinding assay (single-strand breaks) in primary rat hepatocytes	+ (secondary to cytotoxicity)	-	1-10 mM for 4 h	Chang et al. (1992)
Human cell lines: DNA alkaline unwinding assay (single-strand breaks) in CCRF-CEM (human lymphoblastic) cells	+ (weak)	ND	1, 10 mM for 2 h	Chang et al. (1992)
SCGE (Comet) assay in human intestinal epithelial FHs cells (DNA strand breaks)	+	ND	1 – 6.5 mM (estimate from graph)	Attene-Ramos et al. (2010)
Mammalian cell lines: L5178Y tk ⁺ /tk ⁻ mouse lymphoma cell forward mutation assay	+	ND	0.05 – 0.8 mg/ml	(McGregor et al., 1987); NTP (1992)
Sister chromatid exchanges test in CHO cells	+	-	0.05 – 0.5 mg/ml (without S9) 0.05 – 1.6 mg/ml (with S9)	NTP (1992)
Sister chromatid exchanges test in CHO cells	+	-	0.05 – 0.5 mg/ml (without S9) 0.05 – 1.6 mg/ml (with S9)	Galloway et al. (1987)
Sister chromatid exchanges test in CHL cells	-	-	0.06 – 0.25 mg/ml	Sawada et al. (1987)

Assay	Results Without S9	Results With S9	Concentration	Reference
Chromosomal aberrations test in CHO cells	-	-	0.05 – 1.6 mg/ml	NTP (1992)
Chromosomal aberrations test in CHO cells	-	-	0.05 – 1.6 mg/ml	Galloway et al. (1987)
Chromosomal aberrations test in CHL cells	-	-	0.06 – 0.25 mg/ml	Sawada et al. (1987)
SCGE (Comet) assay in CHO cells	+	ND	0.1 – 1.0 mM	Plewa et al. (2002)
SCGE (Comet) assay (DNA repair) in CHO cells	+	ND	6 mM	Komaki et al. (2009)
Non-mammalian cells: Bacterial reverse mutation assay in <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	-	-	Up to 1 mg/plate	McCann and Ames (1976)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA1530, TA1535, G46	-	-	0.1 - 1 mg/plate	Bartsch et al. (1975)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA1535	-	ND	1-100 mM	Rannug et al. (1976)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	-	-	10 µg -3.3 mg/plate	NTP (1992)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA100 (Ames fluctuation test)	-	-	0.3 – 300 µg/ml (without S9) 0.03 – 10 mg/ml (with S9)	Giller et al. (1997)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA104 (Ames microsuspension test)	-	-	1 mg/ml	Nelson et al. (2001)

Assay	Results Without S9	Results With S9	Concentration	Reference
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA98 (Ames preincubation test)	+	-	2-28 mM	Kargalioglu et al. (2002)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA100 (Ames preincubation test)	+	+	2-25 mM	Kargalioglu et al. (2002)
Bacterial reverse mutation assay in <i>S. typhimurium</i> RSJ100 (Ames preincubation test)	-	-	2-26 mM	Kargalioglu et al. (2002)
SOS chromotest in <i>E. coli</i> PQ37	-	-	1 µg/ml – 3 mg/ml	Giller et al. (1997)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	+ (weak)	ND	0.3 – 16 mM	Zhang et al. (2016)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	-	-	Up to 8 mM	Stalter et al. (2016)
Newt micronucleus test in <i>P. waltii</i>	-	ND	10-40 µg/ml	Giller et al. (1997)
Sex-linked recessive lethal test in <i>D. melanogaster</i>	- (feeding) ± (injection)	ND	0, 400 ppm (feeding) 0, 900 ppm (injection)	NTP (1992)

NA, not applicable; ND, not determined

Developmental and Reproductive Toxicity in Animals

MCA does not appear to be a reproductive toxicant via the oral route. Bhat et al. (1991) exposed male Sprague-Dawley rats (5/dose) via drinking water for 90 days to 1.9 mmol/L of neutralized MCA, equivalent to a dose of approximately 19 mg/kg-day. No significant changes were observed in testes weight or morphology, or body, liver, and brain weight, relative to control animals. Toth et al. (1992) reported that a 10-week pilot study of neutralized MCA “revealed no significant reductions in reproductive organ weights, cauda epididymal sperm counts, sperm motility, or sperm motion parameters.”. No further details for the study, including the route and method of exposure, were provided. DeAngelo et al. (1997) exposed male Fischer 344/N rats to drinking water containing neutralized MCA (0, 3.5, 26, or 60 mg/kg-day) for 104 weeks, and found significantly increased relative but not absolute testicular weights, at 26 and 60 mg/kg-day. The authors postulated that the effects were more related to decreased

body weights than to a specific testicular effect of MCA and presented no discussion of potential reproductive effects. However, Bhunya and Das (1987) found an increased number of malformed sperm in male Swiss mice (3/dose) 35 days after i.p. injection with 25 or 50 mg/kg un-neutralized MCA, with no effects at 12.5 mg/kg, the lowest dose tested, or the control group. No animal studies were found on the reproductive effects of MCA on females.

Developmental toxicity of MCA has not been well studied. Johnson et al. (1998) administered 1,570 ppm neutralized MCA in drinking water to female Sprague-Dawley rats from gestation day (GD) 1 until one day before parturition, to evaluate cardio-developmental effects. Other endpoints were not examined and the dose was approximately 193 mg/kg-day. No increase in cardiac defects was observed in fetuses on GD 22 compared to the control group. The review of DPB toxicity by Graves et al. (2001) set a NOAEL of 193 mg/kg-day for no developmental cardiac effects in rats exposed to MCA during gestation based on Johnson et al. (1998).

Hunter et al. (1996) evaluated HAAs for developmental toxicity in vitro. Conceptuses of CD-1 mice at GD 9 were explanted and exposed to 0, 50, 100, 175, 250, 350, or 500 μ M un-neutralized MCA in buffered solution (tissue culture media) for 24-26 hours. Deaths occurred in 14/34, 10/10, and 10/10 embryos cultured at 250, 350, or 500 μ M MCA, respectively, and no deaths occurred in other dose groups, including control. At 175 μ M, 39% of embryos had neural tube defects versus 6% in the control (a statistically significant increase, $p < 0.05$), while heart and pharyngeal defects were 7% versus 0% in the control (not statistically significant). At 250 μ M, 70% of the 20 surviving embryos had malformations, including 65% with heart, 50% neural tube, and 40% pharyngeal arch malformations (all, $p < 0.05$). The authors ranked the HAA potencies as DCA < acetic acid < TBA < TCA < DBA < MCA < MBA. Thus, for this experiment, MCA was considered to be among the most potent of the HAAs for inducing developmental defects in vitro.

Immunotoxicity in Animals

No studies are available on direct immunotoxic effects of MCA, although some effects on immune function might be expected with the effects on spleen weight observed by DeAngelo et al. (1997), as discussed in the *Chronic Toxicity* section.

Neurotoxicity in Animals

Multiple neurotoxic effects were reported in acute animal studies at doses close to the LD₅₀ and in human case studies (as detailed in the *Acute or Short-term Toxicity* section). However, a 90-day oral gavage study by Daniel et al. (1991) did not report neurotoxicity effects in rats exposed to 15-120 mg/kg-day: "No such effects were seen in this gavage experiment nor was abnormal pathology noted in the microscopic examinations of brain and neural tissue." Other longer duration studies (Bhat et al., 1991; NTP, 1992; DeAngelo, 1997) did not examine the animals for neurotoxicity symptoms; however, no histopathology was seen in the brains of rats or mice (Bhat et al., 1991; NTP, 1992).

Chronic Toxicity

Effects in Humans

Human epidemiological evidence of reproductive and developmental effects of HAA exposure is mixed, and none of the studies identified an association of any of the studied outcomes with MCA exposure. These are described in detail in the section *Human Epidemiology Studies on Disinfection Byproducts* and Appendix B-C.

Effects in Animals

The NTP (1992) studies dosed F344/N rats and B6C3F1 mice of both sexes (53-60/sex/dose group) with un-neutralized MCA in ionized water five days a week for 104 weeks. The doses were 0, 11, and 21 mg/kg-day for rats, and 0, 36, and 71 mg/kg-day for mice. An additional 7-10 rats/dose group were designated for interim sacrifices at 6 and 15 months. Animals were observed for morbidity, mortality and clinical signs, and body weights were measured. Complete histopathology examination was performed for all animals in full term experiments. Body weights were slightly decreased (5% decrease) in high-dose male rats but not in any other rat group compared to controls. Survival was significantly decreased for high-dose male rats and high-dose and low-dose female rats. The differences in survival were “due to undetermined causes” (NTP, 1992). There were no changes in organ weights at 2 years, and some organ weight changes observed at 6-month interim sacrifice were not consistent with 15-month groups or the 13-week study (NTP, 1992). The NTP (1992) report notes, “No nonneoplastic lesions were associated with the administration of monochloroacetic acids to rats for 2 years. Although myocardial lesions occurred in dosed rats in the 13-week studies, the incidences of degenerative and inflammatory lesions of the heart in the 2-year studies were similar among dosed and control rats. ... any subtle effects might have been obscured by the development of spontaneous age-related degenerative changes.”

Nasal lesions observed in the 2-year studies in mice (NTP, 1992) likely resulted “from reflux of gavage solution rather than from a direct toxic effect of monochloroacetic acid.”

DeAngelo et al. (1997) exposed male F344/N rats (50/dose) to 0, 3.5, 26, or 60 mg/kg-day neutralized MCA in drinking water for up to two years. A total of 18-21 animals/dose were scheduled for interim sacrifices (3-6 animals/dose at 15, 30, 45, and 60 weeks, only high dose group and control at 60 weeks); 6-14 animals/dose group died prior to study termination from non-treatment related causes; 23-25 animals/dose group were euthanized at 104 weeks. Animals were observed for mortality, morbidity, and any abnormalities of skin, eyes or organ systems. Body weights and water consumption were measured. At the terminal necropsies, full pathologic examination and serum enzyme analysis were performed. Additionally, activity of cyanide-insensitive palmitoyl coenzyme A and rate of hepatocyte proliferation were measured; there were no changes from control for either endpoint at any time. Body weights and relative liver weights were decreased at mid and high doses (26 and 60mg/kg-day, respectively). While at the low dose, absolute and relative spleen weights were increased, at mid and high doses, absolute and relative spleen weights were dramatically decreased compared to the control values. Due to this inconsistent dose-response, US EPA did not include the endpoint of relative

spleen weights in its dose-response analysis of the DeAngelo et al. (1997) study and, in fact, identified the low dose (3.5 mg/kg-day) as a NOAEL based on increased relative liver weight (US EPA, 2006). Increased relative but not absolute testes weight in the same study was likely due to decreased body weights. The authors also reported an increased incidence of myocardial degeneration in the 60 mg/kg-day group at 104 weeks, but did not report incidences (DeAngelo et al., 1997). These chronic toxicity studies are summarized in Table 5.5.

Additionally, Van Duuren et al. (1974) examined carcinogenicity of MCA, as part of a screening study of 17 compounds, in female ICR/Ha Swiss mice (n=30-100/dose), on skin and via subcutaneous injections for 450 days. The skin application dose was 2 mg/animal in the interscapular region 3 times per week; in subcutaneous studies, mice were injected once weekly with 0.5 mg/animal (approximately 24 and 2 mg/kg-day, respectively, based on reference female mouse weight of 0.035 kg). Median survival was not affected with skin treatment, but appeared to decrease with subcutaneous injections (454 vs 504-526 days in control groups). No other endpoints were reported.

Table 5.5 Summary of chronic oral studies of MCA

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male/Female F344/N rats (70/sex/dose)	0, 11, 21 mg/kg-day un-neutralized MCA by gavage for 2 years	Increased mortality in exposed groups due to unidentified causes	LOAEL: 11 mg/kg-day	NTP (1992)
Male/Female B6C3F1 mice (60/sex/dose)	0, 36, 71 mg/kg-day by gavage for 2 years	Increased mortality (males); forestomach squamous cell hyperplasia (males and females)	NOAEL: 36 mg/kg-day	NTP (1992)
Male F344/N rats (50/dose)	0, 3.5, 26, 60 mg/kg-day neutralized MCA in drinking water for 2 years, including interim sacrifices	Systemic toxicity (decreased body weight and relative liver weight) Myocardial degeneration at 60 mg/kg-day	NOAEL ^a : 3.5 mg/kg-day (systemic toxicity)	DeAngelo et al. (1997)

^a DeAngelo et al. (1997) identified the NOAEL as 26 mg/kg-day based on mild pathological alterations and a 13% decrease in body weight compared to control. US EPA, however, considered the NOAEL to be 3.5 mg/kg-day and LOAEL to be 26 mg/kg-day based on decreased body weight and changes in organ weights (US EPA, 2006).

Carcinogenicity

NTP (1992) studied the effects of orally administered MCA in male and female rats and mice, and DeAngelo et al. (1997) evaluated MCA in male rats. Van Duuren et al. (1974) investigated cancer in female mice after chronic dermal or subcutaneous MCA treatments.

The NTP (1992) two-year gavage study, as described in the Chronic Toxicity section, did not show evidence of carcinogenic activity associated with MCA exposure in male or female Fischer 344/N rats or in male or female B6C3F1 mice. The DeAngelo et al. (1997) two-year drinking water study also did not show evidence of carcinogenic activity due to MCA exposure of male Fischer 344/N rats.

Van Duuren et al. (1974) tested 17 direct-acting alkylating agents and related compounds for carcinogenic activity in female ICR/Ha Swiss mice via chronic dermal exposure, subcutaneous injection and i.p. injection. Several of the tested compounds were reported to cause sarcomas at the injection site. However, no increase in tumors was found after the treatments with MCA.

US EPA has classified MCA as a Group D carcinogen, signifying it is 'not classifiable as to its human carcinogenicity' (US EPA, 2003a). MCA was not considered as a carcinogen in US EPA's most recent regulatory action setting the Maximum Contaminant Level Goal (MCLG) for MCA (US EPA, 2006). IARC has not classified the carcinogenicity of MCA. In its *Report on Carcinogens Monograph on Haloacetic Acids Found as Water Disinfection By-Products*, NTP concluded that "evidence of carcinogenicity from studies in experimental animals" was "not sufficient to meet the RoC criteria for listing monochloroacetic acid" (NTP, 2018).

6. TOXICOLOGICAL PROFILE: DICHLOROACETIC ACID

Dichloroacetic acid (DCA) and its salts first came to light in the 1960s for their ability to lower blood glucose (Lorini and Ciman, 1962; Stacpoole and Felts, 1970). DCA was generally found to provide symptomatic relief in several metabolic and cardiovascular conditions; however, its application was hampered by observed adverse side effects such as peripheral neuropathy (Stacpoole et al., 1978; Spruijt et al., 2001). The neurotoxicity of DCA was further examined in animal studies, and various morphological, functional and behavioral adverse effects were demonstrated (Moser et al., 1999; Calcutt et al., 2009). The renewed focus on DCA as a prominent DBP and a metabolite of chlorinated solvents (such as trichloroethylene and tetrachloroethylene) prompted several long-term animal drinking water toxicity studies, which found evidence of carcinogenicity and reproductive toxicity for this compound.

Acute Toxicity

Effects in Humans

Lactic acidosis, a medical condition in which the body produces more lactic acid than it can metabolize, can result in an accumulation of lactic acid in the body, lower blood pH, and life-threatening complications. Due to its ability to stimulate the oxidation of lactic acid to acetyl-coenzyme A, DCA was investigated as a potential treatment for lactic acidosis. Clinical trials have revealed biological and adverse effects of DCA, including metabolic changes in the glycolysis and Krebs cycle, as well as central and peripheral neuropathy (US EPA, 2003a). Reversible elevation of hepatic transaminase, mild liver dysfunction and hypocalcemia were also reported (Stacpoole et al., 1997; Saitoh et al., 1998; Mori et al., 2004).

In a study of 10 healthy volunteers, an intravenous bolus of 50 mg/kg sodium dichloroacetate decreased average lactate concentrations in blood serum approximately 2-fold at 30-60 minutes, increased stroke volume and O₂ availability, and decreased peripheral vascular resistance. These effects increased cardiac output and stimulated myocardial efficiency, and therefore were considered beneficial by the study authors (in the context of potential use of DCA in heart failure). No adverse side effects of DCA treatment were noted (Ludvik et al., 1991). In a different study with healthy volunteers (3-5/dose group), intravenous infusion of 10, 25 or 50 mg/kg sodium dichloroacetate decreased plasma lactate in all subjects at least 2-fold at approximately 2-24 hours into the infusion period (Curry et al., 1985). All four subjects in the high-dose group experienced mild drowsiness or sedation, and one subject experienced temporary nausea on standing.

In a comprehensive review of case studies in children orally administered dichloroacetate (50-100 mg/kg; salt not specified) for lactic acidosis, two out of four patients given a one-day treatment, and all seven patients given multi (2-10-) day treatments responded with decreased blood lactate levels and increased pH (Stacpoole, 1989). In children with lactic acidosis secondary to malaria (9/group), intravenous infusion of 50 mg/kg dichloroacetate (salt not specified) together with quinine, an anti-malarial medication, versus quinine alone, resulted in a more rapid decline in blood lactate levels within 5 hours post treatment (Krishna et al., 1995). Within the timeframe of this study (up to 24 hours post treatment), the authors stated there was

no evidence of toxicity with DCA and noted no DCA-related changes in glucose, pulse, respiratory rate and blood pressure (Krishna et al., 1995). A decrease in plasma lactate levels was observed in ten children with lactic acidosis that received 50 mg/kg DCA by intravenous infusion, and in severely ill patients with sustained hyperlactatemia, there was a highly significant increase in lactate disposal (Agbenyega et al., 2000). In a different study of 124 child patients with malaria and lactic acidosis, 62 patients received quinine and a single dose of intravenous sodium dichloroacetate (50 mg/kg) while 62 patients in the placebo group received quinine alone (Agbenyega et al., 2003). The decrease in plasma lactate relative to the placebo group was significant at 2 hours after treatment and greatest at 4 hours after treatment. Eight deaths occurred in each treatment group, all due to complications of malaria. Among observed adverse effects, generalized seizures and hematocrit reduction occurred with higher prevalence in the DCA treatment group.

Dichloroacetate was further investigated for use in heart disease, based on its ability to improve myocardial efficiency. In nine patients with coronary artery disease, a single intravenous sodium dichloroacetate dose (35 mg/kg) increased the left ventricular stroke volume and myocardial efficiency index, and decreased systemic vascular resistance and arterial lactate (Wargovich et al., 1988). In ten patients with congestive heart failure, a single intravenous sodium dichloroacetate dose (50 mg/kg) similarly exerted inotropic action (increased myocardial efficiency) with maximum effects seen at 1 hour (Bersin et al., 1994). However, in a different study, no improvement in left ventricular function was observed in 25 patients with congestive heart failure followed up to 1 hour after an intravenous infusion of 50 mg/kg dichloroacetate (salt not specified) (Lewis et al., 1998).

Upon exposure to two intravenous infusions of 35-50 mg/kg dichloroacetate (salt not specified) in several human studies consisting of 11 to 126 patients with lactic acidosis, plasma lactate decreased in the majority of subjects at 1-6 hours post treatment, but survival did not consistently improve (Stacpoole et al., 1983a; Stacpoole et al., 1988; Stacpoole et al., 1992). Blood pressure did not increase and mortality did not decrease in DCA-treated patients (Stacpoole et al., 1992). Under these conditions, the authors reported there was no evidence of serious toxicity from dichloroacetate (Stacpoole et al., 1988). However, most patients in these studies were severely ill and were on multiple additional medications, which would make it difficult to distinguish less severe adverse effects due to DCA. In a different case study, intravenous dichloroacetate (salt not specified) did not improve the clinical outcome of one severely ill patient with lactic acidosis when 23 g (body weight not specified) were administered intravenously in several doses over two hours (Irsigler et al., 1977).

Intravenous infusion of 50 mg/kg sodium dichloroacetate significantly reduced blood lactate concentration and peak blood ammonia, and significantly improved peak exercise load and peak oxygen consumption in 18 stable patients with chronic obstructive pulmonary disease (COPD) (Calvert et al., 2008). No adverse effects were noted in this report. Mercken et al. (2009) further examined effects of DCA on exercise-induced oxidative stress in 13 COPD patients previously enrolled in the Calvert et al. (2008) study. Following a single intravenous dose of 50 mg/kg dichloroacetate, oxidized glutathione in the erythrocytes and urinary uric acid

levels were significantly decreased, and plasma interleukin (IL)-6, which modulates oxidative stress, was increased. The results suggested an overall improvement in exercise-induced oxidative stress and inflammation (Mercken et al., 2009).

The metabolic effects of DCA were examined in at least one short-term study involving seven patients with diabetes mellitus and hyperlipoproteinemia, and the findings paralleled those of acute studies. A six- to seven-day daily regimen of 3 or 4 grams orally administered sodium dichloroacetate (patient weights not specified) decreased plasma glucose, lactate, cholesterol and triglyceride levels in adult patients with diabetes mellitus and hyperlipoproteinemia, with mild sedation and increased serum uric acid levels as the only observed adverse effects (Stacpoole et al., 1978; US EPA, 2003a).

Effects in Animals

Oral gavage LD₅₀ values ranged 4.1-5.5 g/kg in mice and 2.8-4.5 g/kg in rats (Woodard et al., 1941; Smyth et al., 1951; Yount et al., 1982). Smyth et al. (1951) reported a dermal LD₅₀ of about 795 mg/kg in rabbits.

Acute and short-term exposures to DCA resulted in a range of effects including changes in glucose and lactate metabolism, neurotoxicity, oxidative stress in the liver and potentiation of chloroform toxicity. Acute and short-term toxicity studies for DCA are summarized in Table 6.1. Several studies that used genetically modified animals are not included in this table (Laughter et al., 2004; Guignabert et al., 2009; Gattone and Bacallao, 2014; Staneviciute et al., 2016; Khan et al., 2017).

Table 6.1 Summary of acute and short-term studies of DCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference [†]
<i>Acute studies (one or several doses within 24 h)</i>				
Wistar CH rats (sex/number of animals not specified), with and without alloxan-induced diabetes	0 or 400 mg/kg DIPA by intraperitoneal (i.p.) injection, euthanized after 18 hours	Decreased blood glucose, transient increase in respiratory quotient in diabetic rats; no change in non-diabetic rats	LOAEL ^a : 400 mg/kg in diabetic rats	Lorini and Ciman (1962)
Male Long-Evans rats, with and without alloxan-induced diabetes (5-9/dose)	0 or 400 mg/kg DIPA by i.p. injection, blood samples collected at 2, 4, 6, and 8 hours post injection	Decreased blood glucose in diabetic rats; no change in non-diabetic rats	LOAEL ^a : 400 mg/kg in diabetic rats	Stacpoole and Felts (1970)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference [†]
Male Ash-Wistar rats; fed or fasted for 24 hours pre-exposure (6-16/dose)	0 or 300 mg/kg neutralized DCA by intravenous (i.v.) infusion; blood sampled at 2-240 min, animals sacrificed 240 min after infusion	Blood: decreases in glucose, lactate, pyruvate, insulin, and free fatty acids; Liver: decreased glucose and increased pyruvate and acetoacetate in fasted animals	LOAEL ^a : 300 mg/kg	Blackshear et al. (1974)
Mongrel dogs (sex not specified), with and without alloxan-induced diabetes (6-10/dose)	0 or 150 mg/kg neutralized DCA by oral gavage, follow-up for 48 hours	Decreased blood glucose, lactate, and pyruvate in non-diabetic and diabetic dogs	LOAEL ^a : 150 mg/kg	Ribes et al. (1979)
Male Holtzman rats (10-20/dose)	0 or 0.4 M sodium DCA administered in two 1 ml i.p. injections; injections 20 or 60 minutes prior to swim time of 210 or 240 seconds	Decreased blood and muscle lactate and pyruvate; increased mean time to exhaustion from swimming; no change in liver glycogen	LOAEL ^a : 446 mg/kg ^b	Schneider et al. (1981)
Male Sprague-Dawley rats (3/dose/time point)	0, 100, 200, or 300 mg/kg un-neutralized DCA (administered as 1, 2 or 3 doses of 100 mg by gastric intubation with 6-hour breaks); single-dose animals sacrificed at 0.5, 1, 3, 6, 12, or 24 hours; multiple-dose animals sacrificed at 3, 6, 12 or 24 hours following each dose)	Liver PDH complex activation	NOAEL: 100 mg/kg	Evans and Stacpoole (1982)
Female C57BL mice, fed or fasted, lean and obese (5-28/dose)	0 or 600 mg/kg sodium DCA (in two i.p. injections of 300 mg/kg each); sacrificed 1 hour (following last dose)	Blood: decreased glucose, lactate and non-esterified fatty acids; Liver: increased glycogen; no differences in DCA effects between lean or obese, fasted or fed	LOAEL ^a : 600 mg/kg	Enser and Whittington (1983)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference [†]
Female Wistar rats, fed or fasted (5-11/dose)	0 or 0.25 ml of 5% (w/v) neutralized DCA solution in 0.15 M sodium chloride (administered in three i.p. injections over 1.5 hours); sacrificed 30 min following last dose	In fasted rats, PDH complex activation in heart and kidney but not in liver; decreased liver lactate, pyruvate, glucose; increased ketone bodies in liver; changes in glucose-induced metabolism	LOAEL ^a : 385 mg/kg ^c	Holness et al. (1986)
Male Wistar rats, recovering from exercise while fasting (8-10/dose)	0 or 1 ml of 0.4 M DCA (salt not specified) by i.p. injection, sacrificed at 3 hours	Blood: decrease in glucose, glycerol and alanine; Liver: decrease in glycogen; Muscle: decrease in glycogen and lactate	LOAEL ^a : 230 mg/kg ^d	Favier et al. (1987)
Male and female Sprague-Dawley rats (5/sex/dose)	0, 120, or 316 mg/kg ^e neutralized DCA by oral gavage in three doses over 24 hours, sacrificed 3 hours after last dose	Decrease in plasma lactate	LOAEL: 120 mg/kg	Davis (1990)
Male F344 rats and male B6C3F1 mice (4-5/dose)	0, 100, 300, or 1,000 mg/kg neutralized DCA by oral gavage, sacrificed at 6 hours	Increased liver lipid peroxidation in rats and mice	NOAEL: 100 mg/kg	Larson and Bull (1992a)
Male and female Long-Evans rats (9-10/sex/dose)	0, 100, 300, 1,000, or 2,000 mg/kg un-neutralized DCA by oral gavage; observations at 4, 24, 168, and 336 hours	Decrease in hindlimb grip (males), decrease in motor activity (males and females); recovery from all endpoints after 7-14 days	NOAEL: 100 mg/kg (hind-limb grip, males)	Moser et al. (1999)
Male Sprague-Dawley rats, with and without induced hindlimb ischemia (16/dose)	0 or 150 mg/kg un-neutralized DCA by i.v. injection, sacrificed at 1 hour	Increased PDH activity in ischemic muscle	LOAEL ^a : 150 mg/kg	Platz et al. (2007)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference [†]
Female New Zealand rabbits with iliac or femoral ischemia with reperfusion (9/dose)	0 or 150 mg/kg un-neutralized DCA by i.v. injection, sacrificed 48 hours	DCA blocked ischemia-dependent increase in serum lactate (in either iliac or femoral ischemia groups) and necrosis (pooled group)	NOAEL ^a : 150 mg/kg	Platz et al. (2007)
<i>Short-term studies (repeated exposure over a period of at least several days)</i>				
Male Long-Evans rats, with and without induced non-ketonic diabetes (5-12/dose)	0 or 12.5 mg/kg sodium dichloroacetate by oral gavage, twice daily for 7 days	Decreased blood glucose in treated diabetic rats	LOAEL ^a : 25 mg/kg-day in diabetic rats	Eichner et al. (1974)
Male Long-Evans rats, with and without induced non-ketonic diabetes (5-12/dose)	0, 25 or 50 mg/kg DIPA by oral gavage, twice daily for 7 days	Decreased blood glucose in treated diabetic rats	LOAEL: 25 mg/kg-day	Eichner et al. (1974)
Mongrel dogs (4/dose), non-diabetic and diabetic (sex not specified)	150 mg/kg-day neutralized DCA by oral gavage for 7 days (animals served as own controls), blood drawn daily	Decreased plasma glucose, pyruvate, lactate, oxaloacetate, and lipids	LOAEL ^a : 150 mg/kg-day	Ribes et al. (1979)
Male Sprague-Dawley rats (3/group)	0 or 100 mg/kg-day neutralized DCA by oral gavage for 7 days, animals sacrificed 3, 6, 12, 24, 48 or 72 hours after last dose	Blood: transient decrease in glucose, pyruvate and lactate; Liver: transiently increased PDH activity; decreased pyruvate decarboxylase activity; Muscle: increased PDH activity	LOAEL ^a : 100 mg/kg-day	Evans (1982); Evans and Stacpoole (1982)
Male and female Sprague-Dawley rats (5-7/sex/dose)	0, 10, 40, 150, or 600 mg/kg-day dichloroacetate (salt not specified) in drinking water for 14 days	Increased urinary ammonia (males)	NOAEL: 40 mg/kg-day	Davis (1986)
Male B6C3F1 mice (6/dose)	0 or 500 mg/kg-day un-neutralized DCA by oral gavage for 10 days	Increased relative liver weight, palmitoyl-CoA oxidation, and peroxisome proliferation	LOAEL ^a : 500 mg/kg-day	Nelson et al. (1989)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference [†]
Male B6C3F1 mice (3-5/dose)	0, 0.1, 0.2, 0.5, or 2 g/L neutralized DCA in drinking water for 2-10 weeks	Decreased serum insulin levels (at highest dose), decreased hepatic IR and PKB α expression	LOAEL: 0.5 g/L (IR and PKB α)	Lingohr et al. (2001)
Male B6C3F1 mice (20-23/dose)	0, 0.3, 1.0, or 2.0 g/L neutralized DCA (0, 57, 190, or 380 mg/kg-day ^e) in drinking water for 14 days	Increased relative liver weight; increased hepatocyte size; increased labeling index and DNA concentration in liver; liver necrosis	NOAEL: 57 mg/kg-day	Sanchez and Bull (1990)
Male and female Swiss Webster mice (4/sex/dose)	0, 1.0 or 2.0 g/L neutralized DCA (0, 190 or 380 mg/kg-day ^e) in drinking water for 14 days	Increased relative liver weight	LOAEL: 190 mg/kg-day	Sanchez and Bull (1990)
Male C57BL/6 mice (5/dose)	0 or 4 g/L (0 or 987 mg/kg-day ^f) un-neutralized DCA in drinking water for 7 days	Increased relative liver weight; slight increase in hepatic triglyceride levels; hepatocyte hypertrophy; altered metabolic profile in urine; increased expression of PPAR α -responsive genes	LOAEL ^a : 987 mg/kg-day	Fang et al. (2013)
Male B6C3F1 mice (4/dose)	0 or 385 mg/kg-day ^g un-neutralized DCA in drinking water for 4 weeks	Increase in relative liver weight, periportal cytoplasmic hepatocellular vacuolization; stearyl-CoA desaturase gene induction, carboxylase and cytochrome b5 gene suppression	LOAEL ^a : 385 mg/kg-day	Thai et al. (2001)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference [†]
Male B6C3F1 mice (8/dose/time-point)	0, 0.5, 1, 2, or 3.5 g/L (0, 123, 247, 494 or 864 mg/kg-day ^f) neutralized DCA in drinking water for 6, 15, or 30 days	Increase in relative liver weight at 30 days; decrease in liver cell proliferation at 6 and 15 days; changes in expression of genes involved in fatty acid degradation, PPAR signaling, protease inhibition, long chain fatty acid synthesis and glucogenesis	LOAEL: 123 mg/kg-day	Wehmas et al. (2017)

DIPA, diisopropylammonium dichloroacetate; 5-HTT, 5-hydroxytryptamine (serotonin) transporter; IR, insulin receptor; NA, not applicable; PDH, pyruvate dehydrogenase; PKB α , protein kinase B α ; 8-OHdG, 8-hydroxydeoxyguanosine; w/v, weight/volume

^a This NOAEL or LOAEL was identified from a single-dose study.

^b Mg/kg dose determined by OEHHHA using a molecular weight of 128.94 g/mol for DCA and a rat body weight of 0.224 kg (female Holtzman rats (male data unavailable), US EPA (1988)).

^c Mg/kg dose determined by OEHHHA using a density of 1.563 g/ml for DCA, and a rat body weight of 0.156 kg (female Wistar rats, from US EPA (1988)).

^d Mg/kg dose determined by OEHHHA using a molecular weight of 128.94 g/mol for DCA and a rat body weight of 0.217 kg (male Wistar rats, from US EPA (1988)).

^e Doses calculated by US EPA (2003b)

^f Estimated by OEHHHA based on default values for body weight and water consumption (US EPA, 1988)

^g Dose calculated by OEHHHA from average of mean daily dose reported for each animal.

[†] Study results were reported in multiple papers when several references are cited

Findings of acute and short-term in vivo studies of DCA are generally supported by in vitro observations. Specifically, the ability of DCA to increase myocardial efficiency in vivo can be attributed to DCA-dependent stimulation of pyruvate dehydrogenase (PDH), which has been observed in vitro. Stimulation of PDH would provide more effective utilization of available glucose in energy-demanding tissues, such as the heart. In in vitro experiments with perfused rat hearts, 100 μ M – 50 mM neutralized DCA stimulated PDH activity (Whitehouse and Randle, 1973; Kerbey et al., 1976), consistent with the report of increased in vivo PDH activity in the heart and other organs and tissues in rats following oral DCA administration (Holness et al., 1986).

One consequence of improved routing of glucose into the energy-producing pathways would be decreased glucose conversion to glycogen (which is a polymer of glucose molecules used to store energy). However, in contrast to the in vivo observations of a glycogen-lowering effect of DCA (Favier et al., 1987), one in vitro study reported that neutralized DCA at concentrations as low as 100 μ M increased glycogen levels, independently of insulin, in hepatocytes isolated from male B6C3F1 mice (Lingohr et al., 2002).

Other miscellaneous cellular effects of DCA treatment in vivo have also been reported. Gap junction intercellular communication was inhibited by 10 mM neutralized DCA in rat hepatocytes in vitro without S9 (cellular fraction containing metabolic enzymes) (Benane et al., 1996). DCA at concentrations up to 24 mM decreased cell viability, total glutathione, superoxide dismutase activity and catalase activity in rat alveolar type II pneumocytes (Valauri-Orton et al., 2015). In J744A.1 macrophage cells, neutralized DCA simulated their activation, induced antioxidant enzymes and decreased glutathione, although the effective concentration (16 mM) was cytotoxic (Hassoun and Ray, 2003; Hassoun and Kini, 2004; Hassoun and Mehta, 2008).

Based on its effects on the mitochondrial enzyme complex, DCA was also considered as a candidate anticancer drug and was demonstrated to sensitize cancer cell lines to apoptosis by mitochondrial-mediated mechanisms (Bonnet et al., 2007; Michelakis et al., 2008; De Preter et al., 2016). DCA tumor growth-inhibiting effects appear to be due to shifting glucose metabolism from glycolysis to glucose oxidation in malignant cells and the resulting release of pro-apoptotic mediators. At low millimolar (mM) concentrations, DCA reduced growth of cancer cells in vitro for lung (Bonnet et al., 2007), colorectal (Madhok et al., 2010), endometrial (Wong et al., 2008), breast (Sun et al., 2010), and head and neck squamous cell carcinoma (Sun et al., 2009) cell lines. DCA at 20 mM did not reduce growth of noncancerous cells but significantly decreased cancer cell proliferation (Madhok et al., 2010). DCA also moderately inhibited the growth of 18 pediatric tumor cell lines, with the strongest effect observed at 50 mM (Heshe et al., 2011).

Anti-cancer effects of DCA in in vivo animal models of tumor growth were also noted, including smaller tumors and fewer metastases in animals injected with cancer cells and treated with 2.5-200 mg/kg-day DCA, administered orally via drinking water, intraperitoneally and intragastrically (Bonnet et al., 2007; Sun et al., 2010; Vella et al., 2012; Duan et al., 2013; Kumar et al., 2013). Neointimal lesion formation in pre-capillary arterioles was prevented in rats receiving 80 mg/kg-day un-neutralized DCA for 20 days by gastric gavage (Li et al., 2014). A decrease in the volume of lung metastases was observed in mice administered oral doses of 0.98 and 1.5 g/kg DCA (Kolesnik et al., 2015; Pyaskovskaya et al., 2016).

Subchronic Toxicity

Effects in Humans

Due to its proposed beneficial effects in severe human pathologies, such as lactic acidosis, subchronic DCA treatments have been reported in the clinical research setting.

Three child patients (ages 14 and 15 months, and 9 years) with mitochondrial encephalopathies (degenerative brain disorders often accompanied by a buildup of lactic acid) received oral un-neutralized DCA (30 mg/kg-day) for 21 months or longer (Kimura et al., 1997). CT scan findings for one patient were also described in a separate case study (Kimura et al., 1995). DCA administration decreased plasma and cerebrospinal fluid lactate, resolved brain stem and basal ganglia lesions, and did not result in reported adverse effects.

Twenty-seven child and adult patients (mean age, 9.8 years; range, 0.8-37.4 years) with congenital lactic acidemia (lactic acid in circulating blood) due to a variety of mitochondrial

diseases, were administered 25-50 mg/kg-day un-neutralized DCA orally for 12 months, with plasma lactate concentrations measured and nerve conduction studies performed at 3, 6 and 12 months of treatment (Spruijt et al., 2001). Peripheral neuropathy was reported in half of all subjects (14/27) and was characterized by decreased nerve conduction velocities. Treatment of each patient started with 50 mg/kg-day DCA, and the dose was reduced to 25 mg/kg-day if symptoms of peripheral neuropathy were noted. The report did not specify whether the symptoms resolved with the lower DCA dose; however, all 27 patients completed the 12-month treatment.

In three child patients with MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes), a rare multi-system and progressive neurodegenerative disorder that begins in childhood, administration of un-neutralized DCA orally (30-50 mg/kg-day; the dosage was adjusted individually to maintain plasma lactate at the normal level) for 10-20 months resulted in decreased plasma lactate levels and clinical improvement (Saitoh et al., 1998). Transient elevations of serum transaminases, hypocalcemia and peripheral neuropathy of the lower extremities were noted as adverse effects. The follow-up for these three patients for up to 5 years plus data for one additional MELAS patient were separately reported (Mori et al., 2004). In addition to the adverse effects observed in Saitoh et al. (1998), liver enlargement was reported in all patients with the average dose of 30 mg/kg-day.

In an open-label study, 37 child and adult patients with mitochondrial disease (including some affected by MELAS) were started on 25-50 mg/kg-day un-neutralized DCA orally. Doses were adjusted according to individual needs (e.g., effectiveness of plasma lactate reduction), and for patients who remained on treatment for ≥ 3 months, doses ranged from 11-51 mg/kg-day (Barshop et al., 2004). Plasma and cerebrospinal fluid lactate levels decreased and positive clinical outcomes were noted in some patients. Moderate increases in serum amino transferases (AST and ALT) observed with initial DCA treatment were sustained over the follow-up period of 4 years on average, indicating mild hepatotoxicity. Neuropathic symptoms were reported in 4/37 (11%) patients.

In a randomized clinical trial involving 30 adult patients with MELAS treated orally with 25 mg/kg-day dichloroacetate (salt not specified), study medication was discontinued in 17 out of 19 patients because of the onset or worsening of peripheral neuropathy, mostly over the initial 24-month period (Kaufmann et al., 2006). Only one subject completed the entire 36-month study.

In a controlled clinical trial involving 43 child patients with congenital lactic acidosis (Stacpoole et al., 2006), un-neutralized DCA orally administered at 25 mg/kg-day over a period of 18 months decreased plasma lactate concentration. Twenty-one patients were started on DCA and were treated for 6 months, 22 patients were started in the placebo group and after the first 6 months both groups were given DCA for 12 months. Several patients were lost to death and, at the end of the study, there were 20 remaining patients in the original placebo group and 16 in the treated group. In some patients, "excessive sleepiness and lethargy, peripheral neuropathy, muscular rigidity of an upper extremity ... and hand tremor" were observed. There were no differences in mean serum concentrations of transaminases.

At the completion of the Stacpoole et al. (2006) study, eligible patients were given the opportunity to continue receiving 25 mg/kg-day DCA with evaluation once every 6 months, and 36 patients elected to participate (Stacpoole et al., 2008a). The median exposure per patient was 2.4 years (range, 0.0 – 9.7 years). Blood and cerebrospinal fluid lactate were decreased due to treatment. Peripheral neuropathy, primarily in the peroneal motor nerve, was reported; however, the authors hypothesized that the observed symptoms of neurotoxicity may have been partially due to the underlying disease. No other adverse effects were reported and, specifically, no changes in serum transaminases were detected.

Additionally, there is an extensive database of case studies describing oral administration of DCA or its sodium salt at 25-50 mg/kg-day given to 1-2 patients of various ages with lactic acidosis, for months to years (Moore et al., 1979; Evans and Stacpoole, 1982; Aynsley-Green et al., 1984; Kuroda et al., 1986; Naito et al., 1989; Stacpoole, 1989; Saijo et al., 1991; Burlina et al., 1993; Kurlemann et al., 1995; Pavlakis et al., 1998; Sudo et al., 2004; Ryu et al., 2009; Brandsma et al., 2010). Clinical findings from these case studies are generally consistent with larger studies (decreased lactate, improved disease outcomes). While adverse effects were generally not defined in these case studies, neuropathy was reported in some (Moore et al., 1979; Kurlemann et al., 1995; Ryu et al., 2009; Brandsma et al., 2010).

After the publication by Bonnet and colleagues proposing DCA as a selective anti-cancer agent, presumably via its ability to inhibit pyruvate dehydrogenase kinase II (PDK II) (Bonnet et al., 2007), a small phase II clinical trial using DCA to treat glioblastoma was initiated in Canada. Five patients with aggressive primary glioblastoma multiforme cancers were treated with dichloroacetate (salt unspecified) orally for up to 15 months (Michelakis et al., 2010). There was no hematologic, hepatic, renal, or cardiac toxicity. However, dose-dependent reversible grade II and III polyneuropathy (damage to multiple nerves outside of the brain and central nervous system) occurred in patients treated with 25 and 50 mg/kg-day DCA, respectively. The maximum dose at which none of the patients had a clinically significant peripheral neuropathy was 6.25 mg/kg orally twice a day for at least 3 months. Serum DCA concentrations were sufficient to inhibit the DCA target enzyme, PDK II, which is highly expressed in all the glioblastomas. Indications of clinical efficacy were present at a dose that did not cause peripheral neuropathy. In a separate experiment, freshly isolated glioblastomas from 49 patients showed mitochondrial hyperpolarization with highly expressed PDK II, which was rapidly decreased by DCA (Michelakis et al., 2010).

Brandsma et al. (2010) observed severe encephalopathy and grade III sensorimotor polyneuropathy after four weeks of dichloroacetate (as unspecified salt) treatment of a 46-year-old melanoma patient. Dichloroacetate was given orally at 15 mg/kg-day (400 mg 3 times a day), taken with 150,000 IU per day of vitamin A.

In a clinical study of 20 adult patients with pulmonary arterial hypertension, dichloroacetate (sodium salt) at 0, 3, 6.25 or 12.5 mg/kg was given orally twice a day for 4 months. After 3 to 11 weeks of 12.5 mg/kg twice daily dichloroacetate treatment, non-demyelinating peripheral neuropathy was observed in 5 patients (Michelakis et al., 2017). Symptoms improved after 1-3

months after either withdrawing from the study or decreasing the dose to 6.25 mg/kg twice daily. The highest tolerated dose in this study was determined to be 6.25 mg/kg twice daily.

Neuropathy was observed in many of the controlled trials (Moore et al., 1979; Stacpoole et al., 1979; Stacpoole et al., 1990; Kurlemann et al., 1995; Saitoh et al., 1998; Stacpoole et al., 1998a; Oishi et al., 2003; Mori et al., 2004; Kaufmann et al., 2006; Brandsma et al., 2010; Michelakis et al., 2010), and the induction of peripheral neuropathy has restricted clinical trials of DCA for congenital lactic acidosis (Stacpoole et al., 1997; Stacpoole et al., 1998a; Stacpoole et al., 1998b; Stacpoole et al., 2003) or for treating patients with mitochondrial disorders (Spruijt et al., 2001; Kaufmann et al., 2006). Other observed side effects included pain, numbness, gait disturbances, and sedation (Stacpoole et al., 1998b). Neuropathy symptoms were observed in some patients at doses as low as 15-25 mg/kg-day and included a decrease or loss of deep tendon reflexes, weakness of the fingers and toes, reduced strength of lower extremity muscles, with the distal muscle groups being most severely affected, ataxia, tremors, and reduced nerve conduction velocity. Adults appear to be more susceptible to this adverse effect than children (Stacpoole et al., 2008a), although children given DCA have also displayed signs of neuropathy (Saitoh et al., 1998). Discontinuation of DCA generally resulted in recovery and improvement of neuropathy symptoms.

The mechanisms of DCA-induced neuropathy are not clear, but Stacpoole et al. (1990) postulated that DCA might deplete tissue thiamine stores by increasing the activity of the pyruvate dehydrogenase complex and other enzymes for which thiamine is a cofactor, based on similarities in the neuropathic signs between DCA administration and thiamine deficiency; co-treatment with thiamine decreased peripheral neuropathy in rats (Stacpoole et al., 1990). This led to trials of thiamine administration in patients being treated with DCA, in order to decrease the potential neuropathic effect. However, thiamine treatment did not prevent slowing nerve conduction in patients treated with DCA (Kurlemann et al., 1995; Spruijt et al., 2001; Kaufmann et al., 2006; Stacpoole et al., 2008a; Stacpoole et al., 2008b).

DCA-treated patients, as well as experimental animals, showed elevated urinary δ -ALA, a heme precursor implicated in porphyria, tyrosinemia type I, and conditions associated with damage of Schwann cells which wrap around nerve cell axons to form a protective myelin sheath (Stacpoole et al., 2006; Felitsyn et al., 2007; Felitsyn et al., 2008). Reversible demyelination was observed in cultured neonatal rat Schwann cells and dorsal root ganglia neurons exposed to DCA for up to 12 days (Felitsyn et al., 2007). DCA had modest adverse effects on neuronal and glial cell vitality, as determined by the release of lactate dehydrogenase. Exposure of myelinating co-cultures of Schwann cells and sensory neurons to δ -ALA reduced the levels of myelin-associated lipids and proteins and increased protein carbonylation and formation of hydroxynonenal and malondialdehyde. The authors suggest that these biochemical changes may change the differentiation state of Schwann cells and could affect neuron vitality.

DCA treatment did not alter steady-state levels of intermediate filament proteins, but promoted formation of anti-neurofilament antibody-reactive whirls, which are present in certain hereditary neuropathies and could potentially disrupt axonal transport (Stacpoole et al., 2008b). A reversible interference with myelin-related proteins may account, at least in part, for the

peripheral neuropathic effects. Increased lipid peroxidation in nerves may also implicate production of excess reactive oxidative species and subsequent oxidative damage in the DCA-induced peripheral neuropathy (Landgraf et al., 2007; Calcutt et al., 2009).

Effects in Animals

In subchronic studies in laboratory animals, DCA consistently induced decreased body weight, increased relative liver weight and liver pathology, and neurotoxic effects (Table 6.2). Other notable effects included ocular toxicity in dogs (Katz et al., 1981; Cicmanec et al., 1991). Developmental, reproductive, and neurotoxic effects due to subchronic exposures are further discussed in subsequent sections of this document and summarized in Tables 6.5-6.8.

Subchronic studies of DCA are summarized in Table 6.2, with NOAELs and LOAELs as identified by OEHHA.

Table 6.2 Summary of subchronic animal studies of DCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference†
Male and female Sprague-Dawley rats (10-15/sex/dose)	0, 125, 500, or 2,000 mg/kg-day sodium dichloroacetate by oral gavage for 3 months followed by a 4-week recovery period ^a	Lethality at high dose (f:2/10, m:2/10); decreased body weight gain; suppression of erythroid parameters; decreased plasma glucose and lactate; increased plasma creatinine and ALP (m); increased relative organ weights (liver, kidneys (f), and adrenals); brain lesions; testicular degeneration (m); decreased plasma total protein, iron, and calcium in males	LOAEL: 125 mg/kg-day (relative liver weight)	Katz et al. (1981)
Male and female beagle dogs (3-4/dose)	0, 50, 75, or 100 mg/kg-day sodium dichloroacetate by oral gavage (gelatin capsules) for 13 weeks followed by a 5-week recovery period ^a	Lethality at high dose (f: 1/3; m: 1/4); ocular anomalies; suppression of erythroid parameters; decreased plasma glucose, pyruvate and lactate; brain lesions; prostate glandular atrophy; hyperplasia of the gall bladder mucosa	LOAEL: 50 mg/kg-day (multiple effects)	Katz et al. (1981)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference†
Male Wistar rats (6/dose)	0 or 0.04 mol/kg in feed (323-516 mg/kg-day ^b) neutralized DCA for 12 weeks	Decreased BW, relative adrenal weight, absolute and relative brain weight, and absolute testis and epididymis weights; increased relative kidney and liver weights; hepatomegaly; increased serum acetoacetate and 3-hydroxybutyrate; decreased nerve conduction velocity	LOAEL ^c : 323 mg/kg-day	Yount et al. (1982)
Male Sprague-Dawley rats (6-15/dose)	Phase 1: 0 or 1,100 mg/kg-day; Phase 2: 0, 50 or 1,100 mg/kg-day sodium dichloroacetate in drinking water for 7 weeks	Decreased BW; hindlimb weakness (control, 1/8; high dose, 5/9); decreased erythrocyte transketolase activity; increased urinary oxalate	NOAEL: 50 mg/kg-day (BW, hindlimb weakness)	Stacpoole et al. (1990)
Male Sprague-Dawley rats (10/dose)	0, 0.05, 0.5, or 5 g/L (0, 3.9, 35.5, or 345 mg/kg-day) neutralized DCA in drinking water for 90 days	Decreased BW; increased relative liver, kidney and spleen weight; enlarged hepatocytes with glycogen accumulation (high dose); degeneration of the tubular epithelium and glomeruli in kidneys (high dose); decreased total serum protein; increased serum ALP and ALT (high dose); increased hepatic β -oxidation (high dose)	NOAEL: 3.9 mg/kg-day (BW and relative organ weights)	Mather et al. (1990)
Male Sprague-Dawley rats (5/dose)	0 or 80.5 mM (1,100 mg/kg-day ^b) neutralized DCA in drinking water for 90 days	Decreased BW; increased absolute and relative liver weight and absolute testis weight; hepatomegaly; morphological alterations in the liver, including vascular changes, collagen deposition and small foci of inflammation; perivascular inflammation in the lung; testicular atrophy of seminiferous tubules and interstitial hyperplasia; brain pathology (vacuolization and gliosis)	LOAEL ^c : 1,100 mg/kg-day	Bhat et al. (1991)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference†
Male and female beagle dogs (5/sex/dose)	0, 12.5, 39.5, or 72 mg/kg-day neutralized DCA in gelatin capsules for 90 days	Dyspnea (forced expiratory effort, coughing); bilateral conjunctivitis; partial paralysis; diarrhea; decreased BW; decreased erythrocyte counts and hemoglobin (high dose); increased lactate dehydrogenase activity (m, high dose); increased relative liver and kidney weights (f); increased relative kidney weight (m); increased relative lung and brain weights (high dose); brain lesions (vacuolization of white myelinated tracts); lung lesions (pneumonia and bronchopneumonia); testicular lesions; hepatic hemosiderosis; mucosal epithelial vacuolization and hyperplasia of the gallbladder; pancreatic acinar degeneration; prostatic glandular atrophy and thymic atrophy (m)	LOAEL: 12.5 mg/kg-day (relative liver weight, testicular degeneration)	Cicmanec et al. (1991)
Male Long-Evans rats (18-19/dose)	0, 31.25, 62.5, or 125 mg/kg-day neutralized DCA by oral gavage for 10 weeks	Decreased BW; decreased absolute and relative epididymis and preputial gland weights; increased relative kidney weight; increased absolute and relative liver weight; increased relative spleen weight; sperm deficiencies (decreased counts and motility, impaired morphology); decreased number of implants/dam	LOAEL: 31.25 mg/kg-day (liver weight)	Toth et al. (1992)
Male B6C3F1 mice (5/dose)	0 or 2 g/L (0 or 466 mg/kg-day ^b) neutralized DCA in drinking water for 4, 8 or 12 weeks	Decreased relative liver weight, increased liver glycogen; decreased serum insulin	LOAEL ^c : 466 mg/kg-day	Kato-Weinstein et al. (2001)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference†
Male Sprague-Dawley rats (6-12/dose)	0, 0.0025, 0.25, or 50 mg/kg-day DCA in drinking water for 1, 4, 8, or 12 weeks	Inhibition of hepatic GSTz specific activity and protein expression; increased relative liver weight (high dose at 8 weeks)	NOAEL: 0.25 mg/kg-day (liver weight)	Guo et al. (2006)
Male B6C3F1 mice (7/group)	0, 7.7, 77, 154, or 410 mg/kg-day neutralized DCA by oral gavage for 4 or 13 weeks	Liver: increased SA production, lipid peroxidation and single-strand DNA breaks; increased relative liver weight (at high dose); decrease in SOD activity; increase in catalase activity. Peritoneal lavage cells: increased SA production, MPO activity, SOD activity and TNF α (at various time points)	LOAEL: 7.7 mg/kg-day (changed enzymatic activities, DNA breaks and lipid peroxidation)	Hassoun et al. (2010a); Hassoun et al. (2010b); Hassoun and Cearfoss (2011)
Male B6C3F1 mice (number not specified)	0 or 77 mg/kg-day neutralized DCA by oral gavage for 13 weeks; on +/- vitamin E diet	Increased SA production, MPO activity, SOD activity and TNF α in peritoneal lavage cells; lipid peroxidation; DNA single strand breaks (on either diet); catalase and GSH-Px activities were increased with vitamin E-deficient diet only	LOAEL: 77 mg/kg-day	Cearfoss and Hassoun (2012); Hassoun and Al-Dieri (2012)
Male B6C3F1 mice (6/dose)	0, 7.5, 15, or 30 mg/kg-day neutralized DCA by oral gavage for 13 weeks	Increased SA production, MPO activity and TNF α in peritoneal lavage cells; increased SA production, lipid peroxidation; DNA single strand breaks; decrease in SOD and GSH-Px activity; decrease in total glutathione in hepatic cells (at the mid and high dose)	LOAEL: 7.5 mg/kg-day (multiple endpoints)	Hassoun et al. (2013); Hassoun et al. (2014)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference†
Male Wistar rats (8/dose)	0, 0.5 or 2 g/L (0, 73.7 or 147.5 mg/kg-day ^d) un-neutralized DCA in drinking water for 2 months	Decrease in weight gain, food intake, water consumption and relative kidney weight; increase in urea, creatinine, and uric acid levels in plasma; increase in SOD activity; decrease in GSH-Px and catalase activity; decrease in glutathione level; alteration in kidney architecture	LOAEL: 73.7 mg/kg-day (multiple endpoints)	El Arem et al. (2014)

ALP, alkaline phosphatase; ALT, alanine amino-transferase; BW, body weight; f, female; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSH-Px, glutathione peroxidase; GSTz, glutathione-S-transferase zeta isoform; IL, interleukin; INF γ , interferon γ ; m, male; MRL, Murphy Roths Large (mouse strain); MPO, myeloperoxidase; NA, not applicable; SA, superoxide anion; SOD, superoxide dismutase; TNF α , tumor necrosis factor alpha.

^a Toxicity endpoints are as reported prior to recovery; most adverse effects were ameliorated following the recovery period.

^b Doses estimated by US EPA (2003b).

^c This LOAEL was identified from a single-dose study.

^d Estimated based on US EPA default value for water consumption (US EPA, 1988).

[†] Study results were reported in multiple papers when several references are cited.

Subchronic studies of higher quality, comprising multiple doses and with duration of 10 weeks or longer, are described in more detail below.

Katz et al. (1981) gavaged adult Sprague-Dawley rats with 0, 125, 500, or 2,000 mg/kg-day aqueous sodium dichloroacetate for three months (10/sex/dose; control and high dose groups had 5 additional animals/sex/dose with a 4-week recovery period). Two of each sex in the 2,000 mg/kg-day groups died during the study. Dose-dependent increases in mean relative liver, kidney, and adrenal gland weights and decreases in body weights, blood glucose and lactate levels were observed at ≥ 125 mg/kg-day. Creatinine levels were significantly increased at all doses. Male rats exhibited statistically significant depressed blood levels of total protein (high dose), triglycerides (mid and high dose), iron (all doses), and calcium (high dose), and elevated levels of total and direct bilirubin (direct bilirubin correlates with conjugated bilirubin; mid and high dose) and potassium (high dose). During the recovery period, absolute and relative organ weights tended to catch up with those of the controls, and the altered biochemical parameters returned to levels similar to control except for creatinine levels, which remained elevated. Based on organ weight changes, CNS effects, and testicular toxicity (discussed in the *Neurotoxicity and Developmental and Reproductive Toxicity* sections), the LOAEL is 125 mg/kg-day (Katz et al., 1981; US EPA, 2003b).

Katz et al. (1981) also evaluated effects of DCA in dogs. Adult beagles, four/sex in the control and highest dose groups and three/sex in the other groups, were gavaged with gelatin encapsulated DCA at 0, 50, 75, or 100 mg/kg-day for 13 weeks, followed by a 5-week recovery

period. Female dogs at all doses showed markedly reduced appetite, and both sexes showed dose-dependent weight loss during treatment. Weight loss and decreased mean blood levels of glucose, lactate, and pyruvate were observed at all doses, which returned to normal during the recovery period. Erythrocyte counts, hematocrit ratios, and hemoglobin levels progressively decreased at all doses, returning to normal during the recovery. An increased incidence of hemosiderin-laden Kupffer cells in the liver and cystic mucosal hyperplasia in the gall bladder were observed at all doses at sacrifice. Treated dogs also showed lung consolidation, a condition in which fluid fills airway spaces instead of air. Bloody stools, vomiting, and paralysis were reported at 100 mg/kg-day. Neurotoxicity in both sexes and reproductive toxicity in males are discussed in the *Neurotoxicity* and *Developmental and Reproductive Toxicity* sections of this document, respectively. A LOAEL of 50 mg/kg-day was determined, based on organ weight changes, neurotoxicity, testicular toxicity, and other effects (Katz et al., 1981; US EPA, 2003b).

Mather et al. (1990) gave 10 male Sprague-Dawley rats per dose neutralized DCA in deionized drinking water for 90 days at 0, 50, 500, or 5,000 ppm, yielding doses of about 0, 3.9, 35.5, or 345 mg/kg-day (US EPA, 2003b). Water consumption was significantly reduced at two months of exposure in the 500 and 5,000 ppm groups. At sacrifice, total blood serum protein was significantly decreased at all doses. At 500 and 5,000 ppm, body weight gain and terminal body weight were significantly reduced, and relative liver and kidney weights were increased, as was alkaline phosphatase. Increased ALT, relative spleen weight and hepatic peroxisomal β -oxidation activity were observed at 5,000 ppm, as were hepatocellular enlargement, intracellular swelling, glycogen accumulation in liver, and diffuse degeneration of the renal tubular epithelium and glomeruli. No consistent effects were seen on liver microsomal enzyme activity, spleen histopathology, or immunological parameters. A NOAEL and LOAEL of 3.9 and 35.5 mg/kg-day, respectively, were derived by US EPA (2003b).

Cicmanec et al. (1991) orally administered 0, 12.5, 39.5, or 72 mg/kg-day DCA in gelatin capsules to five four-month-old beagle dogs/sex/dose for 90 days. Reductions of food and water intake were observed at all doses in both sexes. Body weight gain was decreased 16% in males and 9% in females at 72 mg/kg-day, and 9% and 11% in males and females, respectively at 39.5 mg/kg-day. Histopathological changes in the kidney, liver, and pancreas were observed at all DCA doses in both sexes. Lesions included pale and discolored kidneys, mild vacuolar changes, inflammation, hemosiderosis in the liver, and chronic inflammation and acinar degeneration in the pancreas. Both sexes showed increased relative kidney weights (at the two highest doses), increased relative liver weights (at all doses), and increased relative lung weights at 72 mg/kg-day. Statistically significant decreases in erythrocyte counts and hemoglobin levels were seen in the 72 mg/kg-day group starting at day 30 in males and females. During the first month, 24 of 30 DCA-treated and a few control dogs of both sexes exhibited conjunctivitis, which became more severe later in the study. The adverse ocular effects appeared to be dose-related, with 8 of 10 dogs dosed at 72 mg/kg-day affected. At 72 mg/kg-day, dyspnea was noted starting at day 45 and worsened with time. Dogs in the two higher dose groups experienced sporadic diarrhea, and the most

severely affected required fluid therapy for dehydration. One female and two males in the 72 mg/kg-day group died due to pneumonia and dehydration at days 50, 51, and 74, respectively. Neurotoxic effects were observed in some males and females during the last half of the exposure, as discussed in the *Neurotoxicity* section. Testicular degeneration was also observed, as discussed in the *Developmental and Reproductive Toxicity* section. Based on observed adverse effects, the authors considered the liver, brain, pancreas, and testis as primary targets while kidney and lung lesions were considered as secondary. The severity of observed lesions increased with dose, and a NOAEL was not determined in this study. A LOAEL of 12.5 mg/kg-day was determined based on organ weight changes, liver, kidney, and pancreas toxicity, neurological changes and brain lesions, testicular effects, and other effects (Cicmanec et al., 1991; US EPA, 2003b).

Toth et al. (1992) gavaged male adult Long-Evans rats with sodium dichloroacetate at 0, 31.25, 62.5, or 125 mg/kg-day for 10 weeks. Body weights were reduced, while relative liver, kidney and spleen weights were increased at the two higher doses. As discussed in the *Developmental and Reproductive Toxicity* section, adverse effects were reported on male reproductive parameters. A NOAEL of 31.25 mg/kg-day was determined.

Hassoun et al. (2010a) gavaged male B6C3F1 mice with 0, 7.7, 77 or 410 mg/kg-day DCA for 4 or 13 weeks. Endpoints for oxidative stress in hepatic tissue were measured. An increase in superoxide anion and lipid peroxidation was observed at all doses and both time points. An increase in DNA single strand breaks was observed at 77 mg/kg-day and higher at 4 weeks and all doses at 13 weeks. Increased relative liver weight was observed only at the high dose. A LOAEL of 7.7 mg/kg-day was determined. Hassoun et al. (2013) gavaged male B6C3F1 mice with 0, 7.5, 15 or 30 mg/kg-day DCA for 13 weeks and evaluated biomarkers for phagocytic activation in peritoneal lavage cells. There was a dose-dependent increase in superoxide anion production, myeloperoxidase activity and tumor necrosis factor alpha. A LOAEL of 7.5 mg/kg-day was determined.

Genetic Toxicity

Genotoxicity of DCA in vivo and in vitro are presented in Tables 6.3 and 6.4, respectively. Within each table, studies are organized by experimental system (e.g., human studies before animal studies, primary cells before cell culture), and then by type of genotoxic effect.

No human in vivo studies were located. Mutation frequencies were increased in male transgenic mice harboring the bacterial lacI gene given 1 or 3.5 g/L of DCA in drinking water for 60 weeks, but not at 4 or 10 weeks of treatment (Leavitt et al., 1997). Frequencies of micronuclei in peripheral erythrocytes in male mice were significantly increased following 9 days, 10, 26 or 31 weeks of exposure to 3.5 g/L neutralized DCA in drinking water, while no increase was observed at shorter exposures (9 or 28 days) with lower doses (Fusco et al., 1996). NTP (2007) did not find significantly increased micronucleus frequencies in peripheral erythrocytes in different strains of mice. Several studies reported evidence of DNA strand breaks with different DCA exposure set-ups, primarily in hepatic tissue (Table 6.3). Findings in rats were negative (Table 6.3). Newt larvae did not have increased micronucleus frequency following exposure to

DCA in water (Giller et al., 1997), while positive evidence for micronuclei and DNA breaks was reported for fava bean root tips (Hu et al., 2017).

Evidence of in vitro genotoxicity of DCA is shown in Table 6.4. A statistical increase in micronucleus formation was observed for human peripheral blood lymphocytes treated with 25-100 µg/ml in vitro for 48 hours (Varschney et al., 2013). No DNA strand breaks were observed in human lymphoblastic leukemia CCRF-CEM cells following exposure to 1-10 mM for 2 hours (Chang et al., 1992). DNA mutations and chromosomal aberrations were reported in mouse lymphoma L5178Y/TK⁺ cells following exposure to up to 0.8 mg/ml of DCA (Harrington-Brock et al., 1998). However, a different study did not report significant differences in mutation frequency in this cell line at DCA concentrations as high as 5 mg/ml and with similar exposure duration (Fox et al., 2008). Other studies of chromosomal and DNA damage in mammalian cell lines were also negative.

Several studies employing reverse mutation assays in *S. typhimurium* did not observe genotoxicity of DCA, while other studies employing the same strains and methods reported weak to moderate genotoxicity. DCA was mutagenic in five *S. typhimurium* strains: TA1535 (one positive out of three studies), TA1538 (one positive study), TA98 (two positive out of four studies), TA100 (four positive out of six studies) and RSJ100 (one positive study). DCA was not mutagenic in TA1537 (two negative studies), TA104 (one negative study) or W2P (one negative study). Based on these results, it is not clear what type of mutations DCA would be most likely to produce. DCA also produced mixed results in additional bacterial tests (Table 6.4).

DCA is extensively metabolized in mammals, as described elsewhere in this document. In some studies, addition of metabolic enzyme-containing S9 fraction (a mixture of unfractionated microsomes and cytosol containing a wide variety of metabolic enzymes) eliminated observed genotoxicity (Giller et al., 1997; Kargalioglu et al., 2002), while in many others S9 fraction did not affect observed genotoxicity, and in one study (Ono et al., 1991) DCA was genotoxic only in the presence of S9. Glyoxylic acid, one of DCA's metabolites, was shown to be mutagenic in TA97, TA100, and TA104 without S9 and in TA97, TA100, TA102, and TA104 with S9 (Sayato et al., 1987).

WHO (2005), US EPA (2003b), IARC (2014) and Richardson et al. (2007) reviewed DCA genetic toxicity studies and concluded the evidence was moderate to weak under a variety of conditions. Richardson et al. (2007) suggested that genotoxic mechanism for DCA carcinogenesis may operate only at high doses. After that earlier view, multiple studies have been published with evidence of DCA genotoxicity at low doses in vivo or low concentrations in vitro. Given the available evidence of DCA genotoxicity including at lower doses, a genotoxic mechanism of DCA carcinogenesis cannot be excluded.

Table 6.3 Genetic and related effects of DCA in vivo

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
Mammalian: Mutations in <i>LacI</i> (recoverable target gene)	Male Big Blue B6C3F1 transgenic mice (5-6/dose/time point)	0, 1, or 3.5 g/L neutralized DCA in drinking water for 4, 10, or 60 weeks	+ (1 and 3.5 g/L at 60 weeks)	Leavitt et al. (1997)
Micronucleus assay with peripheral blood erythrocytes (which are normally anucleated)	Male B6C3F1 mice (10/dose/time point)	0, 0.5, 1, 2, or 3.5 g/L neutralized DCA in drinking water for 9 or 28 days	+ (at 9 days, high dose) - (at 28 days)	Fuscoe et al. (1996)
Micronucleus assay with peripheral blood erythrocytes (which are normally anucleated)	Male B6C3F1 mice (10/dose/time point)	0 or 3.5 g/L neutralized DCA in drinking water for 10, 26 or 31 weeks	+ (all time points)	Fuscoe et al. (1996)
Micronucleus assay with peripheral blood erythrocytes	Male and female p53 haplo-insufficient mice (14-15/sex/dose)	0, 0.5, 1, or 2 g/L (0, 45, 80, or 150 mg/kg-day for males; ; 0, 80, 145, or 220 mg/kg-day for females) un-neutralized DCA in drinking water for 26 weeks	-	NTP (2007b)
Micronucleus assay with peripheral blood erythrocytes	Male and female Tg.AC mice ^a (v-Haras transgenic; 8-15/sex/dose)	Study 1: 0, 0.5, 1, or 2 g/L (0, 75, 145, or 240 mg/kg-day for males; 0, 100, 180, or 300 mg/kg-day for females) un-neutralized DCA in drinking water for 26 weeks followed by Study 2: 0, 22.3, 89.3, or 357 mg/kg-day un-neutralized DCA dermally for 26 weeks	-	NTP (2007b)
Micronucleus assay with peripheral blood erythrocytes	Male and female B6C3F1 mice (10/sex/dose)	0, 0.067, 0.125, 0.25, 0.5, or 1 g/L (water consumption and doses not reported) un-neutralized DCA in drinking water for 3 months	+/- (females: positive for trend but no pairwise significance)	NTP (2007b)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male F344 rats (5-27/dose)	0, 15, 41, 113 or 3,300 mg/kg un-neutralized DCA (estimated from graph) by oral gavage, 4 hours	+ (at highest dose)	Nelson and Bull (1988)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (5-28/dose)	0, 0.7, 17 or 6,500 mg/kg un-neutralized DCA (estimated from graph) by oral gavage, 4 hours	+ (at highest dose)	Nelson and Bull (1988)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (6-13/dose/time point)	0, 10, or 500 mg/kg unneutralized DCA by oral gavage (single dose) 1, 2, 4, 8 or 24 hours	+ (at 1, 2 and 4 hours; all doses)	Nelson et al. (1989)
DNA alkaline unwinding assay (DNA strand breaks) hepatic, duodenal and stomach tissues	Male B6C3F1 mice	Single dose: 1-10 mmol/kg (0.13-1.3 g/kg) neutralized DCA by oral gavage, 4 hours (4/dose)	+ (liver cells) - (duodenal and stomach cells)	Chang et al. (1992)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice	Repeated dose: 0, 0.5, or 5 g/L neutralized DCA in drinking water for 7 or 14 days (3/dose/time point)	+	Chang et al. (1992)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male F344 rats	Single dose : 1-5 mmol/kg (0.13-0.65 g/kg) neutralized DCA by oral gavage, 4 hours (4/dose)	-	Chang et al. (1992)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male F344 rats	Repeated dose: 0, 0.05, 0.5, or 2 g/L neutralized DCA in drinking water for 30 days (5/dose)	-	Chang et al. (1992)
SCGE (Comet) assay with leukocytes (DNA strand breaks)	Male B6C3F1 mice (10/dose/time point)	0, 0.5, 1, 2, or 3.5 g/L neutralized DCA in drinking water for 28 days	+ (high dose)	Fusco et al. (1996)
Alkaline elution (DNA single strand breaks) hepatic tissue	Male B6C3F1 mice (8/dose/time point)	0 or 300 mg/kg sodium dichloroacetate by oral gavage (single dose) 6 or 12 hours	+	Hassoun and Dey (2008)
Alkaline elution (DNA single strand breaks) hepatic tissue	Male B6C3F1 mice (6/dose)	0, 12.5, 25, or 50 mg/kg-day sodium dichloroacetate by oral gavage for 13 weeks	+ (at all doses)	Hassoun et al. (2014)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference	
HPLC-EC of digested liver DNA (8-OHdG formation, precursor to point mutations)	male B6C3F1 mice (6/dose/time point)	Single dose: 0 or 300 mg/kg neutralized DCA by oral gavage, 0, 4, 6 or 8 hours	+	(at 4 and 6 hours)	Austin et al. (1996)
Non-mammalian: Newt micronucleus test	Newt (<i>P. waltii</i>) larvae (15/dose)	20-80 µg/ml un-neutralized DCA in water for 12 days	-		Giller et al. (1997)
Micronucleus and SCGE (Comet) assay with <i>V. faba</i> root meristem	<i>V. faba</i> (fava bean) root tips (3/dose)	1 µM-1 mM (micronucleus) or 1-1,000 µM (SCGE) DCA ^a in root water for 5 hours; 24-hour recovery	+	(both assays, at 1,000 µM)	Hu et al. (2017)

8-OHdG, 8-hydroxydeoxyguanosine; HPLC-EC, high performance liquid chromatography with electrochemical detection; SCGE, single cell gel electrophoresis

^aa transgenic strain hemizygous for a mutant v-Ha-ras transgene

Table 6.4 Genetic and related effects of DCA in vitro (human and mammalian cells, non-mammalian experimental systems)

Assay	Results Without S9	Results With S9	DCA Concentration	Reference
Primary cells: Micronucleus induction in human peripheral blood lymphocytes	+	ND	25, 50 or 100 µg/ml	Varshney et al. (2013)
DNA alkaline unwinding assay (DNA strand breaks) in primary hepatocytes from male B6C3F1 mice, male F344 rats; human CCRF-CEM cells	-	ND	1 – 20 mM (mouse cells), 1 – 10 mM (rat cells), 1 – 10 mM (CCRF-CEM cells)	Chang et al. (1992)
Mammalian cells: Mouse lymphoma cell forward mutation assay in L5178Y/TK ^{+/-} cells	+	ND	0.1 – 0.8 mg/ml	Harrington-Brock et al. (1998)
Chromosome aberrations test in L5178Y/TK ^{+/-} cells	+	ND	0.6, 0.8 mg/ml	Harrington-Brock et al. (1998)
Aneuploidy, micronuclei in L5178Y/TK ^{+/-} cells	-	ND	0.6, 0.8 mg/ml	Harrington-Brock et al. (1998)
Mouse lymphoma cell forward mutation assay in L5178Y/TK ^{+/-} cells	-	-	0.125 – 5 mg/ml	Fox et al. (1996)

Chromosome aberration assay in CHO (Chinese hamster ovary) cells	-	-	1.25 – 5 mg/ml	Fox et al. (1996)
SCGE (Comet) assay (genomic DNA damage) in CHO cells	-	ND	1 – 25 mM	Kargalioglu et al. (2002); Plewa et al. (2004); Plewa et al. (2010)
Non-mammalian cells: Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98, TA1538	+	+	1 – 10 µg/plate (0.5 – 5 µg/ml)	Herbert et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100, TA1535, TA1537	-	-	1 – 10 µg/plate (0.5 – 5 µg/ml)	Herbert et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100	+	+	Up to 600 ppm (0.6 mg/ml)	DeMarini et al. (1994)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and <i>E. coli</i> WP2urvA	-	-	0.3 – 5 mg/plate	Fox et al. (1996)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100 (Ames fluctuation test)	+	+	-S9: 0.03 – 3 mg/ml +S9: 0.3 – 10 mg/ml	Giller et al. (1997)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA104	-	-	1 mg/ml	Nelson et al. (2001)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98 (Ames preincubation test)	+	-	-S9: 10 – 60 mM +S9: 5 – 60 mM	Kargalioglu et al. (2002)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100 (Ames preincubation test)	+	+	-S9: 10 – 60 mM +S9: 5 – 60 mM	Kargalioglu et al. (2002)
Bacterial reverse mutation assay, <i>S. typhimurium</i> RSJ100 (Ames preincubation test)	+	-	-S9: 5 – 80 mM +S9: 5 – 60 mM	Kargalioglu et al. (2002)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100, TA1535	+	-	33 µg/plate – 6.7 mg/plate	NTP (2007b)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98	-	-	3 µg/plate – 3.3 mg/plate	NTP (2007b)

umu test in TA1535/pSK1002	-	+	58.5 µg/ml	Ono et al. (1991)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	-	-	Up to 20 mM	Stalter et al. (2016)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	+	ND	0.2 – 17.3 mM	Zhang et al. (2016)
λ prophage induction in <i>E. coli</i> WP2	+/-	+	Up to 5 mg/ml	DeMarini et al. (1994)
SOS chromotest in <i>E. coli</i> PQ37	+	-	0.01 µg/ml – 10 mg/ml	Giller et al. (1997)
DNA repair test in <i>S. typhimurium</i> TS24 <i>recA</i> , TA2322 <i>polA</i> , TA1950 <i>uvrB</i>	-	ND	31 mg/plate	Waskell (1978)

ND, not determined; CHO, Chinese hamster ovary; CCRF-CEM, acute human lymphoma-derived cell line SCGE, single cell gel electrophoresis.

Developmental and Reproductive Toxicity

DCA causes male reproductive toxicity in rats and beagle dogs, including testicular damage and adverse effects on sperm motility and morphology. No female reproductive toxicity effects of DCA have been reported. Developmental toxicity, including delayed fetal development and cardiac anomalies with DCA treatment during gestation, has been reported in rats, as described below. US EPA (2003b) concluded that DCA has adverse effects on the male reproductive system in rats and dogs and causes fetal developmental effects in rats.

DCA was originally added to California’s Proposition 65¹ list as causing reproductive toxicity in 2009, based on its identification as a male reproductive toxicant by ACGIH (American Conference of Governmental Industrial Hygienists). Changes in federal regulations in 2012 eliminated this justification for listing, but DCA still meets the criteria for listing based on the US EPA (2003b) identification of DCA as causing male reproductive toxicity and developmental toxicity.

Developmental toxicity in vitro

Studies in cultured rodent embryos showed developmental defects in the brain, heart, and eye, somite dysgenesis, and various other defects. In vitro developmental toxicity of DCA was observed at concentrations as low as 0.5-1 mM (Saillenfait et al., 1995; Andrews et al., 2004). In vitro studies are summarized in Table 6.5.

¹ <https://oehha.ca.gov/proposition-65/proposition-65-list>

Table 6.5 Summary of in vitro developmental toxicity studies of DCA

Cells/Species	Concentration/ Duration	Endpoints	Reference
B6C3F1 mouse oocytes and epididymal sperm	0, 100, or 1,000 ppm ^a un-neutralized DCA for 24 hours	Decreased fertilization rate	Cosby and Dukelow (1992)
Sprague-Dawley rat whole embryos explanted on GD 9.5	0, 1, 2.5, 3.5, 5, 7.5, or 10 mM un-neutralized DCA for 46 hours	Impaired growth and differentiation; increased number of morphologically abnormal embryos; decreased protein and DNA levels; brain and eye defects	Saillenfait et al. (1995)
CD-1 mouse whole embryos explanted on GD 9	0, 0.73, 1.47, 4.40, 5.87, 7.34, 11.01, or 14.68 mM un-neutralized DCA* for 24 hours	Defects in neural tube, pharyngeal arch, heart, and eye; rotational defects in embryo position; somite dysgenesis	Hunter et al. (1996)
Sprague-Dawley rat whole embryos explanted on GD 9.5	0, 0.5, 1, 2.5, 3.5, or 5 mM un-neutralized DCA for 48 hours	Increased embryo lethality; decrease in somite number, crown-rump length, head length, and developmental score; rotational defects; delayed development; heart and neural tube defects; eye malformations; hypoplasia of prosencephalon and visceral arches	Andrews et al. (2004)

^a Concentrations (ppm) were on v/v basis, chemical density not reported.

* pH of DCA solutions was similar to control

Developmental toxicity in vivo

Adverse developmental effects have been reported in rat pups following gestational exposure to DCA. These included cardiac malformations, ocular toxicity, and decreased body weight. Findings of maternal toxicity were inconsistent: while Smith et al. (1992) identified increased relative liver weight in dams as the most sensitive endpoint in the study (LOAEL = 14 mg/kg-day), Epstein et al. (1992) observed no changes in organ weights with the same strain/exposure route and at higher DCA doses. In a study exposing zebrafish embryos to HAAs, DCA was the only one that induced morphological effects such as pericardial edema at concentrations of 23.5 mM and 46.5 mM when administered for up to 76 hours post-fertilization (Teixido et al., 2015). In a previous study in zebrafish embryos, DCA (administered at 8-32 mM, for up to 144 hours post-fertilization) produced a range of developmental effects, including craniofacial and muscular malformations, yolk sac edema, decreased hatching rate, cardiovascular effects (changes in heart rate and blood flow) and changes in behavior (Hassoun et al., 2005). Although DCA acted as a neurotoxicant in acute and subchronic studies, including in children, no studies on developmental neurotoxicity of DCA were identified by OEHHA.

Mammalian developmental toxicity studies are summarized in Table 6.6. NOAELs and LOAELs are identified by OEHHA, except for single-dose studies. The most sensitive endpoint for

developmental toxicity appears to be the increase in soft tissue anomalies in pups of Long-Evans rats reported by Smith et al. (1992), with a NOAEL of 14 mg/kg-day.

Table 6.6 Summary of in vivo studies of DCA reporting developmental toxicity endpoints

Sex/ Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Female Long-Evans rats, pregnant (19-21/dose)	0, 900, 1400, 1900, or 2400 mg/kg-day neutralized DCA by oral gavage on GD 6 to GD 15	Dams ^a : mortality (at two highest doses); decreased body weight gain; decreased number of live fetuses per litter; increased post-implantation loss; increased relative liver, spleen and kidney weights	LOAEL: 900 mg/kg-day (multiple endpoints for dams and pups)	Smith et al. (1992)
Female Long-Evans rats, pregnant (19-21/dose)	0, 900, 1400, 1900, or 2400 mg/kg-day neutralized DCA by oral gavage on GD 6 to GD 15	Pups: decreased body weight and fetal length; increased cardiovascular, orbital, urogenital and soft tissue anomalies (including cardiovascular and urogenital malformations); increased M/F sex ratio	LOAEL: 900 mg/kg-day (multiple endpoints for dams and pups)	Smith et al. (1992)
Female Long-Evans rats, pregnant (19-21/dose)	0, 14, 140, or 400 mg/kg-day neutralized DCA by oral gavage on GD 6 to GD 15	Dams ^a : decreased total implants/litter, increased relative liver, spleen and kidney weights	LOAEL: 14 mg/kg-day (relative liver weight)	Smith et al. (1992)
Female Long-Evans rats, pregnant (19-21/dose)	0, 14, 140, or 400 mg/kg-day neutralized DCA by oral gavage on GD 6 to GD 15	Pups: decreased length and weight, increased soft tissue anomalies, increased cardiovascular anomalies	NOAEL: 14 mg/kg-day (soft tissue anomalies)	Smith et al. (1992)
Female Long-Evans rats, pregnant (7-17/dose/treatment group)	0 or 1,900 mg/kg neutralized DCA by oral gavage on GDs 6-8, 9-11, or 12-15	Dams: no differences in body or organ weights Pups: intraventricular septal (cardiac) defects ^b	LOAEL ^c : 1,900 mg/kg-day	Epstein et al. (1992)
Female Long-Evans rats, pregnant (7-17/dose/treatment group)	0 or 2,400 mg/kg neutralized DCA by oral gavage on GDs 10, 11, 12, or 13	Dams: no differences in body or organ weights Pups: intraventricular septal (cardiac) defects ^b	LOAEL ^c : 2,400 mg/kg-day	Epstein et al. (1992)

Sex/ Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Female Long-Evans rats, pregnant (7-17/dose/ treatment group)	0 or 3,500 mg/kg neutralized DCA by oral gavage on GDs 9, 10, 11, 12, or 13	Dams: no differences in body or organ weights Pups: intraventricular septal (cardiac) defects ^b	LOAEL ^c : 3,500 mg/kg-day	Epstein et al. (1992)
Female Sprague- Dawley rats, pregnant (18-20/dose)	0 or 300 mg/kg-day neutralized DCA by oral gavage on GD6-15	Dams: decreased body weight Pups: ocular toxicity (decreased lens area, globe area and interocular distance); no cardiotoxicity	LOAEL ^c : 300 mg/kg-day	Fisher et al. (2001); Warren et al. (2006)

GD, gestation day; NA, not applicable

^a Sporadically decreased weight gain in dams was observed over GDs 6-20; however, this effect lacked consistency, trend and dose-response.

^b Poor agreement among time groups within each study

^c This LOAEL was identified from a single-dose study.

Reproductive toxicity in vivo

Reproductive toxicity was observed in male rats and dogs. In general, degeneration of the testicular epithelium; reductions in testis weight, sperm count, and sperm mobility; and morphological changes to sperm were observed. The male reproductive toxicity studies on DCA are summarized in Table 6.7. NOAELs and LOAELs are identified for the studies by OEHHA. Some studies have found no toxic effects of DCA on the male reproductive system in rats (Mather et al., 1990; Stacpoole et al., 1990).

Table 6.7 Summary of in vivo studies of DCA reporting reproductive toxicity endpoints

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague- Dawley rats (10-15/dose)	0, 125, 500, or 2,000 mg/kg-day sodium dichloroacetate by oral gavage for 3 months, followed by 4 week recovery	Testicular germinal epithelial degeneration; syncytial giant cells in germinal epithelium; decreased spermatozoa in epididymis (quantitative data not reported)	LOAEL: 125 mg/kg-day	Katz et al. (1981)
Male beagle dogs (3-4/dose)	0, 50, 75, or 100 mg/kg-day sodium dichloroacetate by oral gavage (gelatin capsules) for 13 weeks, followed by a 5 week recovery period	Prostate glandular atrophy; testicular germinal epithelial lesions (degeneration, syncytial giant cells, vacuolation of Leydig cells; quantitative data not reported)	LOAEL: 50 mg/kg-day	Katz et al. (1981)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Wistar rats (6/dose)	0 or 0.04 mol/kg sodium dichloroacetate (0 or 516 mg/kg-day) in feed for 12 weeks	Decreased combined absolute testis and epididymis weight (dose exceeded MTD)	LOAEL ^a : 516 mg/kg-day	Yount et al. (1982)
Male Sprague-Dawley rats (5/dose)	0 or 80.5 mmol/L (0 or 1,100 mg/kg-day ^b) neutralized DCA in drinking water for 90 days	Decreased absolute testis weight; testicular atrophy and pathology (multinucleated giant cells, enlarged Sertoli cells, interstitial hyperplasia); disrupted spermatogenesis (quantitative data not reported; dose exceeded MTD)	LOAEL ^a : 1,100 mg/kg-day	Bhat et al. (1991)
Male and female beagle dogs (5/sex/dose)	0, 12.5, 39.5, or 72 mg/kg-day neutralized DCA by oral gavage (gelatin capsules) for 90 days	Males: testicular lesions including syncytial giant cells and degeneration of germinal epithelium; prostatic glandular atrophy (0/5,4/5,5/5,5/5); Females: no effect on ovary weights	LOAEL: 12.5 mg/kg-day (testicular lesions)	Cicmanec et al. (1991)
Male and untreated female Long-Evans rats (18-19/dose)	0, 31.25, 62.5, or 125 mg/kg-day sodium dichloroacetate by oral gavage for 10 weeks; untreated female rats were used to study the fertility of exposed males	Males: decreased absolute and relative epididymis and preputial gland weights; increased relative testis weight (high dose); decreased epididymal sperm counts; sperm malformations and impaired motility; decreased implants/dam	LOAEL: 31.25 mg/kg-day (absolute epididymis weight)	Toth et al. (1992)
Male Fischer 344 rats (7-23/dose)	Study 1: 0, 3.6 or 40.2 mg/kg-day neutralized DCA in drinking water (additional dose of 402 mg/kg-day excluded from analysis due to toxicity) for 100 weeks, 21-23/dose; Study 2: 0 or 139 ^c mg/kg-day neutralized DCA in drinking water for 78 weeks, 7/dose	Absolute and relative testis weights were significantly increased in Study 1, at 40.2 mg/kg-day (7.68±0.53 g, 1.93±0.12% BW) relative to control (5.77±0.53 g, 1.39±0.12% BW), but decreased in Study 2, at 139 mg/kg-day (2.85±0.39 g, 0.81±0.14%) relative to control (5.69±0.49 g, 5.77 g, 1.24±0.11% BW); in Study 2, the DCA dose was progressively decreased due to toxicity ^b	NOAEL: 3.6 mg/kg-day	DeAngelo et al. (1996)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague-Dawley rats (6-8/dose)	Single exposure study: 0, 1500 or 3000 mg/kg neutralized DCA by oral gavage, sacrificed at 2, 14 or 28 days after dosing	Delayed spermiation; altered resorption of residual bodies at all time points (results described semi-quantitatively); no changes in serum testosterone or testis and epididymis weights ^d	LOAEL: 1500 mg/kg	Linder et al. (1997)
Male Sprague-Dawley rats (6-8/dose)	Repeated dose study: 0, 18, 54, 160, 480, 1,440 mg/kg-day neutralized DCA by oral gavage for 2, 5, 9 or 14 days	Decreased sperm counts; sperm malformations; impaired sperm motility; delayed spermiation; atypical residual bodies; distorted acrosomes (at various time points)	NOAEL: 54 mg/kg-day (effects on spermatogenesis at 14 days)	Linder et al. (1997)
Male Wistar rats (8/dose)	0, 0.5 or 2 g/L (0, 73.7 or 147.5 mg/kg-day) un-neutralized DCA in drinking water for 2 months	Decrease in absolute testis and epididymis weight; decreased FSH, LH and testosterone levels; increased SOD and CAT activity in testis; increase in lipid peroxidation; decreased GPx and GSH levels; degeneration of seminiferous tubules; depletion of germ cells; disintegration of spermatogenic cells; incomplete spermatogenesis	LOAEL: 73.7 mg/kg-day (multiple endpoints)	El Arem et al. (2014)

BW, body weight; CAT, catalase; FSH, follicle stimulating hormone, GPx, glutathione peroxidase; GSH, glutathione; LH, luteinizing hormone; MTD, maximum tolerated dose; NA, not applicable; SOD, superoxide dismutase

^a This LOAEL was identified from a single-dose study.

^b Doses estimated by US EPA (2003b).

^c The high dose (139 mg/kg-day) was averaged for 8 weeks exposure to 2.5 g/L DCA, 18 weeks to 1.5 g/L DCA and 77 weeks exposure to 1 g/L DCA in drinking water (total exposure 103 weeks). However, testis weights were measured in the interim sacrifice group (78 weeks), thus, the effective average dose would be higher than the reported 139 mg/kg-day.

^d Increased absolute testis weight (at high dose, 28 days) and increased testicular sperm head count “appeared to be spurious results arising from unusually low control values” (Linder et al., 1997).

Immunotoxicity

DCA is one of the metabolites of TCE, and TCE exposure is associated with the development of inflammatory autoimmune diseases such as systemic lupus erythematosus and scleroderma. These diseases may be induced by TCE metabolites, including DCA, dichloroacetyl chloride, and others (Khan et al., 1995; Khan et al., 1997; Khan et al., 2001; Cai et al., 2006; Cai et al., 2007). DCA is also one of the metabolites of tetrachloroethylene, which is associated with induction of scleroderma-like syndromes.

Cai et al. (2007) compared the immunotoxic responses of autoimmune-prone MRL^{+/+} and normal B6C3F1 female mice exposed at 110 mg/kg-day and 92 mg/kg-day, respectively, to

neutralized DCA in drinking water for 12 weeks. DCA significantly increased relative liver weights in both mouse strains versus their respective controls. The serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicators of liver damage, were not significantly altered in either strain. The serum concentrations of immunoglobulin G and M, (IgG and IgM) were significantly increased in the MRL^{+/+} mice, suggesting immune activation, whereas only serum IgG3 was increased in the B6C3F1 mice. DCA treatment did not change the serum levels of inflammatory cytokines in either strain. DCA treatment decreased IL-10 and CXCL-1 (the chemokine C-X-C motif ligand 1, formerly reported as KC) chemokines in the liver of the MRL^{+/+} mice, whereas T-helper cell and pro-inflammatory cytokines were increased in the liver of DCA-treated B6C3F1 mice. Treatment with DCA also increased lipid accumulation in the liver and the effect was more severe in the B6C3F1 than in the MRL^{+/+} mice. Overall, hepatotoxicity was the primary effect of DCA exposure due to enhanced inflammatory response without pronounced immune responses in B6C3F1 mice, while DCA treatment of MRL^{+/+} mice enhanced immune responses with little hepatotoxicity.

Neurotoxicity

Behavioral, functional, and structural indices of neuropathy may be induced in both rats and dogs at DCA doses similar to those used clinically. The DCA-induced symptoms in animals included hind-limb weakness, deficits in gait and righting reflex, tremors, brain and spinal cord demyelination, and paralysis, which would indicate lesions in the peripheral nervous system, the central nervous system, and/or muscle (Katz et al., 1981; Yount et al., 1982; Cicmanec et al., 1991; DeAngelo et al., 1996; Moser et al., 1999; Calcutt et al., 2009).

Moser et al. (1999) reported that the neurotoxic potency of DCA is stronger when it is administered with drinking water than by oral gavage, and that F344 rats were generally more sensitive than Long-Evans rats. In contrast to DCA studies in humans, dogs and rats, no neurotoxicity or morphological changes of the nervous system have been reported in mice to date (US EPA, 2003b).

Animal studies reporting neurotoxicity endpoints are summarized in Table 6.8; NOAELS and LOAELS are identified by OEHHA.

Table 6.8 Summary of in vivo studies of DCA reporting neurotoxicity endpoints

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male and female Sprague-Dawley rats (10-15/sex/dose)	0, 125, 500, or 2,000 mg/kg-day sodium dichloroacetate by oral gavage for 3 months followed by a 4-week recovery period	Vacuolization of white myelinated tracts of the cerebrum and cerebellum (potential artifact; no additional pathology to support an adverse effect)	NA	Katz et al. (1981)
Male and female beagle dogs (3-4/dose)	0, 50, 75, or 100 mg/kg-day sodium dichloroacetate by oral gavage (gelatin capsules) for 13 weeks followed by a 5-week recovery period	Ataxia (female, 1/3); hindlimb weakness (male, 1/4); paralysis (at high dose); vacuolization of white matter in the cerebrum and cerebellum	LOAEL: 50 mg/kg-day (brain lesions)	Katz et al. (1981)
Male Wistar rats (6/dose)	0 or 0.04 mol/kg neutralized DCA in feed (0 or 323-516 mg/kg-day estimated by US EPA (2003b); dose changed as animals gained weight) for 12 weeks	Hindlimb weakness; abnormal gait; decreased nerve conduction velocity; decrease in tibial nerve cross-section area; decreased absolute and relative brain weight	LOAEL ^a : 323 mg/kg-day	Yount et al. (1982)
Male and female beagle dogs (5/sex/dose)	0, 12.5, 39.5, or 72 mg/kg-day neutralized DCA by oral gavage in gelatin capsules for 90 days	Increased relative brain weight; partial hindlimb paralysis (1/5 females, 2/5 males at high dose)	NOAEL: 39.5 mg/kg-day	Cicmanec et al. (1991)
Male Fischer 344 rats (21-28/dose)	Study 1: 0, 0.05, 0.5, or 5 g/L (0, 3.6, 40.2, or 402 mg/kg-day) neutralized DCA in drinking water for 100 weeks (high dose group was started at 5 g/L DCA and progressively reduced to 2.5 g/L, 2 g/L and 1 g/L due to neuropathy); high dose animals were sacrificed at 60 weeks; interim sacrifices at 15, 30, 45 and 60 weeks, 5-7/time point)	Peripheral neuropathy at high dose	NOAEL: 40.2 mg/kg-day	DeAngelo et al. (1996)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Fischer 344 rats (21-28/dose)	Study 2: 0 or 2.5→1.5→1 ^b g/L (0 or 139 mg/kg-day) neutralized DCA in drinking water for 103 weeks; interim sacrifices at 14, 26, 52 and 78 weeks, 6-7/time point	Mild transient neurotoxicity observed initially at 139 mg/kg-day that “was mostly ameliorated with the lowered DCA concentrations”	LOAEL ^a : 139 mg/kg-day	DeAngelo et al. (1996)
Male and female Long-Evans rats (9-10/dose)	Single dose of 0, 100, 300, 1,000 or 2,000 mg/kg neutralized DCA by oral gavage; test times: at 4, 24, 168, and 336 hours	Decreased motor activity (males and females, at 4 and 24 hours); decreased hindlimb grip strength (males, at 4 and 24 hours, no dose-response relationship)	NOAEL ^c : 100 mg/kg-day (hindlimb grip strength)	Moser et al. (1999)
Male Long-Evans rats (10/dose)	0, 30, 100, 300, or 1,000 mg/kg-day neutralized DCA by oral gavage for 10 weeks followed by a 1-week recovery period; animals were tested at 2, 4, 7, and 11 weeks	Gait abnormalities; mild tremors, hypotonia, and decreased forelimb grip strength	NOAEL ^c : 100 mg/kg-day (gait)	Moser et al. (1999)
Male Long-Evans rats (10/dose)	0, 23, 122, or 220 mg/kg-day neutralized DCA in drinking water for 8 weeks followed by a 2-week recovery period; animals were tested at 2, 5, 8, and 10 weeks	Gait abnormalities; decreased forelimb and hindlimb grip strength	NOAEL ^c : 23 mg/kg-day	Moser et al. (1999)
Male Long-Evans rats (10/dose)	Weanling rats: 0, 17, 88, or 192 mg/kg-day neutralized DCA in drinking water for 13 weeks; animals were tested at 3, 6, 9, and 13 weeks	Gait abnormalities; decreased hindlimb grip strength; inhibited pupil response; tremors and hypotonia at high dose	LOAEL ^c : 17 mg/kg-day (gait)	Moser et al. (1999)
Male Fischer 344 rats	0, 18, 91, or 167 mg/kg-day neutralized DCA in drinking water for 8 weeks followed by a 2- week recovery period; animals were tested at 2, 5, 8, and 10 weeks (10/dose)	Gait abnormalities; decreased forelimb and hindlimb grip strength; decreased motor activity; increased foot splay; chest claspings; lack of pupil response	LOAEL ^c : 18 mg/kg-day (gait)	Moser et al. (1999)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Fischer 344 rats	0, 137, or 235 mg/kg-day neutralized DCA in drinking water for 24 months (high dose exposure discontinued at 6 months due to excessive toxicity); tests every month the first year, every two months the second year (18-24/dose)	Severe neurotoxicity at high dose (partial recovery after discontinued exposure); severe gait abnormalities; decreased forelimb and hindlimb grip strength; righting deficits and tremors; chest claspings; inhibited pupil response; increased landing foot splay	LOAEL ^c : 137 mg/kg-day	Moser et al. (1999)
Male Fischer 344 weanling rats (9-12/dose)	0, 162, or 308 mg/kg-day neutralized DCA in drinking water for 12 weeks followed by a 5-week recovery period (high dose exposure discontinued after 3 weeks due to excessive toxicity); animals were tested at 3, 6, 9, 12, and 17 weeks	Gait abnormalities; decreased forelimb and hindlimb strength; altered righting reflex and motor activity; chest claspings	LOAEL ^c : 162 mg/kg-day	Moser et al. (1999)
Male Fischer 344 weanling rats (9-12/dose)	Study 1: 0, 16, 66, or 172 mg/kg-day neutralized DCA in drinking water for 12 weeks; animals were tested at 3, 6, 9, 13, 16, 19, 23, and 27 weeks; Study 2: 0, 16, 89, or 173 mg/kg-day neutralized DCA in drinking water for 13 weeks; animals tested at 3, 6, 9 and 13 weeks; Studies 1 and 2 were conducted as control studies for route (gavage vs. drinking water) and strain comparisons (Fischer vs. Long-Evans), respectively	Gait abnormalities and righting deficits (all doses); transient increase in foot splay (at high dose and mid-dose); decreased motor activity; decreased hind limb grip strength; tremors; chest claspings; inhibited pupil response at high dose; animals generally recovered from these effects within 3-6 weeks (Study 1)	LOAEL ^c : 16 mg/kg-day	Moser et al. (1999)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Fischer 344 weanling rats (9-12/dose)	0 or 176 mg/kg-day (dose was lowered from 246 to 176 mg/kg-day at an unspecified time in the study) neutralized DCA by oral gavage for 12 weeks (averaged from 5/7 exposures); animals were tested at 3, 6, 9, 13, 16, 19, 23, and 27 weeks	Gait abnormalities (but less in comparison to drinking water exposure); little to no effect for tremors, grip strength, motor activity, righting reflex, and pupil response	LOAEL ^a : 176 mg/kg-day	Moser et al. (1999)
Female Sprague-Dawley rats (8-18/dose)	Juvenile rats (4-6 weeks old): Study 1: 0 or 50 mg/kg-day; Study 2: 0, 100, 200, 500 mg/kg-day aqueous dichloroacetate (salt not specified) by oral gavage for 16 weeks	Thermal hypoalgesia; tactile allodynia; decreased nerve conduction velocity; decreased axonal diameter	NOAEL (Study 2): 100 mg/kg-day (nerve conduction velocity, axonal diameter)	Calcutt et al. (2009)
Female Sprague-Dawley rats (8-18/dose)	Adult rats (12-14 weeks old): 0 or 500 mg/kg-day aqueous dichloroacetate (salt not specified) by oral gavage for 8 weeks	Thermal hypoalgesia; tactile allodynia; decreased nerve conduction velocity; decreased axonal diameter; lipid peroxidation in sciatic nerve (adult rats)	LOAEL ^a : 500 mg/kg-day	Calcutt et al. (2009)

NA, not applicable

^a This LOAEL was identified from a single-dose study.

^b DCA treated animals were initially dosed with 2.5 g/L, then lowered to 1.5 g/L at eight weeks and lowered to 1 g/L at 26 weeks due to mild transient neurotoxicity.

^c OEHHA determined NOAEL and LOAEL in (Moser et al., 1999) based on the qualitative description of results. It was not stated whether the differences from control were statistically significant, and the data were not presented in sufficient detail for independent dose-response and statistical analysis.

Among the studies summarized in Table 6.8, (Moser et al., 1999) report gait disruptions resulting from low doses of DCA (16-18 mg/kg-day) administered to rats. These doses are designated as LOAELs by OEHHA for the corresponding studies in Table 6.8, based on the description of results; however, only mean gait scores were reported, and the statistical differences with corresponding controls were not reported and could not be estimated. For each animal, the gait score was ranked as 1 (no abnormalities), 2 (somewhat abnormal), 3 (moderately abnormal) or 4 (severely abnormal). Controls were assigned the score of 1, and at the reported minimal levels of observed effect, the gait scores were generally 1.2-1.3, indicating a generally mild adverse effect. These, and other neurotoxic effects in this study, were reversible within 3-6 weeks of discontinued exposure.

Neurotoxic effects from other studies summarized in Table 6.8 demonstrated much higher NOAEL/LOAEL values. As described in the section above on subchronic toxicity in humans,

subchronic DCA doses of 15-25 mg/kg-day resulted in neuropathy symptoms in some patients, which ameliorated after discontinuation of treatment, or in some cases, after switching to a lower DCA dose. While neurotoxic symptoms in humans are clearly concerning, the available neurotoxicity endpoints in animal studies do not appear to be the most sensitive or of acceptable quality for dose-response analysis compared to other noncancer endpoints of DCA toxicity, as described in the dose-response section below.

Chronic Toxicity

Chronic Toxicity in Humans

There are no studies of chronic exposure to DCA in humans. However, humans are exposed to DCA in drinking water, and epidemiological studies of DBPs, including haloacetic acids, are described in more detail in the *Human Epidemiology Studies on Disinfection Byproducts* section and Appendices B-C.

Chronic Toxicity in Animals

DCA-induced chronic noncancer effects include organ toxicity, mainly in the liver, kidney and testis, and alterations in glucose metabolism. Most of the chronic studies were designed to evaluate DCA carcinogenicity and its mechanisms in mice and rats. Table 6.9 summarizes the chronic DCA studies, with NOAELs and LOAELs identified by OEHHA.

Table 6.9 Summary of chronic noncancer studies of DCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male and female B6C3F1 mice (10-35/group)	Males: 0, 1, or 2 g/L (0, 140, or 300 mg/kg-day) Females: 0 or 2 g/L (0 or 480 mg/kg-day) neutralized DCA in drinking water for 52 weeks	Increased relative liver weight and hepatic multifocal areas of necrosis with frequent infiltration of lymphocytes; cytomegaly; glycogen accumulation in hepatocytes	LOAEL: 140 mg/kg-day	Bull et al. (1990)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male B6C3F1 mice (9-30/dose)	Study 1: 0, 0.05, 0.5, or 5 g/L (0, 7.6, 77, or 486 mg/kg-day) neutralized DCA in drinking water for 60 weeks; some animals in the low and mid dose group were exposed for additional 15 weeks (average doses not reported) Study 2: 0 or 3.5 g/L (410 mg/kg-day) neutralized DCA in drinking water for 60 weeks	Decreased body weight (high dose in either study); increased relative liver weight ^a ; increased relative kidney weight in Study 2	NOAEL: 7.6 mg/kg-day (relative liver weight)	DeAngelo et al. (1991)
Male B6C3F1 mice (20-24/dose)	0 or 0.5 g/L (0 or 88 mg/kg-day) neutralized DCA in drinking water for 104 weeks	Increased relative liver weight; hepatocellular cytomegaly and vacuolization; liver necrosis	LOAEL ^b : 88 mg/kg-day	Daniel et al. (1992)
Female B6C3F1 mice (40-134/dose)	0, 2.0, 6.67, or 20 mmol/L (0, 40, 115, or 330 mg/kg-day calculated by US EPA 2003b) neutralized DCA in drinking water for 360 or 576 days	Decreased body weight (high dose, 576 days); increased relative liver weight ^c ; increased hepatocellular vacuolization at both time points	LOAEL: 40 mg/kg-day	Pereira (1996)
Female B6C3F1 mice (39-25/dose)	0, 0.5, or 3.5 g/L (0, 94, or 438 mg/kg-day) neutralized DCA in drinking water for 104 weeks	Decreased body weight gain; increased relative liver weight (absolute liver weight not reported)	NOAEL: 94 mg/kg-day	Schroeder et al. (1997)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Fischer 344 rats (50-78/dose)	Study 1: 0, 0.05, 0.50, or 5 g/L (0, 3.6, 40.2, or 402 mg/kg-day) neutralized DCA in drinking water for 100 weeks (high dose group was started at 5 g/L DCA and progressively reduced to 2.5 g/L, 2 g/L and 1 g/L due to neuropathy); high dose animals were sacrificed at 60 weeks; interim sacrifices at 15, 30, 45 and 60 weeks, 5-7/ time point)	Increased absolute testis weight (at 40.2 mg/kg-day, 100 weeks)	NOAEL: 3.6 mg/kg-day	DeAngelo et al. (1996)
Male Fischer 344 rats (50-78/dose)	Study 2: 0 or 2.5→1.5→1 ^d g/L (0 or 139 mg/kg-day) neutralized DCA in drinking water for 103 weeks (DCA dose was sequentially reduced due to mild unspecified neurotoxicity); interim sacrifices at 14, 26, 52 and 78 weeks, 6-7/time point	Decreased body weight; decreased absolute testis weight (78 weeks)	LOAEL ^b : 139 mg/kg-day	DeAngelo et al. (1996)
Male B6C3F1 mice (35-88/dose)	0, 0.05, 0.5, 1, 2, or 3.5 g/L (0, 8, 84, 168, 315, or 429 mg/kg-day) neutralized DCA in drinking water for 26, 52, 78, or 100 weeks; interim sacrifices at 26, 52 and 78 weeks at all doses except for the low dose, 10-12/time point	Increased mortality; decreased body weight; increased absolute and relative liver weight; increased hepatic necrosis, hepatic cytomegaly and cytoplasmic vacuolization with glycogen accumulation ^e	LOAEL: 84 mg/kg-day (rel. liver weight at 26 and 52 weeks)	DeAngelo et al. (1999)
Male and female Tg.AC ^f mice	0, 0.5, 1, or 2 g/L (0, 75, 145, or 240 mg/kg-day for males; 0, 100, 180, or 300 mg/kg-day for females) un-neutralized DCA in drinking water for 26 weeks (15/sex/dose)	Decreased body weight; increased absolute and relative liver weights; hepatic cytoplasmic vacuolization	LOAEL: 75 mg/kg-day (rel. liver weight)	NTP (2007b)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male and female Tg.AC ^f mice	0, 0.5, 1, or 2 g/L (0, 75, 150, or 230 mg/kg-day for males; 0, 90, 185, or 265 mg/kg-day for females) un-neutralized DCA in drinking water for 41 weeks (10/sex/dose)	Decreased body weight; increased absolute and relative liver weights	LOAEL: 75 mg/kg-day (rel. liver weight)	NTP (2007b)
Male and female p53 haplo-insufficient ^g mice	0, 0.5, 1, or 2 g/L (0, 45, 80, or 150 mg/kg-day for males; 0, 80, 145, or 220 mg/kg-day for females) un-neutralized DCA in drinking water for 26 weeks (15/sex/dose)	Decreased body weight; increased relative organ weights (heart, kidney, liver, lung, and testis, possibly secondary to body weight decrease); hepatic cytoplasmic vacuolization (at 26 weeks)	LOAEL: 80 mg/kg-day (relative liver weight)	NTP (2007b)
Male and female p53 haplo-insufficient ^g mice	0, 0.5, 1, or 2 g/L (0, 45, 80, or 140 mg/kg-day for males; 0, 65, 140, or 220 mg/kg-day for females) un-neutralized DCA in drinking water for 41 weeks (10/sex/dose)	Decreased body weight; increased relative organ weights (heart, kidney, liver, lung, testis and thymus, possibly secondary to body weight decrease); and ovarian cysts in females (high dose, at 41 weeks)	LOAEL: 65 mg/kg-day (body weight)	NTP (2007b)
Male B6C3F1 mice (25-32/dose)	0 or 5 g/L (0 or 1,000 mg/kg-day) neutralized DCA in drinking water for 61 week; additional groups were initiated with ENU (2.5 or 10 µg/kg) followed by 0, 2, or 5 g/L (0, 0.4, or 1 g/kg-day) neutralized DCA in drinking water for 61 weeks	Decreased body weight; increased absolute and relative liver weight; decreased absolute kidney weight	LOAEL ^b : 1,000 mg/kg-day	Herren-Freund et al. (1987)

ENU, ethylnitrosourea; NA, not applicable.

^a Relative liver weight (liver weight as % of body weight) was increased at 77 and 486 mg/kg-day (6.83%±1.92, and 17.57%±4.37 body weight, respectively, p<0.02) relative to control (5.01%±0.32) and at 410 mg/kg-day (11.63±1.13, p<0.02) relative to control (5.06%±0.32). The large increase measured for the 486 mg/kg-day group “was due in great part to the presence of proliferating nodules and neoplastic lesions” (DeAngelo et al., 1991).

^b This LOAEL was identified from a single-dose study.

^c Data on relative liver weights in Pereira (1996) are presented graphically and statistical significance at individual doses is not indicated.

^d DCA treated animals were initially dosed with 2.5 g/L and then lowered to 1.5 g/L at eight weeks and then to 1 g/L at 26 weeks due to mild transient neurotoxicity observed at 2.5 g/L.

^e No incidence data were shown in the study DeAngelo et al. (1999) for hepatocellular cytomegaly and cytoplasmic vacuolization with glycogen deposition, but prevalence and severity were “dose related and considered significant in all groups examined when compared to the control liver.”

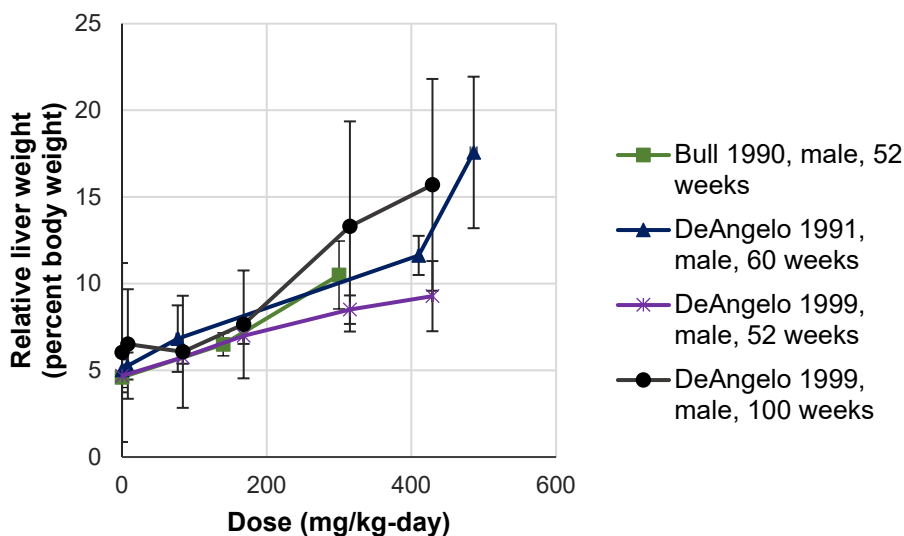
^f Transgenic mice with v-Ha-ras oncogene fused to fetal zeta globin promoter and SV-40 polyadenylation/splice sequence.

^g Transgenic mice with a null mutation in one p53 allele.

Effects in liver (comparison of studies)

Increased relative liver weight and various accompanying liver pathologies, such as vacuolization with glycogen deposition, were the most common noncancer effects in the chronic studies of DCA in mice, and in many studies increased relative liver weight was the most sensitive endpoint. There is good agreement among chronic multi-dose studies regarding increased relative liver weight in male B6C3F1 mice (Fig. 6.1).

Figure 6.1 Relative liver weights in chronic DCA studies in male B6C3F1 mice. Values are mean +/- standard deviation of 8-50 replicates



At doses >300 mg/kg-day, higher increases in relative liver weight are observed in the studies that found high hepatic tumor incidences (Table 6.9: (Schroeder et al., 1997); (DeAngelo et al., 1999) at 100 weeks) compared to the studies that identified fewer tumors (DeAngelo et al., 1999) at 52 weeks). While the increased relative liver weight at higher doses (>300 mg/kg-day) in the former studies may be partially due to tumor mass, this is unlikely to account for the liver weight increases at lower doses, given the various non-neoplastic liver pathologies observed at the range of NOAELs and LOAELs. Two studies with the lowest LOAEL or NOAEL values for increased relative liver weight in B6C3F1 mice, the 52-week subset from DeAngelo et al. (1999) and (DeAngelo et al., 1991) (Table 6.9), are described in more detail below.

DeAngelo et al. (1999), in a study of DCA carcinogenicity, exposed 35 to 71 male B6C3F1 mice per dose to neutralized DCA in drinking water at 0, 0.05, 0.5, 1, 2, or 3.5 g/L for up to 100 weeks. The study authors estimated lifetime average doses as 0, 8, 84, 168, 315, or 429 mg/kg-day, respectively. Ten mice per dose group, except the lowest dose, were sacrificed at 26, 52, and 78 weeks. Dose-dependent absolute and relative liver weight increases were seen at doses ≥ 84 mg/kg-day at 26 and 52 weeks. Mean body weights at 315 and 429 mg/kg-day were significantly reduced at sacrifice. ALT activity was significantly increased at doses ≥ 84 mg/kg-day; liver necrosis was observed at doses ≥ 168 mg/kg-day. There were no interim sacrifices of animals from the 8 mg/kg-day dose group, thus OEHHA identified a LOAEL of 84 mg/kg-day based on increased relative liver weight at 26 and 52 weeks.

DeAngelo et al. (1991) administered neutralized DCA in drinking water at 0, 0.05, 0.5, or 5.0 g/L to 9-30 male B6C3F1 mice per dose for 60 weeks, with interim sacrifices of 5 mice/dose at weeks 4, 15, 30, and 45, yielding time-weighted average doses of 0, 7.6, 77, or 486 mg/kg-day (Study 1). In a separate experiment (Study 2), mice (10-12/dose) were exposed to 0 or 3.5 g/L DCA for 60 weeks, receiving a time-weighted average dose of 410 mg/kg-day. Water

consumption was reduced to 60% that of controls at 486 mg/kg-day in Study 1. Final body weights were decreased to 87% and 83% of respective controls at 486 mg/kg-day in Study 1 and at 410 mg/kg-day in Study 2, but were not affected at the low and mid doses. Relative liver weights were increased with the mid and high doses in Study 1 and with the single dose in Study 2, as indicated in the footnote to Table 6.9 (data from Study 1 and Study 2 from DeAngelo et al. (1991) are combined in Fig. 6.1). Increased kidney weights were seen at 486 mg/kg-day. There were no significant changes in testis or spleen weights. Liver tumors were observed at the highest doses, as discussed in the *Carcinogenicity* section. OEHHA identified a chronic noncancer NOAEL of 7.6 mg/kg-day based on increased relative liver weight.

Effects on testis (comparison of studies)

Male rats and dogs exhibited testicular effects with exposure to DCA. Among the studies presented in Table 6.7, the most common endpoint measured was absolute testis weight, which was increased in some studies, decreased in some studies and did not change in other studies. A single-dose study by Linder et al. (1997) showed an increase in absolute testis weight with a NOAEL of 1500 mg/kg. Other studies, including a subset of the DeAngelo et al. (1996) report (Study 2), indicated a decrease in testis weight at higher doses over a range of exposure times, as well as detailed pathological findings consistent with testicular toxicity of DCA (El Arem et al. (2017); DeAngelo et al. (1996) Study 2; Bhat et al. (1991)). While there are no obvious correlations of the directionality of the effect with the dose or rat strain or duration of the study, potential DCA effects on the testis are of concern since any change in testis weight would be considered adverse (US EPA, 1996). It appears the most sensitive study is by DeAngelo et al. (1996) Study 1, showing increased absolute testis weight in Fischer 344 rats with a NOAEL of 3.6 mg/kg-day. However, no corroborating histopathology of the testis or other reproductive effects were reported in this study. With regard to the increased testis weight observed in DeAngelo et al. (1996), US EPA indicated that “this endpoint was not deemed to be the most sensitive because no histopathological effects were noted” (US EPA, 2003b). Moreover, it is worth noting that older Fischer 344 rats are prone to non-treatment related pathological effects in the testes, which was one of the reasons why this rat strain is no longer used by the NTP for cancer bioassays (King-Herbert and Thayer, 2006; King-Herbert et al., 2010; Maronpot et al., 2016).

Carcinogenicity

State, federal, and international agencies have recognized DCA as a carcinogen or potential carcinogen in humans. In May 1996, based on conclusions from an authoritative body (US EPA), DCA was listed as a carcinogen under Proposition 65. US EPA classified DCA as a “likely human carcinogen” and consequently promulgated a maximum contaminant level goal (MCLG) of zero for DCA in drinking water based on carcinogenicity (US EPA, 1998a). US EPA derived an oral cancer slope factor of $0.05 \text{ (mg/kg-day)}^{-1}$ based on liver adenomas and carcinomas in male B6C3F1 mice (US EPA, 2003b). IARC (2014) concluded that evidence for DCA carcinogenicity was inadequate in humans but sufficient in experimental animals, warranting a classification of Group 2B, possibly carcinogenic to humans. In the preliminary listing recommendation for the Report on Carcinogens, NTP classified DCA as reasonably

anticipated to be a human carcinogen based on sufficient evidence from studies in experimental animals and supporting mechanistic data (NTP, 2018).

A summary of DCA cancer studies reviewed by OEHHA is presented in Table 6.10. The majority utilized neutralized forms of DCA. Hepatic adenomas and carcinomas were reported in both sexes of B6C3F1 mice and in male F344 rats, and bronchiolar/alveolar adenomas and squamous cell papillomas were reported in male Tg.AC mice. Promoter studies were conducted with mice initiated with methylnitrosourea (MNU), ethylnitrosourea (ENU) and vinyl chloride; however, of these, few included uninitiated controls.

Table 6.10 Summary of carcinogenicity and promoter studies of DCA

Sex/ Species[#]	Dose¹/Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male B6C3F1 mice (25-32/dose)	Initiated with 2.5 µg/kg ENU (single i.p. injection); 0, 2, or 5 g/L (0, 400, or 1,000 mg/kg-day) neutralized DCA in drinking water for 61 weeks	hepatic adenoma 1/22, 22/29*, 31/32* hepatic carcinoma 1/22, 19/29*, 25/32*	3/25 animals in control group died	Herren-Freund et al. (1987)
Male B6C3F1 mice (25-32/dose)	Injected (i.p.) with 16.4 µg/kg sodium acetate (as ENU control); 0 or 5 g/L (0 or 1,000 mg/kg-day) neutralized DCA in drinking water for 61 weeks	hepatic adenoma 2/22, 25/26* hepatic carcinoma 0/22, 21/26*	5/27 animals in control group (0 mg/kg-day DCA) died	Herren-Freund et al. (1987)
Male and female B6C3F1 mice (11-35/dose)	Males: 0, 1, or 2 g/L (0, 140, or 300 mg/kg-day ^a) Females: 0 or 2 g/L (0 or 480 mg/kg-day) neutralized DCA in drinking water for 52 weeks	<u>Males:</u> hepatic adenoma 0/35, 0/1, 2/10 hepatic carcinoma 0/35, 0/1, 5/10* (Note: not all animals with lesions were examined histopathologically)	No DCA-dependent increase in tumors in females	Bull et al. (1990)
Male and female B6C3F1 mice (11-35/dose)	For a group of male mice (11/dose), 2 g/L DCA treatment was stopped after 37 weeks, mice were sacrificed at 52 weeks (280 mg/kg-day ^a)	hepatic adenoma 0/35, 2/11 hepatic carcinoma 0/35, 0/11	No DCA-dependent increase in tumors in females	Bull et al. (1990)

Sex/ Species#	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male B6C3F1 mice (50/dose)	<p>Study 1: 0, 0.05, 0.5, 5 g/L (0, 7.6, 77, or 486 mg/kg-day) neutralized DCA in drinking water; 5/dose group were sacrificed at 4, 15, 30 and 45 weeks; at 60 weeks 9 in control, low and mid dose groups each and 30 in high dose group were sacrificed; remaining animals in control, low and mid dose groups were sacrificed at 75 weeks</p> <p>Study 2: 0 or 3.5 g/L (0 or 410 mg/kg-day) neutralized DCA in drinking water for 60 weeks</p>	<p>Data from Study 1 and Study 2 (performed concurrently at the same facility) were combined; at control and two low dose groups, animals were pooled from 60- and 75-week time points. Combined data for 0, 7.6, 77, 410, 486 mg/kg-day groups: hepatic adenoma 0/28, 2/29, 1/27, 12/12*, 24/30* hepatic carcinoma 2/28, 5/29, 2/27, 8/12*, 25/30* hepatic adenoma or carcinoma 2/28, 7/29, 3/27, 12/12*, 27/30*</p>	Data were reported graphically as prevalence; body weights in two high dose groups were 83% and 87% of respective controls	DeAngelo et al. (1991)
Male B6C3F1 mice (33/dose)	0 or 0.5 g/L (0 or 93 mg/kg-day) neutralized DCA in drinking water for 104 weeks	<p>hepatic adenoma 1/20, 10/24* hepatic carcinoma 2/20, 15/24* hepatic adenoma or carcinoma 3/20, 18/24*</p>	Single dose study	Daniel et al. (1992)
Male B6C3F1 mice (10-110/dose)	0 or 5 g/L (0 or 900 mg/kg-day ^a) neutralized DCA in drinking water; all surviving treated animals (89) and ten controls were sacrificed at 76 weeks, additional controls were sacrificed at 96, 103 and 134 weeks (total 14 for three time points)	<p>Tumor incidences for controls were pooled for 76- to 134-week groups; hepatic adenoma 2/24, 83/89* hepatic carcinoma 2/24, 66/89*</p>	Single dose study	Anna et al. (1994)

Sex/ Species#	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male F344 rats (60/dose)	0, 0.05, 0.5, or 2.4 g/L (0, 4, 40, or 296 mg/kg-day ^a) neutralized DCA in drinking water for 15, 30, 45, or 60 weeks (7/dose/time point, 27 at 60 weeks for high dose), or 104 weeks (23-29/dose group, only low and mid doses)	Incidences were summed for 45- to 104-week groups without duration adjustments: hepatic adenoma 1/37, 0/40, 6/43, 8/34* hepatic carcinoma 0/37, 0/40, 3/43, 1/34	Most control animals were sacrificed at 104 weeks to assess spontaneous tumorigenesis	Richmond et al. (1995)
Male B6C3F1 mice (number not specified)	0, 1, or 3.5 g/L (0, 180, or 630 mg/kg-day ^a) neutralized DCA in drinking water for 104 weeks	hepatic carcinoma 19%, 70.6%, 100%	Only percentages for tumor response are reported, no statistical analysis	Ferreira-Gonzalez et al. (1995)
Male Fischer 344 rats (50-78/dose)	Study 1: 0, 0.05, 0.5, or 5 g/L (0, 3.6, 40.2, or 402 mg/kg-day) neutralized DCA in drinking water for 100 weeks (high dose group was started at 5 g/L DCA and progressively reduced to 2.5 g/L, 2 g/L and 1 g/L due to neuropathy); high dose animals were sacrificed at 60 weeks; interim sacrifices at 15, 30, 45 and 60 weeks, 5-7/ time point); Study 2: 0 or 2.5→1.5→1 g/L (0 or 139 mg/kg-day) neutralized DCA in drinking water for 103 weeks (DCA dose was sequentially reduced due to mild unspecified neurotoxicity); interim sacrifices at 14, 26, 52 and 78 weeks, 6-7/time point	Neoplasms were combined for animals surviving ≥78 weeks, without duration adjustment; Study1: hepatic adenoma 1/23, 0/26, 5/29 hepatic carcinoma 0/23, 0/26, 3/29 combined 1/23, 0/26, 7/29 Study 2: hepatic adenoma 0/33, 3/28 hepatic carcinoma 1/33, 6/28* combined 1/33, 8/28*	Mean body weight in treated group in Study 2 was 73% of the control value	DeAngelo et al. (1996)

Sex/ Species [#]	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Female B6C3F1 mice (40-134/dose)	0, 2.0, 6.67, or 20 mM (0, 40, 115, or 330 mg/kg-day ^a) neutralized DCA in drinking water for 360 or 576 days; ^Δ separate group (starting number of animals not specified) exposed to 20 mM for 24 days + 48 days no exposure, repeating this 72-day cycle until sacrifice at 360 or 576 days (averaged dose, 135 mg/kg-day)	hepatic adenoma <u>360 days</u> : 1/40, 0/40, 3/20, 7/20* [^Δ 0/15] <u>576 days</u> : 2/90, 3/50, 7/28*, 16/19* [^Δ 3/34] hepatic carcinoma <u>360 days</u> : 0/40, 0/40, 0/20, 1/20 [^Δ 0/15] <u>576 days</u> : 2/90, 0/50, 1/28, 5/19* [^Δ 1/34]	Mean body weight in the high dose group was approximately 80% of control value	Pereira (1996)
Female B6C3F1 mice (6-39/dose)	Initiated with 25 mg/kg MNU (single i.p. injection); 0, 2.0, 6.67, or 20 mM (0, 50, 167, or 468 mg/kg-day ^a) neutralized DCA in drinking water for 31 or 52 weeks; ^Δ separate recovery group exposed to 20 mM for 31 weeks + 21 weeks no exposure	hepatic adenoma <u>31 weeks</u> : 0/10, 1/10, 0/6, 5/10* <u>52 weeks</u> : 7/38, 2/8, 1/8, 16/22* [^Δ 6/12] hepatic carcinoma <u>52 weeks</u> : 4/38, 3/8, 2/8, 4/22 [^Δ 2/12]	The 360-day subset from (Pereira, 1996) served as saline control for MNU treatments; decreased body weight at high dose	Pereira and Phelps (1996)
Female B6C3F1 mice (25-39/dose)	0, 0.5, or 3.5 g/L (0, 94, or 438 mg/kg-day) neutralized DCA in drinking water for 104 weeks	hepatic carcinoma 1/39, 1/25, 23/25*	Mean body weight in the high dose group was approximately 82% of control value	Schroeder et al. (1997)
Female B6C3F1 mice (20-30/dose)	Initiated with 25 mg/kg MNU (single i.p. injection); 0, 7.8, 15.6, or 25 mM neutralized DCA for 44 weeks	hepatic adenoma incidences are not reported hepatic carcinoma 0/29, 0/17, 0/19, 3/29	Neither body weights, water consumption nor doses were reported	Pereira et al. (1997)

Sex/ Species [#]	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male B6C3F1 mice (35-88/dose)	0 or 0.05 (no interim sacrifice), 0.5, 1, 2, or 3.5 g/L (0, 8, 84, 168, 315, or 429 mg/kg-day) neutralized DCA in drinking water for 26, 52, 78, or 100 weeks (10/dose at 26-, 52- and 78-week interim sacrifices)	hepatic adenoma <u>100 weeks:</u> 5/50, 1/33, 5/25, 18/35*, 9/21*, 5/11* hepatic carcinoma <u>100 weeks:</u> 13/50, 11/33, 12/25, 25/35*, 20/21*, 11/11* hepatic adenoma or carcinoma <u>100 weeks^b:</u> 18/50, 11/33, 14/25, 30/35*, 21/21*, 11/11*; additionally, incidences for hepatic adenomas and/or carcinomas in 52-week and 78- week groups are reported in Table 10.11	Significant early mortality and decreased body weights at two highest doses	DeAngelo et al. (1999)
Male and female B6C3F1 mice (8-29/sex/ dose)	Initiated with 30 mg/kg MNU (single i.p. injection); 0 or 3.2 g/L (males: 0 or 453 mg/kg-day; females: 0 or 483 mg/kg-day ^c) neutralized DCA in drinking water for 31 weeks	<u>Males:</u> hepatic adenoma 2/8, 21/25* hepatic carcinoma 0/8, 7/25 hepatic adenoma or carcinoma 2/8, 23/25* <u>Females:</u> hepatic adenoma 2/29, 17/24* hepatic carcinoma 0/29, 0/24 hepatic adenoma or carcinoma 2/29, 17/24*	Single dose study	Pereira et al. (2001)

Sex/ Species#	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male B6C3F1 mice (20/dose)	0, 0.1, 0.5, or 2 g/L (0, 11, 54, or 216 mg/kg-day ^c) dichloroacetate (salt not specified; pH of solution adjusted to 7.0) in drinking water for 52 weeks	hepatic adenoma 0/20, 1/20, 4/20, 10/19* hepatic carcinoma 0/20, 0/20, 1/20, 1/19 hepatic adenoma or carcinoma 0/20, 1/20, 5/20*, 10/19*	None	Bull et al. (2002)
Male B6C3F1 mice (10/dose)	Initiated with 3 mg/kg vinyl carbamate (route not specified); 0, 0.1, 0.5, or 2 g/L (0, 20, 100, or 400 mg/kg-day) neutralized DCA in drinking water for 18, 24, 30, or 36 weeks	hepatic tumor response (tumors/mouse) and mean tumor volume increased in a dose- and time-dependent manner over 18-36 weeks	Individual tumor incidences not reported	Bull et al. (2004)
Male and female Tg.AC mice (v-Ha-ras transgenic) (10 or 15/sex/dose/ time point)	0, 0.5, 1, or 2 g/L (males: 0, 75, 145, or 235 mg/kg-day; females: 0, 100, 185, or 285 mg/kg-day) un- neutralized DCA in drinking water for 26 or 41 weeks	<u>males:</u> alveolar/bronchiolar adenoma (at 41 weeks) 1/10, 2/10, 7/10*, 3/10	Non- monotonic dose response; no treatment- dependent mortality	NTP (2007b)
Male and female Tg.AC mice (v-Ha-ras transgenic) (10 or 15/sex/dose/ time point)	0, 31.25, 125, or 500 mg/kg un-neutralized DCA dermally 5 days per week (males and females: 0, 22.3, 89.3, or 357 mg/kg-day) for 26 or 39 weeks	squamous cell papilloma (at site of application, 39 weeks) males: 0/10, 0/10, 2/10, 8/10* females: 0/10, 0/10, 0/10, 6/10*	High incidence of non-site- specific squamous cell papilloma in controls; no treatment- dependent mortality	NTP (2007b)
Male and female p53 haplo- insufficient mice (25/sex/dose)	0, 0.5, 1, or 2 g/L (males: 0, 45, 80, or 145 mg/kg-day; females: 0, 75, 145, or 220 mg/kg-day) un- neutralized DCA in drinking water for 26 or 41 weeks	no tumors detected	No evidence of carcino- genicity	NTP (2007b)

Sex/ Species#	Dose ¹ /Route of Exposure/Duration	Tumor Type and Incidence	Notes	Reference
Male and female B6C3F1 mice (30-48/sex/dose; 26-28/sex/dose were examined for tumors)	Males: 0, 136, 232, or 297 mg/kg-day neutralized DCA in drinking water for 10 weeks followed by water only for 84 weeks	hepatic adenoma 5/27, 13/27, 11/27, 15/26* hepatic carcinoma 8/27, 8/27, 6/27, 19/26* hepatoblastoma 0/27, 1/27, 0/27, 0/26 hepatic adenoma, carcinoma or hepatoblastoma 12/27, 15/27, 14/27, 24/26*	Doses averaged over 94 weeks: 0, 14.5, 24.7, or 31.6 mg/kg-day Armitage-Doll adjusted doses ^d : 0, 28.1, 48.0, or 61.4 mg/kg-day	Wood et al. (2015)
Male and female B6C3F1 mice (30-48/sex/dose; 26-28/sex/dose were examined for tumors)	Females: 0, 142, or 253 mg/kg-day neutralized DCA in drinking water for 10 weeks followed by water only for 84 weeks	hepatic adenoma 0/27, 9/26*, 6/28* hepatic carcinoma 0/27, 2/26, 3/28 hepatoblastoma 0/27, 0/26, 0/28 hepatic adenoma, carcinoma or hepatoblastoma 0/27, 10/26*, 9/28*	Doses averaged over 94 weeks: 0, 15.1, or 26.9 mg/kg-day Armitage-Doll adjusted doses ^d : 0, 29.4, or 52.3 mg/kg-day	Wood et al. (2015)
Male B6C3F1 mice (total of 404 mice used were divided into four treatment groups; number per group not specified)	0 or 3.5 g/L neutralized DCA in drinking water for 4, 10, 26, 52, or 93 weeks followed by water only through 93 weeks; interim sacrifices were conducted at multiple points (number of animals not specified) Mean DCA doses reported: 429 (4w), 479 (10w), 423 (26w), 397 (52 w), 377 (93w) mg/kg-day	Pooled animals from different time groups were sacrificed at 52, 57, 78 or 93 weeks, no duration adjustment; hepatic adenoma 12/52 (control), 7/28 (4w), 18/55 (10w), 22/54 (26w), 30/54* (52w), 26/44* (93w) hepatic carcinoma 9/52, 23/28*, 27/55*, 32/54*, 35/54*, 41/44* hepatic adenoma or carcinoma 19/52, 24/28*, 34/55*, 39/54*, 49/54*, 44/44*	Average dose over 93 weeks: 18.5, 51.5, 118.2, 222.0, 337 mg/kg-day Armitage-Doll adjusted doses ^d : 37.9, 99.0, 189.4, 259.6, 269.6 mg/kg-day	Wehmas et al. (2017)

Number of animals (per dose) that were started on treatment is indicated.

¹ Non-control doses are converted to mg/kg-day in parenthesis where possible.

*significantly different from control in Fisher's exact test ($p < 0.05$)

^a Doses estimated by US EPA (2003b).

^b Combined adenoma and carcinoma data from US EPA (2003b).

^c Doses calculated using reference water consumption by Gold and Zeiger (1996).

^d Armitage-Doll dose adjustment factor is made when the experimental period is less than lifetime. It is

calculated as $\frac{(T_e - a)^3 - (T_e - b)^3}{T^3}$, where T_e is time to observation (94 weeks), T is lifetime (assumed 104 weeks), a (0) and b (10) are the boundaries of the treatment interval.

ENU, ethylnitrosourea; i.p. intraperitoneal; mM, mmole/liter; MNU, N-methyl-N-nitrosourea; TCA, trichloroacetic acid;

Among mouse cancer studies that exhibited a dose-response, three are considered high quality due to experimental set-up (includes multiple dose groups, includes one or more low-dose groups, large number of animals per dose group, and quality control of administered DCA) and adequate reporting of results. These studies (DeAngelo et al., 1999; Bull et al., 2002; Wood et al., 2015) are described below in more detail and are further analyzed in the *Dose-Response Assessment* section. Rat studies are not considered for dose-response analysis due to the lower sensitivity of this species as observed in the available studies. No other species of laboratory animals have been analyzed for DCA carcinogenicity.

DeAngelo et al. (1999) administered 0, 0.05, 0.5, 1, 2, or 3.5 g/L DCA to male B6C3F1 mice (10-50/dose) in drinking water for up to 100 weeks, with interim sacrifices at 26, 52 and 78 weeks. For the low dose (0.05 g/L) group, there were no interim sacrifices. High treatment-related mortality was observed in this study, with significantly fewer treated animals at final sacrifice (100 weeks) compared to the control. At 100 weeks, the mean daily doses reported by the authors were 0, 8, 84, 168, 315, and 429 mg/kg-day. Mean daily water consumption over the study period was lower at the highest dose compared to the control; body weights were significantly lower at the two high doses (approximately 80% of the control) at 100 weeks, and the reduced body weight was attributed to malnutrition and physical wasting (cachexia) associated with tumors. Significant hepatic necrosis was observed at the highest dose (429 mg/kg-day) at each sacrificial time point, and also in the 168 and 315 mg/kg-day groups at 26 weeks. Separate hepatic carcinoma and adenoma incidences were reported in the paper, but not the combined incidence of either adenoma and/or carcinoma (DeAngelo et al., 1999). However, OEHHHA requested and obtained individual animal data from the study authors, and derived the combined incidences of hepatic adenomas and carcinomas for those time points when tumors were detected (Table 6.11); 26-week data are not being used for dose-response analysis and are not included here.

Table 6.11 Incidences of combined hepatic adenomas and carcinomas in male mice exposed to DCA in drinking water (DeAngelo et al., 1999)¹

Weeks of treatment	Dose (mg/kg-day)					
	Control	8	84	168	315	429
52	0/10 ²	--	1/10	1/10	2/10	7/10*
78	2/10	--	1/10	4/10	8/10*	9/10*
100	18/50	11/33	14/25	30/35*	21/21*	11/11*

¹ Individual animal data provided to OEHHA by the study authors

² Carcinomas+adenomas/animal at final sacrifice

* Significantly different from control in Fisher's exact test (p<0.05)

Bull et al. (2002) gave 0, 0.1, 0.5, or 2 g/L DCA in drinking water to groups of male B6C3F1 mice (20/dose) for 52 weeks. Mortality was minimal. There was no decrease in body weight at any dose relative to the control group, and water consumption was not reported. Using the body weight in the control group at the end of the study (46.2 g) and assuming daily water consumption at 5 ml (Gold and Zeiger, 1996), OEHHA calculated the administered doses as 0, 10.8, 54.1, and 216.5 mg/kg-day. After 52 weeks, 0/20, 1/20, 4/20 and 10/19 animals, respectively, displayed hepatic adenomas, and 0/20, 0/20, 1/20 and 1/19 animals, respectively, displayed hepatic carcinomas. Combined adenomas and/or carcinomas were 0/20, 1/20, 5/20 and 10/19. Increases in combined adenomas and carcinomas in the mid and high dose groups were statistically significant compared to control (Fisher's Exact test, p<0.05).

In a study designed to examine the effect of early life exposure to DCA, Wood et al. (2015) administered 0, 1, 2, or 3.5 g/L neutralized DCA in drinking water to male and female B6C3F1 mice (30-48/dose) for 10 weeks followed by clean water for 84 weeks. Female mice received 0, 1 and 2 g/L doses only. Mortality was minimal, and mean body weights were similar among control and treated groups at all doses with both sexes. Water intakes were measured, and the time-averaged doses were estimated by the authors as 0, 136, 232 and 297 mg/kg-day in male mice and 0, 142 and 253 mg/kg-day in female mice. Combined incidences of hepatic adenoma, carcinoma and hepatoblastoma were 12/27, 15/27, 14/27 and 24/26 in males; and 0/27, 10/26, 9/28 in females. Incidences at the high dose in males (297 mg/kg-day) and at both doses in females (142 and 253 mg/kg-day) were significantly increased relative to their respective controls when compared using Fisher's exact test (p<0.05).

Mode of Action and Mechanistic Considerations

Oncogene Activation

As described in the *Genetic Toxicity* section, DCA likely possesses genotoxic potential, particularly in in vivo systems. This could lead to the occurrence and accumulation of DNA mutations in animals administered DCA. When genetic mutations occur in proto-oncogenes or tumor suppressor genes, they can activate the former and inactivate the latter, initiating and/or contributing to tumor progression. H-ras, c-jun and other signal transduction proteins in the MAPK/ERK (mitogen activated protein kinase/extracellular signal-regulated kinase) pathway are common targets of genotoxic carcinogens.

Elevated expression of H-ras was closely associated with malignancy (hepatocarcinomas) in male B6C3F1 mice administered neutralized DCA in drinking water (Nelson et al., 1990). Several studies examined the mutation frequency in codon 61 in the *H-ras* proto-oncogene in liver tumors of mice (Anna et al., 1994; Ferreira-Gonzalez et al., 1995; Schroeder et al., 1997). Ferreira-Gonzalez et al. (1995) reported that the frequency of *H-ras* mutations in liver carcinomas of male mice given DCA in drinking water for 104 weeks was similar to that in spontaneously arising carcinomas of untreated controls, although with a different mutation pattern. Similar results in male mice given DCA by oral gavage for 76 weeks were reported by Anna et al. (1994), where mutation frequency was unchanged but mutation pattern was altered. To determine whether DCA acts on the H-ras pathway, Tg.AC mice were dermally exposed to DCA. Squamous cell papillomas were observed in male and female mice after 39 weeks of 357 mg/kg-day DCA exposure suggesting the H-ras gene was associated with DCA carcinogenesis. In contrast, Schroeder et al. (1997) reported finding only one mutation of *H-ras* in 22 hepatic tumors from female mice exposed to DCA in drinking water for 104 weeks. This suggests that *H-ras* mutations, which would result in activation of this proto-oncogene, may not be the sole mechanism responsible for DCA hepatocarcinogenesis in mice, with likely involvement of other proto-oncogenes, or possible DCA promoter effects on pre-existing mutations. This is consistent with the DCA-dependent increased cell proliferation in *c-jun*-positive hepatic foci reported by (Stauber and Bull, 1997). Based on histopathological analysis of liver tumors in DCA-treated male B6C3F1 mice, Carter et al. (2003) proposed multiple pathways of DCA carcinogenesis.

DNA Hypomethylation

Changes in levels, and as a consequence, activity, of proto-oncogenes or tumor suppressor genes can also result from epigenetic changes, i.e., heritable changes in gene regulation that do not involve DNA mutations. One of the most common epigenetic mechanisms affected by environmental chemicals is DNA methylation. Hypomethylation, or decreased addition of methyl residues to the nucleotides within a gene promoter, could increase transcriptional activity of this gene, contributing, in the case of proto-oncogenes, to tumor promotion.

Epigenetic changes were observed in female B6C3F1 mice following oral exposure to DCA. Administration of DCA for 44 weeks following initiation with MNU caused a significant decrease in 5-methyl cytosine in liver adenocarcinoma DNA, but not in DNA from non-tumor hepatic tissue, compared to control animals (Tao et al., 1998; Ge et al., 2001). Hypomethylation of DNA was observed in the promoter regions of the proto-oncogenes *c-jun* and *c-myc* in the liver of treated mice compared to controls. Both mRNA and proteins expressed by the two oncogenes were increased after DCA treatment (Tao et al., 2000a, 2000b; Ge et al., 2001; Tao et al., 2004a; Tao et al., 2004b).

Peroxisome Proliferation

Activation of certain cellular receptors is also a common mechanism of chemically induced carcinogenesis. As an example, activation of peroxisome proliferator-activated receptor α (PPAR α) by prototypical ligands, such as phthalates, leads to peroxisome proliferation, as the name implies, but also oxidative stress, activation of proliferation and tumorigenesis in the liver

of rodents. While TCA has been long characterized as a PPAR α activator (the specifics of the mechanism of activation remain unknown), some evidence of DCA-dependent peroxisome proliferation was also reported.

Peroxisome proliferation has been reported in mice and rats following exposure to DCA in drinking water in some studies (DeAngelo et al., 1989; Austin et al., 1995; DeAngelo et al., 1999), but not in others (Parrish et al., 1996). In vitro studies showed that DCA activates human and mouse PPAR α at concentrations greater than 1 mM (Maloney and Waxman, 1999). Moreover, when observed in cancer studies, peroxisome proliferation following DCA treatment occurred only at high doses and not at lower doses that resulted in a statistically significant increase in liver tumors. These data suggest that peroxisome proliferation is not a critical step in DCA tumorigenesis (DeAngelo et al., 1996; DeAngelo et al., 1999).

Alteration of Cell Proliferation and Apoptosis

Stimulation of cell proliferation and inhibition of apoptosis are necessary steps in tumorigenesis that facilitate clonal expansion of transformed cells and eventual neoplasm formation. A complete carcinogen would be capable of tumor initiation, e.g. through a genotoxic event, and tumor promotion, which stimulates growth of transformed cells. While DCA acted as a complete carcinogen in several cancer bioassays in mice (described in the *Carcinogenicity* section), examination of effects of DCA on proliferation and apoptosis would provide additional insights into the underlying mechanism of its tumorigenesis.

DCA administration promoted proliferation in mouse liver (Sanchez and Bull, 1990; Stauber and Bull, 1997; Ge et al., 2001) and suppressed apoptosis in murine hepatocytes (Snyder et al., 1995; Stauber et al., 1998) and in rat hepatocytes (Walgren et al., 2005). However, several studies reported no change or decreases in mouse liver cell proliferation in response to DCA. The initial increase in hepatic cell division rate in mice reported by Stauber and Bull (1997) was transient, as DCA exposure for ≥ 28 days (up to 38 weeks) caused a significant decrease in cell division. Carter et al. (1995) reported a decrease in hepatic cell proliferation following short-term exposures (5-25 days) of mice to DCA in drinking water. DeAngelo et al. (1999) reported no change in labeling index of hepatocytes (outside proliferative lesions) after 78 or 104 weeks of exposure of mice to DCA in drinking water.

Additionally, DCA treatment did not alter ^3H -thymidine incorporation, a marker of cell proliferation, in cultured hepatocytes from male Long-Evans rats in vitro (Walgren et al., 2005). Canine mammary adenoma and carcinoma cell lines treated with 10 mM un-neutralized DCA showed decreased cell proliferation while no change in apoptotic cells was detected (Harting et al., 2017). Canine prostate adenocarcinoma and transitional cell carcinoma derived cell lines were also treated with 10 mM un-neutralized DCA and a decrease in cell numbers was observed in all but one of the cell lines and no changes in apoptosis were detected (Harting et al., 2016). Duan et al. (2013) reported inhibition of murine C6 glioma cell proliferation and induction of cell apoptosis in vitro. DCA induced apoptosis in human colorectal and prostate carcinoma cells (Li et al., 2014).

Given the inconsistencies in these data, the contributions of altered cell proliferation and apoptosis to DCA-induced tumorigenesis are unclear.

Cellular Metabolism

Cancer cells rely primarily on glycolysis for ATP generation and not on oxidative generation, a much more effective mitochondria-based energy producing process. This phenomenon is termed the Warburg effect, and it is thought that this switch from aerobic to anaerobic glucose oxidation may also inactivate mitochondria-based apoptotic pathways. Thus, normalizing the mitochondrial function and reverting the cellular metabolism to the aerobic mode may counteract tumorigenesis. Interestingly, DCA has long been used as an FDA-approved medication against lactic acidosis, acting at the critical junction of glycolysis and the tricarboxylic acid cycle. It specifically inhibits pyruvate dehydrogenase kinase, with the resulting activation of the pyruvate dehydrogenase complex and diversion of pyruvate into the tricarboxylic acid cycle, away from being converted to lactate (Stacpoole et al., 2008b).

The net result of this action is funneling pyruvate into the mitochondria-based aerobic pathway of glucose utilization, and based on this consideration and some promising preliminary data, DCA was proposed as a novel anticancer agent (Bonnet et al., 2007). The preliminary results of clinical trials with DCA are promising (Michelakis et al., 2010). However, further clinical research will focus on balancing health risks and benefits of this compound, among other areas (Stacpoole, 2011).

In addition to its effect on pyruvate dehydrogenase activity, DCA appears to directly increase glycogen levels in primary murine hepatocytes at concentrations as low as 100 μM (Lingohr et al., 2002), and, importantly, is metabolized by glutathione transferase zeta 1 (GSTz1) to glyoxylate in a metabolic pathway leading to monochloroacetic acid (Tong et al., 1998a). DCA can also act as a mechanistic inhibitor of GSTz1, covalently binding to and inhibiting this enzyme, and repeated exposure to DCA intravenously or orally slows down its metabolism by GSTz1 in animals and humans (Curry et al., 1985; Curry et al., 1991; Tzeng et al., 2000; Ammini et al., 2003). Deficiency of GSTz1 also causes increased oxidative stress, increased expression of other GST forms and decreased glutathione levels (Blackburn et al., 2006). This may increase sensitivity to drug- and chemical-induced toxicity, and in fact, GSTz1^{-/-} mice given acetaminophen demonstrated increased hepatic toxicity compared to wild-type controls (Blackburn et al., 2006). While human GSTz1-1 appeared to have similar affinity for DCA compared to mouse and rat enzymes, it was inactivated about 3.5 times slower (Tzeng et al., 2000), though the extent of GSTz1 inactivation and its relevance to DCA toxicity in humans have not been studied and remain unclear. The biochemical effects, such as GSTz1 inhibition, observed at high DCA concentrations would likely be negligible at exposures to the relatively low environmental DCA concentrations found in drinking water (Li et al., 2008).

The available evidence on DCA effects on cellular metabolism primarily focuses on activation of the pyruvate dehydrogenase complex and inhibition of GSTz1. It is unlikely that either of these effects would play a role in DCA-dependent carcinogenesis.

US EPA's *Guidelines for Carcinogen Risk Assessment* US EPA (2005a) notes: "Knowledge of the biochemical and biological changes that precede tumor development (which include, but are not limited to, mutagenesis, increased cell proliferation, inhibition of programmed cell death, and receptor activation) may provide important insight for determining whether a cancer hazard exists and may help inform appropriate consideration of the dose-response relationship below the range of observable tumor response." Considered in this framework, DCA presents evidence of genotoxicity with positive findings of mutagenesis and DNA damage in several in vivo studies, as well as involvement in epigenetic mechanisms, such as hypomethylation. DCA was able to induce tumors in rodents in the absence of other chemical initiators, indicating that it can act as a complete carcinogen.

DCA's effects on proliferation are mixed. While liver cytotoxicity and cell regeneration have been demonstrated following DCA treatment, these effects occurred at higher doses than those required to induce tumors in rodents. This indicates that cytotoxicity with regenerative proliferation is not likely a key event in the MOA for DCA tumorigenesis. There is only one promoter study for DCA, which included control groups for the initiator ENU, and it is inconclusive. In this study (Herren-Freund et al., 1987), the tumor incidences for the DCA treatment group were high either in the absence or presence of an initiator, likely due to the high DCA dose administered (5 g/L in drinking water).

Conclusions on the Mode of Action for Carcinogenicity of DCA

Overall, the available data suggest that DCA carcinogenesis is complex and may involve multiple modes of action. Using the Key Characteristics of Carcinogens framework (Smith et al., 2016), the evidence suggests that DCA is associated with characteristics 2 and 4 (genotoxicity and epigenetic alterations, respectively), with genotoxicity being the most strongly supported. In vitro genotoxic evidence for DCA is mixed (see *Genetic Toxicity* section; also IARC (2014); NTP (2018)). While some bacterial and in vitro genotoxicity assays produced negative results (Table 6.3), in vivo genotoxicity assays were mostly positive (Table 6.4). Since DCA is an actively metabolized compound, in both humans and animals, in vitro systems may not adequately capture the metabolic conversions responsible for genotoxic events and/or DNA damage in vivo. Given the positive genotoxicity findings with DCA, OEHHHA assumes the default genotoxic mode of action for DCA-mediated increase in hepatic tumors in mice and rats.

7. TOXICOLOGICAL PROFILE: TRICHLOROACETIC ACID

Acute Toxicity

Effects in Humans

Trichloroacetic acid (TCA) is irritating and corrosive to skin and mucous membranes, and it has been used in skin-peeling treatments for a variety of conditions including hyperpigmentation and tattoo removal (US EPA, 2011; WHO, 2004c). At about 15% to 35% TCA, the adverse effects of the TCA skin-peeling formulation (15-35% TCA) are uncommon and include infection, persistent (>1 month) erythema, transient hyperpigmentation and other effects (US EPA, 2011). Applications of 95% TCA in water for tattoo removal can cause a severe chemical burn (Piggot and Norris, 1988; US EPA, 2011). OEHHHA did not identify any acute controlled exposure human studies of ingested TCA.

Effects in Animals

The oral gavage LD₅₀ for neutralized TCA was estimated to be 3,200 to 5,000 mg/kg in rats and 4,970 to 5,640 mg/kg in mice (Woodard et al., 1941; Bailey and White, 1965; NTP, 1991; WHO, 2004c). The oral gavage LD₅₀ for un-neutralized TCA was estimated to be approximately 1,000 mg/kg in male B6C3F1 mice (Miyagawa et al., 1995). Dosed mice and rats quickly went into a narcotic or semi-narcotic state, then either completely recovered or died in a narcotic state (Woodard et al., 1941). The i.p. and subcutaneous LD₅₀s of TCA in mice were 500 mg/kg and 270 mg/kg, respectively (as cited in NTP (1991); chemical form not specified).

Non-lethal effects observed in acute toxicity studies are summarized in Table 7.1. Based on these studies, TCA appears to have mild acute toxicity at the examined doses.

Table 7.1 Summary of acute toxicity studies of TCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Sprague-Dawley rats (4/dose)	Single dose of 1,500 mg/kg neutralized TCA by oral gavage; sacrificed at 5 or 18 hours post-treatment	Induction of hepatic ornithine decarboxylase	LOAEL ^a : 1,500 mg/kg	Parnell et al. (1988)
Male and female Sprague-Dawley rats (5/sex/dose)	Three doses of 0, 0.92 or 2.45 µmol/kg (0, 0.45 or 1.2 mg/kg) neutralized TCA by oral gavage over 24 hours, sacrificed 3 hours after last dose	Decrease in plasma lactate; decrease in liver lactate and plasma glucose (females)	NOAEL: 0.45 (mg/kg)	Davis (1990)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male B6C3F1 mice (6/dose)	Single dose of 0 or 300 mg/kg neutralized TCA by oral gavage; sacrificed at 6, 8 and 10 hours after treatment	Increased 8-OHdG formation in liver	LOAEL ^a : 300 mg/kg	Austin et al. (1996)
Male Sprague-Dawley rats (5/dose)	Single dose of 0 or 200 mg/kg TCA by i.p. injection; blood drawn at 1, 3 and 6 hours after treatment	Increased serum AST, LDH, and CPK	LOAEL ^a : 200 mg/kg	Demir and Çelik (2006)
Male B6C3F1 mice (8/dose)	Single dose of 0, or 300 mg/kg neutralized TCA by oral gavage; sacrificed 6 or 12 hours after treatment	At 12 hours: increased lipid peroxidation, DNA single strand breaks, SA in peritoneal lavage cells and liver cells	LOAEL ^a : 300 mg/kg	Hassoun and Dey (2008)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK, creatine phosphokinase; 8-OHdG, 8-hydroxydeoxyguanosine; i.p., intraperitoneal; LDH, lactate dehydrogenase; NA, not applicable; SA, superoxide anion

^a This LOAEL was identified from a single-dose study.

Short-Term Toxicity in Animals

Short-term effects of TCA administration include decreased body weight, increased liver weight, and increases in peroxisome proliferation in the liver and lipid peroxidation in liver and kidney. The short-term TCA studies are summarized in Table 7.2.

Table 7.2 Summary of short-term toxicity studies of TCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Wistar-derived rats (4-5/dose); male Swiss mice (4-5/dose)	10-200 mg/kg-day TCA ^a by gavage for 10 days	Increased PCO activity and peroxisome proliferation in the liver	NOAEL: 20 mg/kg-day (rat) NOAEL: 50 mg/kg-day (mouse)	Elcombe (1985)
Male Sprague-Dawley rats (4/dose)	0, 30, or 300 mg/kg-day trichloroacetate (salt not specified) by oral gavage for 7 days	Weight loss; decreased food consumption; cyanosis and hyperventilation	NOAEL: 30 mg/kg-day	Davis (1986)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Sprague-Dawley rats (4/dose)	0, 0.3 or 3 g/L (0, 24, or 240 mg/kg-day) trichloroacetate (salt not specified) in drinking water for 21 days	Decreased weight gain; decreased water consumption	NOAEL: 24 mg/kg-day	Davis (1986)
Male F344 rats (5-6/dose)	500 mg/kg un-neutralized TCA in corn oil by oral gavage for 10 days	Increased relative liver weight; increased peroxisome proliferation in liver and kidney in both rats and mice	LOAEL ^b : 500 mg/kg-day	Goldsworthy and Popp (1987)
Male B6C3F1 mice (7-8/dose)	500 mg/kg un-neutralized TCA in corn oil by oral gavage for 10 days	Increased relative liver weight; increased peroxisome proliferation in liver and kidney in both rats and mice	LOAEL ^b : 500 mg/kg-day	Goldsworthy and Popp (1987)
Male Sprague-Dawley rats (6/dose)	0, 6, 12, or 31 mM (0, 212, 327, or 719 mg/kg-day) neutralized TCA in drinking water for 14 days	Decreased body weight; decreased peroxisome volume; induction of peroxisome enzyme CAT, but no effect on PCO activity (marker of peroxisome proliferation)	LOAEL: 212 mg/kg-day (body weight)	DeAngelo et al. (1989)
Male F344 rats (6/dose)	0, 12, or 31 mM (0, 327, or 719 mg/kg-day ^c) neutralized TCA in drinking water for 14 days	Increased PCO activity at high dose	NOAEL: 327 mg/kg-day	DeAngelo et al. (1989)
Male Osborne-Mendel rats (6/dose)	0, 12, or 31 mM (0, 327, or 719 mg/kg-day ^c) neutralized TCA in drinking water for 14 days	Decreased relative liver weight and increased PCO activity at high dose	NOAEL: 327 mg/kg-day	DeAngelo et al. (1989)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male B6C3F1 mice (6/dose)	0, 6, 12, or 31 mM (0, 131, 261, or 442 mg/kg-day) neutralized TCA in drinking water for 14 days	Increased relative liver weight (high dose); increased activity of peroxisomal enzymes and peroxisome proliferation in liver (high dose, the only dose tested for this endpoint)	NOAEL: 261 mg/kg-day (liver weight)	DeAngelo et al. (1989)
Male C3H mice	0, 12, or 31 mM (0, 261, or 442 mg/kg-day ^d) neutralized TCA in drinking water for 14 days, (6/strain/dose)	Increased relative liver weight; increased PCO	LOAEL: 261 mg/kg-day	DeAngelo et al. (1989)
Male C57BL/6 mice	0, 12, or 31 mM (0, 261, or 442 mg/kg-day ^d) neutralized TCA in drinking water for 14 days, (6/strain/dose)	Increased relative liver weight; increased PCO	LOAEL: 261 mg/kg-day	DeAngelo et al. (1989)
Male Swiss-Webster mice	0, 12, or 31 mM (0, 261, or 442 mg/kg-day ^d) neutralized TCA in drinking water for 14 days, (6/strain/dose)	Increased relative liver weight; increased PCO	LOAEL: 261 mg/kg-day	DeAngelo et al. (1989)
Male B6C3F1 mice	0, 12, or 31 mM (0, 261, or 442 mg/kg-day ^d) neutralized TCA in drinking water for 14 days, (6/strain/dose)	Increased relative liver weight; increased PCO	LOAEL: 261 mg/kg-day	DeAngelo et al. (1989)
Male B6C3F1 mice (4-12/dose)	0, 0.3, 1, or 2 g/L (0, 75, 250, or 500 mg/kg-day ^e) neutralized TCA in drinking water for 14 days	Increased relative liver weight (data presented graphically without statistical analysis); increased hepatocyte diameter and increased [³ H]thymidine incorporation in hepatic DNA (high dose)	NOAEL: 250 mg/kg-day	Sanchez and Bull (1990)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male and female B6C3F1 mice (5/sex/dose)	0, 100, 250, 500, or 1,000 mg/kg-day neutralized TCA for 11 days by oral gavage	Increased relative liver weight; increased rate of DNA synthesis in the liver	LOAEL: 100 mg/kg-day	Dees and Travis (1994)
Male B6C3F1 mice (4-18/dose)	0 or 228 mg/kg-day neutralized TCA in drinking water for 14 days followed by single dose (acute challenge) of 0 or 300 mg/kg neutralized TCA by oral gavage on the 15 th day; animals sacrificed 9 hours after the gavage dose	TCA-pretreated mice: increased relative liver weight; increased PCO, CAT and CYP4A (laurate hydroxylase) activity; increased lipid peroxidation; No pretreatment: increased lipid peroxidation (acute effect)	LOAEL ^b : 228 mg/kg-day	Austin et al. (1995)
Female B6C3F1 mice (4-6/dose)	0 or 500 mg/kg-day neutralized TCA by oral gavage, followed 30 minutes later by i.p. injection with 0 or 450 mg/kg methionine, for 5 days	Increased relative liver weight; (not affected by co-treatment with methionine)	LOAEL ^b : 500 mg/kg-day	Tao et al. (2000a)
Female B6C3F1 mice (6/dose)	0 or 500 mg/kg-day neutralized TCA by oral gavage for 5 days; additional groups co-treated with 0, 0.4, 0.8 and 1.6 g/L chloroform in drinking water (for 12 days prior to TCA treatment and throughout TCA treatment)	Increased relative liver weight (not affected by co-treatment with chloroform)	LOAEL ^b : 500 mg/kg-day	Pereira et al. (2001)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Female B6C3F1 mice (6/dose/time point)	0 or 500 mg/kg-day neutralized TCA by oral gavage for 24, 36, 48, 72, and 96 hours	Increased relative liver weight (at 36, 72, 96 hours); increase in mitotic (at 72 hours) and PCNA (at 72, 96 hours) indices in the liver; <i>c-myc</i> promoter hypomethylation in liver, bladder and kidney (at 72, 96 hours)	LOAEL ^b : 625 mg/kg-day ^f	Ge et al. (2001)
SV129 wild-type and PPAR α -null mice (3-5/dose)	0, 0.25, 0.5, 1, or 2 g/L (0, 62.5, 125, 250 or 500 mg/kg-day ^e) neutralized TCA in drinking water for 7 days	Wild-type mice: hepatocyte hypertrophy; induction of lipid metabolic enzymes CYP4A, ACO, and PCO in the liver; these effects were not observed in PPAR α -null mice	NOAEL: 250 mg/kg-day	Laughter et al. (2004)
Male C57BL/6 mice (5/dose)	0 or 4 g/L (0 or 987 mg/kg-day ^g) un-neutralized TCA in drinking water for 7 days	Increased relative liver weight; hepatocyte hypertrophy; altered metabolic profile in urine that is consistent with peroxisome proliferation/fatty acid β -oxidation; increased expression of PPAR α -responsive genes; increased plasma LPC (18:1, 9Z)	LOAEL ^b : 987 mg/kg-day	Fang et al. (2013)

ACO, acyl-CoA oxidase; CAT, carnitine acetyl CoA transferase; CYP4A, cytochrome P450 4A; LPC (18:1, 9Z), lysophosphatidylcholine containing one octadecenoyl moiety with cis-double bond at position 9; NA, not applicable; ND, not determined; PCO, cyanide-insensitive palmitoyl-CoA oxidase; PCNA, proliferating cell nuclear antigen; PPAR α , peroxisome proliferator-activated receptor α

^a Information on whether TCA was neutralized or un-neutralized was not provided.

^b This LOAEL was identified from a single-dose study.

^c Dose estimate is based on doses administered to Sprague-Dawley rats.

^d Dose estimate is based on doses administered to B6C3F1 mice.

^e Doses calculated by US EPA (2011).

^f Average daily dose was used as the LOAEL.

^g OEHHA estimate is based on default values for body weight and water consumption (US EPA, 1988).

Subchronic Toxicity

Effects in Humans

OEHHA did not identify any controlled-exposure subchronic human studies of TCA through ingestion.

Effects in Animals

The available subchronic studies of TCA administered in drinking water are discussed below. Many studies reported concentrations of TCA used in the water, but did not always report the dose to the experimental animals. When not reported, the TCA doses in these studies were estimated either by US EPA (2011) or by OEHHA, based on US EPA (1988) default body weights and water consumption values for subchronic exposures. Subchronic oral exposure to TCA appears to primarily affect liver size and weight, collagen deposition, lipid and carbohydrate metabolism, and peroxisome proliferation, as summarized in Table 7.3. Most of these studies, especially those in mice, were conducted to evaluate the mode of action for TCA-induced liver toxicity.

Table 7.3 Summary of subchronic toxicity studies of TCA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference [†]
Male Sprague-Dawley rats with partial hepatectomy (4-6/dose)	0 or 5,000 mg/L (0 or 726 mg/kg-day ^a) neutralized TCA in drinking water for 0.5, 1, 3 or 6 months	Hepatic PCO activation (peroxisome proliferation) at all time points	LOAEL ^b : 726 mg/kg-day	Parnell et al. (1986)
Male Sprague-Dawley rats (10/dose)	0, 50, 500, or 5,000 mg/L (0, 4.1, 36.5, or 355 mg/kg-day) neutralized TCA in drinking water for 90 days	Increase in relative liver and kidney weights; increase in hepatic β -oxidation	NOAEL: 36.5 mg/kg-day	Mather et al. (1990)
Male Sprague-Dawley rats (5/dose)	0 or 45.8 mM (0 or 825 mg/kg-day ^c) neutralized TCA in drinking water for 90 days	Decreased body weight; collagen deposition and portal vein dilation/extension in liver; perivascular inflammation in lungs	LOAEL ^b : 825 mg/kg-day	Bhat et al. (1991)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference†
Male Wistar rats (5-6/dose)	0 or 25 mg/L un-neutralized TCA (0 or 3.8 mg/kg-day ^c) in drinking water for 10 weeks	Decreased body weight; Plasma: increased SDH activity; increased total triglyceride and glucose; Liver: decreased cholesterol and increased glycogen; Kidney: decreased GSH	LOAEL ^b : 3.8 mg/kg-day	Acharya et al. (1995)
Male Wistar rats (5-6/dose)	0 or 25 mg/L un-neutralized TCA (0 or 3.8 mg/kg-day ^c) in drinking water for 10 weeks	Hepatocyte hypertrophy; hepatic necrosis and altered architecture; renal tubular degeneration and proliferation	LOAEL ^b : 3.8 mg/kg-day	Acharya et al. (1997)
Male B6C3F1 mice (6/dose/time point)	0, 0.1, 0.5, or 2 g/L (0, 25, 125, or 500 mg/kg-day ^c) neutralized TCA in drinking water for 3 or 10 weeks	Increased relative liver weight; increased hepatic activity of PCO and CYP4A (laurate hydroxylase) at both time points	LOAEL: 25 mg/kg-day (PCO induction)	Parrish et al. (1996)
Male B6C3F1 mice (5/dose/time point)	0, 0.3 [#] , 1 [#] or 3 g/L (0, 75, 250, or 750 mg/kg-day ^c) neutralized TCA in drinking water for 4, 8 or 12 weeks; [#] these dose groups were only analyzed at 12 weeks	Increased relative liver weight; decrease in hepatic glycogen at all time points	LOAEL: 75 mg/kg-day	Kato-Weinstein et al. (2001)
Female Sprague-Dawley rats (number not specified)	0 or 2,000 ppm (0 or 300 mg/kg-day ^c) un-neutralized TCA in drinking water for 50 days	Increased SOD (brain, liver, kidney) and CAT (liver, kidney) activities	LOAEL ^b : 300 mg/kg-day	Çelik (2007)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference†
Male Sprague-Dawley rats (6/dose)	0 or 2,000 ppm (0 or 300 mg/kg-day ^c) un-neutralized TCA in drinking water for 52 days	Increased serum bilirubin and platelet count; Decreased serum cholesterol and total protein, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, and hematocrit	LOAEL ^b : 300 mg/kg-day	Çelik and Temur (2009)
Male Sprague-Dawley rats (6/dose)	0 or 2,000 ppm (0 or 300 mg/kg-day ^c) un-neutralized TCA in drinking water for 52 days	Decreased butyrylcholinesterase activity in heart and lung, and adenosine deaminase activity in heart, lung and spleen; Increased MPO activity in brain, liver, heart, kidney and spleen	LOAEL ^b : 300 mg/kg-day	Çelik et al. (2010)
Male B6C3F1 mice (7/dose/time point)	0, 7.7, 77, 154, or 410 mg/kg-day neutralized TCA by oral gavage for 4 or 13 weeks	Liver: increased lipid peroxidation, SA formation, and single strand DNA breaks at both time points; Peritoneal lavage cells: increased SA formation, TNF α , and SOD activity at both time points; increased MPO activity at 4 weeks	LOAEL: 7.7 mg/kg-day	Hassoun et al. (2010a); Hassoun et al. (2010b)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference†
Male B6C3F1 mice (number not specified)	0 or 77 mg/kg-day neutralized TCA by oral gavage for 13 weeks; animals were either on regular diet or vitamin E deficient diet	Only peritoneal lavage cells were examined. Regular diet: increased SA and TNF α ; increased MPO and SOD activities; Vitamin E deficient diet: increased SA and TNF α ; increased MPO, CAT, GSH-Px, and SOD activities (all endpoints significantly greater than those on regular diet, p<0.05)	LOAEL ^b : 77 mg/kg-day	Hassoun and Al-Dieri (2012)
Male B6C3F1 mice (6/dose)	0, 12.5, 25, or 50 mg/kg-day neutralized TCA by oral gavage for 13 weeks	Increase in MPO activity, SA, and TNF α in peritoneal lavage cells	LOAEL: 12.5 mg/kg-day	Hassoun et al. (2013)

CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase; MPO, myeloperoxidase; NA, not applicable; PCO, cyanide-insensitive palmitoyl-CoA oxidase; SA, superoxide anion; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TNF α , tumor necrosis factor alpha

† Study results were reported in multiple papers when several references are cited.

^a Doses estimated by OEHHA based on default values (US EPA, 1988).

^b This LOAEL was identified from a single-dose study.

^c Doses calculated by US EPA (2011).

Genetic Toxicity

Genetic toxicity effects of TCA in vivo are presented in Table 7.4, and genetic toxicity effects of TCA in vitro are presented in Table 7.5. Within each table, studies are organized by experimental system (e.g., human studies before animal studies, primary cells before cell culture), and then by type of genotoxic effect.

No human in vivo studies were located. No mutation animal studies in vivo were located. Frequencies of micronucleus and chromosomal aberrations were increased in bone marrow in mice with TCA doses of 125-500 mg/kg-day and with a variety of exposure scenarios (Bhunya and Behera, 1987). However, other bone marrow studies with comparable doses and exposure durations employing different mouse strains did not find increased frequencies of micronucleus (Mackay et al., 1995) or chromosomal aberrations (Kurinyi, 1984). Evidence of chromosomal aberrations in bone marrow of white Leghorn chickens was inconsistent (Bhunya and Jena, 1996). Following a single oral dose of TCA, several studies reported evidence of DNA strand

breaks, while others did not find significant differences with control (Table 7.4). Two subchronic oral studies in mice reported DNA strand breaks in hepatic tissue of mice at mid-range TCA doses (≥ 25 mg/kg-day) (Hassoun et al., 2010a; Hassoun et al., 2014). Evidence of oxidative damage in hepatic tissue of mice is also mixed, with significantly increased 8-OHdG formation in one single dose study (Austin et al., 1996), but no significant increase in a different repeated exposure study with comparable TCA doses (Parrish et al., 1996). Newt larvae demonstrated increased frequency of micronucleas following exposure to TCA in water (Giller et al., 1997). Increased frequency of micronucleus and DNA breaks was reported for fava bean root tips (Hu et al., 2017). No nuclear DNA damage was detected in *C.elegans* (Zuo et al., 2017). No chromosomal aberrations were detected in two species of seedlings (Kurennyi, 1984).

Regarding the in vitro genetic toxicity of TCA in Table 7.5, some studies found statistically increased frequency of micronucleus and chromosomal aberrations in human peripheral lymphocytes exposed ex-vivo to TCA, while other studies reported negative results at similar concentrations and comparable exposure times. Chang et al. (1992) did not detect increased frequencies of DNA straining breaks in primary rat or mouse hepatocytes following ex-vivo exposure to TCA, or in TCA-exposed human leukemia CCRF-CEM tissue culture cells. Harrington-Brock et al. (1998) reported TCA-dependent increased mutation frequencies in L5178Y/TK^{+/+} cells, while Plewa et al. (2002) did not detect an increase in DNA damage in CHO cells exposed to TCA.

Several studies employing reverse mutation assays in *S. typhimurium* did not observe genotoxicity of TCA, while other studies employing the same strains and methods were positive. Several bacterial tests used TCA concentrations far below the recommended maximum concentration (5 mg/plate), which may contribute to negative findings. TCA was mutagenic in three *S. typhimurium* strains: TA1535 (one positive out of two studies), TA98 (one positive out of four studies) and TA100 (two positive out of eight studies). DCA was not mutagenic in TA1537 (one negative study), TA1538 (one negative study), TA104 (one negative study) or RSJ100 (one negative studies). Based on these results, it is not clear what type of mutations TCA would most likely produce, although evidence is somewhat stronger for *S. typhimurium* strains that favor base-pair changes (TA100, T1535) compared to frameshift mutations (TA98, TA1538, TA1537). TCA also produced mixed results in additional types of bacterial tests (Table 7.5).

Reviews by the National Research Council (1987) and Daniel et al. (1993) did not conclude that there was evidence of TCA mutagenicity with the standard Ames method. The majority of the reverse mutation assays with *S. typhimurium* were negative with or without S9 activation (in vitro incubation in the presence or absence of a mixture of unfractionated microsomes and cytosol containing a wide variety of metabolic enzymes), and overall, the in vitro genotoxicity tests produced generally negative results. However, glyoxylic acid, a metabolite of TCA, was mutagenic in TA97, TA100, and TA104 strains without S9 and in TA97, TA100, TA102, and TA104 strains with S9 (Sayato et al., 1987). Additionally, in vivo administration of TCA produced chromosomal aberrations, DNA single strand breaks, and positive results in the micronucleus assay in mice, rats, and chickens. These data suggest that a genotoxic

mechanism of TCA cannot be excluded, and that metabolism of TCA may be a critical step for genotoxicity.

Table 7.4 Genetic and related effects of TCA in vivo

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
Mammalian: Bone marrow chromosomal aberration and micronucleus assay	Swiss mice (3/dose, sex not specified)	All treatments: TCA ^a by i.p. injection (a, b, d) or by oral gavage (c): (a) 0 or 500 mg/kg; sacrificed at 6, 24 or 48 hours post-treatment; (b) 0, 125, 250 or 500 mg/kg, sacrificed at 24 hours post-treatment; (c) 0 or 500 mg/kg, sacrificed at 24 hours post-treatment; (d) 0 or 100 mg/kg-day for 5 days, sacrificed 24 hours after last dose	+	Bhunya and Behera (1987)
Bone marrow micronucleus assay	Swiss mice (3/dose, sex not specified)	0, 125, 250 or 500 mg/kg TCA ^a by i.p injection, two doses, 24 hours interval between doses, sacrificed 6 hours after the last dose	+	Bhunya and Behera (1987)
Bone marrow micronucleus assay	Male and female C57BL/6JfBL1 0/Alpk mice (10/sex/dose)	0, 337, 675, or 1,080 mg/kg-day (males); 0, 405, 810, or 1,620 mg/kg-day (females) neutralized TCA by i.p. injection for 2 days; sacrificed at 6 and 24 hours post-treatment	-	Mackay et al. (1995)
Bone marrow chromosomal aberration assay	Male white mice (sample size not specified)	0, 1, 10, 100, 500 or 1,000 mg/kg sodium trichloroacetate by oral gavage; 20 hours	-	Kurinnyi (1984)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
Bone marrow chromosomal aberration assay	Male and female white Leghorn chickens (4/dose, sex not specified)	All treatments: TCA ^a by i.p. injection (a, b, d) or by oral gavage (c): (a) 0 or 400 mg/kg; sacrificed at 6, 24 or 48 hours post-treatment, 4 per time point; (b) 0, 100, 200 or 400 mg/kg, sacrificed at 24 hours post-treatment; (c) 0 or 100 mg/kg, sacrificed at 24 hours post-treatment; (d) 0 or 80 mg/kg-day for 5 days, sacrificed 24 hours after last dose	+/- (dose-response and time-response not consistent)	Bhunya and Jena (1996)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male F344 rats (5-27/dose)	Exact doses not reported, 0.7-25 mmol/kg range trichloroacetate (salt not specified) by oral gavage; sacrificed at 4 hours post-treatment	+ (at highest dose)	Nelson and Bull (1988)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (5-28/dose)	Exact doses not reported, 0.001-0.1 mmol/kg range, trichloroacetate (salt not specified) by oral gavage; sacrificed at 4 hours post-treatment	+ (at highest dose)	Nelson and Bull (1988)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (6-13/dose/time point)	0 or 500 mg/kg TCA ^a by oral gavage; sacrificed at 1, 2, 4, 8, or 24 hours post-treatment	+ (at 1, 2 and 4 hours)	Nelson et al. (1989)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (5/dose)	0 or 500 mg/kg neutralized and un-neutralized TCA by oral gavage, sacrificed at 24 hours post-treatment	-	Styles et al. (1991)
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male B6C3F1 mice (5/dose)	One, two or three doses of 0 or 500 mg/kg neutralized TCA by oral gavage; animals sacrificed 1 hour after last dose	-	Styles et al. (1991)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
DNA alkaline unwinding assay (DNA strand breaks) hepatic tissue	Male F344 rats (4/dose)	0, 1 or 5 mmol/kg (0, 164 or 817 mg/kg) neutralized TCA by oral gavage; sacrificed at 4 hours post-treatment	-	Chang et al. (1992)
DNA alkaline unwinding assay (DNA strand breaks) hepatic, stomach and duodenal epithelial tissues	Male B6C3F1 mice (4/dose)	Hepatic cells: 0, 1, 5, or 10 mmol/kg (0, 0.16, 0.82 or 1.6 g/kg) neutralized TCA by oral gavage, sacrificed at 4 hours post-treatment	+ (at highest dose)	Chang et al. (1992)
DNA alkaline unwinding assay (DNA strand breaks) with hepatic, stomach and duodenal epithelial tissues	Male B6C3F1 mice (4/dose)	Stomach and duodenal cells: 0 or 10 mmol/kg (0 or 1.6 g/kg) neutralized TCA by oral gavage, sacrificed at 4 hours post-treatment	-	Chang et al. (1992)
Alkaline elution (DNA single strand breaks) hepatic tissue	Male B6C3F1 mice (8/dose/time point)	0 or 300 mg/kg sodium trichloroacetate by oral gavage; sacrificed at 6 or 12 hours post-treatment	+ (at 12 hours)	Hassoun and Dey (2008)
Alkaline elution (DNA single strand breaks) hepatic tissue	Male B6C3F1 mice (7/dose/time point)	0, 7.7, 77, 154, or 410 mg/kg-day neutralized TCA by oral gavage for 4 or 13 weeks	+ (both time points at ≥ 77 mg/kg-day)	Hassoun et al. (2010a)
Alkaline elution (DNA single strand breaks) hepatic tissue	Male B6C3F1 mice (6/dose)	0, 12.5, 25, or 50 mg/kg-day sodium trichloroacetate by oral gavage for 13 weeks	+ (at ≥ 25 mg/kg-day)	Hassoun et al. (2014)
HPLC-EC of digested liver DNA (8-OHdG formation, a precursor to point mutations)	Male B6C3F1 mice (6/dose/time point)	0, 30, 100, or 300 mg/kg neutralized TCA by oral gavage (single dose); sacrificed at 6, 8, or 10 hours post-treatment	+ (high dose at 8 and 10 hours; data not shown for other doses)	Austin et al. (1996)
HPLC-EC of digested liver DNA (8-OHdG formation, a precursor to point mutations)	Male B6C3F1 mice (6/dose/time point)	0, 0.1, 0.5, or 2 g/L (0, 25, 125, or 500 mg/kg-day ^b) TCA ^a in drinking water for 21 or 71 days	-	Parrish et al. (1996)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
Non-mammalian: Newt micronucleus assay	Newt (<i>P. waltii</i>) larvae (15/dose)	0, 40, 80 or 160 µg/mL (in tank water) TCA ^a for 12 days	+	Giller et al. (1997)
Long amplicon quantitative PCR (nuclear DNA damage)	<i>C. elegans</i> Bristol strain N2	0, 0.2, 0.4, 0.6, 0.8, or 1 mM TCA ^a in well water	-	Zuo et al. (2017)
Chromosomal aberrations in <i>C. tectorum</i> and <i>A. cepa</i> seedlings	-	ND	0.1-10 mg/ml	Kurinnyi (1984)
Micronucleus and SCGE (Comet) assay with <i>V. faba</i> root meristem	<i>V. faba</i> (fava bean) root tips (3/dose)	1 µM-1 mM (micronucleus) or 1-100 µM (SCGE) TCA ^a in root water for 5 hours; 24-hour recovery	+(both assays, at ≥100 µM)	Hu et al. (2017)

8-OHdG, 8-hydroxy-2'-deoxyguanosine; i.p., intraperitoneal; HPLC-EC, high performance liquid chromatography with electrochemical detection; PCR, polymerase chain reaction; SCGE, single cell gel electrophoresis

^a Information on whether TCA was neutralized or un-neutralized was not provided.

^b Doses calculated by US EPA (2011).

Table 7.5 Genetic and related effects of DCA in vitro (human and mammalian cells, non-mammalian experimental systems)

Assay	Results Without S9	Results With S9	TCA Concentration	Reference
Primary mammalian cells: Micronucleus assay with human peripheral lymphocytes	-	-	0.5-5 mg/ml	Mackay et al. (1995)
Micronucleus assay with human peripheral lymphocytes	+	ND	25, 50 or 100 µg/ml	Varshney et al. (2013)
Chromosomal aberrations in human peripheral lymphocytes	-	ND	1-100 µg/ml	Kurinnyi (1984)
Chromosomal aberrations and cytokinesis-block micronucleus assay in human peripheral lymphocytes	+	ND	25, 50 or 100 µg/ml	Varshney et al. (2013)

Assay	Results Without S9	Results With S9	TCA Concentration	Reference
DNA alkaline unwinding assay (DNA single strand breaks) in primary hepatocytes from male B6C3F1 mice, male F344 rats, and CCRF-CEM (human leukemia) cells	-	ND	0.1-10 mM (mouse cells), 1-10 mM (rat cells), 1-10 mM (CCRF-CEM cells)	Chang et al. (1992)
Mammalian cell lines: Mouse lymphoma cell forward mutation assay in L5178Y/TK ^{+/-} cells	+	+	0.75-3.4 mg/ml	Harrington-Brock et al. (1998)
SCGE (Comet) assay (genomic DNA damage) in CHO cells	-	ND	0.1-3 mM	Plewa et al. (2002)
Non-mammalian cells: Bacterial reverse mutation assay, <i>S. typhimurium</i> (eight unspecified mutants), T ₄ bacteriophage mutants AP72, N17	+ (AP72) - (other mutants)	ND	Concentration not specified (<i>S. typhimurium</i>), 100 µg/plate (T ₄ mutants)	Anderson et al. (1972)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98, TA100	-	-	0.45 mg/plate	Waskell (1978)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA1535, TA100	+	-	0.25-4 mg/plate (dissolved in DMSO)	Nestmann et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA1535, TA100	-	-	0.25-4 mg/plate (dissolved in water)	Nestmann et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98	+	-	0.25-4 mg/plate (dissolved in DMSO)	Nestmann et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA1537, TA1538	-	-	0.25-4 mg/plate (dissolved in DMSO)	Nestmann et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100	-	ND	0.1-1,000 µg/plate	Rapson et al. (1980)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98, TA100	-	-	Up to 5 mg/plate	Moriya et al. (1983)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100	-	-	0-10 mg/ml	DeMarini et al. (1994)

Assay	Results Without S9	Results With S9	TCA Concentration	Reference
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA100 (Ames fluctuation test)	+	+	- S9: 0.03-10 mg/ml + S9: 1-10 mg/ml	Giller et al. (1997)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA 104	-	-	1 mg/ml	Nelson et al. (2001)
Bacterial reverse mutation assay, <i>S. typhimurium</i> TA98, TA100, RSJ100	-	-	0.1-100 mM	Plewa et al. (2002)
Bacterial rec-assay in <i>B. subtilis</i> H17 Rec ⁺ and M45 Rec ⁻	-	ND	20 µg/disk (10 mm diameter)	Shirasu et al. (1976)
umu test, <i>S. typhimurium</i> TA1535/pSK1002 (umu test)	+	+	58.5 µg/ml	Ono et al. (1991)
λ prophage induction in <i>E. coli</i> WP2	-	-	Up to 10 mg/ml	DeMarini et al. (1994)
SOS chromotest in <i>E. coli</i> PQ37	-	-	0.01 µg/ml – 10 mg/ml	Giller et al. (1997)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	-	-	0.1-16 mM	Stalter et al. (2016)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	+	ND	0.1-28.1 mM	Zhang et al. (2016)
Chromosomal aberrations in <i>C. tectorum</i> and <i>A. cepa</i> seedlings	-	ND	0.1-10 mg/ml	Kurinyi (1984)

CHO, Chinese hamster ovary; SCGE, single cell gel electrophoresis; ND, not determined

Developmental and Reproductive Toxicity

Human Studies

Epidemiological studies of the association between exposure to DBPs, including TCA, and reproductive health outcomes are summarized in Appendix B. Note that these studies examine treated drinking water that contains many DBPs including HAAs and THMs. As such, it is difficult to attribute adverse reproductive outcomes to a single DBP.

Developmental toxicity in vitro

Hunter et al. reported on effects of TCA in CD-1 mouse embryo cultures. GD 9 embryos (3-6 somites) were exposed to 0 or 0.5 to 5 mM (82-817 mg/L) TCA in buffered medium for 24 hours (10-24/dose), and no deaths were observed (Hunter et al., 1996). Significantly increased malformations and impaired differentiation were observed at 2 mM (327 mg/L) and higher, including neural tube defects (22.2% at 2 mM compared to none at control) and a significantly

decreased number of somites. At ≥ 3 mM, defects of the eye, pharyngeal arch and heart were reported. The heart defects included “incomplete looping, a reduction in the length of the heart beyond the bulboventricular fold, and a marked reduction in the caliber of the heart tube lumen” (Hunter et al., 1996).

Saillenfait et al. studied effects of TCE, PCE and their oxidative metabolites, including TCA, in embryos from Sprague-Dawley rats explanted on GD 10 (Saillenfait et al., 1995). Embryos (12-22/dose) were cultured for 46 hours in buffered medium with TCA concentrations ranging from 0.5 to 6 mM (82-980 mg/L) and remained viable (heart beat present) at concentrations up to 5 mM (817 mg/L). Growth and differentiation of embryos were negatively impacted by TCA in a concentration-dependent manner. The most sensitive growth parameter was head length, significantly reduced at ≥ 0.5 mM, and somite number was significantly reduced at ≥ 2.5 mM. Embryo malformations occurred at 2.5 mM and 3.5 mM (no data reported for higher concentrations) and included brain defects, eye defects, reductions in embryonic axis and first branchial arch, otic system defects and absence of hindlimb bud. The authors noted the lack of TCA effects on rat embryonic heart development.

Developmental toxicity in vivo

Developmental cardiotoxicity of TCA was observed in two independent rat studies (Smith et al., 1989; Johnson et al., 1998); however no cardiac or cardiovascular defects were observed in another study employing a similar dose (Fisher et al., 2001). Additionally, testes, brain and ocular effects were observed in several rat studies (Singh, 2005, 2006; Warren et al., 2006). No in vivo developmental toxicity studies of TCA in mice or rabbits were found. Developmental in vivo toxicity studies of TCA in rats are summarized in Table 7.6.

Table 7.6 Summary of in vivo developmental toxicity studies of TCA

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference
Female Long Evans rats, pregnant (20-26/dose)	0, 330, 800, 1,200, or 1,800 mg/kg-day neutralized TCA by oral gavage on GD 6-15	Dams: Decreased live fetuses per litter, increased percent post-implantation loss, decreased body weight, increased relative spleen and kidney weights Fetuses: Decreased body weight, decreased crown-rump length, increased cardiovascular and skeletal malformations	LOAEL: 330 mg/kg-day	Smith et al. (1989)
Female Sprague-Dawley rats, pregnant (11-55/dose)	0 or 2,730 ppm (0 or 291 mg/kg-day) neutralized TCA in drinking water throughout pregnancy	Dams: Increased number of implantation sites per litter, increased resorption sites per litter Pups: Increase in cardiac defects	LOAEL ^a : 291 mg/kg-day	Johnson et al. (1998)

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference
Female Sprague-Dawley rats, pregnant (19/dose); about 70% of fetuses examined in Warren et al. (2006)	0 or 300 mg/kg-day neutralized TCA by oral gavage on GD 6-15	Dams: Decreased body weight and absolute uterine weight Fetuses: Decreased body weight	LOAEL ^a : 300 mg/kg-day	Fisher et al. (2001)
Female Sprague-Dawley rats, pregnant (19/dose); about 70% of fetuses examined in Warren et al. (2006)	0 or 300 mg/kg-day neutralized TCA by oral gavage on GD 6-15	Fetuses: Decreased body weight, ocular endpoints relative to body weight (increased ratio of fetal lens area to body weight, increased ratio of fetal globe area to body weight, increased ratio of medial canthus distance to body weight, increased ratio of interocular distance to body weight)	LOAEL ^a : 300 mg/kg-day	Warren et al. (2006)
Female Sprague-Dawley rats, pregnant (number not stated)	0, 2.75, or 27.3 mg/L (0, 361, or 3,588 mg/kg-day) neutralized TCA in drinking water on GD 0-11	Changes in expression of stress response and calcium-ATPase genes in embryonic heart tissue	LOAEL: 361 mg/kg-day	Collier et al. (2003)
Female Charles-Foster rats, pregnant (6-12/dose)	0, 1,000, 1,200, 1,400, 1,600, or 1,800 mg/kg-day neutralized TCA by oral gavage on GD 6-15	Dams: Increased post-implantation loss Pups: Decreased fetal testes weight; decreased seminiferous tubule diameter; increased apoptosis of gonocytes	LOAEL: 1,000 mg/kg-day	Singh (2005)
Female Charles-Foster rats, pregnant (25/dose)	0, 1,000, 1,200, 1,400, 1,600, or 1,800 mg/kg-day neutralized TCA by oral gavage on GD 6-15	Dams: Decreased weight gain; increased post-implantation loss Pups: Decreased body weight; decreased absolute brain weight; decreased length of the whole brain; increased percent of brain vacuolation, brain hemorrhages and hydrocephalus	LOAEL: 1,000 mg/kg-day	Singh (2006)

GD, gestation day; NA, not applicable

^a This LOAEL was identified from a single-dose study.

Reproductive toxicity

The reproductive toxicity database for TCA is very limited, with only a few observations in two subchronic studies and one chronic study in male rats and mice. No studies in females or multigenerational studies were identified.

Two subchronic (90-day) studies in male rats reported no adverse effects in testes. At doses as high as 355 mg/kg-day neutralized TCA administered in drinking water, Mather and colleagues did not observe any changes in gross pathology or histopathology in the testes of adult male Sprague-Dawley rats (10/dose) (Mather et al., 1990). Similarly, no changes in weight or histology were observed in testes from five adult male Sprague-Dawley rats exposed to 825 mg/kg-day neutralized TCA in drinking water relative to controls (Bhat et al., 1991).

In contrast, a study by DeAngelo et al. reported testicular tubular degeneration in B6C3F1 mice (50/dose) given 0.5 or 5 g/L of neutralized TCA (68 or 602 mg/kg-day, respectively) in drinking water for 60 weeks (DeAngelo et al., 2008). Incidence at the mid- and high-dose was not significantly different from control but a significant dose-related trend was observed.

In an in vitro reproductive toxicity study, Cosby and Dukelow (1992) evaluated the effects of 0, 100, or 1,000 ppm TCA (0, 0.1 or 1 g/L) on in vitro fertilization of oocytes from B6D2F1 mice. Mouse oocytes and sperm were incubated in the in vitro fertilization reaction in the presence of TCA (in buffered medium) or control medium for 24 hours, and successful oocyte fertilization was determined by an increase in nuclear DNA staining. Significant interference with fertilization was found with 1,000 ppm TCA, which produced a 53.1% fertilization rate compared to 82.4% in controls ($p < 0.001$) (Cosby and Dukelow, 1992).

Immunotoxicity

Neutralized TCA did not alter immune parameters (antibody production, delayed-type hypersensitivity, natural killer cell cytotoxicity, and production of PGE₂ and IL2) when administered to Sprague-Dawley rats (10/dose) in drinking water at doses of 0, 4.1, 36.5, or 355 mg/kg-day for 90 days (Mather et al., 1990). Un-neutralized TCA demonstrated moderate activity in the dermal guinea pig maximization test, in which dermal allergic reactions are scored at 21 days following two intradermal injections and a topical application of a 2-5% solution of the tested compound (Tang et al., 2002).

Neurotoxicity

No neurotoxicity studies of TCA were identified and available chronic and subchronic studies of TCA have generally not reported observations of neurotoxic endpoints. Narcosis was observed prior to death after very high doses in mice and rats in one LD₅₀ study (Woodard et al., 1941). Several tissue enzymes were measured as putative indicators of neurotoxicity and immunotoxicity in Sprague-Dawley rats of unspecified sex (6/dose) exposed to 0 or 2,000 ppm (300 mg/kg-day) un-neutralized TCA in drinking water for 52 days (Çelik et al., 2010). While butyrylcholinesterase activity was significantly decreased in the heart and lungs (but not in the brain, liver, kidney or spleen), acetylcholinesterase activity in these organs did not change compared to controls.

Chronic Toxicity

Effects in Humans

OEHHA did not identify any controlled human studies of chronic oral TCA exposure.

Epidemiological studies of DBPs in drinking water, as groups comprising THMs or HAAs or as individual DBP chemicals, are described in the *Human Epidemiology Studies* section.

Effects in Animals

The available chronic oral toxicity studies on TCA were primarily designed to evaluate carcinogenicity in the liver and are summarized in Table 7.7. Many studies reported the concentration of TCA used in the water, but did not always report the dose to the experimental animals. Doses, where not reported in the original study, were calculated by US EPA (2011) or by OEHHA using US EPA default values for body weight and/or water consumption (US EPA, 1988).

Table 7.7 Summary of chronic toxicity studies of TCA

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference†
Male B6C3F1 mice (22/dose)	0 or 5 g/L (0, or 1,000 mg/kg-day) neutralized TCA in drinking water for 61 weeks; additional dose groups were initiated with ENU (2.5 or 10 µg/kg) followed by 0, 2.5 or 5 g/L neutralized TCA in drinking water for 61 weeks	Decreased body weight, increased relative liver weight	LOAEL ^a : 1,000 mg/kg-day	Herren-Freund et al. (1987)
Male and female B6C3F1 mice (10-35/sex/dose)	Males: 0, 1, or 2 g/L (0, 164, or 329 mg/kg-day ^b); females: 0 or 2 g/L (0 or 329 mg/kg-day ^c) neutralized TCA in drinking water for 52 weeks (15-, 24-, and 37-week interim sacrifices of 5 males/dose at 0 and 2 g/L)	Increase in relative liver weight; necrotic degenerative lesions in liver (low incidence); moderate hepatomegaly and glycogen accumulation; lipofuscin accumulation in the liver	LOAEL: 164 mg/kg-day	Bull et al. (1990); Nelson et al. (1990)
Male and female B6C3F1 mice (10-35/sex/dose)	Males (11/dose): 2 g/L (329 mg/kg-day ^b) for 37 weeks, sacrificed at 52 weeks	Necrotic degenerative lesions in liver (low incidence); moderate hepatomegaly and glycogen accumulation	LOAEL ^a : 329 mg/kg-day	Bull et al. (1990); Nelson et al. (1990)

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference [†]
Female B6C3F1 mice (4/dose)	0, 2, 6.67, or 20 mM (0, 78, 262, or 784 mg/kg-day ^d) neutralized TCA in drinking water for 52 weeks	Only livers from the control and high dose groups were examined: hypomethylation of promoter regions and protein expression of <i>c-jun</i> and <i>c-myc</i> ; increased expression of <i>IGFII</i> gene and protein levels	NA	Pereira and Phelps (1996); Tao et al. (2000b); Tao et al. (2004a)
Female B6C3F1 mice (18-90/dose)	0, 2, 6.67, or 20 mM (0, 44, 155, or 453 mg/kg-day ^d) neutralized TCA in drinking water for 51 or 82 weeks	Decreased body weight; increased relative liver weight (significance not indicated); increase in liver lesions, increased hepatic proliferation (BrdU labeling index)	NOAEL: 155 mg/kg-day	Pereira (1996)
Male F344/N rats (19-24/dose)	0, 0.05, 0.5, or 5 g/L (0, 3.6, 32.5, or 364 mg/kg-day) neutralized TCA in drinking water for 104 weeks (interim sacrifices of 3-6/dose at 15, 30, 45, and 60 weeks)	High dose: decreased body weight; increased serum ALT; increased PCO activity (peroxisome proliferation) in the liver; mild liver inflammation and necrosis	NOAEL: 32.5 mg/kg-day	DeAngelo et al. (1997)
Male B6C3F1 mice (12-22/dose)	0 or 2 g/L (0 or 480 mg/kg-day ^d) neutralized TCA in drinking water for 50 weeks followed by 0, 0.02, 0.5, 1.0, or 2.0 g/L (0, 5, 115, 230, or 460 mg/kg-day ^d) neutralized TCA in drinking water for 2 more weeks	Transient increase in proliferation rate in normal hepatocytes	LOAEL ^a : 479 mg/kg-day ^e	Stauber and Bull (1997)
Male and female B6C3F1 mice (14-29/dose)	Initiated with 30 mg/kg MNU (i.p. injection); 0 or 4.0 g/L (females: 0.96 g/kg-day ^d ; males: 1 g/kg-day ^d) neutralized TCA in drinking water for 31 weeks	Increased relative liver weight (more potent effect in males)	LOAEL ^a : 1 g/kg-day	Pereira et al. (2001)

Sex/Species	Dose/Route of Exposure	Endpoints	NOAEL/LOAEL	Reference [†]
Male B6C3F1 mice (20/dose)	0 or 2 g/L (0 or 238 mg/kg-day ^f) neutralized TCA in drinking water for 52 weeks	Only the liver was examined: increase in relative liver weight	LOAEL ^a : 238 mg/kg-day	Bull et al. (2002)
Male B6C3F1 mice (20/dose)	0, 0.5, or 2 g/L (0, 55, or 238 mg/kg-day ^f) neutralized TCA in drinking water for 52 weeks	Only the liver was examined: increase in relative liver weight	LOAEL: 55 mg/kg-day	Bull et al. (2002)
Male B6C3F1 mice (30/dose)	Study 1: 0, 0.05, 0.5, or 5 g/L (0, 7.7, 68.2, or 602.1 mg/kg-day) neutralized TCA in drinking water for 60 weeks (interim sacrifices of 5/dose at 4, 15, 31, and 45 weeks)	Decreased body weight; increased relative liver weight; liver necrosis; inflammation and centrilobular cytoplasmic alterations; increased serum LDH activity (at week 30); increased PCO activity in the liver; increased hepatic proliferation at 20-60 weeks	NOAEL: 7.7 mg/kg-day (relative liver weight)	DeAngelo et al. (2008)
Male B6C3F1 mice (57/dose)	Study 2: 0 or 4.5 g/L (0 or 572 mg/kg-day) neutralized TCA in drinking water for 104 weeks (interim sacrifices of 5/dose at 15, 39, and 45 weeks; 10 control animals at 60 weeks)	Increased relative liver weight (weeks 15-45); increased PCO activity in the liver; increased hepatic proliferation	LOAEL ^a : 572 mg/kg-day	DeAngelo et al. (2008)
Male B6C3F1 mice (72/dose)	Study 3: 0, 0.05, or 0.5 g/L (0, 6.7, or 81.2 mg/kg-day ^g) neutralized TCA in drinking water for up to 104 weeks (interim sacrifices of 8/dose at 26, 52, and 78 weeks)	Increased hepatocyte proliferation (at week 78) without significant increase in relative liver weight at low dose only (no significant difference at high dose)	NA (inconsistent effect)	DeAngelo et al. (2008)

[†] Study results were reported in multiple papers when several references are cited.

ALT, alanine aminotransferase; BrdU, 5-bromo-2'-deoxyuridine; ENU, ethylnitrosourea; GGT+, γ -glutamyltranspeptidase-positive; LDH, lactate dehydrogenase; MNU, N-methyl-N-nitrosourea; NA, not applicable; PCO, cyanide insensitive palmitoyl CoA oxidase

^a This LOAEL was identified from a single-dose study.

^b Doses for male mice and LOAEL in the Bull et al. (1990) study were estimated by the study authors according to US EPA (2011); no dose estimate for female mice was provided.

^cDose for female mice (Bull et al., 1990) was calculated by OEHHA using default values (US EPA, 1988b).

^dDoses calculated by US EPA (2011).

^eThe time-weighted average was used to calculate the LOAEL.

^fDoses calculated based on default water consumption (5 ml/animal-day) and reported weights at the end of the study (Bull et al., 2002).

^gDoses in the DeAngelo et al. (2008) study have been calculated by OEHHA based on reported water consumption rates and measured TCA concentrations, and are different from the doses reported in the original study and by US EPA (2011), which were 6 and 58 mg/kg-day for the 0.05 and 0.5 g/L dose groups.

Different hepatic endpoints, including increased relative liver weight, increased proliferation, necrosis, inflammation and peroxisome proliferation, and decreased body weight were common findings in the chronic oral studies of TCA toxicity. All of these effects were reported in the 60-week study in male B6C3F1 mice (DeAngelo et al., 2008), in which 30 animals/dose were exposed to 0, 7.7, 68.2, or 602.1 mg/kg-day neutralized TCA in drinking water. This study examined several organs including the liver, the kidneys, the spleen and the testes, in contrast to most other TCA studies that focused exclusively on the liver. In addition to the adverse effects in the liver, the 60-week study (DeAngelo et al., 2008) also reported increased testicular tubular degeneration (a significant dose trend and incidences at 0.5 and 5 g/L TCA) and increased serum LDH (lactate dehydrogenase) activity, likely caused by increased inflammation and necrosis in the liver. Mortality was low and concentrations of the TCA dosing solutions were verified. A NOAEL of 7.7 mg/kg-day for DeAngelo et al. (2008) is based on increased liver weights and is health-protective for other adverse effects observed in this study, such as testicular tubular degeneration.

The liver appears to be the main target organ of TCA toxicity in DeAngelo et al. (2008). However, hepatocellular necrosis in this study was transient and abated by week 60. The authors hypothesized this could be due to “front-loading” of the animals (DeAngelo et al., 2008), i.e., animals receiving a higher relative dose early in the study due to decreasing water consumption later in the study. Among other adverse liver effects, hepatic inflammation was increased at week 60 with the high dose. While (2008) also reported significant centrilobular cytoplasmic alteration in the liver at week 60 in all dose groups, this effect may or may not be adverse. Acharya et al. (1997) also observed hepatic necrosis at a lower dose (3.8 mg/kg-day) in a 10-week mechanistic study in Wistar rats.

All three studies in DeAngelo et al. (2008) also reported increased hepatic proliferation, although the variability of data was very high. Study 1 demonstrated significantly increased hepatic proliferation in the 68.2 mg/kg-day dose group at 60 weeks (but not at 31 or 45 weeks), and in the 602.1 mg/kg-day dose group at 31 and 45 weeks (but not at 60 weeks). Study 2 demonstrated increased hepatic proliferation at 45 weeks with 572 mg/kg-day TCA, while the difference with control at 30 weeks was not significant. Finally, in Study 3 the only significant difference was observed with 6.7 mg/kg-day at 78 weeks, while shorter exposure and/or higher dose (81.2 mg/kg-day) did not demonstrate significant differences from control. In fact, in Study 3, the overall dose trend in hepatic proliferation was reversed at 52 vs. 78 weeks, with most data points not significantly different from respective controls. Given the high data variability

and the inconsistent results between studies, increased hepatic proliferation reported in DeAngelo et al. (2008) is not considered as a candidate critical endpoint.

The hepatotoxicity endpoints of peroxisome proliferation, mild necrosis, and inflammation, in addition to reduced body weight and increased serum ALT, were also reported in male F344/N rats (12-22/dose) exposed to 0, 3.6, 32.5, 364 mg/kg-day neutralized TCA in drinking water (DeAngelo et al., 1997). The NOAEL was 32.5 mg/kg-day, with all adverse effects observed at high dose only. At the end of this two-year study, mortality in the high dose group was higher compared to control, but the difference was not significant ($p=0.08$).

Pereira and Phelps investigated the toxic effects of neutralized TCA in female B6C3F1 mice, finding minor toxicity at high doses (Pereira, 1996; Pereira and Phelps, 1996). Only the liver was examined in these studies, and due to the high reported NOAEL of 155 mg/kg-day for Pereira (1996) and LOAEL of 784 mg/kg-day and lack of reporting for all dose groups for Pereira and Phelps (1996), these studies are of limited use for dose-response analysis.

Two multi-dose studies by Bull et al. (1990; 2002) in male B6C3F1 mice also only examined the liver and both found increased relative liver weight with 52-week exposures to neutralized TCA in drinking water. Due to poor data reporting, Bull et al. (1990) cannot be considered for dose-response analysis. Bull et al. (2002) exposed male B6C3F1 mice (12-40/dose) to neutralized TCA in drinking water (0, 120, or 480 mg/kg-day) and reported increased relative liver weight at all doses (LOAEL=120 mg/kg-day); these data are amenable to dose-response analysis. The increase in relative liver weight was not due to tumor burden (Bull et al., 2002). Mortality in this study appeared to be minimal.

Carcinogenicity

There are several chronic studies in mice of both sexes, and most demonstrate a statistically significant increase in liver tumors at doses as low as 68.2 mg/kg-day (DeAngelo et al., 2008) and/or a significant positive trend in tumor incidences with dosage.

TCA can act as a liver tumor promoter in rats or mice pretreated with a carcinogenic initiator before chronic exposure to TCA in drinking water (Parnell et al., 1986; Herren-Freund et al., 1987; Parnell et al., 1988; Pereira and Phelps, 1996; Latendresse and Pereira, 1997; Pereira et al., 1997; Pereira et al., 2001). In addition to hepatic tumors, TCA-dependent promotion of renal tumors was observed in male mice (Pereira et al., 2001). One study in rats showed no significant increase in tumors at any dose (DeAngelo et al., 1997). These studies are summarized in Table 7.8.

TCA is also a peroxisome proliferator, and some reports proposed a possible link between TCA-induced peroxisome proliferation and tumorigenesis (DeAngelo et al., 1989). While results from in vitro genotoxicity studies are mostly negative, in vivo administration of neutralized TCA appears to be genotoxic in rodents (OEHHA, 1999; US EPA, 2005b).

US EPA (2011, 2013) concluded that "there is suggestive evidence of carcinogenic potential for TCA based on significantly increased incidences of liver tumors" in male and female B6C3F1

mice, and “lack of treatment-related tumors in a study of male F344/N rats.” There are no carcinogenic studies of TCA in humans. US EPA (1998a) promulgated an MCLG of 0.3 mg/L for TCA in drinking water based on developmental toxicity and possible carcinogenicity.

IARC classified TCA as group 2B, possibly carcinogenic to humans, based on sufficient evidence for carcinogenicity in animals, and inadequate evidence in humans (Guha et al., 2012; IARC, 2014). TCA was listed as a carcinogen under Proposition 65 in September 2013, based on the IARC identification of TCA as a carcinogen (OEHHA, 2017). However, in its preliminary recommendation in the monograph for the Report on Carcinogens (RoC), NTP considered existing evidence of TCA carcinogenicity as not sufficient for RoC listing (NTP, 2018).

Table 7.8 Summary of carcinogenicity and promoter studies of TCA

Sex/ Species [#]	Dose/Route of Exposure/Duration	Tumor Incidence	Notes	Reference
Male B6C3F1 mice (23-33/ dose)	Injected (i.p.) with 2 µl/g sodium acetate as ENU control; 0 or 5 g/L (0 or 1 g/kg-day) neutralized TCA in drinking water for 61 weeks	hepatic adenoma 2/22, 8/22* hepatic carcinoma 0/22, 7/22*	TCA acted as a complete carcinogen; survival at 61 weeks: 22/27, 22/25	Herren-Freund et al. (1987)
Male B6C3F1 mice (23-33/ dose)	Initiated with 2.5 µg/kg ENU (i.p. injection); 0, 2, or 5 g/L (0, 0.4, or 1 g/kg-day) neutralized TCA in drinking water for 61 weeks	hepatic adenoma 1/22, 11/33*, 6/23* hepatic carcinoma 1/22, 16/33*, 11/23*	Survival at 61 weeks: 22/25, 33/33, 23/24	Herren-Freund et al. (1987)
Male B6C3F1 mice (23-33/ dose)	Initiated with 10 µg/kg ENU (i.p. injection); 0 or 5 g/L (0 or 1 g/kg-day) neutralized TCA in drinking water for 61 weeks	hepatic adenoma 9/23, 11/28 hepatic carcinoma 9/23, 15/28	Survival at 61 weeks: 23/23, 28/29; terminal body weight at high dose was 88% of control	Herren-Freund et al. (1987)
Male and female B6C3F1 mice (10-35/ sex/dose)	Males: 0, 1, or 2 g/L (0, 164, or 329 mg/kg-day ^a); females: 0 or 2 g/L (0 or 482 mg/kg-day ^a) neutralized TCA in drinking water for 52 weeks; (15-, 24-, and 37-week interim sacrifices of 5 males/dose at 0 and 2 g/L); ^Δ 11 males in the 2 g/L group were treated for 37 weeks and sacrificed at 52 weeks (recovery group)	Males: hepatic adenoma 0/35, 2/11, 1/11 [^Δ 0/11] hepatic carcinoma 0/35, 2/11, 4/11* [^Δ 3/11*] Females: no tumors	Not all mice were examined histologically	Bull et al. (1990)

Sex/ Species [#]	Dose/Route of Exposure/Duration	Tumor Incidence	Notes	Reference
Male B6C3F1 mice (number not specified)	0 or 4.5 g/L (0 or 583 mg/kg-day) neutralized TCA in drinking water for 94 weeks	hepatic adenoma ^b 0%, 43.3%* hepatic carcinoma ^b 12.3%, 72.8%* hepatic adenoma and/or carcinoma ^b 11.4%, 86.7%*	Only percentages for tumor response are reported	DeAngelo et al. (1991)
Male B6C3F1 mice (number not specified)	0 or 4.5 g/L (0 or 1,080 mg/kg-day ^c) neutralized TCA in drinking water for 104 weeks	hepatic carcinoma 19%, 73.3%	Only percentages for tumor response are reported, no statistical analysis	Ferreira- Gonzalez et al. (1995)
Female B6C3F1 mice (6- 40/dose)	Injected (i.p.) with 4 ml/kg sterile saline as MNU control; 0, 2, 6.67, or 20 mM (0, 78, 262, or 784 mg/kg- day ^c) neutralized TCA in drinking water for 31 or 52 weeks	31 weeks: hepatic adenoma 0/15, 0/10, 0/10, 0/15 52 weeks: hepatic adenoma 1/40, 3/19, 3/19, 2/40 hepatic carcinoma 0/40, 0/19, 0/19, 5/40*	TCA acted as complete carcinogen; carcinoma data at 31 weeks not reported	Pereira and Phelps (1996)
Female B6C3F1 mice (6- 40/dose)	Initiated with 25 mg/kg MNU (i.p. injection); 0, 2, 6.67, or 20 mM (0, 78, 262, or 784 mg/kg-day ^c) neutralized TCA in drinking water for 31 or 52 weeks; ^Δ recovery group: 20 mM treatment suspended at 31 weeks and animals were sacrificed at 52 weeks	31 weeks: hepatic adenoma 0/10, 1/8, 3/8, 6/10* 52 weeks: hepatic adenoma 7/38, 3/10, 5/6*, 15/22* [^Δ 7/11*] hepatic carcinoma 4/38, 0/10, 5/6*, 18/22* [^Δ 4/11]	Carcinoma data at 31 weeks not reported except for the 20 mM group, which contained two mice with carcinomas	Pereira and Phelps (1996)
Female B6C3F1 mice (18- 90/dose)	0, 2, 6.67, or 20 mM (0, 44, 155, or 453 mg/kg-day ^c) TCA in drinking water for 360 or 576 days	360 days: hepatic adenoma 1/40, 3/40, 3/19, 2/20 hepatic carcinoma 0/40, 0/40, 0/19, 5/20* 576 days: hepatic adenoma 2/90, 4/53, 3/27, 7/18* hepatic carcinoma 2/90, 0/53, 5/27*, 5/18*	Mortality appeared to have been minimal	Pereira (1996)

Sex/ Species#	Dose/Route of Exposure/Duration	Tumor Incidence	Notes	Reference
Female B6C3F1 mice (20- 30/dose)	Initiated with 25 mg/kg MNU (i.p. injection); 0, 6 or 25 mM neutralized TCA in drinking water for 44 weeks	hepatic adenomas incidence not reported hepatic carcinoma 0/29, 0/20 4/29	None	Pereira et al. (1997)
Male F344/N rats (50/dose)	0, 0.05, 0.5, or 5 g/L (0, 3.6, 32.5, or 364 mg/kg-day) neutralized TCA in drinking water for 104 weeks; interim sacrifices at 15, 30, 45, and 60 weeks (number not specified)	hepatic adenoma 1/23, 1/24, 3/20, 1/22 hepatic carcinoma 0/23, 0/24, 0/20, 1/22 (denominator represents number of animals surviving 80- 104 weeks)	No significant increase in tumors at any dose	DeAngelo et al. (1997)
Male and female B6C3F1 mice (8- 29/dose)	Initiated with 30 mg/kg MNU (i.p. injection); 0 or 4.0 g/L (females: 0 or 0.96 g/kg-day ^c , males: 0 or 1 g/kg- day ^c) neutralized TCA in drinking water for 31 weeks	Females: hepatic adenoma 2/29, 2/14 hepatic carcinoma ^d 0/29, 4/14 combined liver tumors 2/29, 6/14 Males: hepatic adenoma 2/8, 12/16 hepatic carcinoma ^d 0/8, 10/16* combined liver tumors 2/8, 13/16* renal tumors (cystic adenomas, tubular cell carcinomas) 0/8, 14/16*	Only liver and kidney were examined	Pereira et al. (2001)
Male B6C3F1 mice (20/dose)	Experiment 1: 0, or 2 g/L (0 or 238 mg/kg-day ^e) neutralized TCA in drinking water for 52 weeks	Combined hepatic nodules, adenoma and carcinoma: 4/12, 33/40*	None	Bull et al. (2002)
Male B6C3F1 mice (20/dose)	Experiment 2: 0, 0.5, or 2 g/L (0, 55, or 238 mg/kg-day ^e) neutralized TCA in drinking water for 52 weeks	hepatic adenoma 0/20, 5/20, 6/20 hepatic carcinoma 0/20, 3/20, 3/20 hepatic adenoma and carcinoma 0/20, 6/20*, 8/20*	None	Bull et al. (2002)

Sex/ Species [#]	Dose/Route of Exposure/Duration	Tumor Incidence	Notes	Reference
Male B6C3F1 mice (30/dose)	Study 1: 0, 0.05, 0.5, or 5 g/L (0, 7.7, 68.2, or 602.1 mg/kg-day ^f) neutralized TCA in drinking water for 60 weeks; interim sacrifices (5/dose) at 4, 15, 31, and 45 weeks	hepatic adenoma and/or carcinoma (45-60 weeks) ^{g,h} 4/35, 5/32, 12/34*, 19/34* hepatic adenoma and/or carcinoma (60 weeks) ^g 4/30, 4/27, 11/29*, 16/29*	Controls were given 2 g/L sodium chloride	DeAngelo et al. (2008)
Male B6C3F1 mice (57/dose)	Study 2: 0 or 4.5 g/L (0 or 572 mg/kg-day ^f) neutralized TCA in drinking water for 104 weeks; interim sacrifices (5/dose) at 15, 39, 45 weeks; 10 control animals sacrificed at 60 weeks	hepatic adenoma (104 weeks): 0/25, 21/36* hepatic carcinoma (104 weeks): 3/25, 28/36* hepatocellular adenoma and/or carcinoma (104 weeks): 3/25, 32/36*	Controls were given 1.5 g/L neutralized acetic acid	DeAngelo et al. (2008)
Male B6C3F1 mice (72/dose)	Study 3: 0, 0.05, or 0.5 g/L (0, 6.7, or 81.2 mg/kg-day ^f) neutralized TCA in drinking water for 104 weeks; interim sacrifices (8/dose) at 26, 52, and 78 weeks	hepatic adenoma and/or carcinoma (52-104 weeks) ^{g,i} 31/56, 21/48, 36/51 hepatic adenoma and/or carcinoma (104 weeks) ^g 27/42, 19/35, 32/36*	Controls were given deionized water	DeAngelo et al. (2008)

[#]Number of animals (per dose) that were started on treatment is indicated

ENU, ethylnitrosourea; i.p., intraperitoneal; MNU, methylnitrosourea

*significantly different from control (p<0.05) using Fisher's exact test

^aDoses for male mice in the Bull et al. (1990) study were estimated by the study authors according to US EPA (2011); dose for female mice was calculated by OEHHHA using default values (US EPA, 1988)

^bTumor data were presented in graphs as prevalence, or % of animals developing tumors

^cDoses calculated using reference body weight values from US EPA (1988)

^dLabeled as adenocarcinomas in original report

^eDoses were calculated using default water consumption of 5 ml/animal-day and the reported body weights (Bull et al., 2002)

^fDoses in the DeAngelo et al. (2008) study have been calculated by OEHHHA based on reported water consumption rates and measured TCA concentrations

^gIncidences from original data provided by US EPA; incidences were reported incorrectly in DeAngelo et al. (2008)

^hFirst occurrence of tumor was at 45 weeks

ⁱFirst occurrence of tumor was at 52 weeks

With the exception of the DeAngelo et al. (2008) study, most TCA cancer bioassays focused on the liver and were shorter than lifetime duration. DeAngelo et al. (2008) appeared to be the best quality study overall, due to the large number of animals employed (30-72/dose), multiple measured endpoints including water consumption and complete pathology, lifetime exposure of

several subsets (Study 2 and Study 3) and consistent findings of liver neoplasms in several independent experiments. This study also had added emphasis on peroxisome proliferation and specifically found correlated significant increases for both hepatic peroxisome proliferation and hepatic neoplasms at 68.2 mg/kg-day.

Mode of Action and Mechanistic Considerations

A number of mechanisms have been proposed for the liver tumors in rodents caused by TCA and they are discussed in this section.

Peroxisome Proliferation

In rodents, PPAR α activation and resulting peroxisome proliferation are thought to increase oxidative stress and proliferation in the liver, leading to tumors. It was found early on that TCA activated PPAR α in rodent liver in vivo (Table 7.9) and it was hypothesized that TCA would possess other properties of typical peroxisome proliferators including hepatocarcinogenicity. The involvement of PPAR α activation in TCA-mediated carcinogenesis was extensively reviewed in Bull and Stauber (1999) following a suite of studies attempting to analyze similarities and differences in DCA- and TCA-driven carcinogenesis. While these authors noted that “tumors induced by TCA appear to express a phenotype similar to that induced by other peroxisome proliferators,” they also concluded that there seemed to be no “causal link between increases in peroxisome numbers and the induction of cancer.”

Table 7.9 In vivo activation of peroxisome proliferation^a by TCA in the liver

Species	Effective doses ^b in mg/kg-day	Duration (number of animals)	Reference
Mice	(50 [^]), 100, 200	10 days (n=4-5)	Elcombe (1985)
	500 (only dose)	10 days (n=8)	Goldsworthy and Popp (1987)
	261, 442	14 days (n=6)	DeAngelo et al. (1989)
	228 (only dose)	14 days (n=6-18)	Austin et al. (1995)
	500 (high dose)	1 week (n=3-5)	Laughter et al. (2004)
	25, 125, 500	3 or 10 weeks (n=6)	Parrish et al. (1996)
	68, 602	4-60 weeks (Study 1, n=30)	DeAngelo et al. (2008)
	572 (only dose)	26-104 weeks (Study 2, n=57)	DeAngelo et al. (2008)
Rats	50, 100, 200	10 days (n=4-5)	Elcombe (1985)
	726 (only dose)	2 weeks – 6 months (n=4-6)	Parnell et al. (1988)
	500 (only dose)	10 days (n=6)	Goldsworthy and Popp (1987)
	719 (high dose)	14 days (n=6)	DeAngelo et al. (1989)
	355 (high dose)	13 weeks (n=10)	Mather et al. (1990)

^aAs measured by increase in activity of the specific marker enzyme cyanide-insensitive palmitoyl-CoA oxidase (PCO)

^bDose(s) at which a statistically significant increase above control values was observed

[^]Two-fold increase over control, but not statistically significant

Based on early negative reports of the in vitro genotoxicity of TCA and observations of peroxisome proliferation and liver carcinogenesis, specifically in mice, several authors

suggested that TCA may induce liver tumors in mice through PPAR α activation (Komulainen, 2004; Corton, 2008; DeAngelo et al., 2008). Laughter et al. (2004) showed that several toxic effects of TCA, such as increases in liver weight and hepatocyte proliferation, are mediated through PPAR α , as PPAR α -null mice were unaffected by TCA.

However, at least two considerations suggest that the tentative PPAR α -dependent MOA may be more nuanced in the case of TCA compared to conventional peroxisome proliferators. First, unlike conventional peroxisome proliferators, TCA does not cause hepatic tumors in rats even though it is capable of activating peroxisome proliferation in the rat liver (Table 7.9). Second, there are currently no reports on ligand binding and direct activation of PPAR α by TCA, which appears to be an unlikely PPAR α ligand based on its small size and structural dissimilarity with conventional PPAR α ligands such as phthalates. Although the available evidence does not exclude the possibility that at least some TCA tumors could originate with PPAR α activation, there is evidence suggesting it is not the only MOA for TCA carcinogenesis.

Furthermore, US EPA concluded in its toxicological assessment of TCA that “PPAR α agonism may not be the sole MOA for TCA-induced tumors in mice” (US EPA, 2011). This was based on a detailed review of the literature on TCA-induced foci and tumors, and the observed differences in mutation frequencies and spectra, phenotypic characteristics and immunostaining characteristics compared to those from other peroxisome proliferators. US EPA also noted, “there is *suggestive evidence of carcinogenic potential* for TCA” and has derived CSFs from available bioassays based on linear low-dose extrapolation from the BMDL₁₀ for liver tumors in B6C3F1 mice (US EPA, 2011).

Cytotoxicity and Cell Proliferation

Cytotoxicity and compensatory cell proliferation has been proposed as another possible MOA for TCA carcinogenesis. In this proposed MOA, TCA-induced hepatic cytotoxicity would generate necrotic foci in the liver and stimulate compensatory proliferation in the surrounding tissue. This effect would accelerate the process of spontaneous carcinogenesis, ultimately leading to the formation of liver neoplasms.

TCA was mildly to moderately cytotoxic in a number of in vitro and ex vivo systems, including mouse liver slices, mammalian cell lines and bacteria (Pravecsek et al., 1996; Plewa et al., 2002; Plewa et al., 2010; Zhang et al., 2010; Stalter et al., 2016; Zhang et al., 2016). TCA consistently possessed weaker cytotoxic properties compared to other DBPs, including other HAAs (Plewa et al., 2002; Plewa et al., 2010; Zhang et al., 2010; Stalter et al., 2016; Zhang et al., 2016), and occasionally was not cytotoxic at the examined concentrations, such as in the (Kadry et al., 1989) study in rat hepatocytes (at 5.7 mM). Bruschi and Bull (1993) applied TCA at concentrations of up to 5 mM to suspensions of hepatocytes isolated from Sprague-Dawley rats or B6C3F1 mice but found no evidence of cytotoxicity. Under cytotoxic concentrations, TCA appears to inhibit protein synthesis and increase cell membrane permeability (Channel and Hancock, 1992). Based on a review of the available evidence, Bull et al. (2004) concluded that TCA, together with DCA, “are effective carcinogens at doses that do not produce cytotoxicity.”

Increases in labeling index and cell proliferation have been observed in mice following acute or short-term exposure to either neutralized or un-neutralized TCA at relatively high doses (Dees and Travis, 1994; Miyagawa et al., 1995; Sanchez and Bull, 1990). Stauber and Bull (1997) exposed male B6C3F1 mice to neutralized TCA in drinking water (2 g/L or 480 mg/kg-day) for 14-350 days, and observed an initial increase in hepatocyte cell division, at 14 and 28 days. However, the increase in hepatocyte proliferation was transient, and TCA substantially inhibited division of normal hepatocytes at the end of treatment (at 350 days). Additionally, at 0.01-1 mM, TCA did not alter ³H-thymidine incorporation in cultured hepatocytes from male Long-Evans rats in vitro (Walgren et al., 2005), leading to the conclusion that TCA was not a direct mitogen in this system.

Finally, as noted above, data for cell proliferation in three chronic studies by DeAngelo et al. (2008) were highly variable, and dose-response was inconsistent among the studies. The proliferation rates were sporadically increased (significant difference with control) at some doses and time points; however, at least in one instance the proliferation rate was much lower compared to that of control. The study did not specify how many animals were used for each experiment, and the utilized method, i.e., subcutaneous implantation of an osmotic pump containing a small amount of radioactively labeled thymidine, appears to be prone to high experimental variability. As a result, evidence of altered proliferation from DeAngelo et al. (2008) and its possible relation to carcinogenesis is ambiguous.

Based on the available data, it appears unlikely that cytotoxicity or cell proliferation would be the primary driver for TCA liver carcinogenesis.

Oncogene Activation

Proto-oncogenes encode cellular proteins that control cell cycle and proliferation. DNA mutations can cause increased expression and/or increased activity of these proteins, leading to stimulation of proliferation, inhibition of apoptosis and cellular differentiation, and other processes contributing to cellular transformation and tumorigenesis. *C-myc* and *Hras* are proto-oncogenes that encode signal transduction proteins in the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway. Overexpression and/or mutations of *c-myc* and *Hras* can result in constitutive (signal-independent) activity and are commonly observed in cancer. Mutations in the promoter or coding regions of proto-oncogenes, such as *c-myc* and *Hras*, may contribute to changes in gene expression and protein levels in TCA-dependent neoplasms. Expression of *c-myc* was significantly increased in hyperplastic nodules and carcinomas in TCA-treated male B6C3F1 mice compared to non-tumor tissues in either exposed or control animals after 52 weeks of exposure via drinking water and remained elevated at the end of the experiment (at 52 weeks) even when the treatment was suspended after 37 weeks (Nelson et al., 1990). Expression of *Hras* was significantly increased in carcinomas only and abated with suspended treatment. In male B6C3F1 mice, Ferreira-Gonzalez et al. (1995) examined patterns and frequency of *Hras* mutations in spontaneously arising carcinomas, comparing them to those from carcinomas following a 104-week administration of 1,080 mg/kg-day of neutralized TCA in drinking water. Surprisingly, the frequency and pattern of *Hras* mutations were not different between the two groups.

In addition to gene expression tools, immunohistochemical examination of protein expression is commonly used to characterize tumors and determine their etiology. Using a subset of TCA-treated female B6C3F1 mice from a prior study (Pereira and Phelps, 1996), Latendresse and Pereira (1997) found that TCA-induced neoplastic foci and tumors were predominantly basophilic, negative for GST- π and TGF- β , and positive for c-jun proteins, while DCA-dependent tumors had a different protein expression profile. The authors also noted marked heterogeneity of the examined TCA-dependent neoplasms, suggesting that several underlying mechanisms may be responsible. In contrast with the Latendresse and Pereira (1997) study, Stauber and Bull (1997) and Bull et al. (2002) reported a lack of c-jun immunoreactivity with TCA-induced hepatic tumors.

The currently available data on specific mutations, gene expression and protein levels for TCA-induced neoplasms do not provide strong support for any specific proto-oncogene as the cause of TCA-dependent tumorigenesis.

Epigenetic Alterations (Hypomethylation)

Although the exact mechanisms of TCA carcinogenicity are presently unclear, epigenetic changes in particular genes may contribute to tumorigenesis. Hypomethylation of DNA in the liver and kidneys of mice exposed to TCA has been reported in several studies from one research group (Tao et al., 1998; Tao et al., 2000a, 2000b; Ge et al., 2001; Tao et al., 2004a; Tao et al., 2005). The in vivo studies of TCA-dependent hypomethylation are summarized in Table 7.10.

Table 7.10 In vivo hypomethylation studies of TCA.

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	Reference [†]
Female B6C3F1 mice (8-16/dose)	Initiated with 25 mg/kg MNU (i.p. injection); 0 or 25 mM neutralized TCA in drinking water for 11 days or 44 weeks	Decreased DNA methylation in liver at 11 days; decreased DNA methylation in liver tumors at 44 weeks	Pereira et al. (1997); (Tao et al., 1998)
Female B6C3F1 mice (4-6/dose)	0 or 500 mg/kg-day neutralized TCA by oral gavage, followed 30 minutes later by i.p. injection with 0 or 450 mg/kg methionine, for 5 days	Hypomethylation of <i>c-jun</i> and <i>c-myc</i> promoters in liver; increased expression of <i>c-jun</i> and <i>c-myc</i> mRNA and protein in liver (reversed with methionine treatment)	Tao et al. (2000a)
Female B6C3F1 mice (4/dose)	0 or 20 mM (784 mg/kg-day*) neutralized TCA in drinking water for 52 weeks *dose calculated by US EPA (2011)	Hypomethylation of <i>c-jun</i> and <i>c-myc</i> promoters in liver; increased expression of <i>c-jun</i> (protein), <i>c-myc</i> (protein) and <i>IGFII</i> (mRNA, protein)	Pereira and Phelps (1996); Tao et al. (2004a); Tao et al. (2000b)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	Reference [†]
Female B6C3F1 mice (6/dose)	0 or 500 mg/kg-day neutralized TCA by oral gavage for 5 days; additional groups co-treated with 0, 0.4, 0.8 and 1.6 g/L chloroform in drinking water (for 12 days prior to TCA treatment and throughout TCA treatment)	<i>c-myc</i> promoter hypomethylation in liver and increased <i>c-myc</i> mRNA expression in liver (these effects were not affected by co-treatment with chloroform)	Pereira et al. (2001)
Female B6C3F1 mice (6/dose/time point)	0 or 500 mg/kg-day neutralized TCA by oral gavage; animals sacrificed at 24, 36, 48, 72, and 96 hours after the start of treatment	<i>c-myc</i> promoter hypomethylation in liver, bladder and kidney (at 72, 96 hours)	Ge et al. (2001)
Male and female B6C3F1 mice (6/sex/dose)	0, 0.4 or 4 g/L (100 or 1,000 mg/kg-day) neutralized TCA in drinking water for 7 days; each dose group was concurrently treated with 0, 0.1, 0.3, 1.0, or 1.6 g/L chloroform in drinking water; the 4 g/L group also concurrently received 0, 4 or 8 g/kg methionine in diet	<i>c-myc</i> promoter hypomethylation in male kidney (reversed by methionine in the diet, but not affected by chloroform in drinking water) Effects were observed at both doses.	Tao et al. (2005)

[†] Study results were reported in multiple papers when several references are cited.

Administration of neutralized TCA for 11 days in drinking water (25 mM or 1 g/kg-day) to female B6C3F1 mice caused significant DNA hypomethylation in the liver (Tao et al., 1998). A significant decrease in methylated DNA was also observed in liver adenomas and carcinomas in female mice initiated with MNU and exposed to 25 mM neutralized TCA in drinking water for 44 weeks (Tao et al., 1998). At the gene level, the promoter regions of the proto-oncogenes *c-jun*, *c-myc* and insulin growth factor II (*IGF-II*) had lower levels of DNA methylation, which possibly contributed to increased expression of these genes in TCA-treated mice (Tao et al., 2000a, 2000b; Ge et al., 2001; Tao et al., 2004a; Tao et al., 2005).

Other In Vitro Studies

Benane et al. (1996) exposed rat clone 9 cells (normal liver epithelial cell line) to 0, 0.5, 1, 2.5 or 5 mM neutralized TCA for 1-168 hours and measured gap junction intercellular communication via dye transfer. TCA significantly inhibited intercellular communication at ≥ 1 mM at all times. Klaunig et al. (1989) also observed decreased gap junction intercellular communication in primary B6C3F1 mouse hepatocytes at applied TCA concentrations of 0.1-1 mM for 4 hours, while no difference was observed at later time points. No decrease was observed in primary F344 rat hepatocytes in this study, in contrast with the results observed with rat liver epithelial cells (Benane et al., 1996).

Conclusions on the Mode of Action for Carcinogenicity of TCA

Overall, the available data suggest that TCA carcinogenesis is complex and may involve multiple modes of action. Given the positive findings of TCA genotoxicity *in vivo*, as well as its metabolic conversion into genotoxic DCA, genotoxicity and/or alteration of DNA repair appear as viable TCA cancer MOAs. Other contributing mechanisms may include PPAR α activation and hypomethylation of proto-oncogenes *c-myc* and *c-jun*.

Based on this analysis, OEHHA concludes that TCA could act as a genotoxic carcinogen and that the available bioassay data are sufficient to assess the carcinogenic potential of TCA to humans using linear low-dose extrapolation.

8. TOXICOLOGICAL PROFILE: MONOBROMOACETIC ACID

Acute Toxicity

Monobromoacetic acid (MBA) is irritating and corrosive to skin and mucous membranes due to its relatively strong acidic properties (The Merck Index Online, 2017). An oral LD₅₀ (median lethal dose) of 100 mg/kg was determined for neutralized MBA in white mice (Morrison 1946). Le Poidevin (1965) estimated an LD₅₀ of about 85 mg/kg neutralized MBA in adult white female mice via i.p. injection. Linder et al. (1994a) estimated the oral gavage LD₅₀ for neutralized MBA in adult male Sprague-Dawley rats to be about 177 mg/kg (95% confidence limit, 156 to 226 mg/kg).

Harrestrup Andersen et al. (1955) injected neutralized MBA solution (pH 7) intravenously into three conscious, unrestrained 7- to 48-month-old dogs of unknown breed at doses of 2, 4, 8, 16, and 24 mg/kg. One dog received all doses; one dog received 8 and 24 mg/kg doses; one dog received a single dose of 4 mg/kg. For animals with multiple exposures, treatments were done once a week; each dog served as its own control. All intravenous doses caused ataxia; diarrhea was observed at ≥4 mg/kg, and vomiting at ≥8 mg/kg. Electrocardiography (ECG) effects (changes in T-waves) were noted only at 24 mg/kg in the two dogs given this dose. Muscle rigidity and hind-limb paralysis were also noted at the highest dose. The study also tested one dog with sequential oral bolus doses of neutralized MBA at 8, 24 and 48 mg/kg (with recovery period of at least eight days between doses). While the 24 mg/kg oral dose caused ECG changes, the 48 mg/kg bolus dose did not. The same dog was also given 48 mg/kg MBA as six separate 8 mg/kg doses at 1.5-hour intervals. ECG changes were observed after 16 mg/kg in this repeated dosing experiment. Doses ≥24 mg/kg caused ataxia and vomiting.

Reproductive toxicity effects such as absolute weight of testes, seminal vesicles, and ventral prostate, as well as serum testosterone, testicular sperm head counts, and sperm motility were unaffected in male Sprague-Dawley rats (8/group) 2 or 14 days after a single oral gavage dose of 100 mg/kg neutralized MBA in water (Linder et al., 1994a).

An acute study in five rabbits found severe retinal damage after a single intravenous injection of 32 mg/kg sodium bromoacetate (27.6 mg/kg MBA equivalent) 3-21 days post exposure (Lucas et al., 1957). The retinal damage was similar to that observed with iodoacetate, as reported in the literature. Lower doses, 16-23 mg/kg, either as single or double doses, did not lead to retinal damage, although only three animals were employed for these dose groups. There were no controls in the study, and no other toxic effects were reported.

Subchronic Toxicity

No published standard subchronic toxicity studies (e.g., 90-day rodent studies) were found for MBA.

Dalgaard-Mikkelsen et al. (1955) administered MBA mixed with feed to pigs of Danish Country breed in a multigenerational study. Three generations of pigs of both sexes were exposed to varying doses of MBA, and general toxicity, as well as pathological, neurological and

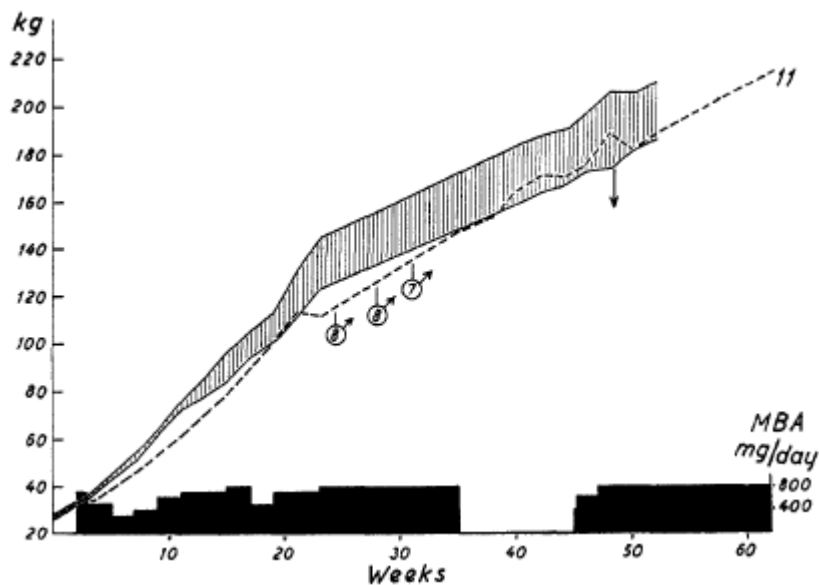
reproductive endpoints were examined. The study was undertaken to estimate a probable toxic dose in larger animals and not specifically as a reproductive toxicity study.

The first generation comprised 13 pigs, divided into the control and treatment groups at the age of two months. The details of the study are summarized in Table 8.1. Treatment was administered via a 10% aqueous MBA solution added to the feed. The concentration of MBA in the feed and food consumption rates were not reported. The study has further limitations, such as poor study design, small number of animals per dose group, lack of controls with most dosing regimens, variable dosage and treatment durations over time with details of the changes not adequately reported, gaps in exposure, and inadequate pathology examination and reporting. On average, animals were treated for 13.8 months. Animals in the first generation were typically started on 400 mg/day (with maximum daily doses of up to 20 mg/kg-day), with gradual increases to 800 or 1200 mg/day, which resulted in the average daily dose at killing of only 3.6 mg/kg-day, due to much higher body weights. Thus, administered doses dramatically decreased over the course of the experiment. Average daily doses (in mg/kg-day) were not reported, and were calculated as a ratio of a reported total dose (Tables 5 or 10 of the original manuscript, Dalgaard-Mikkelsen et al., 1955) to the time-weighted average body weight for the corresponding first-generation animal, which was calculated as follows.

Figure 8.1 presents an example of a reported weight curve (dashed line graph, left y-axis) and dosing information (black histogram at the bottom, right y-axis) for one of the treated animals from the first generation (Figure 2 in Dalgaard-Mikkelsen et al., 1955). The hatched area represents the growth curves for all the control sows. The weight curves for treated animals from this and other figures were digitally quantitated with GetData Graph Digitizer, and the resulting integrated averaged weights were used by OEHHA to calculate average daily doses in mg/kg-day for treated animals in the first generation. Weight data were reported only for surviving treated animals of the first generation; therefore, only this subset yielded data amenable to dose-response analysis.

The arithmetic mean of the averaged daily doses for surviving animals in the first generation was 5 mg/kg-day (Table 8.1). It is important to note that there is a high level of uncertainty in the derivation of this value due to poor reporting in the original study and to what appears to be a wide range of effectively applied doses throughout the course of the experiment.

Figure 8.1 Growth curve for one first-generation sow as presented in Dalgaard-Mikkelsen et al. (1955)



Apart from two animals that died during the study (from non-treatment-related causes according to the authors of the study), none of the surviving treated animals from the first generation displayed general toxicity (such as vomiting, diarrhea, altered behavior) or “revealed pathological changes, either on naked-eye inspection or on histological examination” upon final sacrifice (Dalgaard-Mikkelsen et al., 1955).

Progeny from the treated sows and a control boar were treated with MBA in the second generation, again, with highly variable and inconsistent dosing regimens. A variety of adverse effects and pathologies were reported in the second generation, including slow movement, paralysis, degeneration of skeletal muscles and liver, hematuria, dyspnea, cyanosis, muscular degeneration and various lung pathologies, among others. However, no controls for the second and third generation were used in this experiment and weight information was not reported. Details for the first two generations of the study are provided in Table 8.1.

Table 8.1 Summary of the first and second generation pig studies in Dalgaard-Mikkelsen et al. (1955)

	First Generation	Second Generation^a
Animals at start of study	Controls: 6 (1 boar, 5 sows) Treatment: 7 (2 boars, 5 sows)	Controls: none Treatment ^b : 9 (2 boars, 7 sows)
Animals at end of study ^c	Controls: 6 Treatment: 5	Controls: none Treatment: 6
Average exposure duration in all treated animals	313 days	285 days
Average exposure duration in surviving animals	414 days	384 days
Toxic effects in surviving animals (examined: general signs of toxicity, neurological effects, pathologies upon final sacrifice)	No symptoms or pathology	Multiple toxic effects, most common: muscular degeneration and pulmonary edema (7/9 animals). No symptoms in two animals (offspring of controls ^a) that were treated with about half the dose compared to other 2 nd generation animals.
Time-weighted average body weight in surviving animals	131 kg (estimated from body weight graphs) ^d	Weight data not reported
Average daily dose in surviving animals	5.0 mg/kg-day ^e	Not calculated ^f

^a Second generation consisted of 7 offspring of a control boar and treated sows, and 2 offspring of a control boar and sow.

^b Six animals were treated with the same dose as the first generation and 3 animals were treated with half the dose given to the first generation.

^c Two treated animals died in the first generation, with the cause of death reported as unrelated to MBA exposure. Three treated animals died in the second generation. Postmortem evaluation of second generation animals showed muscle degeneration and bleeding, pulmonary edema, and dystrophy of the liver.

^d For each surviving exposed animal in the first generation, body weights, which are reported in the form of graphs (example is given in Fig. 8.1), were integrated using GetData Graph Digitizer and the corresponding time-weighted body weight was calculated. The presented value is an average of time-weighted body weights for all surviving treated animals.

^e Calculated using reported total grams MBA per animal, average lifetime body weight, and total experimental days.

^f Could not be calculated due to the lack of body weight data.

In the Dalgaard-Mikkelsen et al. (1955) study, time-weighted average daily doses could only be calculated for animals in the first generation. The exact body weights (change over time) were not reported for animals in the second generation; therefore, time-weighted average doses could not be calculated. Since animals in the second generation that demonstrated toxicity upon MBA exposure were exposed both in utero, presumably through lactation and directly, it is not possible to determine whether the effects resulted from a developmental perturbation.

There were no controls in the second generation and the overall quality of reporting was poor. No NOAEL or LOAEL was determined by the study authors.

Dalgaard-Mikkelsen et al. (1955) also made several observations on reproductive toxicity, which are covered in the *Developmental and Reproductive Toxicity* section of this document.

Furthermore, three animals of the third generation were exposed to much higher oral doses of MBA (approximately ten-fold higher than doses applied in the first generation) and died at 39-40 days of exposure; following their deaths animals were examined, and similar adverse effects including muscular degeneration and lung pathologies were observed. Due to small numbers of animals, high doses used, lack of controls and uncertainty of developmental effects of MBA, the data from the third generation were not considered for dose-response analysis (not included in Table 8.1).

Genetic Toxicity

Genetic toxicity studies of MBA are summarized in Table 8.2 and 8.3. Within each table, studies are organized by experimental system (e.g., human studies before animal studies, primary cells before cell culture), and then by type of genotoxic effect. No human *in vivo* studies of MBA genotoxicity were located. *In vivo* genotoxicity studies in newt larvae and nematodes were negative (Table 8.2). Increased frequency of micronuclei, chromosome aberrations and DNA damage was reported for peripheral human lymphocytes in *ex vivo* studies (Ali et al., 2014; Escobar-Hoyos et al., 2013). DNA strand damage following exposure to DBA was reported in nine human and animal cell culture studies (Table 8.3), and no study in these experimental systems was negative. Bacterial-based studies were few and mostly negative, with gain of signal in the presence of the S9 fraction in two studies (Giller et al., 1997; Kargalioglu et al., 2002). This may indicate the need for MBA metabolism in exerting genotoxic effects in cell-based systems. Finally, genotoxic MBA effects were reported for plant-based systems.

Table 8.2 In vivo genetic toxicity studies of MBA

Assay	Species	Dose/Route of Exposure	Result	Reference
Non-mammalian: Micronucleus test	Newt larvae (<i>P. waltii</i>) (15/dose)	10-40 µg/ml unneutralized MBA in tank water for 12 days	-	Giller et al. (1997)
LA-QPCR ^a in total nuclear DNA	Nematode (<i>C. Elegans</i>) (5,000/dose)	0.2 – 1 mM unneutralized MBA for 24 hours	-	Zuo et al. (2017)
Micronucleus assay	<i>V. faba</i> root tips	0.1-100 µM (0.014-13.9 µg/ml)	+	Hu et al. (2017)
Comet assay	<i>V. faba</i> root tips	1-100 µM (0.14-13.9 µg/ml)	+	Hu et al. (2017)

^a Long amplicon quantitative PCR

Table 8.3 Genetic and related effects of MBA in vitro

Assay	Results Without S9	Results With S9	Concentration	Reference
Primary mammalian cells: Micronucleus assay in peripheral blood lymphocytes	+	ND	0.1 – 6.25 μ M (0.014-0.875 μ g/ml)	Ali et al. (2014)
Chromosome aberrations assay in human lymphocytes	+	ND	4-1100 μ M (0.6 - 153 μ g/ml)	Escobar-Hoyos et al. (2013)
SCGE (Comet) assay (DNA strand breaks) in human lymphocytes	+	ND	4 – 270 μ M (0.6 - 38 μ g/ml)	Escobar-Hoyos et al. (2013)
SCGE (Comet) assay (DNA strand breaks) in peripheral blood lymphocytes and sperm	+	ND	25 μ M (3.5 μ g/ml)	Ali et al. (2014)
Human cell lines: Micronucleus assay in human lymphoblast TK6 ⁱ cells	-	ND	0.5-20 μ M (0.07-2.8 μ g/ml)	Liviach et al. (2010)
SCGE (Comet) assay in human epithelial FHS ^e cells (DNA strand breaks)	+	ND	30 – 150 μ M ^f (4.2 - 21 μ g/ml)	Muellner et al. (2010)
SCGE (Comet) assay in human epithelial FHS ^e cells (DNA strand breaks)	+	ND	4.5-14 μ g/ml (estimate from graph)	Attene-Ramos et al. (2010)
SCGE (Comet) assay in HepG2 ^g cells (DNA strand breaks)	+	ND	0.01 – 100 μ M (1.3 ng/ml - 13.4 μ g/ml)	Zhang et al. (2012)
Mammalian cell lines: HGPRT ^j mutation assay in CHO ^d -K1 cells (DNA mutations)	+	ND	10-800 μ M (1.4-111 μ g/ml)	Zhang et al. (2010)
Alkaline elution (DNA strand breaks) in mouse leukemia L-1210 ^b cells	+	ND	0.1 mM (13.9 μ g/ml)	Stratton et al. (1981)

Assay	Results Without S9	Results With S9	Concentration	Reference
SCGE ^c (Comet) assay in CHO ^d cells (DNA strand breaks)	+	ND	2.5 –25 µM (0.35 – 3.5 µg/ml)	Plewa et al. (2002); Plewa et al. (2004); Plewa et al. (2010)
SCGE (Comet) assay (DNA repair ^h) in CHO ^d cells	+	ND	60 µM (8.3 µg/ml)	Komaki et al. (2009)
SCGE (Comet) assay in CHO ^d cells (DNA strand breaks)	+	ND	60 µM (8.3 µg/ml)	Dad et al. (2013)
SCGE (Comet) assay DNA strand breaks) in CHO ^d cells	+	ND	50, 100 µM (6.9, 13.9 µg/ml)	Pals et al. (2016)
Non-mammalian cells: Bacterial reverse mutation assay in <i>S. typhimurium</i> TA100 (Ames fluctuation test)	-	+	0.03-30 µg/ml (without S9) 0.3-300 µg/ml (with S9)	Giller et al. (1997)
Bacterial reverse mutation assay in <i>S. typhimurium</i> TA98, TA100 (Ames preincubation test)	-	+	5 – 550 µM (0.7 – 76 µg/ml)	Kargalioglu et al. (2002)
SOS-umuC assay ^a in <i>S. typhimurium</i> TA1535/pSK1002	+	ND	0.1 – 1.6 mM (13.9 – 222 µg/ml)	Zhang et al. (2016)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	-	-	Up to 0.2 mM (28 µg/ml)	Stalter et al. (2016)
SOS chromotest in <i>E. coli</i> PQ37	-	-	1 µg/ml – 3 mg/ml	Giller et al. (1997)

^a Genotoxicity assay (ISO 13829) originally developed by the International Organization for Standardization designed to test for DNA damage

^b Mouse lymphocytic leukemia

^c Single cell gel electrophoresis

^d Chinese hamster ovary

^e Nontransformed human fetal small intestinal epithelial cells

^f Positive results were observed starting at the 30 µM

^g Human liver hepatocellular carcinoma

^h Assay was modified to examine DNA repair

ⁱ Thymidine kinase heterozygote

^j Hypoxanthine-guanine phosphoribosyltransferase

ND, not determined

In in vitro studies of more than 70 DBPs by Plewa and colleagues (Kargalioglu et al., 2002; Plewa et al., 2004; Komaki et al., 2009; Attene-Ramos et al., 2010; Muellner et al., 2010), MBA was the most cytotoxic and genotoxic among chlorinated and brominated DBPs (THMs and HAAs). The rank order of genotoxic response among monosubstituted HAAs was MIA (monoiodoacetic acid) > MBA > MCA in *S. typhimurium* (Kargalioglu et al., 2002), CHO cells (Plewa et al., 2004), and small intestinal epithelial FH cells (Attene-Ramos et al., 2010). MBA was ranked more genotoxic than DCA and TCA, which are classified as carcinogenic (Chang et al., 1992; Kargalioglu et al., 2002). Escobar-Hoyos et al. (2013) also compared genotoxic effects between the mono-HAAs and found that MBA was more genotoxic than MCA in human lymphocytes and CHO cells. Two in vivo studies were identified and the results were negative for genotoxicity (Giller et al., 1997; Zuo et al., 2017).

MBA altered transcriptome profiles for genes involved in cell cycle regulation and in DNA repair, especially repair of DNA double strand breaks, similar to the effects of ionizing radiation (Attene-Ramos et al., 2010; Muellner et al., 2010). A toxicogenomic study by Pals et al. (2013) showed that MBA generated oxidative stress and alteration of oxidative stress responsive genes in a non-transformed human epithelial cell line (FHs 74 Int). In Caco-2 cultured human colorectal cancer cells, MBA activated adaptive stress responses and DNA damage-responsive p53 pathways (Prochazka et al., 2015). Oxidative stress is one of IARC's key characteristics of carcinogens, and is a major player in the generation of DNA mutations (Smith et al., 2016).

Developmental and Reproductive Toxicity

Developmental toxicity in vitro

Hunter et al. (1996) studied the developmental toxicity of MBA (among other HAAs) in CD-1 mouse embryo cultures exposed to 0-50 μM un-neutralized MBA in buffered solution (tissue culture media) for 24 hours. The pH of the MBA tissue culture medium was similar to control medium (pH 8.36 ± 0.04). Neural tube malformations, rotational defects, pharyngeal arch defects and heart defects were significantly increased at 6 μM MBA. The combined number of malformations at 2 μM MBA was significantly increased compared to controls, with increasing dose-response up to 10 μM . At 10 μM , 100 percent of embryos demonstrated malformations (eye defects, somite dysmorphology, and decreased somite numbers) and at 50 μM , all cultured embryos were dead. MBA was the most toxic of the HAAs tested, with a BMDL of 2.7 μM for a 5% increase in neural tube defects, as reported by the study authors. While findings in Hunter et al. (1996) support the potential developmental toxicity of MBA, there are no in vivo studies of developmental toxicity in mice.

MBA slightly inhibited differentiation of human neural stem cells (detected as change in nestin expression) in culture with 0.5 μM exposure for 12 days; no significant inhibition was observed with 0.1 μM MBA (Fu et al., 2017).

Developmental toxicity in vivo

Dalgaard-Mikkelsen et al. (1955) conducted a multigenerational feed study with pigs, which included a limited number of observations in second-generation animals. Dosing regimens

varied widely due to a number of factors, including periodic increases and decreases in dosing and interruptions in treatment. The male and female pigs in the first generation did not display toxicity with an average dose of 5.0 mg/kg-day. The second-generation animals, bred from MBA-treated sows and a control boar, demonstrated multiple toxic effects at 800 mg/day (approximately 11-47 mg/kg-day doses at death). Some second-generation animals were exposed to 400 mg/day and showed less toxic effects. The most prominent adverse effect was muscle degeneration. There were no controls in the second generation, and the average doses could not be calculated by OEHHA. Because the second-generation animals were exposed to MBA in utero, presumably through lactation and directly with oral doses, one cannot determine if the observed adverse effects were due to developmental toxicity.

The authors examined litters from the first- and second-generation sows, and reported lower birth weights and decreased survival in offspring in some cases. Of the second generation, four sows, all with different treatment histories, were bred with a treated second-generation boar, producing offspring with low survival rates (past weaning stage) or characterized as "less vitally resistant than those of normal sows." While not definitive, the Dalgaard-Mikkelsen et al. (1955) study suggests MBA may have potential for developmental toxicity.

Reproductive toxicity in vitro

Jeong et al. (2016) studied the reproductive effects of MBA in mouse ovarian antral follicle cultures exposed to 0, 2, 5, 10, 15 μM un-neutralized MBA for 96 hours. Antral follicle growth was inhibited at concentrations of 5 μM and higher, between 72 and 96 hours of exposure. Secreted estradiol levels were decreased at 10 and 15 μM of MBA. A recovery experiment in which follicles were exposed to 10 or 15 μM MBA for 48 hours and incubated for another 48 and 96 hours without MBA showed inhibition of antral follicle growth and reduced estradiol levels. In comparison with other mono-HAAs, MBA had a similar potency to monoiodoacetic acid and displayed about 20-fold higher potency in this in vitro system compared to monochloroacetic acid.

Reproductive toxicity in vivo

Linder et al. (1994a) gavaged adult male Sprague-Dawley rats (8/dose) with an aqueous solution of neutralized MBA in distilled water with two dosing regimens, a single 100 mg/kg dose with 2 or 14 days follow-up, or a daily 25 mg/kg dose for 14 days. Control animals were treated with water. MBA had no effect on weights of the testes, epididymis, seminal vesicles, and ventral prostate. Serum testosterone, epididymal sperm counts, sperm morphology and motility, and histopathology of the testes and epididymis were also not affected by MBA. Based on the lack of reproductive effects in this study, a NOAEL of 25 mg/kg-day can be determined for the 14-day study.

Dalgaard-Mikkelsen et al. (1955) crossed various combinations of treated and untreated pigs and noted pregnancies, numbers of live and dead piglets at birth, and numbers of surviving piglets at weaning. Two control sows were bred with control boars (three and two breeding attempts, respectively). One sow produced 8 live and 2 dead piglets; 6 were alive at weaning. The other sow produced 11 live and 1 dead piglets; 6 were alive at weaning. Two control sows

bred with an MBA-treated boar (one breeding attempt in either case) did not conceive. One control sow bred with an MBA-treated boar (two breeding attempts) produced 4 live and 3 dead piglets, with 2 alive at weaning. Two MBA-treated sows were bred with a control boar (three and one breeding attempts). One sow produced 11 live piglets, with 6 alive at weaning. The other sow produced 9 live piglets, with 6 alive at weaning. One MBA-treated sow bred with an MBA-treated boar produced 11 dead piglets. While these data suggest some reproductive toxicity with MBA exposure, they cannot be analyzed statistically due to small numbers of examined animals.

Immunotoxicity

No studies were found on the immunological effects of MBA.

Neurotoxicity

Harrestrup Andersen et al. (1955) observed apathy and ataxia in dogs given intravenous neutralized MBA at 2-24 mg/kg, with muscular rigidity and hindlimb paralysis at the highest dose. With oral dosing, brief ataxia occurred after 24 mg/kg, while at 48 mg/kg apathy and ataxia were observed for 7 days. The oral acute LOAEL for ataxia was 24 mg/kg.

Carcinogenicity

No studies on MBA carcinogenicity were located. However, as indicated in the *Genetic Toxicity* section, many studies of its genotoxic effects are positive.

US EPA and IARC have not published reviews of MBA carcinogenicity. WHO (2004a) considered the database for MBA inadequate to develop guideline values in drinking water. Based on the number and variety of positive genotoxicity studies, and the observation that MBA is more potent in genotoxicity assays than DCA and TCA, which are both classified as carcinogens, OEHHA concludes there is reason for concern that MBA may be a carcinogen.

Unpublished studies

Due to the MBA database scarcity, unpublished studies may be of some interest in discussion of approaches to PHG derivation.

Randall and co-workers investigated developmental toxicity of MBA in pregnant Long-Evans rats, as reported in a conference abstract (Randall et al., 1991). Animals were treated by oral gavage on gestation days 6-15 with 0, 25, 50 or 100 mg/kg-day, and were sacrificed on day 20. A number of fetal defects were noted at the high dose (100 mg/kg-day) including cardiovascular and craniofacial malformations, and the authors noted that the toxicity level was comparable to chloroacetic acid (MCA).

The assessment report from the European Commission on regulation of MBA as a food and feed area disinfectant contains references to a host of unpublished studies on MBA toxicity submitted by Sopura N.V. and Albemarle SPRL (European Commission, 2013). Of particular note are the following two toxicity studies:

“In a 28 day oral toxicity study in rats the NO(A)EL on the basis of reduction of water and food intake and liver relative decreased weight in males and kidney relative increased weight in females is 7 mg/kg bw/day.”

“In a 90 day oral (drinking water) study, rats displayed signs of toxicity such as reduction of food and water intake and body weight in both males and females. Changes in haematological parameters (such as increased mean corpuscular volume, decrease in thrombocyte, increased alkaline phosphatase activity, decreased plasma levels of total protein, cholesterol and phospholipids, increased plasma level of bilirubin in females and increased plasma level of chloride), decreased volume and increased density of the urine, decreased number of crystals in the urinary sediments, and in liver, brain and kidney weights were also reported. Based on the reduction of food intake and body weight in both males and females, a NOAEL of 10.3 mg/kg bw/day is established for male rats and a NOAEL of 14 mg/kg bw/day is established for females.”

No further details are provided on these reports. Since these studies are neither published nor peer-reviewed, OEHHA cannot use them as critical studies for PHG derivation. However, it is notable that the NOAELs identified in these studies are within two-fold of the NOAEL of 5 mg/kg-day derived from the Dalgaard-Mikkelsen et al. (1955) study.

In Vitro Studies and Mechanistic Considerations

In addition to genotoxicity, a number of other MBA-dependent in vitro endpoints were examined and used for ranking of in vitro potencies among DBPs (the most informative studies are summarized in Table 8.4).

Table 8.4 MBA in DBP in vitro studies (excluding genotoxicity)

Study	Experimental system	DBP concentrations	Endpoint(s)	DBP in vitro ranking ^a
Hunter et al. (1996)	CD1 mouse whole embryo culture	1 µM – 17mM for 24-26 hours	Neural tube defects	DFA<TFA<DCA<TBA~TCA<DBA<MCA<MBA
Kargalioglu et al. (2002)	<i>S. typhimurium</i>	0.2-50 mM for 1 hour	Cytotoxicity	DCA~TCA<bromoform~DBA~TBA~MCA~KBrO₃<< MBA<<MX
Plewa et al. (2002)	CHO cells	0.0025-43 mM for 72 hours	Cytotoxicity	TCA<DCA<TBA<KBrO₃<MCA<DBA<MX<MBA
Plewa et al. (2004)	CHO cells	2 µM – 20 mM for 72 hours	Cytotoxicity	MCA<<MBA<MIA
Plewa et al. (2004)	<i>S. typhimurium</i> TA100	0.1-100 mM for 1 hour	Cytotoxicity	MCA<<MBA<MIA
Plewa et al. (2010)	CHO cells	0.1 µM – 20 mM for 72 hours	Cytotoxicity	DCA<TCA<BIA<MCA<BCA<BDCA<DBA<DIA<CDBA<TBA<MBA<MIA

Study	Experimental system	DBP concentrations	Endpoint(s)	DBP in vitro ranking ^a
Zhang et al. (2010)	CHO-K1 cells	10 µM – 20 mM for 72 hours	Cytotoxicity	DCA~TCA<DBA~MCA<MBA<MIA
Pals et al. (2011)	CHO cells (intact and homogenate)	10 µM – 20 mM for 10-60 minutes	GADPH inhibition	MCA<<MBA<MIA
Dad et al. (2013)	CHO cells	3 µM – 6 mM for 4 hours	Decrease in ATP levels	MCA<MBA<MIA
Pals et al. (2013)	HepG2 cells-based reporter assay	0.01 µM – 0.1 mM for 16 hours	Expression of ARE-controlled reporter	MCA<MBA<MIA
Michalowicz et al. (2015)	Peripheral blood mononuclear cells (human)	0.1 – 20 mM for 1 to 4 hours (depending on endpoint)	Increased necrotic changes	No clear ranking among HAAs
Michalowicz et al. (2015)	Peripheral blood mononuclear cells (human)	0.1 – 20 mM for 1 to 4 hours (depending on endpoint)	Increased transmembrane mitochondrial potential; increased activity of caspases	MCA~MBA<DCA~DBA
Michalowicz et al. (2015)	Peripheral blood mononuclear cells (human)	0.1 – 20 mM for 1 to 4 hours (depending on endpoint)	Increased apoptosis, ROS generation	MCA~MBA<DBA<DCA
Prochazka et al. (2015)	Caco-2 cells	Concentration ranges are not reported; for 4 hours	Cytotoxicity	MCA<DCBQ~DBBQ<MBA~MIA (based on reported EC ₅₀ s)
Prochazka et al. (2015)	HepG2-derived cells	Concentration ranges are not reported; for 15 hours	Nrf2/ARE activation	MCA<DCBQ~DBBQ<MBA~MIA (based on reported EC ₅₀ s)
Prochazka et al. (2015)	HCT116-derived cells	Concentration ranges are not reported; for 16 hours	P53 activation	MCA<DCBQ~DBBQ<MBA~MIA (based on reported EC ₅₀ s)
Stalter et al. (2016) <i>Only HAA results are presented</i>	<i>A. vischeri</i> (bacterium)	2.2 µM-25 mM for 0.5 hours	Cytotoxicity	TCA<DCA~MCA<DBCA~BDCA~BCA~DBA<TBA~BIA<CIA~MBA<MIA

Study	Experimental system	DBP concentrations	Endpoint(s)	DBP in vitro ranking ^a
Stalter et al. (2016) <i>Only HAA results are presented</i>	MCF and HepG2 cells-based reporter assays	0.1 µM-62 mM time not reported	Oxidative stress (ARE induction)	TCA (no effect)< DCA ~ DBCA < TBA ~ MCA ~ BCA ~ DBA < BIA ~ CIA < MBA ~ MIA
Stalter et al. (2016) <i>Only HAA results are presented</i>	HCT-116 cells-based reporter assay	0.11 µM-25 mM time not reported	P53 activation	TCA , DCA , DBCA , BDCA , TBA (no effect)< MCA ~ BCA ~ DBA ~ BIA ~ CIA < MBA < MIA
Zhang et al. (2016)	<i>S. typhimurium</i> TA1535/pSK1002	0.1-91.8 mM for 2 hours	Cytotoxicity	CH < TCA < CN < DBA < DCA < MCA < DCA < TCN < MBA < BCN < DBN < MIA < MX
Dad et al. (2017)	CHO cells	3 µM-0.9 mM for 4 hours	GAPDH inhibition	BDCA < CDBA < TCA < TBA < DCA < DBA < BCA < MCA < MBA < MIA
Dad et al. (2017)	CHO cells	3 µM-0.9 mM for 4 hours	PDC activation	BDCA < BCA < CDBA ~ DCA ~ TCA < DBA ~ MCA < TBA < MBA < MIA
Dad et al. (2017)	CHO cells	3 µM-0.9 mM for 4 hours	Increased cellular ATP levels	other DBPs (no effect) < DCA
Dad et al. (2017)	CHO cells	3 µM-0.9 mM for 4 hours	Decreased cellular ATP levels	BDCA < MCA < MIA < MBA
Fu et al. (2017)	Human neural stem cells	0.1 or 0.5 µM for 12 days	Decreased differentiation	MCA ~ MBA

^a HAA5 compounds are in bold

DBPs: BCA, bromochloroacetic acid; BCN, bromochloroacetonitrile; BDCA, bromodichloroacetic acid; BIA, bromoiodoacetic acid; CDBA, chlorodibromoacetic acid; CH, chloral hydrate; CIA, chloroiodoacetic acid; CN, chloroacetonitrile; DBA, dibromoacetic acid; DBN, dibromoacetonitrile; DCA, dichloroacetic acid; DCN, dichloroacetonitrile; DFA, difluoroacetic acid; DIA, diiodoacetic acid; MBA, monobromoacetic acid; MCA, monochloroacetic acid; MIA, monoiodoacetic acid; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone; TBA, tribromoacetic acid; TCA, trichloroacetic acid; TCN, trichloroacetonitrile; TFA, trifluoroacetic acid

Other abbreviations: Caco-2, colorectal adenocarcinoma; CHO, Chinese hamster ovary; GADPH, glyceraldehyde 3-phosphate dehydrogenase; HCT, human colorectal carcinoma; HepG2, hepatocellular carcinoma; MCF-7, Michigan cancer foundation-7; PDC, pyruvate dehydrogenase complex.

As demonstrated in Table 8.4, among the five HAAs that are currently regulated (HAA5; TCA, DCA, MCA, DBA and MBA), MBA possessed the highest potencies in most in vitro studies, with Michalowicz et al. (2015) as the only exception. However, it is difficult to view these results in the context of possible in vivo MBA toxicity, since among very few available animal studies, only a limited number of endpoints were examined, and only general conclusions about the underlying mechanisms could be formulated based on these data. Additionally, based on a

single rat study (Saghir and Schultz, 2005), MBA appears to be metabolized and/or excreted at a dramatically higher rate compared to DCA, TCA or DBA. Therefore, the higher potency of MBA in toxicological mechanisms of interest (Table 8.4) may or may not be compensated for by increased metabolic clearance in the context of in vivo toxicity.

Two potential underlying mechanisms of noncancer toxicity of MBA are suggested by in vitro studies: (1) mild alkylating action and (2) inhibition of glycolysis (GADPH inhibition and decreased ATP). Due to bromine being a good leaving group, MBA possesses mild alkylating properties (Desai and Miller, 2010; Dad et al., 2013). Alkylating agents are generally toxic to rapidly dividing cells, with hematopoietic, epithelial (GI tract) and spermatogenic tissues as common targets. Interestingly, Linder et al. (1994a) did not observe any deleterious effects on spermatogenesis in a targeted 14-day study in rats, suggesting that the alkylating potential of MBA may not be of concern, at least in this organ system and at examined doses.

In contrast, the limited available animal data are supportive of the toxic effects of MBA in energy-dependent organs, such as the visual, the heart and the nervous systems (Dalgaard-Mikkelsen et al., 1955; Harrestrup Andersen et al., 1955; Lucas et al., 1957), indicating that the proposed mechanism of GADPH inhibition and decreased ATP levels by MBA in vitro may underlie these effects. Among other HAA5 compounds, only MCA appears to share this mechanism, and with a dramatically lower potency.

Taken together, the available in vitro evidence is not sufficient to provide support for extrapolation of in vivo toxicity among MBA and other HAA5 compounds. While many in vitro studies suggest that MBA would have the highest potency among the HAA5 for the examined endpoints, the relative potencies may not be similar for toxicities in vivo. Moreover, different HAA5 compounds appear to possess distinct biological mechanisms complicating the comparisons of noncancer toxic endpoints among them as a group.

9. TOXICOLOGICAL PROFILE: DIBROMOACETIC ACID

Acute Toxicity

Effects in Humans

Dibromoacetic acid (DBA) is expected to be irritating and corrosive to skin and mucous membranes due to its relatively strong acidity (NTP, 1996, 2007a). No human studies of acute or subacute toxicity of DBA were identified.

Effects in Animals

In adult male Sprague-Dawley rats, the oral gavage LD₅₀ was reported to be 1,737 mg/kg (Linder et al., 1994a). In this study, rats (5/dose) were administered a single dose of 1,000 to 2,000 mg/kg DBA via oral gavage. Although this study was designed to primarily examine the acute spermatogenic effects of DBA, it was the only study located that identified an LD₅₀. Other symptoms of toxicity included excessive drinking water intake, hypomobility, respiratory depression, labored breathing, mild diarrhea, and ataxia. Most deaths occurred within 48 hours (Linder et al., 1994a).

A summary of acute and short-term toxicity studies in animals is presented in Table 9.1. Concentrations (in mg/L) are converted to doses (in mg/kg-day) using the animal body weight and the following relationship:

$$(1) \quad \text{Dose} = \text{Concentration} \times \text{L/BW}$$

where L is water consumption in liters/day, and BW is the body weight of the animal in kilograms. Water consumption is determined based on body weight using the following equation:

$$(2) \quad \text{L/day} = 0.10 \times \text{BW}^{0.7377} \text{ (US EPA, 1988).}$$

Table 9.1 Summary of acute and short-term animal studies of DBA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Sprague-Dawley rats (5/dose)	Single dose of 1,000-2,000 mg/kg neutralized DBA by gavage; examined 14-21 days after dosing	LD ₅₀ of 1,737 mg/kg; difficulty moving limbs and mild ataxia; excessive water consumption; labored breathing; hypomobility; mild diarrhea; misshapen sperm; abnormal retention of Step 19 spermatids	LOAEL ^a : 1,000 mg/kg	Linder et al. (1994a)
Male B6C3F1 mice (6/dose)	0, 100, 500, or 2,000 mg/L (0, 26, 130, or 519 mg/kg-day) ^b DBA in drinking water for 21 days	Increased absolute and relative liver weights; peroxisome proliferation (cyanide-insensitive acyl-CoA oxidase activity)	NOAEL: 26 mg/kg-day	Parrish et al. (1996)
Male Fischer 344 rats (8/dose)	0, 1,000, or 2,000 mg/L (0, 156, or 311 mg/kg-day) ^c neutralized DBA in drinking water for 2, 4, 7, or 28 days	Liver effects: increased DNA hypomethylation at ≥7 days; increased glycogen accumulation at ≥; increased peroxisome proliferation at ≥2	LOAEL: 156 mg/kg-day	Tao et al. (2004b)
Female B6C3F1 mice (8/dose)	0, 1,000, or 2,000 mg/L (0, 264, or 528 mg/kg-day) ^d neutralized DBA in drinking water for 2, 4, 7, or 28 days	Liver effects: increased DNA hypomethylation at ≥7 days; increased glycogen accumulation at ≥7; increased peroxisome proliferation at ≥4 days	LOAEL: 264 mg/kg-day	Tao et al. (2004b)
Male Fischer 344 rats (8/dose)	0, 1,000, or 2,000 mg/L (0, 156, or 311 mg/kg-day) ^c neutralized DBA in drinking water for 5, 7, or 28 days	Hypomethylation of the <i>c-myc</i> gene in kidney at ≥ 7 days	LOAEL: 156 mg/kg-day	Tao et al. (2005)
Male B6C3F1 mice (8/dose)	0, 1,000, or 2,000 mg/L (0, 247, or 494 mg/kg-day) ^e neutralized DBA in drinking water for 5, 7, or 28 days	Hypomethylation of the <i>c-myc</i> gene in kidney at ≥ 7 days	LOAEL: 247 mg/kg-day	Tao et al. (2005)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male and female F344/N rats (5/sex/dose);	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 17, 31, 67, 134, or 270 mg/kg-day for males; 0, 17, 33, 67, 135, or 257 mg/kg-day for females) neutralized DBA in drinking water for 2 weeks	<u>Males and females:</u> increased absolute and relative liver weight; decreased relative heart weight; hepatocyte cytoplasmic alteration; <u>Males:</u> testicular lesions (delayed spermiation, presence of large residual bodies ^f)	LOAEL: 17 mg/kg-day (liver effects)	NTP (2007a)
Male and female B6C3F1 mice (5/sex/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 24, 47, 95, 178, or 370 mg/kg-day for males; 0, 22, 53, 88, 166, or 309 mg/kg-day for females) neutralized DBA in drinking water for 2 weeks	<u>Males and females:</u> increased absolute and relative liver weight; decreased thymus weight; thymus atrophy <u>Males:</u> morphological changes to testicular germinal epithelium (spermatid retention and atypical residual bodies)	NOAEL: 53 mg/kg-day (relative liver weight in female mice)	NTP (2007a)

^a This LOAEL was identified from a single-dose study.

^b mg/L is converted to mg/kg-day using male mouse body weight of 0.027 kg (Parrish et al., 1996) and the water consumption rate of 0.007 L/day, calculated from equation 2.

^c mg/L is converted to mg/kg-day using male rat body weight of 0.180 kg (US EPA, 1988) and the water consumption rate of 0.028 L/day, calculated from equation 2.

^d mg/L is converted to mg/kg-day using female mouse body weight of 0.0246 kg (US EPA, 1988) and the water consumption rate of 0.0065 L/day, calculated from equation 2.

^e mg/L is converted to mg/kg-day using male mouse body weight of 0.0316 kg (US EPA, 1988) and the water consumption rate of 0.0078 L/day, calculated from equation 2.

^f Residual bodies are cytoplasmic fragments that are shed during sperm maturation and are normally resorbed by the Sertoli cells. Large or atypical residual bodies can indicate abnormal spermiation and diminished Sertoli cell function.

NA, not applicable

Subchronic Toxicity

Effects in Humans

No subchronic human studies of DBA toxicity were identified.

Effects in Animals

Limited published data are available on the subchronic toxicity of DBA in animals (Table 9.2). In general, decreased terminal body weights, changes in absolute or relative organ weights (e.g., heart, brain, thymus, liver, and kidney), and adverse testicular effects were observed.

Table 9.2 Summary of subchronic animal studies of DBA

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male B6C3F1 mice (5/dose)	0, 300, 1,000, or 2,000 mg/L (0, 72, 240, or 480 mg/kg-day) ^a neutralized DBA in drinking water for 4, 8, or 12 weeks	Reduced terminal body weight; increased absolute and relative liver weight; increase in liver glycogen; decrease in serum insulin and glucose	LOAEL: 72 mg/kg-day	Kato-Weinstein et al. (2001)
Male and female Crl Sprague-Dawley rats (30/dose); two-generation study	P: 0, 50, 250, or 650 mg/L (0, 4.4, 22.4, or 52.4 mg/kg-day for males for 92 days; 0, 6.9, 32.4, or 79.4 mg/kg-day for females) in drinking water for 120 days F ₁ : exposure during gestation and lactation, and exposure at same concentrations as P generation for a minimum of 71 days post weaning, continuing through mating (14 days), gestation (21 days) and lactation (15 days)	P and F ₁ : reduced food and water consumption; reduced body weight gains, relative adrenal weight, and terminal body weight; increased absolute and relative brain, liver, kidney, pituitary, and spleen weights in males and females; reduced absolute adrenal weight in females; altered relative thymus weight in P females and F ₁ males; abnormal/increased residual bodies in the testes and delayed spermiation in P and F ₁ males	LOAEL: 4.4 mg/kg-day (increased relative liver and kidney weights, delayed spermiation in P and F ₁ males)	Christian et al. (2002)
Male and female F344/N rats (10/sex/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 10, 20, 40, 90, or 166 mg/kg-day for males; 0, 12, 23, 48, 93, or 181 mg/kg-day for females) neutralized DBA in drinking water for 3 months	Increased absolute and relative liver and kidney weights; decreased terminal body weight (high dose); decreased absolute heart and thymus weights; hepatocyte vacuolization <u>Males</u> : decreased absolute testis weight; testicular lesions/atrophy; increased liver labeling index <u>Females</u> : increased erythrocytes; hematopoietic cell proliferation in spleen	LOAEL: 10 mg/kg-day (males, relative liver weight) 12 mg/kg-day (females, relative liver weight)	Melnick et al. (2007); NTP (2007a)

Sex/Species	Dose/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male and female B6C3F1 mice (10/sex/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 16, 30, 56, 115, or 230 mg/kg-day for males; 0, 17, 34, 67, 132, or 260 mg/kg-day for females) neutralized DBA in drinking water for 3 months	Increased relative liver weight and severity of hepatocyte vacuolization; <u>Males</u> : reduced mean cell hemoglobin, platelets and white blood cell counts; decreased liver labeling index; abnormal testicular morphology <u>Females</u> : increased lung weights	NOAEL: 56 mg/kg-day (males, liver and testes effects) 34 mg/kg-day (females, relative liver weight)	Melnick et al. (2007); NTP (2007a)

^a mg/L is converted to mg/kg-day using male mouse body weight of 0.035 kg (Kato-Weinstein et al., 2001) and the water consumption rate of 0.0084 L/day, calculated from equation 2.

P, parental generation; F₁, first filial generation

Among the studies listed in Table 9.2, Christian et al. (2002) generated the lowest LOAEL of 4.4 mg/kg-day, based on increased relative liver and kidney weights in the parental (P) generation males and females. In this two-generation study, Sprague-Dawley rats were exposed to 0, 50, 250, or 650 mg/L DBA in drinking water for 92 days, corresponding to 0, 4.4, 22.4, or 52.4 mg/kg-day for males and 0, 6.9, 32.4, or 79.4 mg/kg-day for females. First filial generation (F₁) pups were exposed in utero, during lactation, and through drinking water at the same concentrations as the P generation for a minimum of 71 days post weaning. In addition to the changes in relative liver and kidney weights (Table 9.3), absolute and/or relative weights of several other organs (e.g., adrenal gland, brain, thymus, spleen, and pituitary gland) were also changed in males and/or females in the P and F₁ generations. However, these changes occurred at exposures greater than 4.4 mg/kg-day.

Changes in absolute and relative liver and kidney weights were also observed in the Kato-Weinstein et al. (2001) and NTP (2007a) studies but at higher doses. It should be noted that water consumption was decreased in treated animals, which may account for the reduction in body weight.

Table 9.3 Relative liver and kidney weights^a in P generation male and female rats exposed to DBA in drinking water^b, from Christian et al. (2002)

Concentration	0 mg/L	50 mg/L	250 mg/L	650 mg/L
Male Doses (mg/kg-day)	0	4.4	22.4	52.4^c
Sample size	n=30	n=30	n=30	n=29
Relative liver weight	3.572 ± 0.322	4.251 ± 0.260**	4.495 ± 0.263**	4.456 ± 0.281**
Relative left kidney weight	0.364 ± 0.029	0.424 ± 0.038**	0.418 ± 0.031**	0.444 ± 0.041**
Relative right kidney weight	0.367 ± 0.030	0.421 ± 0.032**	0.435 ± 0.065**	0.441 ± 0.037**
Female Doses (mg/kg-day)	0	6.9	32.4	79.4

Concentration	0 mg/L	50 mg/L	250 mg/L	650 mg/L
Sample size	n=27	n=27	n=23	n=25
Relative liver weight	4.622 ± 0.647	5.330 ± 0.652**	5.276 ± 0.880**	5.932 ± 0.804**
Relative left kidney weight	0.448 ± 0.036	0.492 ± 0.048**	0.478 ± 0.040*	0.506 ± 0.050**
Relative right kidney weight	0.458 ± 0.049	0.499 ± 0.049**	0.488 ± 0.045*	0.526 ± 0.057**

^a ratio of organ weight to body weight

^b exposure was for 120 days in P generation females (cohabitation through lactation) and for 92 days in P generation males (cohabitation)

^c terminal body weight was decreased in males at high dose; absolute liver and kidney weights were significantly increased in this group demonstrating that the observed increase in relative weights were not due to decreased body weight

** p ≤ 0.01, as determined by Christian et al. (2002) using analysis of variance.

Genetic Toxicity

Several studies have shown that DBA is genotoxic in vitro and in vivo. Although the mechanism of carcinogenicity of DBA is unknown, IARC noted, “Several comparative genotoxicity and mutagenicity studies ... have demonstrated that dibromoacetic acid is more potent than its chlorinated analogue, dichloroacetic acid, and that they have several molecular and biochemical activities in common” (IARC, 2013). Genetic toxicity studies are summarized in Tables 9.4 and 9.5. Within each table, studies are organized by experimental system (e.g., human studies before animal studies, primary cells before cell culture), and then by type of genotoxic effect. DBA was active in three out of three available mammalian genotoxicity assays in vivo, and two inactive assays in vivo were in *C. elegans* and newt (Table 9.4). DBA elicited a positive mutagenic response in vitro, in both bacterial and mammalian cells (Table 9.5). Based on this evidence, OEHHA concludes that DBA could act as a genotoxic carcinogen.

Table 9.4 Genetic and related effects of DBA in vivo

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
Mammalian: Micronucleus assay with peripheral blood erythrocytes	Male and female B6C3F1 mice (9-10/sex/dose)	0, 0.125, 0.25, 0.5, 1, or 2 g/L (0, 16, 30, 56, 115, or 230 mg/kg-day for males; 0, 17, 34, 67, 132, or 260 mg/kg-day for females) neutralized DBA in drinking water for 3 months	+ (males) - (females)	NTP (2007a)
HPLC-EC of digested liver DNA (8-OHdG formation, a precursor to point mutations)	Male B6C3F1 mice (6/dose/time point)	0, 30, 100, or 300 mg/kg neutralized DBA by oral gavage (single dose); sacrificed at 1, 3, 5, 7, 9, or 12 hours post-treatment	+ (high dose at all time points; data not shown for other doses)	Austin et al. (1996)

Assay	Sex/Species	Dose/Route of Exposure/Duration	Results	Reference
HPLC-EC of digested liver DNA (8-OHdG formation, a precursor to point mutations)	Male B6C3F1 mice (6/dose/time point)	0, 0.1, 0.5, or 2 g/L (0, 29, 144, or 578 mg/kg-day) ^a unneutralized DBA in drinking water for 21 days	+	Parrish et al. (1996)
Non-mammalian: Newt micronucleus test	Newt (<i>P. waltii</i>) larvae (15/dose)	0, 20, 40 or 80 µg/ml unneutralized DBA in tank water for 12 days	-	Giller et al. (1997)
Long amplicon quantitative PCR (nuclear DNA damage)	<i>C. elegans</i> Bristol strain N2	0, 0.2, 0.4, 0.6, 0.8, or 1 mM unneutralized DBA in well water	-	Zuo et al. (2017)
Micronucleus assay	<i>V. faba</i> roots	0.1 µM – 1 mM unneutralized DBA in soaking water	+	Hu et al. (2017)
SCGE (Comet) assay	<i>V. faba</i> roots	0.1 µM – 1 mM unneutralized DBA in soaking water	+	Hu et al. (2017)

8-OHdG, 8-hydroxy-2'-deoxyguanosine; HPLC-EC, high performance liquid chromatography with electrochemical detection; PCR, polymerase chain reaction

^a mg/L converted to mg/kg-day using male mouse drinking rate of 0.0078 L/day (US EPA, 1988) and body weight of 0.027 kg (Parrish et al., 1996)

Table 9.5 Genetic and related effects of DBA in vitro (human and mammalian cell lines, non-mammalian experimental systems)

Assay	Results Without S9	Results With S9	DBA Concentration	Reference
Human cell line: SCGE (Comet) assay in HepG2 cells	+	ND	1 µM – 1 mM	Zhang et al. (2012)
Mammalian cell lines: Gene mutation in Hprt locus in CHO-K1 cells	+	ND	0.02 – 1 mM	Zhang et al. (2010)
SCGE (Comet) assay in CHO-AS52 cells	+	ND	0.25 – 5.0 mM	Plewa et al. (2002)
Non-mammalian cells: Reverse mutation assay in <i>S. Typhimurium</i> TA100 (Ames fluctuation test)	+	+	-S9: 0.003 – 3 mg/ml +S9: 0.01 – 10 mg/ml	Giller et al. (1997)
Reverse mutation assay in <i>S. Typhimurium</i> TA100 (Ames microsuspension test)	+	+	1 mg/ml	Nelson et al. (2001)

Assay	Results Without S9	Results With S9	DBA Concentration	Reference
Reverse mutation assay in <i>S. Typhimurium</i> TA98	-	-	5 mg/plate	Fang et al. (2001) as cited by IARC (2013)
Reverse mutation assay in <i>S. Typhimurium</i> TA100	+	+	0.5 mg/plate	Fang et al. (2001) as cited by IARC (2013)
Reverse mutation assay in <i>S. Typhimurium</i> TA98 (Ames preincubation test)	+	+	0.15 – 16 mM	Kargalioglu et al. (2002)
Reverse mutation assay in <i>S. Typhimurium</i> TA100 (Ames preincubation test)	+	+	-S9: 0.15 – 16 mM +S9: 0.015 – 15.5 mM	Kargalioglu et al. (2002)
Reverse mutation assay in <i>S. Typhimurium</i> RSJ100 (Ames preincubation test)	-	-	0.015 – 15.5 mM	Kargalioglu et al. (2002)
Reverse mutation assay in <i>S. Typhimurium</i> TA98	-	-	33 µg – 10 mg/plate	NTP (2007a)
Reverse mutation assay in <i>S. Typhimurium</i> TA100	+	+	33 µg – 10 mg/plate	NTP (2007a)
SOS chromotest in <i>E. coli</i> PQ37	+	+	-S9: 0.2 – 0.75 mg/ml +S9: 0.1 – 3 mg/ml	Giller et al. (1997)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	+	ND	1 – 14 mM	Zhang et al. (2016)
SOS-umuC assay in <i>S. typhimurium</i> TA1535/pSK1002	+	ND	0.018 – 2.3 mM	Stalter et al. (2016)

ND, not detected; CHO, Chinese hamster ovary; SCGE, single cell gel electrophoresis.

Developmental and Reproductive Toxicity

Developmental toxicity in vitro

There were no in vitro developmental toxicity studies identified for DBA.

Developmental toxicity in vivo

Developmental toxicity of DBA was observed in rats and rabbits of both sexes in studies that combined in utero and F₁ exposures (Table 9.6). The observed adverse effects include delays in preputial separation and vaginal patency in F₁ rats (Christian et al., 2002; Klinefelter et al., 2004), as well as reduced ovarian primordial follicles in F₁ female rabbits and impaired testicular development in F₁ male rabbits (Bodensteiner et al., 2004; Veeramachaneni et al., 2007). In

contrast, rat studies with relatively shorter duration of exposure did not report developmental effects. In an oral rat study with doses up to 250 mg/kg-day for the first eight days of pregnancy, no adverse effects were seen on day 9 or day 20 (Cummings and Hedge, 1998). Another oral rat study with exposure from GD 17 to PND 7 also did not report any adverse effects in F₁ follicular populations (e.g., number of each type of ovarian follicle: primordial, primary, small preantral, large preantral, small antral, polyovular in F₁) at doses up to 50 mg/kg-day (teRiele and Bodensteiner, 2006). These studies are not included in Table 9.6. While mixtures of the HAAs have been observed to cause pregnancy loss and eye malformations in rats, it is not possible to attribute observed adverse effects to specific compounds in the mixture (Narotsky et al., 2011). Thus, these type of studies are not considered in this evaluation.

A summary of selected developmental toxicity studies is presented in Table 9.6.

Table 9.6 Summary of in vivo studies of DBA reporting developmental toxicity endpoints

Sex/ Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male and female Crl Sprague- Dawley rats (30/sex/ dose)	P: 0, 50, 250, 650 mg/L (males: 0, 4.4, 22.4, or 52.4 mg/kg-day in drinking water for 92 days; females: 0, 6.9, 32.4, or 79.4 mg/kg- day) in drinking water for 120 days (including exposure through gestation and lactation) F ₁ : exposure during gestation and lactation, and exposure at same concentrations as P generation for a minimum of 71 days post weaning, continuing through mating (14 days), gestation (21 days) and lactation (15 days)	F ₁ : delays in preputial separation and vaginal patency; reduced viability index; increased gestation duration; reproductive tract malformations in males; delayed spermiation	NOAEL: 4.4 mg/kg-day	Christian et al. (2002)

Sex/ Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Sprague-Dawley rats, pregnant dams and offspring (3/dose)	0, 400, 600, or 800 mg/L (0, 49, 74, or 99 mg/kg-day for dams ^a ; 0, 66, 99, or 132 mg/kg-day for male pups ^b) neutralized DBA from GD15 through PND21 in drinking water; F ₁ pups continued to be exposed until PND120	Decreased body weight in pups; F ₁ males: delayed preputial separation; decreased absolute testis and epididymis weights; delayed spermiation, atrophy in seminiferous tubules; altered sperm motion parameters; reduced fertility F ₁ females: delayed vaginal opening	LOAEL: 66 mg/kg-day	Klinefelter et al. (2004)
Sprague-Dawley rats, pregnant dams and offspring (12/dose)	0, 4, 40, or 400 mg/L (0, 0.49, 4.9, or 49 mg/kg-day for dams ^a ; time-weighted average of 0, 0.6, 6.3, 66 mg/kg-day for male pups) neutralized DBA from GD15 to PND21 in drinking water; F ₁ pups continued to be exposed until PND56 or PND120	F ₁ males: decreased body weight; delayed preputial separation; altered sperm morphology; F ₁ females: delayed vaginal opening	NOAEL: 6.3 mg/kg-day	Klinefelter et al. (2004)
Dutch-Belted rabbits, pregnant dams and offspring (≥10 dams/dose; 6-10 pups/dose)	0, 1-1.2, 5.5-6.7, or 50-58 mg/kg-day ^c neutralized DBA in drinking water from GD15 to weaning at 6 weeks, continuing in offspring to 12 or 24 weeks	F ₁ females: reduced ovarian primordial follicles	NOAEL: 1 mg/kg-day	Bodensteiner et al. (2004)
C57B1/6J mice, pregnant dams and offspring (10 dams/dose; 15-17 pups/dose)	0, 6.6, or 65.9 mg/kg-day neutralized DBA in drinking water from GD15 to 3 weeks postpartum (necropsy at 3 or 7 weeks)	Increased absolute testes and kidney weights in F ₁ males; increased absolute liver and kidney weights in F ₁ females	NOAEL: 6.6 mg/kg-day	Weber et al. (2006)

^a Doses were calculated using equations 1 and 2, and a pregnant Sprague-Dawley rat body weight of 0.406 kg (Leavens et al., 2006).

^b Data for female pups were not reported.

^c A dose range is presented because there were slight differences in administered dose between dams and pups, and in pups receiving DBA for 12 or 24 weeks.

P, parental generation; F₁, first filial generation; GD, gestation day; PND, postnatal day

The two-generation study by Christian et al. (2002) reported developmental effects in rat pups in the F₁ generation. Delayed preputial separation and vaginal patency was observed indicating delayed sexual maturity. In females, there was reduced viability index and increased duration of gestation. In males, delayed spermiation and reproductive tract malformations were observed. The lowest NOAEL reported in this study is 4.4 mg/kg-day for reduced viability index. Klinefelter et al. (2004) reported developmental delays and other signs of developmental and reproductive toxicity in rat pups exposed to DBA during gestation, lactation, and post-weaning. Male animals displayed delays in preputial separation and altered sperm morphology, whereas female animals displayed delays in vaginal opening. Additionally, the sperm membrane proteome was altered in male pups, and pup body weight was significantly decreased in both sexes. The lowest NOAEL reported for this study is 6.3 mg/kg-day for delayed vaginal opening and delayed preputial separation.

Reproductive toxicity in vitro

One in vitro reproductive toxicity study reported reduced testosterone production in primary rat Leydig cells exposed to 100 µM DBA (Carr et al., 2011).

Reproductive toxicity in vivo

Adult male rats exposed to DBA had various reproductive effects, including sperm damage and decreased sperm production (Linder et al., 1994a; Linder et al., 1994b; Linder et al., 1995; Tsuchiya et al., 2000; Christian et al., 2002; Klinefelter et al., 2004), compromised fertility (Linder et al., 1994a; Linder et al., 1994b; Linder et al., 1995; Linder et al., 1997; Tsuchiya et al., 2000; Christian et al., 2002; Kaydos et al., 2004; Klinefelter et al., 2004), and decreased absolute testis and epididymis weight (Linder et al., 1994a; Linder et al., 1994b; Tsuchiya et al., 2000; Klinefelter et al., 2004; NTP, 2007a). Delayed spermiation was observed in adult rats in several studies following DBA treatment (Linder et al., 1994a; Linder et al., 1995; Linder et al., 1997; Tsuchiya et al., 2000; Christian et al., 2002; Klinefelter et al., 2004; NTP, 2007a). Decreases in seminiferous tubule proteins were observed after acute in vivo and ex vivo exposures to DBA (Holmes et al., 2001). Histopathological changes in the testes and epididymis were also observed (Linder et al., 1994a; Linder et al., 1994b; Linder et al., 1997; NTP, 2007a). Additionally, rats exposed to DBA in utero and postnatally showed developmental delays, reproductive tract malformations, and delayed spermiation (Christian et al., 2002; Klinefelter et al., 2004; NTP, 2007a). DBA also reduced testosterone levels, and mRNA and protein levels of CYP17, an enzyme essential for testosterone production, in male rats (Carr et al., 2011). Delayed spermiation data are presented in Table 9.7.

Table 9.7 Delayed spermiation data in male rats from Christian et al. (2002)

	P Generation				F1 Generation			
Dose (mg/kg-day)	0	4.4	22.4	52.4	0	4.4	22.4	52.4
Retention of step 19 spermatids	4/30	3/30	13/30*	23/30***	0/30	1/30	12/30***	20/30***
Abnormal/increased residual bodies	3/30	5/30	15/30**	25/30***	1/30	2/30	10/30**	14/30***

* p<0.05, ** p<0.01, *** p<0.001 using Fisher's exact test, calculated by OEHHHA
F1, first filial; P, parental

DBA treatment of female rats increased circulating concentrations of estradiol (Cummings and Hedge, 1998; Balchak et al., 2000; Murr and Goldman, 2005) and estrone (Murr and Goldman, 2005; Goldman et al., 2007), possibly due to a suppression of their hepatic catabolism (Goldman and Murr, 2003). A summary of positive reproductive toxicity studies is presented in Table 9.8.

Studies in other species have also shown effects of DBA on male and female reproductive systems. Weber et al. (2006) found increased absolute testis and ovary weights in mice treated from gestation through puberty. NTP (2007a) found changes in testicular morphology in male mice treated with DBA in drinking water for 2 or 14 weeks.

Veeramachaneni et al. (2007) and Bodensteiner et al. (2004) reported reproductive toxicity in rabbits treated with DBA. In a study reported by Veeramachaneni et al. (2007), pregnant Dutch-Belted rabbits were administered 0, 1-1.25, 5.2-5.8, or 50-61 mg/kg-day DBA in drinking water from GD15 through weaning at 6 weeks, with exposure of the offspring continuing for an additional 12 or 24 weeks. Thus, F₁ animals were exposed to DBA in utero, via lactation, and in drinking water post weaning. Although sperm count was not impacted, an increase in morphologically abnormal sperm was observed, with the predominant defects occurring in the acrosome and the nucleus (Table 9.9). At 24 weeks, testicular lesions were found in all groups, including controls, but the frequency and severity of lesions was greater at higher doses (Table 9.10). Lesions in seminiferous tubules were characterized by pyknosis of germ cells, syncytia of spherical spermatids, and vacuolization of seminiferous epithelium. F₁ males also displayed impaired sexual function and decreases in conception, which may stem from the testicular and sperm effects. Testicular lesions and increased morphologically abnormal sperm in F₁ male rabbits from the study were the most sensitive reproductive endpoints reported, with a LOAEL of 1 mg/kg-day (Veeramachaneni et al., 2007).

A study conducted by the same laboratory assessed effects of DBA on ovarian follicles in female offspring of pregnant Dutch-Belted rabbits. Rabbits were exposed to 0, 1-1.2, 5.5-6.7, or 50-58 mg/kg-day DBA in drinking water from GD15 through weaning at 6 weeks, and continuing

exposure for offspring for 12 or 24 weeks (Bodensteiner et al., 2004). F₁ females showed a decrease in the number of ovarian primordial follicles and a decrease in healthy follicles at 12 and 24 weeks. OEHHA identified a NOAEL of 1 mg/kg-day for reduced ovarian primordial follicles (Bodensteiner et al., 2004).

Table 9.8 Summary of in vivo studies of DBA reporting reproductive toxicity endpoints

Sex/Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague-Dawley rats (5-8/dose)	Single dose of 0 or 1,250 mg/kg neutralized DBA by gavage; effects measured 2, 14, or 28 days after exposure	Decreased absolute testis and epididymis weights; reduced cauda sperm count; reduced testosterone on day 2; delayed spermiation; presence of residual bodies; altered sperm morphology and motility	LOAEL ^a : 1,250 mg/kg	Linder et al. (1994a)
Male Sprague-Dawley rats (6-8/dose)	0, 10, 30, 90, or 270 mg/kg-day neutralized DBA by gavage for 14 days	Histopathological alterations in the testis and epididymis; decreased testicular and epididymal weights; decreased sperm counts; sperm morphology and motility changes	LOAEL: 10 mg/kg-day	Linder et al. (1994b)
Male Sprague-Dawley rats (5-10/dose) plus an unspecified number of untreated female rats for breeding	0, 2, 10, or 50 mg/kg-day neutralized DBA by gavage for 31 or 79 days and 0 or 250 mg/kg-day by gavage for 2, 5, 9, 16, or 42 days with a recovery period up to 186 days	Decreased sperm counts; changes in sperm morphology; decreased epididymis weight; increased fetal death, ossification of metatarsals and microphthalmia in offspring	NOAEL: 10 mg/kg-day	Linder et al. (1995)
Male Sprague-Dawley rats (6/dose)	0, 2, 10, or 50 mg/kg-day neutralized DBA by gavage for 31 or 79 days or 250 mg/kg-day by gavage for 2, 5, 9, 16, or 42 days with a recovery period up to 186 days	Histopathological changes in the testis and epididymis; presence of atypical residual bodies; delayed spermiation; seminiferous tubule atrophy	NOAEL: 2 mg/kg-day	Linder et al. (1997)
Male Sprague-Dawley rats (6/dose)	0, 5, or 50 mg/kg-day neutralized DBA by gavage for 2 or 4 weeks and 250 mg/kg-day by gavage for 2 weeks	Altered sperm morphology; presence of atypical residual bodies; delayed spermiation	NOAEL: 5 mg/kg-day	Tsuchiya et al. (2000)

Sex/Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
Female Sprague-Dawley rats (8-11/dose)	0, 10, 30, 90, or 270 mg/kg-day neutralized DBA by gavage for 14 days	Disrupted estrous cyclicity at 90 and 270 mg/kg-day; increased estradiol release	NOAEL: 30 mg/kg-day	Balchak et al. (2000)
Male and female Crl Sprague-Dawley rats (30/sex/dose)	P: 0, 50, 250, 650 mg/L (males: 0, 4.4, 22.4, or 52.4 mg/kg-day in drinking water for 92 days; females: 0, 6.9, 32.4, or 79.4 mg/kg-day) in drinking water for 120 days (including exposure through gestation and lactation)	Altered sperm production and morphology; delayed spermiation	NOAEL: 4.4 mg/kg-day (delayed spermiation in P and F ₁)	Christian et al. (2002)
Male and female Crl Sprague-Dawley rats (30/sex/dose)	F ₁ : exposure during gestation and lactation, and exposure at the same concentrations as the P generation for a minimum of 71 days post weaning, continuing through mating (14 days), gestation (21 days) and lactation (15 days)	Reproductive tract malformations and delayed spermiation in males	NOAEL: 4.4 mg/kg-day (delayed spermiation in P and F ₁)	Christian et al. (2002)
Male Sprague-Dawley rats (8/dose)	0, 2, or 4 mg/kg-day neutralized DBA by gavage for 14 days	Reduced fertility (percentage of number of implants/ number of corpora lutea); reduced Sp22 (sperm membrane protein correlated with fertility)	LOAEL: 2 mg/kg-day	Kaydos et al. (2004)
Dutch-Belted rabbits, pregnant dams and offspring (≥10 dams/dose; 6-10 pups/dose)	0, 1-1.2, 5.5-6.7, or 50-58 mg/kg-day ^a neutralized DBA in drinking water from GD15 to weaning at 6 weeks, continuing in offspring to 12 or 24 weeks	Reduced ovarian primordial follicles in F ₁ females	NOAEL: 1 mg/kg-day	Bodensteiner et al. (2004)
Female Sprague-Dawley rats, nonpregnant (10/dose)	0, 50, 150, or 300 mg/L (0, 5, 16, or 33 mg/kg-day) neutralized DBA in drinking water for 20 weeks	Elevated serum estradiol and estrone	LOAEL: 5 mg/kg-day	Murr and Goldman (2005)

Sex/Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/ LOAEL	Reference
C57B1/6J mice, pregnant dams and offspring (10 dams/dose; 15-17 pups/dose)	0, 6.6, or 65.9 mg/kg-day neutralized DBA in drinking water from GD15 to 3 weeks postpartum (necropsy at 3 or 7 weeks)	Increased absolute testes and kidney weights in F ₁ males; increased absolute liver and kidney weights in F ₁ females	NOAEL: 6.6 mg/kg-day	Weber et al. (2006)
Male F344/N rats (5/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 17, 31, 67, 134, or 270 mg/kg-day) neutralized DBA in drinking water for 15 days	Testicular lesions; delayed spermiation	NOAEL: 31 mg/kg-day	NTP (2007a)
Male F344/N rats (10/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 10, 20, 40, 90, or 166 mg/kg-day) neutralized DBA in drinking water for 3 months	Decreased absolute testis weight; testicular lesions and atrophy; delayed spermiation	NOAEL: 20 mg/kg-day	NTP (2007a)
Male B6C3F1 mice (5/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 24, 47, 95, 178, 370 mg/kg-day) neutralized DBA in drinking water for 15 days	Morphological changes in the testis; atypical residual bodies in seminiferous tubules; spermatid retention	NOAEL: 95 mg/kg-day	NTP (2007a)
Male B6C3F1 mice (10/dose)	0, 125, 250, 500, 1,000, or 2,000 mg/L (0, 16, 30, 56, 115, 230 mg/kg-day) neutralized DBA in drinking water for 3 months	Abnormal testicular morphology; atypical residual bodies in seminiferous tubules; spermatid retention	NOAEL: 56 mg/kg-day	NTP (2007a)
Dutch-Belted rabbits, pregnant dams and male offspring (≥10 dams/dose; 10-22 pups/dose)	0, 1-1.25, 5.2-6.7, or 55-61 mg/kg-day ^b neutralized DBA in drinking water from GD15 to weaning at 6 weeks, continuing in offspring to 12 or 24 weeks	F ₁ males: Impaired sexual functions (failure to achieve erection or ejaculate); decreased conception rates; disrupted sperm morphogenesis; reduced fertility; testicular lesions	LOAEL: 1 mg/kg-day	Veeramach aneni et al. (2007)

Sex/Species	Doses/Route of Exposure/Duration	Endpoints	NOAEL/LOAEL	Reference
Male Sprague-Dawley rats (3-4/dose)	0 or 250 mg/kg-day DBA by gavage for 1 or 4 days	Retention of Step 19 spermatids with enlarged residual bodies in testes at 4 days; reduced CYP17 mRNA and protein levels in testes at 4 days; reduced testosterone at 4 days	LOAEL ^a : 250 mg/kg-day	Carr et al. (2011)

^a This LOAEL was identified from a single-dose study.

^b A dose range is presented because there were slight differences in administered dose between dams and pups, and in pups receiving DBA for 12 or 24 weeks.

NA, not applicable

Table 9.9 Morphological changes in sperm of DBA-treated male rabbits from Veeramachaneni et al. (2007)

Treatment group (mg/kg-day)	N	Total sperm/ejaculate ($\times 10^6$) ^a	Sperm morphology ^a		
			Morphologically normal sperm (%)	Acrosomal defects (%)	Nuclear defects (%)
0	10	145.08±11.84	86.56±0.60	2.11±0.36	2.51±0.17
1	9	164.95±11.09	63.22±4.91*	18.33±2.84*	13.67±1.45*
5	9	140.88±10.80	71.04±3.63*	13.38±3.94*	9.18±2.02*
50	9	150.83±22.57	61.18±2.31*	15.95±1.40*	16.26±1.35*

^a Values represent mean \pm standard error of mean

*Values significantly different from control ($p < 0.05$) as reported (Veeramachaneni et al., 2007)

Table 9.10 Histopathological changes in testes of DBA-treated male rabbits from Veeramachaneni et al. (2007)

Treatment group (mg/kg-day)	N	Percent of seminiferous tubules graded as ^a				Degree of germinal epithelial loss ^{a,c}
		0 ^b	1	2	3	
0	9	91.1±0.8	7.7±0.8	1.1±0.6	0.1±0.1	2.6±0.3
1	10	77.9±2.7*	13.0±1.7	6.7±1.5*	2.7±1.4	8.6±1.5*
5	10	79.5±3.1*	16.6±2.5*	3.9±1.3	-	6.1±1.0
50	8	76.0±2.8*	17.9±2.2*	5.1±1.2*	0.5±0.3	7.9±0.9*

^a Values represent mean \pm standard error of mean

^b Grading as described by the study authors: 0 – normal, intact seminiferous epithelium; 1 – the seminiferous epithelium with pyknotic germ cells and desquamation or focal vacuolation; 2 – seminiferous epithelium intermediate between grades 1 and 3; 3 – seminiferous epithelium with premeiotic germ cells and Sertoli cells

^c weighted sum of percentages in each grade times the grade value (i.e., 0, 1, 2 or 3)

*Values significantly different from control ($p < 0.05$) as reported (Veeramachaneni et al., 2007)

Immunotoxicity

DBA induced suppression of the immune response characterized by thymus atrophy and splenomegaly, reduction in the response to polyclonal mitogens, and increased apoptosis of

thymus and spleen cells in mice (Gao et al., 2008; Gao et al., 2016). Immune-cell apoptosis mediated by the pathway of death receptors Fas and FasL was postulated to be the mechanism underlying this immunotoxicity (Gao et al., 2008). On the other hand, Smith et al. (2010) found no effect of DBA on several relevant immune responses in a similar study in mice.

Gao et al. (2008) gavaged groups of male and female BALB/c mice (≥ 5 /dose) with neutralized DBA at 0, 5, 20, or 50 mg/kg-day for 28 days. Immunotoxicity was indicated by a dose-dependent decrease in absolute and relative thymus weight and increased absolute and relative spleen weight (Table 9.11). Proliferation of thymocytes stimulated with concanavalin A was significantly decreased in a dose-dependent manner. Histological changes included cortical atrophy of the thymus, white pulp shrinkage of the spleen, and apoptosis of many splenic and thymic lymphocytes. Spleen cell death via apoptosis and expression of apoptosis-related genes such as *Fas*, *Traf2*, *Bcl-2*, and *Bax* increased with DBA dose. Increased expression of the Fas and FasL proteins in the thymus and spleen suggested that DBA might mediate cell death through the Fas and FasL death receptor pathway. A LOAEL of 5 mg/kg-day for increased absolute and relative spleen weight in female mice was identified by OEHHA. Treatment of thymocytes from male BALB/c mice with neutralized DBA in vitro induced decreased cellular proliferation, changes in cytokine production, and increased cytotoxicity and apoptosis (Gao et al., 2016). Furthermore, treatment of mouse Cl.Ly1+2/-9 T-cells from the spleen reduced cellular viability and increased apoptosis (Zhou et al., 2018).

Table 9.11 Spleen and thymus weight in mice following DBA exposure for 28 days (Gao et al., 2008)

Dose	0 mg/kg-day	5 mg/kg-day	20 mg/kg-day	50 mg/kg-day
Males^a				
Absolute spleen weight ^b	80.5 ± 2.7	88.7 ± 3.7	91.2 ± 3.4**	94.0 ± 2.5***
Relative spleen weight ^c	3.68 ± 0.14	3.87 ± 0.22	4.18 ± 0.20**	4.23 ± 0.09*
Absolute thymus weight ^b	43.1 ± 3.4	37.6 ± 2.0	33.3 ± 2.8**	33.4 ± 2.3**
Relative thymus weight ^c	1.85 ± 0.14	1.65 ± 0.09	1.50 ± 0.13**	1.50 ± 0.11**
Females^a				
Absolute spleen weight	78.9 ± 2.2	100.1 ± 7.7**	102.4 ± 5.0**	101.2 ± 4.8**
Relative spleen weight	3.99 ± 0.18	5.33 ± 0.45**	5.29 ± 0.27**	5.22 ± 0.19**
Absolute thymus weight	46.9 ± 3.7	47.3 ± 3.9	35.8 ± 2.3*	29.5 ± 3.3***
Relative thymus weight	2.39 ± 0.17	2.41 ± 0.18	1.87 ± 0.11*	1.55 ± 0.17***

* p<0.05, ** p<0.01, *** p<0.001, determined by Gao et al. (2008) using analysis of variance.

^a 10/dose

^b Units in mg

^c Units in mg/g body weight

Smith et al. (2010) administered 125, 500, or 1,000 mg/L DBA in drinking water to female B6C3F1 mice for 28 days and evaluated innate, humoral, and cell-mediated immune responses as well as host resistance. These exposures were equivalent to 0, 32, 130, or 260 mg/kg-day, calculated by OEHHA using equations 1 and 2, and a mouse body weight of 0.0258 kg, from Smith et al. (2010). Relative liver weights were significantly elevated in the two highest

concentration groups, but absolute and relative lung, kidney, and spleen weights were not significantly different. Absolute thymus weight was also significantly decreased in the two highest concentration groups. Sporadic statistically significant changes in both percent and absolute splenocyte surface markers were observed for several splenocyte subsets, but were reported as not biologically relevant due to lack of a dose-response (Smith et al., 2010). DBA exposures did not significantly affect the IgM plaque assay, serum IgM anti-sRBC (sheep red blood cells) titers, mixed-leukocyte response, interferon-gamma-induced in vitro macrophage cytotoxic activity, basal natural killer (NK) cell activity or the host resistance assays.

Neurotoxicity

Limited information on the neurotoxicity of DBA has been reported.

Linder et al. (1994a) reported that a single gavage dose of 1,000 to 2,000 mg/kg of neutralized DBA given to male Sprague-Dawley rats produced excessive drinking, difficulty moving hindlimbs, mild ataxia and labored breathing. Linder et al. (1994b) also administered neutralized DBA to Sprague-Dawley rats via gavage for 14 days with doses up to 270 mg/kg-day, and observed mild lethargy during the first week at the highest dose. In another study (Linder et al., 1995), a 42-day exposure to 250 mg/kg-day neutralized DBA produced signs of excitability, awkward gait, atypical limb movements and posturing, progressing to tremors and immovable hindlimbs. By the second week of treatment, male rats appeared to have a behaviorally mediated decrease in fertility without a decrease in serum testosterone. No such effects were observed at the next lower dose of 50 mg/kg-day.

Moser et al. (2004) reported neurotoxicity in male and female Fischer 344 rats given DBA in drinking water for 6 months at 0, 0.2, 0.6, or 1.5 g/L, for average doses of 0, 20, 72 or 161 mg/kg-day. The averaged doses reflect the mean value for males and females combined at each dose level. Functional observational battery and motor activity tests were given before dosing and at 1, 2, 4, and 6 months. DBA produced dose-related neuromuscular toxicity at 72 and 161 mg/kg-day characterized by limb weakness, mild gait abnormalities, and hypotonia. Sensorimotor depression was observed at all doses, with decreased responses to the tail-pinch and click tests. Other signs of toxicity at 161 mg/kg-day included decreased activity and a behavior the authors describe as chest claspings in females. Neurotoxicity was evident as early as one month, but did not progress with continued exposure. The major neuropathological finding was degeneration of spinal cord nerve fibers at 72 and 161 mg/kg-day. Cellular vacuolization, mostly in spinal cord gray matter and occasionally in white matter tracts, was also observed. No treatment-related changes were seen in brain, eyes, peripheral nerves, or peripheral ganglia. The authors determined a neurobehavioral LOAEL of 20 mg/kg-day for sensorimotor depression, and a NOAEL of 20 mg/kg-day for neuropathological changes.

Jiang et al. (2017) administered neutralized DBA to weanling Sprague-Dawley rats via intragastric injection at 0, 20, 50, or 125 mg/kg-day for 28 days. DBA negatively affected spatial learning and memory in the Morris water maze. Elevated levels of lipid peroxidation and oxidative stress were observed in the hippocampus and pre-frontal cortex of rat brains. Additionally, mRNA and protein levels of several pro-inflammatory cytokines were elevated in

the hippocampus and pre-frontal cortex. OEHHA identified a NOAEL of 20 mg/kg-day based on the induction of oxidative stress in the pre-frontal cortex and hippocampus.

Chronic Toxicity

Effects in Humans

No human studies of chronic toxicity of DBA were identified.

Effects in Animals

Chronic effects of DBA have been identified in carcinogenicity studies in rodents (Melnick et al., 2007; NTP, 2007a). NTP (2007a) exposed groups of 50 male and female Fischer 344 rats to drinking water containing 0, 50, 500, or 1,000 mg/L neutralized DBA for 2 years. Average doses were about 0, 2, 20, or 40 mg/kg-day for males and 0, 2, 25, or 45 mg/kg-day for females. Survival was not affected by DBA. Mean body weights were decreased at the middle and high doses for about the last half of the exposure in both sexes. Water consumption at the high dose was also decreased in both sexes during the second year. In addition to neoplasms (discussed in the *Carcinogenicity* section), increased hepatic cystic degeneration was observed in male rats in all treatment groups. Nephropathy (characterized by a thickened basement membrane, glomerular thickening, tubular protein casts, and chronic inflammatory infiltrates with fibrosis) was observed in females from all exposure groups, and alveolar epithelial hyperplasia (characterized by focal thickening of the alveolar septa due to an increased number of cuboidal type II pneumocytes) was significantly increased at the two higher doses. The mean severities of these non-neoplastic endpoints were rated between minimal and mild. These results are presented in Table 9.12. OEHHA identified the lowest DBA dose, 2 mg/kg-day, as a LOAEL for the liver effects in males and kidney effects in females.

Table 9.12 Non-neoplastic chronic toxicity in rats from NTP (2007a)

Concentration	0 mg/L	50 mg/L	500 mg/L	1,000 mg/L
Male Doses (mg/kg-day)	0	2	20	40
Hepatic cystic degeneration	3/50	9/50*	11/50*	15/50**
Female Doses (mg/kg-day)	0	2	25	45
Nephropathy	18/50	32/50**	37/50**	40/50**
Alveolar epithelial hyperplasia	3/50	7/50	13/50**	14/50**

* p<0.05, ** p<0.01, determined by NTP (2007) using the Poly-3 test

NTP (2007a) also exposed groups of 50 male and female B6C3F1 mice to drinking water containing 0, 50, 500, or 1,000 mg/L DBA for 2 years, for average doses of 0, 4, 45, or 87 mg/kg-day for males and 0, 4, 35, or 65 mg/kg-day for females. Survival was not affected by DBA exposure. Mean body weights of males at the two lower doses were greater than those of the controls after week 85. Water consumption was not affected. Liver and lung neoplasms are discussed below in the *Carcinogenicity* section. Little non-neoplastic toxicity was observed. Increased splenic hematopoietic cell proliferation and an increased incidence of cataracts was reported in male mice at 1,000 mg/L DBA. For males, 45 and 87 mg/kg-day of DBA can be considered the NOAEL and LOAEL, respectively, while for females, the highest dose of 65 mg/kg-day is the NOAEL.

Carcinogenicity

NTP (2007a) (also reported in Melnick et al. (2007)) exposed groups of 50 male or female Fischer 344 rats and B6C3F1 mice to 0, 50, 500, or 1,000 mg/L DBA in water in 2-year cancer bioassays. This corresponds to average doses of: 0, 2, 20, or 40 mg/kg-day for male rats; 0, 2, 25, or 45 mg/kg-day for female rats; 0, 4, 45, or 87 mg/kg-day for male mice; and 0, 4, 35, or 65 mg/kg-day for female mice.

An increase in malignant mesothelioma was observed in high dose male rats ($p=0.07$). Additionally, an increase in mononuclear cell leukemia was observed in high dose female rats and low dose male rats (Table 9.13).

Table 9.13 Incidence of neoplasms in rats treated with DBA from NTP (2007a)

Concentration	0 mg/L	50 mg/L	500 mg/L	1,000 mg/L
Male Doses (mg/kg-day)	0	2	20	40
Malignant mesothelioma	3/47 ^a	1/46	0/45	10/47
Mononuclear cell leukemia	17/49	31/50**	24/48	13/49
Female Doses (mg/kg-day)	0	2	25	45
Mononuclear cell leukemia	11/49 ^a	13/49	16/50	22/49*

* $p<0.05$, ** $p<0.01$, determined by OEHA using Fisher's exact test

^a Test for trend, significant ($p<0.05$)

DBA treatment resulted in a statistically significant dose-related increase in hepatocellular adenomas and carcinomas in both sexes and hepatoblastomas in male mice (Table 9.14). Hepatocellular adenomas were significantly increased in males at 50, 500 or 1,000 mg/L DBA, and in females at 500 or 1,000 mg/L DBA. Hepatocellular carcinomas were significantly increased in the male high-dose group and in females receiving 500 mg/L. Hepatoblastomas were significantly increased in males at 500 or 1,000 mg/L but not significantly increased in females. The incidences of adenoma or carcinoma and of combined hepatic tumors in male mice were significantly increased at all doses in a dose-dependent manner. Hepatocellular adenomas or carcinomas were significantly increased in female mice that received 500 or 1,000 mg/L compared to control.

Table 9.14 Incidence of hepatocellular neoplasms in B6C3F1 mice treated with DBA from NTP (2007a)

Concentration	0 mg/L	50 mg/L	500 mg/L	1,000 mg/L
Male Doses (mg/kg-day)	0	4	45	87
Adenoma	18/46	37/49**	37/48**	42/50**
Carcinoma	14/48	9/49	19/48	26/50*
Hepatoblastoma	0/46	0/49	3/48*	2/50**
Adenoma or Carcinoma or Hepatoblastoma	28/48	41/49**	43/48**	48/50**
Female Doses (mg/kg-day)	0	4	35	65
Adenoma	19/46	26/47	32/47**	35/48**
Carcinoma	3/45	3/44	12/46	8/46
Adenoma or Carcinoma	22/46	28/47	37/47**	37/48**

* $p<0.05$, ** $p<0.01$, determined by OEHA using Fisher's exact test

Statistically significant increases in lung alveolar/bronchiolar adenomas were observed in male mice treated with 500 mg/L (Table 9.15). There were modest, non-significant increases in lung tumors in females.

Table 9.15 Incidence of lung neoplasms in B6C3F1 mice treated with DBA from NTP (2007a)

Concentration	0 mg/L	50 mg/L	500 mg/L	1,000 mg/L
Male Doses (mg/kg-day)	0	4	45	87
Alveolar/Bronchiolar Adenoma	7/46 ^a	5/49	17/48*	12/48
Alveolar/Bronchiolar Carcinoma	5/39	8/45	8/40	7/41
Alveolar/Bronchiolar Adenoma or Carcinoma	12/46	12/49	22/48*	17/48
Female Doses (mg/kg-day)	0	4	35	65
Alveolar/Bronchiolar Adenoma	1/49	3/48	3/49	6/48
Alveolar/Bronchiolar Carcinoma	1/44	2/44	2/44	2/46
Alveolar/Bronchiolar Adenoma or Carcinoma	2/49	5/48	5/49	7/48

*p<0.05 determined by OEHHA using Fisher's exact test

^a Test for trend, significant (p=0.044)

NTP (2007a) concluded that there was some evidence of DBA carcinogenicity in male rats based on an increased incidence of malignant mesothelioma. Mononuclear cell leukemia observed in male rats may have also been related to DBA exposure. Similarly, NTP concluded that there was some evidence of carcinogenicity of DBA in female rats based on an increased incidence and positive trend of mononuclear cell leukemia. In male and female mice, there was clear evidence of DBA carcinogenicity based on increased incidences of hepatocellular neoplasms and hepatoblastoma. Lung neoplasms were considered by NTP to be DBA-related in male mice and may have been related in female mice.

Melnick et al. (2007) reiterated the conclusions from NTP (2007), calling DBA a multiple organ carcinogen in laboratory animals based on tumors observed in the abdominal cavity mesothelium of male rats, hematopoietic system in female rats, and the liver and lung of mice. Although the mode of action for DBA carcinogenicity is not known, Melnick et al. (2007) posited that an early increase in hepatocyte proliferation is not likely a key event since there was no increase in the hepatocyte DNA labeling index observed in mice exposed for 26 days while a slight increase that did occur in male F344 rats was not accompanied by an increase in liver tumor response. The authors further asserted that the carcinogenicity of DBA may involve a genotoxic mechanism since the chemical induces DNA damage.

US EPA has not published a review of DBA toxicity. WHO (2004a), before release of the NTP (2007) study, cited the lack of studies on DBA in declining to establish guideline values for DBA in drinking water.

Conclusions on the Carcinogenicity of DBA

OEHHA concludes that positive results for hepatic adenomas and carcinomas in male and female mice, and alveolar/bronchiolar adenomas in male mice provide sufficient evidence of

carcinogenicity to develop a health-protective value for DBA based on cancer. The most robust and sensitive data for estimating cancer potency are the incidences of combined tumors of the liver and lung in male and female B6C3F1 mice from NTP (2007a), which is evaluated using BMDS modeling. Furthermore, OEHHA concludes that the preponderance of positive evidence in both in vitro and in vivo genetic toxicity assays supports DBA as a genotoxic carcinogen and that its cancer potency can be estimated using linear low-dose extrapolation.

10. DOSE-RESPONSE ASSESSMENT

To determine a health-protective level, the most sensitive endpoints from studies determined to be relevant to human health are selected, and analyses of the dose-response relationships are performed. The adverse effect, or a measure of response that leads to an adverse effect, that occurs at the lowest dose is selected as the critical effect from which a health-protective concentration is derived.

A PHG can be derived using general equations for calculating health-protective concentrations in drinking water for either cancer or noncancer endpoints, which utilize different calculations, described in more detail below. A single PHG for the five HAAs combined was not developed due to the distinct potencies and critical effects of the individual chemicals.

Noncancer Dose-Response Analyses and Acceptable Daily Dose Calculations

For noncancer dose-response analysis, an acceptable daily dose (ADD), in units of milligrams per kilogram of body weight per day (mg/kg-day), is established for each HAA. The ADD is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without any anticipated adverse effects.

Method for Calculating ADD

Point of Departure

The point of departure (POD) is the 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 calculation of the ADD. The POD is typically established by fitting a dose-response model to the toxicology data. This is done using the US EPA Benchmark Dose Software (BMDS version 2.7) when appropriate. This software is publicly available (<http://www.epa.gov/ncea/bmds/>). OEHHA considered a web-based Bayesian benchmark dose (BBMD) modeling system developed by Shao and colleagues (Shao and Shapiro, 2018), however, a more thorough evaluation is needed to ensure its compatibility with OEHHA's risk assessment guidelines and practice before it can be considered as an alternative to BMDS. BMDS uses mathematical models to fit data and determines the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). The BMR is typically set at 5% above the background or the response of the control group for dichotomous data. OEHHA's peer-reviewed risk assessment guidelines (OEHHA, 2008) state, "[Reference concentration] determinations for various endpoints by the U.S. EPA have used either 5% or 10% as the benchmark response rate, depending on the statistical uncertainty in the data (U.S. EPA, 2002a; U.S. EPA, 2004). OEHHA has used the 5% response rate in several chronic [reference exposure levels], and showed that the lower 95% confidence bound on the BMC_{05} typically appears equivalent for risk assessment purposes to a NOAEL in well designed and conducted animal studies where a quantal measure of toxic response is reported." Therefore, OEHHA typically uses a BMR of 5% when analyzing dichotomous data unless there is a significant reason to use a different response rate.

For continuous data, a BMR of one standard deviation from the control mean is typically used when there are no data to indicate what level of response is biologically significant (US EPA,

2012). To account for uncertainty in the data, the model also calculates the 95% lower confidence limit of the BMD, known as the BMDL (L stands for lower confidence limit). For PHG development, OEHHA uses the BMDL as the POD for the calculation of a health-protective drinking water concentration when the data are amenable to BMD modeling.

OEHHA does not generally model datasets consisting of a single treatment group (in addition to controls) because such modeling provides limited information regarding the dose-response relationship, and thus is not optimal for POD determination. When these data are presented in the study summary tables above, footnotes are included to indicate that the LOAELs come from single-dose studies.

In this document, the phrase “data not amenable to BMD modeling” indicates that the selected dataset should not be modeled with BMDS because the data do not meet suitability criteria as outlined in Davis et al. (2011) and US EPA (2012), including biological or statistical significance, sufficient dose groups to support modeling, and appropriate dose-response relationships (for example, datasets with doses with non-saturating responses). Additionally, continuous datasets where the BMDS software was unable to appropriately model the variance were also considered “not amenable to modeling.”

The phrase “poor model fit” indicates cases where BMDS did not produce a model with acceptable fit for a given dataset. This may be due to the goodness-of-fit p-value being below the OEHHA default of 0.05 and/or failure to pass variance tests (for continuous data). Additionally, several other considerations are applied to judging the utility of the model for POD derivation, including visual fit, BMD/BMDL ratio, scaled residuals, relative position of the BMDL to the lowest dose, and general characteristics of the dataset that can make it suitable or unsuitable for BMD modeling (Davis et al., 2011).

Model selection criteria when comparing outputs of different models for the same endpoint/dataset were: the lowest Akaike’s information criterion (AIC), goodness of fit p-value ≥ 0.05 , scaled residual \leq the absolute value of 2, and visual inspection of the dose-response curve.

Application of BMD modeling for noncancer effects mitigates some of the limitations of the NOAEL/LOAEL approach, including:

- dependence on dose selection and sample size,
- uncertainty in the estimate of the dose-response due to the characteristics of the study design,
- the need to use a ten-fold uncertainty factor when a NOAEL cannot be determined in a study, and
- inability to account for the shape of the dose-response curve when selecting an experimentally-derived NOAEL or LOAEL.

The results presented in the following section are for the datasets analyzed with BMDS for each HAA. Appendix D presents model selection criteria, the complete output profiles from the BMD modeling and further details on the modeling.

Uncertainty Factors

When developing health-protective levels for noncancer effects based on animal studies, OEHHA generally applies a combined UF of 300 (10 for interspecies extrapolation and 30 for intraspecies variability, consisting of $\sqrt{10}$ for pharmacokinetics, $\sqrt{10}$ for pharmacodynamics, and $\sqrt{10}$ for differences in developmental pharmacokinetics to protect infants and children) (OEHHA, 2008). The $\sqrt{10}$ developmental pharmacokinetic subfactor is applied unless data are available to indicate that infants and children are not at higher risk due to differences in pharmacokinetics. However, if the critical endpoint is derived from a developmental study in which the fetus or juvenile animal is exposed, or if the chemical's site of action is the point of contact, the developmental pharmacokinetic subfactor is typically not applied. Additional adjustments may be included depending on the limitations of available data, including 10 for extrapolation from a subchronic to chronic exposure, 10 for extrapolation from a LOAEL to a NOAEL, and possibly an additional factor of 3 or 10 for missing or deficient studies, with a maximum combined UF of 3,000. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results.

Table 10.1 below is adapted from OEHHA's *Technical Support Document for the Development of Noncancer Reference Exposure Levels* (OEHHA, 2008).

Table 10.1 Default uncertainty factors for PHG derivation

Uncertainty Factor	Value
<i>LOAEL uncertainty factor (UFL)</i>	
<i>Values used:</i>	10 LOAEL, any effect 1 NOAEL or benchmark dose modeling used
<i>Interspecies uncertainty factor (UFA)</i>	
<i>Combined interspecies uncertainty factor (UFA):</i>	1 human observation $\sqrt{10}$ animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
<i>Toxicokinetic component (UFA-k) of UFA:</i>	1 where animal and human PBPK models are used to describe interspecies differences $\sqrt{10}$ non-primate studies with no chemical- or species-specific kinetic data

Uncertainty Factor	Value
<i>Toxicodynamic component (UFA-d) of UFA:</i>	1 where animal and human mechanistic data fully describe interspecies differences. (<i>This is unlikely to be the case.</i>) 2 for residual susceptibility differences where there are some toxicodynamic data $\sqrt{10}$ non-primate studies with no data on toxicodynamic interspecies differences
<i>Intraspecies uncertainty factor (UFH)</i>	
<i>Toxicokinetic component (UFH-k) of UFH:</i>	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 $\sqrt{10}$ 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
<i>Toxicodynamic component (UFH-d) of UFH:</i>	1 human study including sensitive subpopulations (e.g., infants and children) $\sqrt{10}$ studies including human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)
<i>Subchronic uncertainty factor (UFS)¹</i>	
<i>Values used:</i>	1 study duration >12% of estimated lifetime $\sqrt{10}$ study duration 8-12% of estimated lifetime 10 study duration <8% of estimated lifetime
<i>Database deficiency factor (UFD)</i>	
<i>Values used:</i>	1 no substantial data gaps $\sqrt{10}$ substantial data gaps including, but not limited to, developmental toxicity

¹Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008).

A detailed discussion of OEHHA's default uncertainty factors and how they were derived can be found in OEHHA's peer-reviewed *Technical Support Document for the Derivation of Noncancer Reference Exposure Levels* (OEHHA, 2008).

Acceptable Daily Dose

The ADD is an estimated maximum daily dose (in mg/kg-day) that can be consumed by humans for an entire lifetime without toxic effects. This is similar to the term "reference dose" (RfD) used by the US EPA. To determine the ADD, the POD must be divided by a factor which incorporates uncertainties in the risk assessment, such as differences between animals and

humans, and accounts for differences among humans in response to the toxicant. This combined factor is referred to as a total uncertainty factor (UF).

The ADD is calculated using the following equation:

$$ADD = POD \div UF$$

Monochloroacetic Acid

Systemic and cardiovascular toxicities appear to be the sensitive endpoints for MCA. Two chronic rat studies (NTP, 1992; DeAngelo et al., 1997) describe adverse systemic effects while two subchronic studies (Daniel et al., 1991; NTP, 1992) and one chronic study (DeAngelo et al., 1997) show adverse cardiovascular effects of MCA exposure. However, dose-response of heart inflammation in either male or female rats (Daniel et al., 1991) did not demonstrate pairwise significance compared to controls despite a significant trend. Therefore, this study/endpoint is ultimately not considered for the development of the PHG.

Four candidate critical studies are listed in Table 10.2. The Daniel et al. (1991) study supports the findings of cardiomyopathy in the 13-week NTP (1992) report and findings of myocardial degeneration in the DeAngelo et al. (1997) report, since all three studies were executed at comparable dose ranges.

Cardiotoxicity has been associated with MCA exposures, including case reports of accidental transdermal human exposure to highly concentrated MCA, and animal oral exposure studies. Therefore, cardiotoxic effects were considered in the selection of a critical study for MCA. Myocardial degeneration was reported in a chronic study in male rats (DeAngelo et al., 1997) with a NOAEL of 26 mg/kg-day, which was higher than the NOAEL of 3.5 mg/kg-day for systemic toxicity in the same study (Table 10.2). BMD modeling could not be performed on the myocardial degeneration reported by DeAngelo et al. (1997) because incidence data were not provided. Cardiomyopathy was also reported in a 13-week study in rats (NTP, 1992). This study suggested a NOAEL of 21 mg/kg-day. OEHHA derived BMDL₀₅s of 23 mg/kg-day and 20 mg/kg-day for males and females, respectively from the NTP study (Table 10.3).

Table 10.2 Candidate critical studies for MCA noncancer effects

Reference	Sex/ Species	Dose/Route of Exposure	Endpoint	NOAEL/LOAEL/ BMDL ₀₅ (mg/kg-day)
<i>Systemic Toxicity</i>				
DeAngelo et al. (1997)	Male F344/N rats (50/sex/dose ^a)	0, 3.5, 26, 60 mg/kg-day in drinking water for 2 years	Systemic toxicity (decreased body weight and relative liver weight) Relative liver weight (g) ^b : 4.35 ± 1.01, 4.56 ± 0.88, 3.51 ± 0.96*, 3.22 ± 0.20* Final body weight (g) ^c : 434.5, 419.8, 364.3, 266.0*	NOAEL: 3.5 (final body weight data could not be modeled)

Reference	Sex/ Species	Dose/Route of Exposure	Endpoint	NOAEL/LOAEL/ BMDL ₀₅ (mg/kg-day)
NTP (1992)	Male and female F344/N rats (53/sex/dose)	0, 11, 21 mg/kg-day by gavage for 2 years	Increased mortality in exposed groups due to unidentified causes Incidence: M: 1/53, 4/53, 12/53* F: 0/53, 4/53, 12/53*	NOAEL (F): 11 BMDL ₀₅ (M): 7.8 BMDL ₀₅ (F): 3.4
<i>Cardiovascular Toxicity</i>				
NTP (1992)	Male and female F344/N rats (9-17/ sex/dose)	0, 21, 43, 64, 86, 107 mg/kg- day by gavage for 13 weeks	Cardiomyopathy Incidence: M: 0/10, 0/10, 5/10*, 9/9*, 13/13*, 15/15* F: 0/10, 0/10, 6/9*, 10/10*, 15/15*, 17/17*	NOAEL: 21 BMDL ₀₅ (M): 23.4 BMDL ₀₅ (F): 20.1
DeAngelo et al. (1997)	Male F344/N rats (50/sex/dose)	0, 3.5, 26, 60 mg/kg-day in drinking water for 2 years	Myocardial degeneration (incidence data not reported)	NOAEL: 26

*Significantly different versus controls (p<0.05)

^a Numbers of animals at terminal sacrifice were 23, 24, 23, and 25 for the 0, 3.5, 26, and 60 mg/kg-day groups, respectively.

^b Values are presented as mean ± standard deviation.

^c Body weights throughout the experimental period were reported graphically and standard deviations were not reported.

Table 10.3 BMD modeling results^a for candidate critical studies of MCA

Study Reference (study duration)	Endpoint	Model	Goodness of fit p-value	BMD ₀₅ (mg/kg-day)	BMDL ₀₅ (mg/kg-day)
<i>Systemic Toxicity</i>					
NTP (1992) (2 years)	Increased mortality in male rats	Logistic	0.98	10.2	7.8
NTP (1992) (2 years)	Increased mortality in female rats	Quantal- Linear	0.6395	4.95	3.4
<i>Cardiovascular Toxicity</i>					
NTP (1992) (13 weeks)	Cardiomyopathy in male rats	LogLogistic	1.0000	36.5	23.4
NTP (1992) (13 weeks)	Cardiomyopathy in female rats	LogLogistic	1.0000	35.1	20.1

^a Only the best model, based on criteria described in Appendix D, is shown for each endpoint; the goodness of fit p-value ≥0.1 indicates the model describes observed data sufficiently well.

The chronic NTP (1992) study was of comparable quality to the DeAngelo et al. (1997) study and reported mortality due to unidentified causes with a NOAEL of 11 mg/kg-day. BMD modeling of mortality in male rats produced a BMD of 10.2 mg/kg-day and BMDL₀₅ of 7.8 mg/kg-day and in female rats a BMD of 4.95 mg/kg-day and BMDL₀₅ of 3.4 mg/kg-day (Table 10.3). The latter value is similar to the NOAEL of 3.5 mg/kg-day based on the DeAngelo et al. (1997) study. However, it is generally more appropriate to develop guidance values based on less severe effects than mortality.

The DeAngelo et al. (1997) study is chosen as the critical study for PHG derivation. It employed a reasonable number of animals (23-25 male rats at termination), administered neutralized MCA in drinking water for a lifetime exposure, and included comprehensive pathological examination and serum analysis. In the mid- and high-dose groups (26 and 60 mg/kg-day), this study reported decreased body weight (13% and 38%, respectively, compared to control), and decreased absolute and relative liver weight. However, the data were not amenable to BMD modeling, since statistical analysis in BMD (version 2.7) indicated that the assumption of neither constant nor modeled variance was appropriate for this dataset. In addition, decreased absolute kidney weight, and increased relative testes weight were reported, although relative kidney weight and absolute testes weight were not changed compared to controls. Based on the decreased body weight and changes in relative liver weights, US EPA considered 26 mg/kg-day as a LOAEL, and the low dose, 3.5 mg/kg-day as a NOAEL (US EPA, 2006). OEHHHA concurs with this determination and selects 3.5 mg/kg-day as the point of departure (POD) for MCA.

A combined UF of 1,000 is applied, which includes a factor of 10 for interspecies extrapolation, and 30 for variation in the human population ($\sqrt{10}$ for toxicodynamics and 10 for toxicokinetics, which accounts for diversity, including infants and children, with no human kinetic data (OEHHHA, 2008). In addition, a UF of $\sqrt{10}$ is applied for database deficiency. While developmental toxicity is suggested by an in vitro study (Hunter et al., 1996), there are no female reproductive studies and no multi-generation reproductive studies. Further, the NOAEL for mortality from the NTP study and resulting BMDLs are only a few fold higher than the NOAEL for systemic toxicity in the DeAngelo et al study. This results in the following Acceptable Daily Dose (ADD) calculation:

$$\text{ADD} = 3.5 \text{ mg/kg-day} \div 1,000 = 0.0035 \text{ mg/kg-day}$$

Dichloroacetic Acid

Liver toxicity

Liver toxicity was reported in chronic DCA studies in mice, and the most common endpoints included increased relative liver weight and vacuolization with glycogen deposition (Table 6.9). Liver toxicity was also reported in subchronic studies in rats, dogs and humans, and therefore, it is considered in the development of a health-protective concentration (HPC) for noncancer effects. Among six chronic studies in mice, only DeAngelo et al. (1991) and DeAngelo et al. (1999) are considered for dose-response assessment because they employed multiple doses, reported liver effects at relatively low levels and observed consistent adverse effects (several liver endpoints, significant trends in response). Among remaining chronic studies that were not

considered for dose-response analysis, Bull et al. (1990) employed relatively large doses, Daniel et al. (1992) was a single-dose study, Pereira (1996) reported data in a graphical way that could not be used for quantitative analysis (i.e., statistical information was masked by symbols and could not be extracted), and NTP (2007b) employed transgenic animal models targeted to dermal toxicity and cancer. Given the small number of chronic studies acceptable for dose-response analysis, and in light of the finding of liver toxicity (liver enlargement) in a subchronic human study (Mori et al., 2004), hepatic toxicity endpoints in subchronic animal studies are also considered for POD derivation. Specifically, the data sets for increased relative liver weight in the following subchronic studies are considered: Mather et al. (1990), Cicmanec et al. (1991) and Toth et al. (1992). The details of these studies are presented in Table 10.4.

The studies that generated acceptable BMD models for liver toxicity include Mather et al. (1990), Cicmanec et al. (1991) and Toth et al. (1992), while DeAngelo et al. (1991) and DeAngelo et al. (1999), as well as the female dog subset in Cicmanec et al. (1991) did not (Table 10.5).

Reproductive Toxicity and Neurotoxicity

DCA demonstrated a range of reproductive toxicity effects in male rats (Table 6.7), and is on the Proposition 65 list for male reproductive and developmental toxicity. Since LOAELs for reproductive adverse effects in Cicmanec et al. (1991) and Toth et al. (1992) are within the range of values considered for liver toxicity, these studies are included as candidate studies for POD derivation and analyzed with BMDS (Tables 10.4, 10.5).

Among studies with neurotoxicity endpoints (Table 6.8), Moser et al. (1999) reported gait changes in rats from exposures as low as 16 mg/kg-day. However, the reported data are not amenable to statistical or dose-response analysis. Therefore, the Moser et al. (1999) study is not deemed appropriate for dose-response analysis. Katz et al. (1981) qualitatively reported mild to moderate vacuolization of the brain in all DCA-exposed dogs, suggesting that the lowest dose, 50 mg/kg-day, would be a LOAEL for this adverse effect. However, as presented in Table 10.4, several candidate critical studies had LOAEL values lower than 50 mg/kg-day, indicating that a POD derived from these candidate critical studies would be protective of the effects reported by (Katz et al., 1981). Therefore, the latter study, as well as other studies with higher LOAEL values, were not chosen as candidate critical studies.

The candidate studies are summarized in Table 10.4, and their BMDS analyses are presented in Table 10.5.

Table 10.4 Candidate critical studies for noncancer effects of DCA

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)
<i>Liver Toxicity</i>				
Toth et al. (1992)	Male Long-Evans rats (18-19/dose)	0, 31.25, 62.5, 125 mg/kg-day by oral gavage for 10 weeks	Relative liver weight increase	LOAEL: 31.25

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)
Mather et al. (1990)	Male Sprague-Dawley rats (10/dose)	0, 3.9, 35.5, 345 mg/kg-day in drinking water for 90 days	Relative liver weight increase	NOAEL: 3.9
Cicmanec et al. (1991)	Male and female beagle dogs (5/sex/dose)	0, 12.5, 39.5, 72 mg/kg-day in gelatin capsules by oral gavage for 90 days	Relative liver weight increase	LOAEL: 12.5
DeAngelo et al. (1991)	Male B6C3F1 mice (9-30/dose)	0, 7.6, 77, 410, or 486 mg/kg-day in drinking water for 60 weeks	Relative liver weight increase	NOAEL: 7.6
DeAngelo et al. (1999)	Male B6C3F1 mice (8-50/dose; 10/dose at interim sacrifices)	0, 8 (no interim sacrifice), 84, 168, 315, or 429 ^a mg/kg-day in drinking water for 100 weeks; interim sacrifices at 26, 52 and 78 weeks	Relative liver weight increase	NOAEL (100 w): 168 LOAEL (26 & 52 w): 84
<i>Reproductive Toxicity</i>				
Toth et al. (1992)	Male Long-Evans rats (18-19/dose)	0, 31.25, 62.5, 125 mg/kg-day by oral gavage for 10 weeks	Absolute epididymis weight decrease	LOAEL: 31.25
Cicmanec et al. (1991)	Male and female beagle dogs (5/sex/dose)	0, 12.5, 39.5, 72 mg/kg-day in gelatin capsules by oral gavage for 90 days	Testicular degeneration in male dogs Incidence: 0/5, 4/5*, 5/5*, 5/5*	LOAEL: 12.5

^a Mean daily doses were calculated for 100-week exposure;

* Values are statistically different from control (p<0.05).

Table 10.5 BMD modeling results^a for candidate critical studies of DCA

Study Reference	Endpoint	Model	Goodness of fit p-value	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
<i>Liver Toxicity</i>					
Toth et al. (1992)	Relative liver weight increase in male rats	Exponential ⁴	0.4785	27.4	18.5
Mather et al. (1990)	Relative liver weight increase in male rats	Hill	0.2381	9.67	6.75

Study Reference	Endpoint	Model	Goodness of fit p-value	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
Cicmanec et al. (1991)	Relative liver weight increase in male dogs	Exponential4	0.1802	0.592	0.329
Cicmanec et al. (1991)	Relative liver weight increase in female dogs	Poor model fit			
DeAngelo et al. (1991)	Relative liver weight increase in male mice	Poor model fit			
DeAngelo et al. (1999)	Relative liver weight increase at 100 weeks in male mice	Poor model fit			
<i>Reproductive Toxicity</i>					
Toth et al. (1992)	Absolute epididymis weight decrease in male rats	Exponential4	0.4337	29.5	17.9
Cicmanec et al. (1991)	Testicular degeneration male dogs	Quantal Linear	0.9986	0.382 ^b	0.169 ^b

^a Only the best model, based on criteria described in Appendix D, is shown for each endpoint; the goodness of fit p-value ≥ 0.1 indicates the model describes observed data sufficiently well.

^b Values are BMD₀₅ and BMDL₀₅ determined with BMR of 0.05.

The BMDL₀₅ for testicular degeneration in Cicmanec et al. (1991) is 0.169 mg/kg-day. However, the response levels are similar for all of the non-control doses and are near or at saturation, indicating very large uncertainty in model prediction and extrapolation to the low end of dose-response. Benchmark dose guidance notes, “A dataset in which all non-control doses have essentially the same response level ... provides limited information about the dose-response relationship since the complete range of response from background to maximum must occur somewhere below the lowest dose; thus, the BMD may be just below the first dose, or orders of magnitude lower.” Furthermore, the BMD₀₅ and BMDL₀₅ are more than 10-fold lower than the lowest dose (12.5 mg/kg-day), in which case BMDS guidance would flag this model as questionable (US EPA, 2016). In this case, the BMD values are not suitable because the BMDL is far below the lowest tested dose, 70-fold lower. Moreover, the response levels were similar for all non-control doses had plateaued. Absolute testicular weight in male Fischer 344 rats in DeAngelo et al. (1996) was significantly increased at mid-dose (40.2 mg/kg-day) but was significantly lower than controls at the high dose (139 mg/kg-day); this lack of consistency in the adverse effect precluded BMD modeling of the DeAngelo et al. (1996) dataset. Overall, while several studies have reported male reproductive effects from DCA exposure (Table 6.7), there are also issues that contributed to the exclusion of these studies for POD derivation:

- Quantitative data were not reported in some studies.

- Use of the LOAEL would introduce additional uncertainty in the derivation of a health protective concentration when there are equally valid and sensitive studies reporting a NOAEL.
- Varying effects on testis weight were observed but a reliable candidate POD study was lacking.

Study and Endpoint Selection

The Cicmanec et al. (1991) data produce a BMDL_{1SD} of 0.329 mg/kg-day for increased relative liver weight, a value almost 40-fold lower than 12.5 mg/kg-day, which is the lowest dose used in the study. The low BMDL_{1SD} results from the supralinear fit of the model, which is in turn driven by the high level of response at the low dose (>50% increase in relative liver weight compared to control). In this dataset, as with the data for testicular degeneration, all response levels were at or near maximal response. Thus, for the same reasons outlined above for the testicular degeneration data, the BMDL for increased relative liver weight is not chosen as the POD. Furthermore, the 90-day study duration for Cicmanec et al. (1991) would require an additional UF of 10 for extrapolating from <8% of lifetime to a lifetime exposure. US EPA (2003b) opted for the LOAEL/NOAEL approach in the derivation of a reference dose and selected the LOAEL of 12.5 mg/kg-day for increased relative liver weight and testicular degeneration Cicmanec et al. (1991).

When considering the different studies reporting increases in relative liver weight, although Mather et al. (1990) produced a lower BMDL_{1SD} (6.75 mg/kg-day) and Cicmanec et al. (1991) obtained a LOAEL of 12.5 mg/kg-day, these studies are not selected due to greater uncertainty in extrapolating from subchronic to chronic duration, which would require application of an additional uncertainty factor. Compared to the Mather et al. (1990) and Cicmanec et al. (1991) studies, the DeAngelo et al. (1991) study was chronic in duration, employed a greater number of animals per dose and animals were exposed to drinking water rather than gelatin capsules as in Cicmanec et al. (1991), making it a more suitable study for PHG derivation. By selecting DeAngelo et al. (1991), there is less uncertainty in extrapolating to an acceptable daily dose (ADD). Although lower overall uncertainty is not the sole criterion used for critical study selection, it is generally preferable over studies with greater uncertainty. Thus, OEHHA is selecting the NOAEL of 7.6 mg/kg-day for increased relative liver weight from the chronic drinking water study by DeAngelo et al. (1991) as the POD. Because several studies (Table 6.7) showed that reproductive toxicity is a concern for DCA exposure, and there is a wide range of NOAELs and LOAELs from these studies, a database UF of $\sqrt{10}$ is applied to account for the lack of studies to adequately characterize the doses that result in these effects, particularly for changes in testicular weight. Human genetic variations in the enzymes responsible for DCA metabolism, presumably to its active form, have been reported (Stacpoole et al., 2008b). OEHHA's default UF of 30 for human variability (OEHHA, 2008) would account for these differences.

A combined UF of 1,000 (consisting of 10 for interspecies extrapolation, 30 for human variability, including infants and children, and to account for genetic polymorphisms in metabolism, and $\sqrt{10}$ for database deficiency) is applied in the following ADD calculation:

$$\text{ADD} = 7.6 \text{ mg/kg-day} \div 1,000 = 0.0076 \text{ mg/kg-day}$$

Trichloroacetic Acid

As outlined in the *Chronic toxicity in animals* section, which includes study descriptions and the rationale for suitability of studies for dose-response analysis, DeAngelo et al. (2008), Bull et al. (2002) and DeAngelo et al. (1997), provide noncancer data sets of acceptable quality for dose-response analysis (i.e., there were no issues with the study that would preclude a reasonable dose-response assessment). The details for the most sensitive endpoints in these studies are provided in Table 10.6.

Table 10.6 Candidate critical studies for TCA noncancer effects

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL/BMDL ₀₅ (mg/kg-day)
DeAngelo et al. (2008) Study 1	Male B6C3F1 mice (10 or 30/dose)	0, 7.7, 68.2, 602.1 mg/kg-day in drinking water for 60 weeks	Hepatic necrosis (transient, observed at 30-45 weeks): 0/10, 0/10, 3/10, 5/10*	NOAEL: 68.2 ^a BMDL: 8.45
DeAngelo et al. (2008) Study 1	Male B6C3F1 mice (10 or 30/dose)	0, 7.7, 68.2, 602.1 mg/kg-day in drinking water for 60 weeks	Increased relative liver weight (30/dose) ^b : 5.3±1.0, 5.0±1.0, 6.6±2.0**, 8.5±1.7**	NOAEL: 7.7
Bull et al. (2002)	Male B6C3F1 mice (20/dose)	0, 55, 238 mg/kg-day in drinking water for 52 weeks	Increased relative liver weight ^b : 4.6±0.9, 6.3±2.2**, 7.0±3.1**	LOAEL: 55
DeAngelo et al. (1997)	Male F344/N rats (19-24/dose)	0, 3.6, 32.5, 364 mg/kg-day in drinking water for 104 weeks	Decreased body weight (data presented graphically ^c)	NOAEL: 32.5

* Statistically significant from control with Fischer's Exact test, p<0.05

** Statistically significant from control with Student's t-test, p<0.05

^a The high and mid doses are identified as the LOAEL and the NOAEL, respectively. While there was a 30% incidence of necrosis at the mid-dose, due to the small sample size, there was not enough statistical power to detect significant differences at this dose.

^b Values represent mean ± standard deviation.

^c Statistical significance was noted at high dose on the graph.

BMD modeling was performed on the TCA noncancer datasets presented in Table 10.6 using a BMR of 5% for dichotomous data or 1 standard deviation from the control mean for continuous data. The modeling results are presented in Table 10.7. Model selection criteria include a goodness of fit p-value >0.05, the lowest AIC, and a scaled residual value no greater than the absolute value of two. In several instances no acceptable model fit could be achieved and those are not further evaluated in this assessment.

Table 10.7 BMD modeling results^a for candidate critical studies of TCA

Study reference (study duration)	Endpoint	Model	Goodness of fit p-value	BMD ₀₅ (mg/kg-day)	BMDL ₀₅ (mg/kg-day)
DeAngelo et al. (2008) Study 1 (60 weeks)	Hepatocellular necrosis (transient)	LogLogistic	0.4965	19.2	8.45
DeAngelo et al. (2008) Study 1 (60 weeks)	Increased relative liver weight	Poor model fit			
Bull et al. (2002) (52 weeks)	Increased relative liver weight	Poor model fit			
DeAngelo et al. (1997)	Decreased body weight	Data were presented in a graph and could not be modeled ^b			

^a Only the best model, based on criteria described in Appendix D, is shown for each endpoint; the goodness of fit p-value ≥ 0.1 indicates the model describes observed data sufficiently well.

^b Only means presented in the graph, and no measure of variance.

Several chronic toxicity studies (Table 7.7) have demonstrated that the liver is the target organ for TCA toxicity. DeAngelo et al. (2008) Study 1 is a well-executed chronic study in mice reporting several toxicological effects consistent with liver pathology. The endpoints included increased relative liver weight, hepatocellular necrosis and inflammation, and peroxisome proliferation. Increased relative liver weight provides the lowest NOAEL (7.7 mg/kg-day), but the model fit was poor. The hepatocellular necrosis data provides the only BMDL (8.45 mg/kg-day) among the candidate critical endpoints. The NOAEL for this endpoint (68.2 mg/kg-day) is higher than the BMDL₀₅, and this is likely due to the small number of animals examined (10/dose), which would result in decreased statistical power and lower sensitivity to detect a dichotomous effect. Note that at the NOAEL, there were 3 out of 10 animals that exhibited liver necrosis.

Serum LDH was also increased at the mid and high dose in Study 1 at 30 weeks and similar to hepatocellular necrosis, abated by week 60. With measurable increases in LDH activity in serum it seems reasonable to conclude that cellular injury and death is occurring at significant levels in the only organ (the liver) where lesions were found in TCA-exposed animals.

While hepatocellular necrosis is reported as ‘mild’ in the original report (DeAngelo et al., 2008), it is worth noting that 25-50% of the liver was affected by necrosis (observed as a fraction of the microscopic field of the analyzed liver samples). Hepatocellular necrosis describes autolytic cellular death in the liver, an organ responsible for several critical vital functions in the body; therefore, substantial morphological damage to this organ would be considered adverse. Increased relative liver weight and hepatocellular necrosis were observed at comparable doses and indicate the liver as a target organ of TCA noncancer toxicity. Since BMD analysis is OEHHA’s preferred approach, the BMDL₀₅ of 8.45 mg/kg-day for hepatocellular necrosis is chosen as the point of departure (POD) for PHG determination. The choice of hepatocellular necrosis as the critical endpoint is consistent with the approach used by US EPA in its

toxicological assessment for TCA (US EPA, 2011). Although the observed hepatic necrosis was transient, specifically observed at 30-45 weeks and not at 60 weeks, the concern for potential relevance in human health supports the use of this endpoint for PHG derivation.

A BMR of 5% was chosen for dose-response analysis of this noncancer endpoint (see *Point of Departure* section). The BMDL₀₅ of 8.45 mg/kg-day for hepatocellular necrosis (DeAngelo et al., 2008) is chosen as the POD for noncancer effects. A combined UF of 1,000 includes UFs of 10 for interspecies extrapolation, 30 for human variability ($\sqrt{10}$ for pharmacodynamics and 10 for pharmacokinetics, to allow for diversity, including infants and children with no kinetic data (OEHHA, 2008)), and $\sqrt{10}$ for database deficiencies. The UF of 10 for interspecies extrapolation would account for the apparent differences in elimination kinetics between mice and humans. Severe adverse developmental effects (e.g., decreased live fetuses per litter, increased percent post-implantation loss) were found in several developmental/reproductive toxicity studies employing relatively high TCA concentrations (Smith et al., 1989; Johnson et al., 1998; Fisher et al., 2001), and the occurrence of developmental effects at lower concentrations remains unknown. There is no multi-generation reproductive and developmental toxicity study. This limitation in the developmental toxicity database prompted the use of the database deficiency UF of $\sqrt{10}$. This combined UF of 1,000 is applied to the following ADD calculation:

$$\text{ADD} = 8.5 \text{ mg/kg-day} \div 1,000 = 0.0085 \text{ mg/kg-day}$$

Monobromoacetic Acid

No chronic studies with MBA are available. However, there are two published oral MBA studies of subacute to subchronic duration, summarized in Table 10.8. Linder et al. (1994a) determined a NOAEL of 25 mg/kg-day for male reproductive effects in rats exposed to this dose of neutralized MBA for 2 weeks. The limitations of this study include short duration, limited number of examined effects, single dose and the overall lack of toxic effects.

The Dalgaard-Mikkelsen et al. (1955) study is a multigenerational study in pigs, in which the first (F₀) generation control group comprised one boar and 5 sows and the exposed group comprised 2 boars and 5 sows. Five F₀ animals did not have adverse effects following the 15-month exposure to an estimated average dose of 5 mg/kg-day MBA, while two sows died earlier in the study and were removed because the cause of death was not determined to be treatment-related. Animals in the second generation (F₁) demonstrated an array of toxic effects, including skeletal muscle degeneration. When doses were raised to 800 mg/day for some animals, severe toxicities were reported. At 133 days, this dose was equivalent to approximately 12 mg/kg-day. For other animals at 400 mg/day, no toxicity was reported for two and skeletal muscle degeneration was reported for the third animal.

OEHHA selects Dalgaard-Mikkelsen et al. (1955) as the critical study. The average dose of 5 mg/kg-day, calculated as outlined above, for the five surviving animals in the first generation is determined to be the NOAEL. This value is supported by the limited data provided in the second generation study.

Table 10.8 Candidate studies for MBA noncancer effects

Reference	Sex/Species	Dose/Route of Exposure	Endpoint	NOAEL/LOAEL (mg/kg-day)
Linder et al. (1994a)	Male Sprague-Dawley rats (8/dose)	0, 25 mg/kg-day gavage for 2 weeks	Weight of testes, epididymis, seminal vesicles, and ventral prostate, serum testosterone, testicular sperm head counts, sperm morphology and motility were not affected (quantitative data not presented in paper)	NOAEL: 25
Dalgaard-Mikkelsen et al. (1955)	Male and female pigs (F ₀ generation: 6 in control group, 7 in MBA group)	F ₀ : average of 5 mg/kg-day in feed for 15 months	No toxic effects in first generation animals	NOAEL: 5

The proposed NOAEL of 5 mg/kg-day has been calculated with a high level of uncertainty, due to the non-optimal design and poor reporting of the Dalgaard-Mikkelsen et al. (1955) study. However, this remains the most complete study of MBA toxicity among available reports, due to its in vivo nature, examination of multiple toxic endpoints, employment of replicate subjects in treatment, and longer exposure duration. The proposed NOAEL (5 mg/kg-day) would be health-protective against endpoints examined in other MBA reports, including Linder et al. (1994a) (NOAEL of 25 mg/kg-day for male rat reproductive endpoints) and the unpublished study by Joniker (1998) (NOAEL of 10.3 mg/kg-day for male rat reductions in body weight and food intake (as reported in European Commission (2013))).

While the developmental and reproductive toxicity results reported by Dalgaard-Mikkelsen et al. (1955) indicate these effects could occur at relatively low doses, no LOAELs/NOAELs were determined for these endpoints because the study suffered from small sample sizes, poor study design, limited number of endpoints evaluated, and limited reporting of dose and body weight information. The only other study reporting on reproductive toxicity is Linder et al. (1994a), which only examined male rats for up to two weeks.

The average exposure duration in the 1st generation (Dalgaard-Mikkelsen et al. (1955)) was 13.8 months, or approximately 4% of the 28-year average lifespan of pigs (US EPA, 1988). This exposure duration would require a subchronic uncertainty factor of 10 (Table 10.1). However, due to OEHHA's policy for the composite UF not to exceed 3,000, the subchronic UF for this assessment based on Dalgaard-Mikkelsen et al. (1955) was set at $\sqrt{10}$.

Additionally, MBA is not assessed for carcinogenicity in this document due to the absence of carcinogenicity studies. However, MBA demonstrated genotoxic potential in several in vitro studies, including positive findings in a chromosomal aberration assay and in DNA damage and mutation assays (Table 8.2). Because there are limited data indicating the potential for both cancer and adverse developmental/reproductive effects, a database deficiency uncertainty factor of $\sqrt{10}$ is applied to account for this in the ADD (acceptable daily dose) derivation.

Thus, the combined uncertainty factor for MBA noncancer ADD derivation is 3,000, consisting of 10 for interspecies extrapolation, 30 for intraspecies variability, $\sqrt{10}$ for subchronic to chronic extrapolation, and $\sqrt{10}$ for database deficiency.

An alternative approach to risk assessment of DBPs where the toxicity database is poor is to evaluate the available data from in vitro toxicity tests, and metabolism and other mechanistic considerations to try to develop relative potencies (NTP, 2018). However, among HAA5, tri- and di-halogen substituted compounds (TCA, DCA, DBA) appear to have distinct mechanism(s) of toxicity from that of MBA, and while MCA appears somewhat similar to MBA in most in vitro tests, its potency is much lower than that of MBA (Table 8.4). Therefore, it is OEHHA's opinion that the current in vitro data would not provide a convincing rationale for an in vivo extrapolation of a health-protective ADD from another HAA5 compound to MBA.

The ADD is calculated as follows:

$$\text{ADD} = 5 \text{ mg/kg-day} \div 3,000 = 0.0017 \text{ mg/kg-day}$$

Dibromoacetic Acid

Adverse effects at specific organs as well as reproductive and developmental effects have been reported in several subchronic and chronic studies, and those indicating relatively low NOAELs/LOAELs/BMDLs are considered as candidate critical studies for POD selection. These studies are summarized in Table 10.9.

A LOAEL of 2 mg/kg-day was identified for nephropathy in female rats in the NTP (2007) chronic toxicity study. However, based on histopathological similarities, the nephropathy observed in the NTP (2007) study likely represents chronic progressive nephropathy (CPN), which occurs spontaneously, mostly in older rats. This effect was not observed in other subchronic or chronic studies of DBA. It is worth noting that the lesions observed in female rats were ranked as minimum to mild and there was no dose-dependent increase in severity. For these reasons (lack of this effect in other long-term studies, mild severity, lack of a dose-dependent increase in severity, and high background incidence), OEHHA is not considering nephropathy in female rats as a candidate critical endpoint.

Table 10.9 Candidate critical studies for noncancer effects of DBA

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL/ BMDL (mg/kg-day)
NTP (2007a)	Male and female F344/N rats (50/sex/dose)	0, 50, 500, or 1,000 mg/L (0, 2, 20, or 40 mg/kg-day for males; 0, 2, 25, or 45 mg/kg-day for females) neutralized DBA in drinking water for 2 years	Hepatic cystic degeneration in males	NOAEL: 2 ^a BMDL ₀₅ : 4.0
NTP (2007a)	Male and female F344/N rats (50/sex/dose)	0, 50, 500, or 1,000 mg/L (0, 2, 20, or 40 mg/kg-day for males; 0, 2, 25, or 45 mg/kg-day for females) neutralized DBA in drinking water for 2 years	Alveolar epithelial hyperplasia in females	NOAEL: 2 BMDL ₀₅ : 4.3
Veeramach aneni et al. (2007)	Dutch-Belted rabbits, pregnant dams and offspring (≥10 dams/dose; 10-22 pups/dose)	0, 1-1.25, 5.2-6.7, or 55-61 mg/kg-day ^b neutralized DBA in drinking water from GD15 to weaning at 6 weeks, continuing in offspring to 12 or 24 weeks	F ₁ : Decrease in morphologically normal sperm, lesions in seminiferous epithelium	LOAEL: 1
Bodenstein er et al. (2004)	Dutch-Belted rabbits, pregnant dams and offspring (≥10 dams/dose; 6-10 pups/dose)	0, 1-1.2, 5.5-6.7, or 50-58 mg/kg-day ^b neutralized DBA in drinking water from GD15 to weaning at 6 weeks, continuing in offspring to 12 or 24 weeks	Reduced primordial follicles in F ₁ at 24 weeks	NOAEL: 1

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL/ BMDL (mg/kg-day)
Christian et al. (2002)	Male and female ^c Crl Sprague-Dawley rats (30/sex/dose)	P: 0, 50, 250, or 650 mg/L (0, 4.4, 22.4, or 52.4 mg/kg-day for males; 0, 6.9, 32.4, or 79.4 mg/kg-day for females) DBA in drinking water for 92 days F ₁ : exposure during gestation and lactation, and exposure at same concentrations as P generation for a minimum of 71 days post weaning, continuing through mating (14 days), gestation (21 days) and lactation (15 days)	Increase in relative liver weight	LOAEL: 4.4 BMDL _{1SD} : 0.82 (P males)
Christian et al. (2002)	Male and female ^c Crl Sprague-Dawley rats (30/sex/dose)	P: 0, 50, 250, or 650 mg/L (0, 4.4, 22.4, or 52.4 mg/kg-day for males; 0, 6.9, 32.4, or 79.4 mg/kg-day for females) DBA in drinking water for 92 days F ₁ : exposure during gestation and lactation, and exposure at same concentrations as P generation for a minimum of 71 days post weaning, continuing through mating (14 days), gestation (21 days) and lactation (15 days)	Altered sperm production and delayed spermiation	NOAEL: 4.4

* Statistically significant from control with Fischer's Exact test, p<0.05

^a This dose is reported as LOAEL in the original study (NTP, 2007a) using poly-3 test, however the difference from control is not significant when the exact Fisher's exact test is applied.

^b A dose range is presented because there were slight differences in administered dose between dams and pups, and in pups receiving DBA for 12 or 24 weeks.

^c Christian et al. (2002) also examined female rats in reproductive experiments. These animals were sacrificed following gestation and lactation phases. However, these data were not included because of dramatically changed water intake and dose during lactation.

BMD modeling was performed on the datasets from the studies presented in Table 10.9. The only studies that generated acceptable BMD models were NTP (2007a) and Christian et al. (2002). The Bodensteiner et al. (2004) and Veeramachaneni et al. (2007) datasets did not produce adequate model fits, or generated BMDL values that were >250 fold below the BMD, and thus were not considered for POD determination. Delayed spermiation in the F1 generation were not modeled because individual animal data were not available, and thus there was no way to account for litter effects. Details of the models and the outputs are provided in Appendix D. Where available, the BMDL values are presented in Table 10.10 for comparison of sensitivity of endpoints in the candidate critical studies and the corresponding detailed BMDS results are presented in Table 10.10.

Table 10.10 BMD modeling results^a for candidate critical studies of DBA

Study/ species/ sex	Endpoint	Model	Goodness of fit p-value	BMD ₀₅ (mg/kg-day)	BMDL ₀₅ (mg/kg-day)
NTP (2007) Male rat	Hepatic cystic degeneration	LogLogistic	0.2406	7.3	4.0
NTP (2007) Female rat	Alveolar epithelial hyperplasia	LogLogistic	0.4119	7.5	4.3
Christian et al. (2002) Male rat	Relative liver weight	Exponential4	0.5828	1.2 ^b	0.82 ^b
Christian et al. (2002) Male rat	Retention of step 19 spermatids	Probit	0.5029	5.3	4.1
Christian et al. (2002) Male rat	Abnormal/increased residual bodies	Probit	0.5852	4.4	3.5

^a Only the best model, based on criteria described in Appendix D, is shown for each endpoint; the goodness of fit p-value ≥ 0.1 indicates the model describes observed data sufficiently well.

^b For continuous data, the benchmark response (BMR) is 1 standard deviation (SD) from the control mean. Thus, these values are BMD_{1SD} and BMDL_{1SD}, respectively.

BMD modeling of the increased relative liver weight data in rats in the Christian et al. (2002) study obtained a BMDL_{1SD} of 0.82 mg/kg-day. However, the response for increased relative liver weight at the low dose is approximately 80% of the maximum, and these types of datasets, i.e., high response at the lowest dose, are not ideal for producing reliable BMDL estimates.

The relatively high BMDLs from two of the NTP (2007) datasets in Table 10.10 are close to or higher than some of the NOAELs (Table 10.9) from the datasets that are not amenable to BMDS modeling, thus they are not selected as PODs. The Bodensteiner et al. (2004) study indicates a NOAEL of 1 mg/kg-day for reduced primordial follicles in female rabbits, but the authors note that DBA exposure in this study did not affect patterns of antral follicular growth or the ovulatory response and further studies are needed to determine effects later in the

reproductive cycle. Veeramachaneni et al. (2007) and Christian et al. (2002) indicate LOAELs of 1 mg/kg-day and 4.4 mg/kg-day, respectively, for male reproductive effects.

Testicular lesions and decreased incidence of morphologically normal sperm in male rabbits (Veeramachaneni et al., 2007) appear to be the most sensitive endpoints and are supported by several other reports of reproductive toxicity in male rats (Linder et al., 1994a; Linder et al., 1994b; Linder et al., 1995; Linder et al., 1997; Tsuchiya et al., 2000; Christian et al., 2002; Klinefelter et al., 2004; NTP, 2007a) and male mice (NTP, 2007a). Other endpoints such as nephropathy and reduced primordial follicles lack corroborating studies and are somewhat less sensitive, with NOAEL or BMDL values at approximately the same level as the LOAEL in Veeramachaneni et al. (2007). This supports selection of the LOAEL of 1 mg/kg-day from the Veeramachaneni et al. (2007) study as the POD for determining the noncancer health-protective concentration.

The LOAEL of 1 mg/kg-day for male reproductive toxicity, specifically testicular lesions and increased morphologically abnormal sperm in F₁ male rabbits (Veeramachaneni et al., 2007), is selected as the POD. This determination is supported by many reproductive and developmental toxicity studies showing DBA also caused adverse effects in sperms and testis in rodents (Table 9.8). Morton (1988) reports that exposure to a toxicant should occur for at least 6 cycles of the seminiferous epithelium (each cycle is 10.7 days), or 64 days, to best assess toxicity in reproductive studies in male rabbits. Male rabbits in the Veeramachaneni et al. (2007) study were exposed for 24 weeks, and although this duration would be considered subchronic for typical animal studies, it is sufficiently long to evaluate the chronic effects of DBA on sperm in rabbits. Thus, a subchronic to chronic extrapolation uncertainty factor is not applied. A combined UF of 3,000 is applied for ADD calculation, consisting of 10 for LOAEL to NOAEL extrapolation, 10 for interspecies extrapolation, and 30 for human variability ($\sqrt{10}$ for pharmacodynamics and 10 for pharmacokinetics, to allow for diversity, including infants and children with no kinetic data (OEHHA, 2008)).

The ADD is calculated using the following equation:

$$\text{ADD} = 1 \text{ mg/kg-day} \div 3,000 = 0.0003 \text{ mg/kg-day}$$

Cancer Dose-Response Analyses and Cancer Potency Derivation

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-day)⁻¹). 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), but are inadequate for use in estimating the cancer potency of HAAs due to the confounding presence of other carcinogenic DBPs in drinking water. Therefore, the cancer potency estimation of HAA5 relies on data from animal studies testing individual HAA5 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. Also, adenoma and carcinoma incidences in the same tissue may be combined, as adenomas may represent early stages of tumor development, and may progress to carcinomas.
- Routes of exposure from tap water use. As shown in previous sections, HAAs are not volatile and have relatively low permeability through skin. Inhalation and dermal exposures are considered negligible for calculation of health-protective values for the HAAs.
- 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 HAAs are evaluated to determine whether human risk should be estimated assuming low-dose linearity or otherwise. This evaluation was conducted in the carcinogenicity section of DCA, TCA and DBA. 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 linearized multistage 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):

$$p(d) = \beta + (1 - \beta) \times \exp[-(q_1d + q_2d^2 + \dots + q_id^i)]$$

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 (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 HAAs using the previously described US EPA BMDS (Version 2.7), and the dose associated with a benchmark response (BMR) 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 ≤ 2 ; and
- The dose-response curve is inspected visually for adequacy of fit.

In modeling cancer datasets, all available LMS models are analyzed (at least 1st degree LMS and 2nd degree LMS for a 3-dose study). Among the LMS models with acceptable fit ($p > 0.05$), the model with the fewest parameters is chosen based on the scientific principle of parsimony, which is consistent with BMDS guidance. It should be noted that the Multistage Weibull (MSW) Time-to-Tumor model does not report a p-value or scaled residuals.

The results presented here are for the acceptable fits to the datasets analyzed for each HAA. 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 BMR of 5%, or 0.05, by the $BMDL_{05}$. (The result is typically a value close to the upper 95% confidence bound on the parameter q_1 .)

$$CSF_{animal} = BMR \div BMDL_{05} = 0.05 \div BMDL_{05}$$

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 (Portier et al., 1986):

$$CSF_{\text{animal}}^{\text{adj}} = CSF_{\text{animal}} \times (T \div T_e)^3.$$

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 (OEHHA, 2009). 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-day)⁻¹:

$$CSF_{\text{human}} = CSF_{\text{animal}}^{\text{adj}} \times (bw_h \div bw_a)^{1/4}.$$

Animal body weights used in calculating CSF_{human} were averaged weights of the control group provided in the study.

Dichloroacetic Acid

There are several rodent cancer bioassays with exposure to DCA alone showing evidence of tumorigenesis (summarized in Table 6.10). The most sensitive and consistent endpoint was hepatic tumors (combined adenomas and carcinomas) in male B6C3F1 mice (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Daniel et al., 1992; Anna et al., 1994; Ferreira-Gonzalez et al., 1995; DeAngelo et al., 1999; Bull et al., 2002; Wood et al., 2015; Wehmas et al., 2017). Three bioassays in female B6C3F1 mice (Pereira, 1996; Schroeder et al., 1997; Wood et al., 2015) found a significant increase in hepatic adenomas and carcinomas as well, at higher DCA doses.

The available male F344 rat studies (Richmond et al., 1995; DeAngelo et al., 1996) observed significant toxicity at the highest dose, resulting in early sacrifice and/or progressively decreased dose. While the multi-dose study of Richmond et al. (1995) observed significantly increased hepatic adenomas at the highest dose (296 mg/kg-day), and the single dose (plus control) study of DeAngelo et al. (1996) observed significantly increased hepatic carcinomas, and adenomas and carcinomas at the highest dose (139 mg/kg-day), neither is considered as a candidate critical study for DCA carcinogenesis due to increased toxicity at the doses where tumors were observed, and due to lower sensitivity of the studies.

Among cancer studies in B6C3F1 mice, only three reports employed a multi-dosing experimental design and demonstrated a statistically significant increase in tumor incidences at two or more doses. These studies include DeAngelo et al. (1999), Bull et al. (2002) and Wood et al. (2015) and are considered as candidate studies for PHG derivation (summarized in Table 10.11). The DeAngelo et al. (1999) study included two interim sacrifice groups (at 52 and 78 weeks) that are considered separately from the 100 week group. The experimental design in the Wood et al. (2015) study (exposure over first 10 weeks in a 94-week study) requires the

Armitage-Doll dose adjustment, which takes into consideration the effects of discontinuous treatment earlier in the cancer study.

Table 10.11 DCA candidate cancer studies (summary)

Reference (study duration)	Species/ Sex	Doses (#/dose)	Tumor (hepatic)	Incidence	Notes
DeAngelo et al. (1999) (52 weeks)	B6C3F1 mice male	0, 84, 168, 315, 429 mg/kg-day (10/dose)	adenoma or carcinoma	0/10 ^a , 1/10, 1/10, 2/10, 7/10*	Small number of animals used, duration adjustment required
DeAngelo et al. (1999) (78 weeks)	B6C3F1 mice male	0, 84, 168, 315, 429 mg/kg-day (10/dose)	adenoma or carcinoma	2/10 ^a , 1/10, 4/10, 8/10*, 9/10*	Small number of animals used, duration adjustment required
DeAngelo et al. (1999) (100 weeks)	B6C3F1 mice male	0, 8, 84, 168, 315, 429 mg/kg-day (11-50/dose)	adenoma or carcinoma	18/50 ^a , 11/33, 14/25, 30/35*, 21/21*, 11/11*	Treatment-related mortality, decreased body weights (at two highest doses)
DeAngelo et al. (1999) (52- 100 weeks)	B6C3F1 mice male	0, 8, 84, 168, 315, 429 mg/kg-day	adenoma or carcinoma	20/70 ^a , 11/33, 16/45, 35/55*, 31/41*, 27/31*	Treatment-related mortality
Bull et al. (2002) (52 weeks)	B6C3F1 mice male	0, 11, 54, 216 mg/kg-day (20/dose)	adenoma or carcinoma	0/20 ^a , 1/20, 5/20*, 10/19*	Dichloroacetate, salt unspecified; duration adjustment required
Wood et al. (2015) (94 weeks: 10 weeks treatment+ 84 weeks recovery)	B6C3F1 mice male	0, 136, 232, 297 mg/kg-day (26-27/dose)	adenoma, carcinoma or hepatoblastoma	12/27 ^a , 15/27, 14/27, 24/26*	Treatment was only during first 10 weeks; doses require Armitage-Doll adjustment
Wood et al. (2015) (94 weeks: 10 weeks treatment+ 84 weeks recovery)	B6C3F1 mice female	0, 142, 253 mg/kg-day (26-27/dose)	adenoma, carcinoma or hepatoblastoma	0/27 ^a , 10/26*, 9/28*	Treatment was only during first 10 weeks; doses require Armitage-Doll adjustment

*Statistically different from control using Fisher's exact test (p<0.05)

^aSignificant trend (p<0.05) in Cochran Armitage trend test

Combined cancer incidence data for hepatocellular adenomas and carcinomas were analyzed using the linear multi-stage (LMS) cancer model (BMDS version 2.7, US EPA) to estimate the

lower 95% confidence limit of the dose associated with a 5% increased risk of developing a tumor (BMDL₀₅). The results of BMDS LMS analyses of the candidate DCA cancer data sets are provided in Table 10.12.

Table 10.12 DCA candidate cancer studies (BMDS analysis and CSF calculation)

Reference (study duration)	Species/ Sex	Tumor type (hepatic)	Model	BMD ₀₅ / BMDL ₀₅ mg/kg-day (LMS model polynomial) p-value ^a	Animal CSF (mg/kg-day) ⁻¹	Human CSF (mg/kg-day) ⁻¹
DeAngelo et al. (1999) (52 weeks)	B6C3F1 mice male	adenoma or carcinoma	LMS	36.6/23.0 (1 st degree) p=0.37	0.002176	0.0139 (0.11 ^b)
DeAngelo et al. (1999) (78 weeks)	B6C3F1 mice male	adenoma or carcinoma	LMS	19.2/12.3 (1 st degree) p=0.28	0.00406	0.026 (0.062 ^b)
DeAngelo et al. (1999) (52-100 weeks combined data)	B6C3F1 mice male	adenoma or carcinoma	MSW	32.7/7.86	0.00636	0.041
Bull et al. (2002) (52 weeks)	B6C3F1 mice male	adenoma or carcinoma	LMS	12.9/8.7 (1 st degree) p=0.89	0.00574	0.036 (0.29 ^b)
Wood et al. (2015) (94 weeks)	B6C3F1 mice male	adenoma, carcinoma or hepatoblastoma	Poor model fit (LMS)			
Wood et al. (2015) (94 weeks)	B6C3F1 mice female	adenoma, carcinoma or hepatoblastoma	LMS ^c	2.52/1.76 (1 st degree) p=0.21	0.02835	0.217 (0.29 ^b)

^a The polynomial degree of the linear multistage (LMS) model is indicated in parenthesis followed by the p-value for model fit

^b adjusted for shorter exposure

^c 1st degree polynomial LMS model using time-averaged doses

NA, not applicable

Mathematical cancer models: LMS, linear multi-stage; MSW, multi-stage Weibull.

While Bull et al. (2002) and Wood et al. (2015) showed tumors induced at comparable doses to DeAngelo et al. (1999), these studies had limitations in terms of shorter exposure duration (52 weeks, and 10 weeks treatment with 84 weeks follow-up, respectively). As a result, these studies introduce additional uncertainty in requiring extrapolation to lifetime exposure. Bull et al. (2002) and Wood et al. (2015) produce higher slope factors, but in doing so require the application of additional models to the data, such as a poly-3-based duration adjustment and the

Armitage-Doll-based dose correction. Thus, the combined 52-100 weeks dataset by DeAngelo et al. (1999) is chosen as the critical study.

To incorporate tumor data from dose groups with different durations of exposure and to account for treatment-related mortality, US EPA's Multistage Weibull (MSW) Time-to-Tumor Model software² (2010) was applied to the DeAngelo et al. (1999) data set of combined time points (52-100 weeks). The MSW model does not report a χ^2 goodness-of-fit table (p-value or scaled residuals). Similar to the poly-3 method, the MSW model adjusts tumor rates for possible underestimates due to early treatment-dependent mortality. Contrary to the Bull et al. (2002) study however, the majority of animals in the combined DeAngelo et al. (1999) data set survived to the near-lifetime term of 100 weeks, decreasing uncertainty in the applied survival adjustment. Tumors are modeled as incidental since no cause of death was established. Unlike the poly-3 method used on the independently derived BMDL₀₅ and CSF_{animal} (as described above), the MSW method produces the BMDL₀₅ value already adjusted for time-to-tumor differences. The dose associated with a 5% increased risk of developing tumors (BMD₀₅) and the 95% lower confidence limit on that dose (BMDL₀₅) are 32.7 and 7.86 mg/kg-day, respectively, for the 2nd-degree polynomial. The model output is shown in Appendix E. The animal CSF was calculated as follows:

$$\text{CSF}_{\text{animal}} = \text{BMR} \div \text{BMDL}_{05} = 0.05 \div 7.86 \text{ (mg/kg-day)}^{-1} = 0.0064 \text{ (mg/kg-day)}^{-1}.$$

Since the MSW method was used for BMDL₀₅ derivation in this case, no additional time adjustment was needed. The human CSF was calculated based on allometric conversion using the average human weight (70 kg) and the average animal weight in the control group (0.041 kg) in DeAngelo et al. (1999):

$$\text{CSF}_{\text{human}} = 0.0064 \text{ (mg/kg-day)}^{-1} \times (70 \text{ kg} \div 0.041 \text{ kg})^{1/4} = 0.041 \text{ (mg/kg-day)}^{-1}.$$

This value is utilized for derivation of a human health-protective concentration for DCA based on cancer.

Trichloroacetic Acid

The most consistent findings of the carcinogenicity of TCA were hepatocellular adenomas and carcinomas in male B6C3F1 mice, reported in at least six studies (Herren-Freund et al., 1987; Bull et al., 1990; Ferreira-Gonzalez et al., 1995; Pereira et al., 2001; Bull et al., 2002; DeAngelo et al., 2008). Several studies, summarized in Table 7.8, also reported hepatocellular adenomas and carcinomas in female B6C3F1 mice, but some studies either did not find any evidence of such tumors at a relatively high dose and with chronic administration of TCA (Bull et al., 1990), or found very small incidences at high doses only (Pereira, 1996; Pereira and Phelps, 1996). Since it appears that the endpoint of hepatocellular tumors in female B6C3F1 mice was less

² Available for download at: <https://cfpub.epa.gov/ncea/bmds/recordisplay.cfm?deid=217055>

sensitive in comparison to male mice, only studies of hepatocellular tumors in male mice were considered for dose-response assessment and PHG derivation.

Of the six studies with male B6C3F1 mice, only three included multiple doses (Bull et al., 1990; Bull et al., 2002; DeAngelo et al., 2008), and of these three, the study of Bull et al. (1990) was the least sensitive, based on the observed tumor response. For this reason, hepatocellular tumor subsets from Bull et al. (2002) and multi-dose subsets from DeAngelo et al. (2008) (Study 1 and Study 3) were analyzed as TCA candidate cancer studies (Table 10.13 and 10.14). Because there was no significant treatment-related mortality and individual animal data (obtained from US EPA) were available (DeAngelo et al., 2008), the number of animals alive at the first occurrence of tumor was used as the denominator in calculating tumor incidence. The earliest time points for hepatocellular tumor detection were week 45 and week 52 for Studies 1 and 3, respectively. To increase the number of animals per dose and therefore the statistical power of the study, animals from the main time groups (60 weeks and 104 weeks for Studies 1 and 3, respectively) and intermediate groups with detected hepatocellular tumors (≥ 45 and ≥ 52 weeks, respectively) were pooled, resulting in the incidences presented in Table 7.8. The Bull et al. (2002) study lacked individual animal data, and no mortality adjustment was performed.

Combined cancer incidence data for hepatocellular adenomas and carcinomas were analyzed using the linear multi-stage (LMS) cancer model (BMDS version 2.7, US EPA) to estimate the BMDL₀₅. Results of BMD analysis and calculated animal and human cancer slope factors are shown in the Table 10.14.

Table 10.13. TCA candidate cancer studies

Reference (study duration)	Species/ Sex	Doses (#/dose)	Tumor	Incidence	Notes
Bull et al. (2002) 52 weeks	Male B6C3F1 mice (20/dose)	0, 55, or 238 mg/kg-day	hepatic adenoma and carcinoma	0/20 ^a , 6/20*, 8/20*	Duration adjustment required
DeAngelo et al. (2008) 45-60 weeks	Male B6C3F1 mice (30/dose)	Study 1: 0, 7.7, 68.2, or 602.1 mg/kg-day	hepatic adenoma and/or carcinoma	4/35 ^a , 5/32, 12/34*, 19/34*	Duration adjustment required
DeAngelo et al. (2008) 104 weeks	Male B6C3F1 mice (72/dose)	Study 3: 0, 6.7, or 81.2 mg/kg-day	hepatic adenoma and/or carcinoma	31/56 ^a , 21/48, 36/51	None

*Statistically different from control using Fisher's exact test ($p < 0.05$)

^aSignificant trend ($p < 0.05$) in Cochran Armitage trend test

Table 10.14 TCA candidate cancer studies (BMD5 analysis and CSF calculation)

Reference/ Study Duration	Species/ Sex	Tumor Type	Model	BMD ₀₅ / BMDL ₀₅ mg/kg-day (LMS model polynomial) p-value ^a	Animal CSF (mg/kg- day) ⁻¹	Human CSF ^b (mg/kg- day) ⁻¹
DeAngelo et al. (2008) Study 1 60 weeks ^c	B6C3F1 mice male	Hepatocellular adenoma or carcinoma	LMS	45.4/27.4 (1 st degree) p=0.15	0.0018	0.061 ^{c,d,e}
DeAngelo et al. (2008) Study 3 104 weeks ^d	B6C3F1 mice male	Hepatocellular adenoma or carcinoma	LMS	8.1/4.4 (1 st degree) p=0.16	0.011	0.071
Bull et al. (2002) 52 weeks	B6C3F1 mice male	Hepatocellular adenoma or carcinoma	LMS	16.9/11.2 (1 st degree) p=0.11	0.0045	0.22 ^e

^a The polynomial degree of the linear multistage (LMS) model is indicated in parenthesis followed by the p-value for model fit

^b For the calculation of the human CSF from the animal CSF, the mouse weights are either the lifetime averaged weight in the control group (DeAngelo et al., 2008) or the final body weight in the control group (Bull et al., 2002)

^c Pooled surviving animals from 45-week and 60-week groups

^d Pooled surviving animals from 52-104 weeks

^e Adjusted for exposure duration to 104 weeks, with $(104/T_e)^3$, where $T_e = 60$ weeks (DeAngelo et al. (2008), Study 1), $T_e=52$ weeks (Bull et al. (2002))

Among the three candidate critical studies, the 104-week Study 3 from DeAngelo et al. (2008) produced the lowest BMDL₀₅ and, unlike the 60-week Study 1 by DeAngelo et al. (2008) and the 52-week study by Bull et al. (2002), Study 3 did not require a time adjustment to 104 weeks for a lifetime study, which would introduce additional uncertainty. Therefore, the Study 3 subset of DeAngelo et al. (2008) was chosen as the critical study for TCA cancer dose-response analysis. The output of all BMD5 runs is shown in Appendix E. The animal CSF was calculated as follows:

$$CSF_{\text{animal}} = BMR \div BMDL_{05} = 0.05 \div 4.4 \text{ (mg/kg-day)}^{-1} = 0.0011 \text{ (mg/kg-day)}^{-1}.$$

The human CSF was calculated based on allometric conversion using the average human weight (70 kg) and the lifetime-averaged animal weight in the control group (0.045 kg) in DeAngelo et al. (2008) Study 3:

$$CSF_{\text{human}} = 0.0011 \text{ (mg/kg-day)}^{-1} \times (70 \text{ kg} \div 0.045 \text{ kg})^{1/4} = 0.071 \text{ (mg/kg-day)}^{-1}.$$

This value is utilized for derivation of a human health-protective concentration for TCA based on cancer.

Both Study 1 (60 weeks) and Study 3 (104 weeks) subsets from DeAngelo et al. (2008) produce comparable CSF estimates. However, since the 104-week Study 3 does not require the time

adjustment, it is chosen as the critical study for TCA cancer dose-response analysis, and the resulting CSF, $0.071 \text{ (mg/kg-day)}^{-1}$, is used for PHG derivation.

While the Bull et al. (2002) study results in a three-fold higher CSF estimate compared to the chosen critical study (Table 10.14), the derivation included the time adjustment from 52 weeks to 104 weeks for a lifetime study, which introduces additional uncertainty. Since Study 3 from DeAngelo et al. (2008) is a high-quality two-year study, Bull et al. (2002) is not chosen as a critical study.

Dibromoacetic Acid

The NTP (2007a) report comprises four 2-year cancer bioassays (male and female mice, male and female rats) and provides evidence of DBA carcinogenesis, primarily based on mouse data. Although male and female rats demonstrated significantly increased incidences and/or significant trends in some tumor types (Table 9.13), the tumor responses observed in the rat studies were modest compared to those observed in the mouse studies. Therefore, only male and female mouse data from the NTP (2007a) report are considered for dose-response assessment. Because there was no significant treatment-dependent early mortality observed in either mouse study, the number of animals alive at the first occurrence of tumor was used as the denominator in calculating the tumor incidences presented in Tables 9.13 and 9.14. Doses used are those reported in the studies.

Combined incidence data for indicated tumor types (either combined liver or alveolar/bronchiolar tumors) are analyzed using the linear multi-stage (LMS) cancer model (US EPA, 2015) to estimate the lower 95% confidence limit (BMDL_{05}) of the dose (BMD) associated with a 5% increased risk of developing a tumor. For the lung tumors in both male and female mice, treatment-related increases in alveolar/bronchiolar adenomas were observed with a statistically significant trend, thus were modeled. Additionally, tumor incidences from both sites (liver and lung) are analyzed with a combined multisite approach, wherein BMDS (MS_Combo) is used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models for the different sites and/or cell types. This multisite model provides a basis for estimating the cumulative risk of carcinogen treatment-related tumors. The results of BMDS LMS and multisite analysis are provided in Table 10.15.

Table 10.15 DBA candidate cancer endpoints in NTP (2007a) study (BMDS analysis and CSF calculation).

Reference/ Study Duration	Species/ Sex	Tumor Type	Model	BMD ₀₅ / BMDL ₀₅ mg/kg-day (LMS model polynomial) p-value ^a	Animal CSF (mg/kg- day) ⁻¹	Human CSF (mg/kg-day) ⁻¹
NTP (2007a) 2 years	B6C3F1 mice male	Hepatocellular adenoma, carcinoma and/or hepato- blastoma	LMS	2.05/1.34 (1 st degree) p=0.051	0.037	0.23
	B6C3F1 mice male	Alveolar/ bronchiolar adenoma	LMS	18.5/10.5 (1 st degree) p=0.064	0.0048	0.030
	B6C3F1 mice male	<i>Multisite</i> ^b : Hepatocellular adenoma, carcinoma and/or hepato- blastoma + Alveolar/ bronchiolar adenoma	BMDS multi- site cancer model	1.85/1.25	0.040	0.25
	B6C3F1 mice female	Hepatocellular adenoma, carcinoma and/or hepato- blastoma	LMS	3.60/2.30 (1 st degree) p=0.27	0.022	0.13
	B6C3F1 mice female	Alveolar/ bronchiolar adenoma	LMS	38.5/18.5 (1 st degree) p=0.55	0.0027	0.017
	B6C3F1 mice female	<i>Multisite</i> ^b : Hepatocellular adenoma, carcinoma and/or hepato- blastoma + Alveolar/ bronchiolar adenoma	BMDS multi- site cancer model	3.29/2.16	0.023	0.14

^a The polynomial degree of the linear multistage (LMS) model is indicated in parenthesis followed by the p-value for model fit

^b The multisite cancer model does not produce a p-value

As shown in Table 10.15, male mice were the most sensitive to the carcinogenic effects of DBA, with BMDS multisite analysis for combined hepatocellular and alveolar/ bronchiolar tumors providing the highest CSF. Therefore, the PHG calculation is based on the human CSF derived

from the male mice data in the 2-year NTP (2007) carcinogenesis study. The animal CSF was calculated as follows:

$$CSF_{\text{animal}} = \text{BMR} \div \text{BMDL}_{05} = 0.05 \div 1.25 \text{ (mg/kg-day)}^{-1} = 0.040 \text{ (mg/kg-day)}^{-1}.$$

The human CSF was calculated based on allometric conversion using the average human weight (70 kg) and the lifetime-averaged animal weight in the control group (0.045 kg) in NTP (2007):

$$CSF_{\text{human}} = 0.040 \text{ (mg/kg-day)}^{-1} \times (70 \text{ kg} \div 0.045 \text{ kg})^{1/4} = 0.25 \text{ (mg/kg-day)}^{-1}.$$

This value is utilized for derivation of a human health-protective concentration for DBA based on cancer.

11. HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS

Health-protective concentrations of HAAs are derived from the ADDs for noncancer effects and from cancer potency factors for cancer effects previously calculated in the *Dose-Response Assessment* section. As discussed in more detail below, calculation of noncancer health-protective concentrations takes into account daily water intake from multiple routes of exposure and relative source contribution for noncancer effects. For cancer effects, age sensitivity factors are applied to daily water intake to account for increased susceptibility of infants and children to carcinogens.

Noncancer Health-Protective Drinking Water Concentrations

Daily Water Intake Equivalent

To calculate a drinking water public health goal, the ADD is converted to a concentration in drinking water that accounts for the exposure to the chemical in tap water. The exposure may include intake of contaminants in tap water via multiple routes, including oral ingestion, inhalation, and dermal contact from household uses (e.g., drinking, cooking, bathing, and showering). This is necessary because inhalation exposure can occur when a chemical volatilizes out of the water and dermal exposure when a chemical is absorbed through the skin. The daily water intake equivalent (DWI) is expressed in the units liters or liter equivalents per kilogram of body weight per day (L/kg-day or $L_{eq}/kg\text{-day}$, respectively). Liter equivalents represent the equivalent 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.

For oral ingestion rates, OEHHA uses age-specific water ingestion estimates (OEHHA, 2012) 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). These age-specific intake rates are normalized to body weight and expressed as L/kg-day. The updated water ingestion rates indicate that drinking water ingestion per unit body weight is higher in infants than in adults. Updates of previous PHGs using default ingestion rates of 2 L/day for adults and 1 L/day for a 10 kg child are using these more refined estimates. For noncancer endpoints, the time-weighted average daily water ingestion rate for a 70-year lifetime for the general population is generally used. However, if the critical effect occurs during exposure of a particularly sensitive age group or other subgroup, the high end estimates of the age-specific water ingestion rate for the subgroup will be used in the PHG calculations (OEHHA, 2012). OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects due to exposure to drinking water contaminants than the general population. These improvements in water ingestion estimates are crucial to the assessment of risk to sensitive subgroups as well as the general population. The lifetime average drinking water consumption rates are calculated as shown in Table 11.1.

Table 11.1 Drinking Water Consumption Rates

Life Stage	Age range (years)	Fractional duration (years)	Oral ingestion (L/kg-day)	Fractional Contribution
3 rd Trimester (pregnancy)	NA	0.75/70	0.047	0.00050
Infant	0-2	2/70	0.196	0.0056
Child	2-16	14/70	0.061	0.0122
Adult	16-70	54/70	0.045	0.0347
Time-weighted average (L/kg-day)				0.0530

NA, not applicable

As noted above, exposure can occur from pathways such as inhalation and dermal absorption while bathing or showering, in addition to ingestion. For example, volatile organic compounds (VOCs) are released from tap water in the shower and can be inhaled. However, since the HAA5 are not volatile and have relatively low permeability through skin, these exposures are considered negligible for calculation of health-protective values for the HAA5.

Relative Source Contribution

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (which may include inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80), and are determined based on available exposure data. The RSC helps to ensure that the PHG identifies a level of a drinking water contaminant that would pose no significant health risk after taking into account exposures to the chemical from food, air pollution and other sources.

Derivation of Noncancer Value

Following determination of the ADD, the health-protective concentration (C, in milligrams/liter, mg/L) in drinking water can be derived by incorporating the total equivalent daily drinking water intake of the chemical (DWI) from tap water and other sources, using the following equation:

$$C = (ADD \times RSC) \div DWI$$

An RSC of 80% is selected for calculation of the health-protective concentration for each of the five HAAs. The use of the 80% value to represent the percentage of total multiroute exposures attributable to drinking water reflects the conclusion that most exposure to these compounds will occur as a result of their formation during drinking water disinfection. Although the available data suggest that exposure from other environmental media is relatively low, use of 80% is considered prudent given the uncertainty regarding exposure from media other than disinfected tap water.

Monochloroacetic Acid

The public health protective level for MCA is calculated as:

$$C = (0.0035 \text{ mg/kg-day} \times 0.8) \div 0.053 \text{ L/kg-day} = 0.0528 \text{ mg/L, rounded to 53 ppb}$$

In accordance with this calculation, a noncancer public health-protective level of 53 ppb is determined for MCA in drinking water. This value takes into account possible sensitive subpopulations. Since there is currently no evidence to indicate MCA is a carcinogen, this value for noncancer effects is selected as the PHG for MCA.

Dichloroacetic Acid

The public health protective level for DCA is calculated as:

$$C = (0.0076 \text{ mg/kg-day} \times 0.8) \div 0.053 \text{ L/kg-day} = 0.115 \text{ mg/L, or 115 ppb}$$

The estimated health-protective concentration for noncancer effects is 115 ppb for DCA.

Trichloroacetic Acid

The public health protective level for TCA is calculated as:

$$C = (0.0085 \text{ mg/kg-day} \times 0.8) \div 0.053 \text{ L/kg-day} = 0.128 \text{ mg/L or 128 ppb}$$

The estimated health-protective concentration for noncancer effects is 128 ppb for TCA.

Monobromoacetic Acid

The public health protective level for MBA is calculated as:

$$C = (0.0017 \text{ mg/kg-day} \times 0.8) \div 0.053 \text{ L/kg-day} = 0.025 \text{ mg/L, or 25 } \mu\text{g/L or 25 ppb.}$$

Thus, it is estimated that a health protective concentration of 25 ppb for MBA in drinking water would be protective against all noncancer effects, including potential effects in sensitive subpopulations. Since there is currently no evidence to indicate MBA is a carcinogen, this value for noncancer effects is selected as the PHG for MBA.

Dibromoacetic Acid

The public health-protective level for DBA is calculated as:

$$C = (0.0003 \text{ mg/kg-day} \times 0.8) \div 0.053 \text{ L/day} = 0.005 \text{ mg/L or 5 ppb}$$

The estimated health-protective concentration for noncancer effects is 5 ppb for DBA.

Cancer Health-Protective Drinking Water Concentrations

When determining cancer risk, OEHHA applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children 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 through 15 years of age. These factors are applied regardless of the mechanism of action, unless chemical-specific data exist to better guide the risk assessment. ASFs are incorporated into the total daily exposure by multiplying the ASF by the total daily water intake (DWI) and the fractional duration of the life stage. Current practice is to use 70 years as the lifetime for humans. The sum of the ASF-

adjusted exposures is the daily lifetime exposure (in L/kg-day) used to derive the PHG (Table 11.2).

Table 11.2 Calculation of ASF-adjusted exposures by life stage

Life Stage	Age Sensitivity Factor (ASF)	Duration (d)	Daily Water Intake (DWI, L/kg-day)	ASF × d × DWI (L/kg-day)
3 rd trimester (Pregnancy)	10	0.25/70	0.047	0.0017
Infant (0-2 yr)	10	2/70	0.196	0.0560
Child (2-16 yr)	3	14/70	0.061	0.0366
Adult (16-70 yr)	1	54/70	0.045	0.0347
Total Lifetime Exposure = $\sum_j[\text{ASF}_j \times d_j \times \text{DWI}_j]$ (L/kg-day)				0.129

Because exposure to HAAs is mainly by oral ingestion, the calculation of a health-protective concentration is based only on exposure through ingestion of drinking water, as follows:

$$C = R \div (p \times \sum_j[\text{ASF}_j \times d_j \times \text{DWI}_j])$$

Where:

- R = default risk level of one in one million, or 10^{-6}
- p = cancer potency in $(\text{mg}/\text{kg}\text{-day})^{-1}$
- \sum_j = sum of drinking water intake contributions at each life stage
- ASF_j = age sensitivity factors for the 3rd trimester fetus, infants, children, and adults
- d_j = duration of exposure for 3rd trimester fetal, infant, child, and adult life stages
- DWI_j = equivalent water intake values for each life stage.

Dichloroacetic Acid

The study of DeAngelo et al. (1999) provides the most appropriate dose-response data for DCA-dependent hepatic tumorigenesis. This study is chosen as the critical study for cancer, and the resulting human CSF of $0.041 (\text{mg}/\text{kg}\text{-day})^{-1}$ is used to calculate the risk-specific concentration for a 1 in 10^6 lifetime cancer risk, which would serve as a basis for the DCA health-protective concentration value for cancer effects.

The health-protective concentration (C) that protects against the carcinogenic effects of DCA in tap water is:

$$C = 10^{-6} \div (0.041 (\text{mg}/\text{kg}\text{-day})^{-1} \times 0.129 \text{ L}/\text{kg}\text{-day}) = 0.2 \times 10^{-3} \text{ mg}/\text{L} \text{ or } 0.2 \text{ }\mu\text{g}/\text{L} \text{ or } 0.2 \text{ ppb}$$

The health-protective concentration of 0.2 $\mu\text{g}/\text{L}$ or 0.2 ppb for cancer is selected as the PHG. Since this value is lower than the health-protective concentration of 115 ppb derived for noncancer effects, the PHG should protect against both cancer and noncancer effects of DCA.

Trichloroacetic Acid

The study of DeAngelo et al. (2008) provides the most appropriate dose-response data for TCA-dependent hepatic tumorigenesis. The Study 3 subset of this report is chosen as the critical study for cancer, and the resulting CSF of $0.071 \text{ (mg/kg-day)}^{-1}$ is used to calculate the risk-specific concentration for a 1 in 1 million lifetime cancer risk, which would serve as a basis for the TCA PHG value.

The health-protective concentration (C) that protects against the carcinogenic effects of TCA in tap water is:

$$C = 10^{-6} \div (0.071 \text{ (mg/kg-day)}^{-1} \times 0.129 \text{ L/kg-day}) = 0.1 \times 10^{-3} \text{ mg/L or } 0.1 \text{ } \mu\text{g/L or ppb}$$

Thus, 0.1 $\mu\text{g/L}$ or ppb is selected as the PHG. Since this value is lower than the health-protective concentration of 128 ppb derived for noncancer effects, the PHG of 0.1 ppb should protect against both cancer and noncancer effects of TCA.

Dibromoacetic Acid

The male mouse data set in the NTP (2007a) studies provides the most appropriate dose-response data for DBA-dependent carcinogenesis. The human CSF of $0.25 \text{ (mg/kg-day)}^{-1}$ is used to calculate the risk-specific concentration for a 10^{-6} lifetime cancer risk, which would serve as the basis for the health-protective concentration for cancer.

The health-protective concentration (C) that protects against the carcinogenic effects of DBA in tap water is:

$$C = 10^{-6} \div (0.25 \text{ (mg/kg-day)}^{-1} \times 0.129 \text{ L/kg-day}) = 0.03 \times 10^{-3} \text{ mg/L or } 0.03 \text{ } \mu\text{g/L or } 0.03 \text{ ppb}$$

The health-protective concentration of 0.03 $\mu\text{g/L}$ or 0.03 ppb for cancer is selected as the PHG. Since this value is lower than the health-protective concentration of 5 ppb derived for noncancer effects, the PHG of 0.03 ppb should protect against both cancer and noncancer effects of DBA.

12. RISK CHARACTERIZATION

Mechanistic Evidence and Other Considerations

Mechanistic evidence and some key uncertainties applicable to the PHGs and health-protective concentrations for the HAA5 are summarized below:

- **Genotoxicity.** For two carcinogenic HAA5, DCA and TCA, evidence of genotoxicity was mixed. While most in vitro genotoxicity studies for TCA were negative, the large majority of in vivo studies were positive, including multiple in vivo liver studies in mice and rats. For DCA genotoxicity, in vitro studies with higher doses and most in vivo studies were positive. The more frequent observations of in vivo genotoxicity with these compounds may result from metabolic conversions, which cannot be readily reproduced under in vitro conditions. Indeed, glyoxylic acid, which is a metabolite of DCA, was genotoxic in vitro. DCA itself is a minor metabolite of TCA, adding to TCA genotoxic potential. Taken together, these data are consistent with a genotoxic mode of action in DCA-mediated or TCA-mediated carcinogenesis. The evidence of in vitro and in vivo genotoxicity of DBA is more compelling compared to chlorinated HAA5.
- **Lack of cancer studies for MBA.** While MBA was strongly genotoxic, no carcinogenicity studies were identified. Only noncancer toxicity studies were available for this compound, and the PHG was based on multiple noncancer endpoints in a subchronic pig study (Dalgaard-Mikkelsen et al., 1955). A database deficiency uncertainty factor of $\sqrt{10}$ was applied in the PHG calculation to account, in part, for the potential carcinogenicity of MBA.
- **Linear model of cancer dose-response.** Because TCA activated peroxisome proliferation in mice and rats and owing to early mostly negative reports on TCA genotoxicity, the scientific literature extensively discussed the hypothesis that TCA carcinogenicity maybe fully mediated by PPAR α activation. However, detailed histopathological, gene expression and in vivo genotoxicity evidence for TCA argue against this limited view and indicate that following chronic TCA exposures, at least some tumors arise from a mechanism that is distinct from PPAR α activation. OEHHA's default linear extrapolation to low dose is appropriate in this situation. A similar hypothesis was advanced for DCA, a weaker PPAR α activator compared to TCA, and similar conclusions apply.
- **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 rates during these early life periods using 95th percentile drinking water consumption rates (see *Cancer Health-Protective Drinking Water Concentrations* section).

- **Interspecies extrapolation.** To estimate the risk of any human cancer based on animal data, OEHHA uses the interspecies scaling factor of body weight to the $\frac{3}{4}$ power to account for toxicokinetic and toxicodynamic differences between rodents and humans that might result in differences in tumorigenic response to HAA exposure. The difference between scaled and unscaled cancer slope factors was approximately seven-fold for mouse-based values.
- **Dose metric.** The exact mode of action (MOA) for toxic effects of HAA5 remains unknown, with likely involvement of metabolites. However, none of the available PBPK models (DCA, TCA, DBA) incorporate likely metabolites, and the appropriate dose metrics for PBPK-assisted interspecies extrapolations in the mechanisms of toxicity for individual compounds remain unclear. Due to these uncertainties, weight-based interspecies conversions were used instead of PBPK-assisted extrapolation, and administered doses were used as dose metrics.
- **Interactions of HAAs.** In drinking water, HAA5 necessarily occur as a mixture with each other and other DBPs, likely leading to mixture effects. The effect of the mixture of DCA and TCA on lipid peroxidation and single strand DNA breaks was greater than additive at certain HAA levels (Hassoun et al., 2014). Additionally, certain HAAs can inhibit the common metabolizing enzymes, such as GST-zeta, and have been demonstrated to change gene expression of a host of other metabolic enzymes (Thai et al., 2003). Despite the potential for mixture effects, the precise mechanisms have not been established, and the toxicity database for HAA mixtures is poor.
- **Epidemiological evidence.** Human exposures to multiple DBPs through drinking water are highly correlated (Inoue-Choi et al., 2015). Therefore, the developmental, reproductive and carcinogenic effects observed in human epidemiological studies (as described in the corresponding section of this document) cannot be attributed to exposure to a single HAA with certainty. It is likely that the observed effects are due to a mixture of DPBs, and the underlying toxic interaction mechanisms remain unknown.
- **Updated water ingestion rates.** OEHHA risk calculations now include age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake. These age-specific intake rates acknowledge that drinking water ingestion per unit body weight is higher in infants than in adults. For noncancer endpoints, the time-weighted average daily water ingestion rate for a 70-year lifetime for the general population is generally used. However, if the critical effect occurs in a particularly sensitive age group or other subgroup, the high end estimates of the age-specific water ingestion rate for the subgroup can be used (OEHHA, 2012).

- **Sensitive subgroups.** OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects than the general population due to exposure to drinking water contaminants. These improvements in water ingestion estimates and age sensitivity are crucial to the assessment of risk to these sensitive subgroups as well as the general population. Both of these adjustments increase the presumed toxicity of chemicals and thus lower the estimated health-protective concentrations of drinking water contaminants.

For noncancer effects, the estimated public health-protective concentration also reflects a relative source contribution (RSC) of 80% of the total HAA5 exposure coming from drinking water. This is a matter of professional judgment based on somewhat limited exposure information, but is consistent with available data on exposures to HAAs from various environmental sources, including exposure to common solvents which produce these chemicals as metabolites and foods that may contain HAA5 residues. US EPA, as a matter of long-standing practice, has used 20% as the RSC for HAAs in drinking water (US EPA, 2006).

Monochloroacetic Acid

The PHG of 53 ppb for MCA is based on systemic toxicity following chronic exposure of rats to MCA in drinking water (DeAngelo et al., 1997). No carcinogenic effects of MCA have been identified (NTP, 1992; DeAngelo et al., 1997; US EPA, 1998a, 2006). Noncancer toxic effects of MCA include metabolic alterations, neurotoxicity, cardiotoxicity, as well as liver, kidney, lung, spleen, and blood toxicity (Bhat et al., 1991; Daniel et al., 1991; NTP, 1992; DeAngelo et al., 1997). No epidemiological studies were identified that specifically linked exposure to MCA with adverse effects in humans. While accidental dermal or oral exposures to highly concentrated or solid MCA have caused deaths in humans, MCA concentrations in drinking water are several orders of magnitude lower and are not likely to cause adverse effects in humans. Thus, no adverse effects in three human volunteers were reported after daily oral exposure to approximately 2.1 mg/kg-day of MCA in water for 60 days (Morrison and Leake, 1941 as cited in NAS (2009)), a dose that is approximately 1,000 times higher than the exposure from drinking water at the level of the PHG.

The PHG is expected to be health-protective in regards to all possible adverse effects in humans.

Dichloroacetic Acid

The PHG of 0.2 ppb for DCA is based on liver cancer observed in mice by DeAngelo et al. (1999). Tumors were induced at a number of sites including liver, kidney, and large intestine in male and female B6C3F1 mice as well as in male Fischer 344 rats in multiple studies (Herren-Freund et al., 1987; Bull et al., 1990; Nelson et al., 1990; Sanchez and Bull, 1990; DeAngelo, 1991; DeAngelo et al., 1991; Daniel et al., 1992; Anna et al., 1994; Richmond et al., 1995; DeAngelo et al., 1996; Pereira, 1996; Stauber and Bull, 1997; Bull and Stauber, 1999; DeAngelo et al., 1999; Bull et al., 2002). Evidence of in vitro genotoxicity of DCA is inconsistent, and there have been suggestions in the literature that DCA is genotoxic only at very high doses and would unlikely be mutagenic at the levels found in drinking water.

Nonetheless, in vitro studies with higher doses and most in vivo studies were positive. There are no epidemiological studies directly linking DCA exposure to cancer, although increased cancer rates are associated with exposures to DBPs as a group. IARC classified DCA as "possibly carcinogenic to humans (Group 2B)" on the basis of sufficient evidence for carcinogenicity in animals (Guha et al., 2012). These evaluations met the criteria of listing DCA as a carcinogen under California's Proposition 65.

At doses higher than those associated with increased tumors, several other adverse effects have been documented, including liver toxicity, reproductive toxicity and neurotoxicity in animals (Katz et al., 1981; Cicmanec et al., 1991; DeAngelo et al., 1991; DeAngelo et al., 1999; Hassoun et al., 2010a; Hassoun et al., 2010b) and neurotoxicity in humans (Stacpoole et al., 1979; Stacpoole et al., 1990; Kurlemann et al., 1995; Stacpoole et al., 1997; Stacpoole et al., 1998a; Stacpoole et al., 1998b; Spruijt et al., 2001; Stacpoole et al., 2003; Kaufmann et al., 2006; Stacpoole et al., 2008a; Weimer and Sachdev, 2009; Brandsma et al., 2010; Michelakis et al., 2010). The most sensitive noncancer toxic effect in humans, reversible neuropathy, is not expected at or below the animal-based health-protective level in drinking water (115 ppb).

Trichloroacetic Acid

The PHG of 0.1 ppb for TCA is based on liver cancer observed in mice by DeAngelo et al. (2008) following chronic exposure to TCA in drinking water. Increased liver tumors in mice after TCA exposure have also been observed in several other studies (Herren-Freund et al., 1987; Bull et al., 1990; Pereira, 1996; Bull et al., 2002). While previous studies have attempted to explain why TCA appears to produce liver tumors in mice but not in rats, this question has not yet been resolved. Although evidence on the genotoxicity of TCA is mixed, published studies suggest that TCA is genotoxic in vivo, and that metabolism of TCA may be a critical step for genotoxicity. There are no epidemiologic data that directly link exposure to TCA in drinking water with increases in cancer in humans, although increased cancer rates are correlated with exposure to DBPs as a whole, as described in the *Human Epidemiology Studies on Disinfection Byproducts* section .

US EPA (2011) concluded there is suggestive evidence of carcinogenic potential based on the consistent positive evidence in both sexes of B6C3F1 mice. IARC recently classified TCA as "possibly carcinogenic to humans (Group 2B)" on the basis of sufficient evidence for carcinogenicity in animals (Guha et al., 2012). These evaluations met the criteria for listing TCA as a carcinogen under Proposition 65.

Despite the limited available database, noncancer effects would not be expected from exposure to TCA in drinking water at the low levels that are protective against cancer risk.

Monobromoacetic Acid

The PHG of 25 ppb for MBA is based on a NOAEL of 5 mg/kg-day in pigs from the study of Dalgaard-Mikkelsen et al. (1955) . The adverse effects in this study included slow movements, liver and skeletal muscle degeneration, and emaciation. This NOAEL is supported by studies

by Harrestrup Andersen et al. (1955) in dogs, which showed ataxia, vomiting, and diarrhea with an acute oral LOAEL of 24 mg/kg.

There are no carcinogenicity studies on MBA, but there is strong evidence of genotoxicity in vitro. Comparisons of relative mutagenicity of the HAAs find the brominated HAAs including MBA to be more mutagenic and cytotoxic than their chlorinated analogs (Kargalioglu et al., 2002; Plewa et al., 2002; Plewa et al., 2004; Attene-Ramos et al., 2010; Muellner et al., 2010; Plewa et al., 2010). In view of these reports and because cancer studies are positive for the less genotoxic HAAs DCA, TCA and DBA, there is a concern about possible carcinogenicity of MBA.

The major limitations of the database include the absence of any standard protocol toxicity studies, including carcinogenicity, reproductive, and developmental studies. No extra uncertainty factor has been incorporated into the risk assessment for MBA, because the combined UF is 3,000, the maximum recommended value based on recommendations of CalEPA Risk Assessment Advisory Committee (1996) and the US EPA (2002).

Dibromoacetic Acid

The PHG of 0.03 ppb for DBA is based on liver and lung tumors in mice chronically exposed to DBA in drinking water (NTP, 2007a). Carcinogenicity has been observed at a number of sites, including leukemia and mesotheliomas in rats and liver and lung neoplasms in mice (Melnick et al., 2007; NTP, 2007a). Evidence of DBA genotoxicity is mixed.

Developmental and reproductive toxicity have been observed in rabbits at higher doses than those associated with significant increases in tumors (Bodensteiner et al., 2004; Veeramachaneni et al., 2007). The concentration of DBA in drinking water selected to protect against cancer effects is expected to be fully protective against the potential noncancer effects.

To summarize, the table below provides the PHGs, based on cancer or noncancer effects, depending on the specific HAA compound, and health protective concentrations for the noncancer effects of carcinogenic HAAs. 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 ($\mu\text{g/L}$).

Table 12.1. PHGs and Noncancer Health-Protective Concentrations (HPCs)

Chemical Name	PHG (ppb)	PHG Effect	HPC (ppb)	HPC Effect
Monochloroacetic acid	53	Systemic toxicity	-	-
Dichloroacetic acid	0.2	Liver cancer	115	Liver toxicity
Trichloroacetic acid	0.1	Liver cancer	128	Liver toxicity
Monobromoacetic acid	25	Muscular degeneration	-	-
Dibromoacetic acid	0.03	Liver and lung cancer	5	Male reproductive toxicity

ppb, parts per billion

Disinfection Benefits Versus HAA Risk

In interpreting the results of this risk assessment for five HAAs, 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 (2006) attempted to balance the benefits of chlorination versus risks of exposure to DBPs when it established a drinking water MCL of 60 ppb for HAAs. 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 PHG development process does not include a quantitative risk-benefit analysis comparing risks from exposure to DBPs to risks from exposure to microorganisms in water, as that type of analysis falls outside of the scope of this assessment. This task is conducted by SWRCB in its description of best practices for drinking water disinfection and development of California MCLs.

Other Regulatory Standards

Other regulatory standards from federal, state and foreign agencies are summarized in the following table (Table 12.1). Currently, there are no regulatory standards for HAA5 chemicals in

the European Union. In WHO assessments for MBA and DBA, available data were considered inadequate to permit derivation of health-based guideline values (WHO, 2017).

Table 12.2 Other select regulatory standards for HAA5 or individual HAAs

Chemical or group	Agency or country	PHG or equivalent (ppb) ^a	MCL or equivalent (ppb) ^b	Reference
HAA5	US EPA State agencies		60	US EPA (1998a)
HAA5	Health Canada		80	Health Canada (2017)
TCA	US EPA	20		US EPA (1998a)
TCA	WHO	200		WHO (2017)
TCA	Japan		30	MHLW (Japan) (2015)
TCA	Australia, New Zealand		100	NHMRC and NRMCC (2011)
TCA	China		100	Wang et al. (2014)
DCA	US EPA	0		US EPA (1998a)
DCA	WHO	50		WHO (2017)
DCA	Japan		30	MHLW (Japan) (2015)
DCA	Australia New Zealand		100	NHMRC and NRMCC (2011)
DCA	China		50	Wang et al. (2014)
MCA	US EPA	70		US EPA (2006)
MCA	WHO	20		WHO (2017)
MCA	Japan		20	MHLW (Japan) (2015)
MCA	Australia New Zealand		150	NHMRC and NRMCC (2011)

^a Health-protective level such as PHG (OEHHA) or MCLG (US EPA), or the health-based guideline value (WHO)

^b Maximum contaminant level (MCL) in drinking water, regulatory value

US EPA (2011) has updated the review of TCA with a new noncancer calculation, yielding an RfD of 0.02 mg/kg-day based on hepatocellular necrosis (DeAngelo et al., 2008), with a combined UF of 1,000. US EPA (2011) concluded that the data are "suggestive" of a cancer risk to humans, and calculated an oral cancer slope factor of 0.067 per mg/kg-day. An updated MCLG has not yet been published. The US EPA IRIS program has not published reviews of MBA or DBA.

The Agency for Toxic Substances and Disease Registry (ATSDR) has not conducted a risk assessment or prepared a toxicological profile on any of the HAA5. In addition, none of the HAA5 is on the Candidate Priority List of Hazardous Substances awaiting review under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, Superfund).

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APPENDIX A. 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.³ 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 $L_{eq}/kg\text{-day}$) of multiroute exposure to tap water for each life stage. The lifetime daily multiroute intake rate of tap water in $L_{eq}/kg\text{-day}$ is the time-weighted average of these life-stage specific tap water intake rates.⁴ The liter equivalent ($L_{eq}/kg\text{-day}$) 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 A1 shows the descriptions and values of parameters applied in the exposure equations. Tables A2 and A3 show life-stage specific exposure parameter values.

Table A1. 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				
I_{fl}	fluid (water) intake, normalized to body weight	0.045 to 0.196 ^b	L/kg-day	OEHHA, 2012

³ 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). Available at: <https://dtsc.ca.gov/caltox-download-instructions/>

⁴ 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., $0.75/70 \times 0.047 + 2/70 \times 0.196 + 14/70 \times 0.061 + 54/70 \times 0.045 = 0.053$ L/kg-day for exposure via oral ingestion) in calculating the noncancer 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.

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
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 A2. OEHHA Calculated Exposure Parameters (OEHHA, 2012⁵)

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 m³/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 (m²/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 A3. 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:⁶

$$\text{Intake}_{\text{oral}} = C_{\text{tap,water}} \times \text{IfI}$$

B. Inhalation Intake: Inhalation of Indoor Air in Active State, Resting State, and Shower/Bath

⁵ 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.

⁶ 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 ($\text{Intake}_{\text{inh}}$) 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:

$$\text{Intake}_{\text{inh}} = C_{\text{air}} \times (\text{BR}_a \times \text{ET}_{\text{ai}} + \text{BR}_r \times \text{ET}_{\text{ri}} - \text{BR}_a \times \text{ET}_{\text{sb}}) + C_{\text{bath_air}} \times \text{BR}_a \times \text{ET}_{\text{sb}}$$

The chemical concentration in indoor air and bathroom air are derived from the two equations below:

$$C_{\text{air}} = \frac{3 \times 10^6 \times 0.7 \times \left(\frac{W_{\text{house}}}{\text{VR}_{\text{house}}}\right) \times C_{\text{tap_water}}}{\frac{2.5}{(D_{\text{water}}/86400)^{2/3}} + \frac{R_{\text{gas}} \times 298 \times Z_{\text{water}}}{(D_{\text{air}}/86400)^{2/3}}}$$

and

$$C_{\text{bath_air}} = \frac{3 \times 10^6 \times 0.6 \times \left(\frac{W_{\text{shower}}}{\text{VR}_{\text{bath}}}\right) \times C_{\text{tap_water}}}{\frac{2.5}{(D_{\text{water}}/86400)^{2/3}} + \frac{R_{\text{gas}} \times 298 \times Z_{\text{water}}}{(D_{\text{air}}/86400)^{2/3}}}$$

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 skin⁷ (t_{lag}):

a. Exposure time \ll diffusion lag time, i.e. $\frac{t_{\text{lag}} \times 2}{\text{ET}_{\text{sb}}} > 3$:

$$\text{Uptake}_{\text{dermal}} = C_{\text{tap_water}} \times \left(\frac{\delta_{\text{skin}} \times K_m}{2}\right) \times f_s \times \text{CF} \times \text{SA}_b \times \frac{\text{ET}_{\text{sb}}}{2 \times t_{\text{lag}}} \times \frac{1 \text{ event}}{\text{day}}$$

b. For $1 \leq \frac{t_{\text{lag}} \times 2}{\text{ET}_{\text{sb}}} \leq 3$:

$$\text{Uptake}_{\text{dermal}} = C_{\text{tap_water}} \times \left(\frac{\delta_{\text{skin}} \times K_m}{2}\right) \times f_s \times \text{CF} \times \text{SA}_b \times \frac{1 \text{ event}}{\text{day}}$$

⁷ 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. $\frac{t_{lag} \times 2}{ET_{sb}} < 1$:

$$\text{Uptake}_{\text{dermal}} = C_{\text{tap_water}} \times \left[\frac{\delta_{\text{skin}} \times K_m}{2} + \left(\frac{ET_{sb}}{2} - t_{lag} \right) \times K_p^w \right] \times f_s \times CF \times SA_b \times \frac{1 \text{ event}}{\text{day}}$$

where the chemical-specific t_{lag} is obtained from:

$$t_{lag} = \frac{\delta_{\text{skin}} \times K_m}{6 \times K_p^w}$$

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:

$$K_p^w = \frac{1}{(MW)^{0.6}} \times \frac{2.4 \times 10^{-6} + 3 \times 10^{-5} \times (K_{ow})^{0.8}}{\delta_{\text{skin}}}$$

- b. Chemicals with MW \geq 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 $\text{Intake}_{\text{oral}}$, $\text{Intake}_{\text{inh}}$, and $\text{Uptake}_{\text{dermal}}$ as follows:

Relative Contribution from Oral Ingestion (%)

$$= \frac{\text{Intake}_{\text{oral}}}{\text{Intake}_{\text{oral}} + \text{Intake}_{\text{inh}} + \text{Uptake}_{\text{dermal}}} \times 100\%$$

Relative Contribution from Inhalation⁸ (%)

$$= \frac{\mathbf{Intake}_{inh}}{\mathbf{Intake}_{oral} + \mathbf{Intake}_{inh} + \mathbf{Uptake}_{dermal}} \times 100\%$$

Relative Contribution from Dermal Uptake (%)

$$= \frac{\mathbf{Uptake}_{dermal}}{\mathbf{Intake}_{oral} + \mathbf{Intake}_{inh} + \mathbf{Uptake}_{dermal}} \times 100\%$$

⁸ 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.

APPENDIX B. EPIDEMIOLOGIC STUDIES OF REPRODUCTIVE OUTCOMES

Abbreviations:

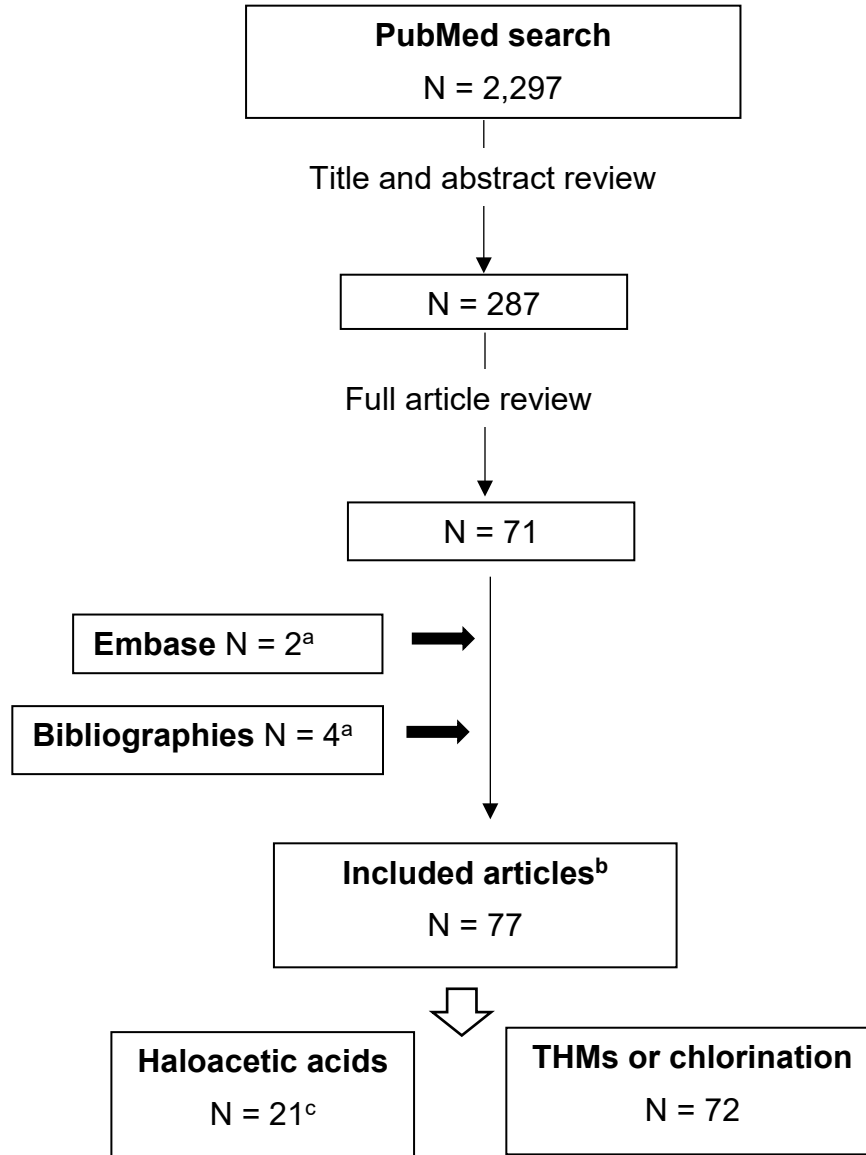
BDCM, bromodichloromethane
BMI, body mass index
BW, birthweight
CI, confidence interval
CM, congenital malformation
CNS, central nervous system
CYP, cytochrome p450
DBA, dibromoacetic acid
DBCM, dibromochloromethane
DCA, dichloroacetic acid
FGR, fetal growth restriction
GST, glutathione-S-transferase
HAA, haloacetic acid
HAA3, DCA, TCA and bromodichloroacetic acid
HAA5, MCA, DCA, TCA, MBA and DBA
HAA9, HAA5, plus bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid and tribromoacetic acid
IUGR, intrauterine growth retardation
LBW, low birthweight
LIN, linearity
LMP, last menstrual period
MCA, monochloroacetic acid
MTHFR, methylene tetrahydrofolate reductase
NA⁺, sodium
NBDPS, National Birth Defects Prevention Study
NTD, neural tube defect
OR, odds ratio
POR, prevalence odds ratio
ppb, parts per billion
R, correlation coefficient
ref, reference
RR, relative risk estimate
SAB, spontaneous abortion
SES, socioeconomic status
SGA, small for gestational age
SNP, single nucleotide polymorphism
TCA, trichloroacetic acid
THM, trihalomethane
THMBr, brominated trihalomethanes
TTHM, total trihalomethanes, sum of BDCM, chloroform, DBCM, and bromoform
UK, United Kingdom
VCL, curvilinear velocity
VLBW, very low birth weight
VSL, straight line velocity

Databases searched: PubMed and Embase

Search string:

(disinfection byproducts OR chlorination OR haloacetic acid OR trihalomethane OR monochloroacetic acid OR dichloroacetic acid OR trichloroacetic acid OR monobromoacetic acid OR dibromoacetic acid OR tribromoacetic acid OR bromochloroacetic acid OR bromodichloroacetic OR dibromochloroacetic acid OR chloroform OR bromodichloromethane OR dibromochloromethane OR bromoform) AND (pregnancy OR fetus OR birthweight OR intrauterine growth retardation OR small for gestational age OR gestational age OR fetal growth retardation OR preterm OR stillbirth OR pregnancy loss OR spontaneous abortion OR neonatal death OR sperm OR congenital malformation OR neural tube defect OR spina bifida OR anencephaly OR hypospadias OR Tetralogy of Fallot OR cleft palate OR cleft lip OR ventricular septal defect OR time to pregnancy OR fertility OR menstruation OR fecundability).

Literature search results for epidemiologic studies of THMs and HAAs and reproductive outcomes



^a Additional studies identified through Embase or from the bibliographies of the included articles or relevant reviews

^b Studies meeting the inclusion criteria discussed in Section 4

^c Sixteen of these studies also provide data for THMs or water chlorination

Table B1. Epidemiologic Studies of Haloacetic Acids and Fetal Growth (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hinckley et al. (2005)	LBW, IUGR	Arizona (24 zip codes) 1998-2003	Retrospective cohort	48,119 pregnant women; 3,760 IUGR and 859 LBW	HAA5 (total and individual)	HAA concentrations in 3 water facilities measured quarterly in 1998-2002, averaged over the 3rd trimester and assigned to zip code of residence listed in birth records.	<p><u>IUGR:</u></p> <p>DCA: ORs = 1.00 (ref), 1.15 (95% CI: 0.97-1.36) and 1.28 (95% CI: 1.08-1.51) for water concentrations of <6, 6-8, and ≥8 µg/L; OR = 1.05 (1.02-1.09) for each 1 µg/L increase</p> <p>TCA: ORs = 1.00 (ref), 1.00 (95% CI: 0.84-1.18), and 1.19 (95% CI: 1.01-1.41) for water concentrations of <4, 4-6, and ≥6 µg/L; OR = 1.04 (1.02-1.07) for each 1 µg/L increase</p> <p>Other: ORs for HAA5 and other individual HAAs near 1.0</p> <p><u>Term LBW:</u></p> <p>DBA: ORs = 1.00 (ref), 1.01 (95% CI: 0.72-1.41), and 1.49 (95% CI: 1.09-2.04) for concentrations of <4, 4-5, and ≥5 µg/L. OR = 1.17 (95% CI: 1.03-1.32) for each 1 µg/L increase; highest ORs for exposures at weeks 33-36 of gestation</p> <p>HAA5: ORs near 1.25 but not statistically significant and OR for HAA5 as a continuous variable near 1.00</p>	Age, parity, education, race, ethnicity, smoking, and prenatal care	<ul style="list-style-type: none"> • No personal interviews • Data on birth outcomes and co-variates from birth records • Only term births (≥37 weeks gestation) included in the analyses of LBW • Increases in ORs are small • Exposure period: 3rd trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hoffman et al. (2008a)	SGA	US (three study sites) 2000-2004	Prospective cohort	1,958 live births; 113 SGA	HAA5 (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on residence and water intake.	<p><u>SGA:</u></p> <p>HAA5: ORs near 1.0</p> <p>Individual HAA5: ORs up to 1.4 in the higher categories but not statistically significant</p> <p><u>BW:</u></p> <p>HAA5: No clear association</p> <p>Individual HAA5: Birth weight losses up to 50-60 grams in the upper exposure categories but not statistically significant; some evidence of a monotonic dose-response pattern with decreasing birth weight for increasing levels of exposure but formal statistical tests for dose-response patterns not presented</p>	Age, race, ethnicity, education, income, employment, marital status, BMI, parity, and caffeine use	<ul style="list-style-type: none"> • Selection: few details provided • Possible major differences in sociodemographic characteristics seen across the 3 sites (see Hoffman et al., 2008b) • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011 • No adjustments for smoking • Exposure period: each trimester
Horton et al. (2011)	SGA	US (two study sites) 2000-2004	Prospective cohort	31,008 births; 1,543 SGA	HAA5	Weekly samples collected from a single representative location in the water distribution systems during 2000-2004. Further details on exposure assessment provided in Savitz et al., 2006.	<p>Chlorinated site:</p> <p>HAA5: all ORs near 1.0</p> <p>Brominated site:</p> <p>HAA5: all ORs near 1.0</p>	Age, race, education, smoking, marital status, alcohol, and parity	<ul style="list-style-type: none"> • Data on outcomes and co-variates from birth records • Includes 2 of the 3 sites in Hoffman et al., 2008a and related publications • Exposure period: 3rd trimester • Only term births used in the SGA analysis • No personal interviews • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Levallois et al. (2012)	SGA	Quebec City, Canada 2006-2008	Case-control	571 cases and 1,925 controls	HAAs (total and individual)	Data from monthly sampling in 46 sites during 2006-2008 used to develop spatio-temporal models linked to personal questionnaire data on water consumption, filter use, boiling water, showering habits, and other factors.	<p><u>Total HAAs:</u></p> <p>OR = 1.4 (95% CI: 1.1-1.9) for >60 vs. <60 µg/L, p-trend = 0.03 although unclear trend in categorical analyses</p> <p><u>Individual HAAs:</u></p> <p>TCA: OR = 1.4 (95% CI: 1.0-1.8) for concentrations of >17.78 vs. <5.03 µg/L (p-trend = 0.01)</p> <p>DCA: ORs up to 1.2 but not statistically significant (p-trend = 0.11); higher ORs when intakes considered (OR = 1.4; 95% CI: 1.1-1.9 for intakes of >14.80 vs. <1.09 µg/day; p-trend = 0.01)</p>	Age, calendar week of the year of birth, education, income, BMI, parity, history of LBW, smoking, second hand tobacco smoke, coffee, alcohol, chronic disease, and preeclampsia	<ul style="list-style-type: none"> • Selection: cases and controls selected from birth records; controls randomly selected, matched to cases on period of birth • Exposure period: 3rd trimester • Possible interactions were seen with CYP2E1 SNPs although not statistically significant (Levallois et al., 2016) • No clear interaction with haloacetaldehydes or haloacetonitriles (Ileka-Priouzeau et al., 2015) • Some evidence of interaction with CYP17A1 polymorphisms (Bonou et al., 2017)
(Porter et al., 2005)	IUGR	Maryland 1998-2002	Retrospective cohort	15,315 births; 1,114 IUGR	HAAs (total and individual)	Monthly HAA concentrations from 1997-2000 collected at 4 sampling points from a local water utility in the study county.	<p>HAA5 (3rd trimester exposure): ORs = 1.00 (ref), 1.29 (95% CI: 1.01-1.66), 1.41 (95% CI: 1.11-1.81), 1.15 (95% CI: 0.89-1.49), and 1.34 (95% CI: 1.04-1.71) for each quintile of exposure (cut-off points not given); ORs for other trimesters and for overall pregnancy average near 1.0</p> <p>TCA: somewhat similar results to HAA5</p> <p>Other HAAs: All ORs near 1.0</p>	Prenatal care, marital status, age, and smoking	<ul style="list-style-type: none"> • Outcome and co-variate data from birth certificates • Large seasonal variations seen in HAA concentrations • Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
(Rivera-Nunez and Wright, 2013)	SGA	Massachusetts (whole state) 1996-2004	Retrospective cohort	68,409 SGA	HAA5 (total and individual)	Quarterly town averages linked to addresses.	<p><i>For 3rd trimester exposure:</i></p> <p><u>SGA:</u></p> <p>Many unadjusted ORs near 1.2-1.4 and statistically significant but are close to 1.0 and not statistically significant after adjustments, including adjustments for THM)</p> <p><u>BW:</u></p> <p>Decreased BW with higher HAA5, DCA and TCA levels but marked reduction in associations with statistical adjustments</p> <p><i>For 2nd trimester exposure:</i></p> <p>Similar results</p>	Source, disinfection, maternal age, race, education, marital status, payment method, and income (census tract)	<ul style="list-style-type: none"> •Exposure period: 2nd and 3rd trimesters •Adjustments for “source” and “disinfection” are unclear
(Smith et al., 2016)	BW	Northern England (Bradford cohort) 2007-2010	Prospective cohort	12,453 women	HAA3 (total and individual)	Routine water monitoring data linked to personal questionnaire information on water consumption, hot and cold beverage consumption, consumption of other fluids, filter use, and showering and bathing. This information was then used to develop exposure models for time weighted average water concentrations.	No clear associations; difference in mean BW = -0.6 g (95% CI: -25.5-24.4) comparing HAA3 concentrations ≥ 38.83 to < 23.82 $\mu\text{g/L}$ (whole pregnancy average); similar results seen for individual trimester exposures	Caffeine intake, SES, education, glucose levels, ethnicity, smoking, parity, age, BMI, gestational age, and sex	<ul style="list-style-type: none"> •BWs obtained from clinical records •Mean HAA3 concentration = 34.5 $\mu\text{g/L}$ •In some analyses major changes in results are seen after statistical adjustments •The authors state that, “Only three HAA3 had detectable data points to be modeled...” •Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Wright et al. (2004)	SGA	Massachusetts (109 towns) 1995-1998	Retrospective cohort	194,827 births; 17,359 SGA	HAAs (total and individual)	Weekly to quarterly HAA monitoring data from 1995-1998. Town averages calculated and linked to subject residences.	All ORs near 1.0	Income, prenatal care, race, education, smoking, age, parity, and maternal medical history	<ul style="list-style-type: none"> •Exposure period: 3rd trimester •Includes towns with populations of >10,000 people •Birth outcomes and co-variates from birth certificates •Gestational age based on clinician estimate •Residence based on maternal zip code
Zhou et al. (2012)	BW	Wuhan, China 2008-2009	Cross-sectional	398 women	HAA: urinary TCA	Urine collected near the time of hospital admission for delivery	Increasing quartiles of urinary TCA associated with decreased BW but results not statistically significant	Sex, age, maternal health, education, parity, BMI, income, smoking, second hand tobacco smoke, and alcohol	<ul style="list-style-type: none"> •Birth outcomes obtained from birth records •Recruitment strategy unclear •Unclear when urine sample was collected •Creatinine adjustment done •Exposure period: unclear

Table B2. Epidemiologic Studies of Haloacetic Acids and Other Reproductive Outcomes (studies sorted by outcome then author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Wright et al. (2017)	CM (cardiac)	Massachusetts (68 towns) 1999-2004	Case-control	904 CM (cardiac) cases and 9,040 controls	HAA5 (total and individual)	Quarterly water measurements and disinfection treatment information from 1999-2004 linked to town of residence and data on month of birth. Averaged concentrations for the 1st trimester used.	<p><u>Tetralogy of Fallot:</u> ORs = 1.00 (ref), 2.13 (95% CI: 0.53-8.65), 4.98 (95% CI: 1.02-24.35), 5.88 (95% CI: 1.06-32.57), and 6.51 (95% CI: 1.23-34.59) for HAA5 concentrations of ≤8.17, >8.17-19.33, >19.33-25.79, >25.79-33.97, >33.97-100.00 µg/L of HAA5 elevated ORs for TCA and DCA but not statistically significant ORs for MCA and DBA below 1.0.</p> <p><u>Other outcomes:</u> TCAs and HAA5 have some ORs above 2.0 for conotruncal defects but not statistically significant and no clear dose-response patterns</p>	Water source and treatment, BW, income, prenatal care, maternal health and reproductive health risk factors	<ul style="list-style-type: none"> • Selection: cases obtained from the state birth defects monitoring program; controls randomly selected from all live births in Massachusetts, matched to cases by week of conception • Information on co-variables obtained from birth records or census data (e.g., income) • Mean HAA5 level = 22.4 µg/L (±14.89 µg/L) • Exposure period: 1st trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Kaufman et al. (2017)	CM (cranio-facial)	Massachusetts (113 towns) 1999-2004	Case-control	366 CM (cranio-facial) cases and 3,660 controls	HAAs (total and individual)	Quarterly water measurements and disinfection treatment information from 1999-2004 linked to town of residence and data on month of birth. Averaged concentrations for the 1st trimester used.	<p><u>Cleft palate:</u> OR of 3.94 (95% CI: 1.08-14.39) for HAA5 >34.20 vs. ≤7.38 µg/L Similar results for TCA and DCA ORs decrease somewhat with additional adjustment for THMs</p> <p><u>Cleft lip:</u> ORs near 1.0</p> <p><u>Eye defects:</u> OR of 2.59 (95% CI: 0.74-9.14) for HAA5 >31.62 vs. ≤9.96 µg/L Similar result for TCA U-shaped dose-response pattern</p> <p><u>Ear defects:</u> All ORs near or below 1.0</p>	Water source and treatment type, income (zip code), race, and prenatal care	<ul style="list-style-type: none"> • Selection: cases obtained from the state birth defects monitoring program; controls randomly selected from all live births in Massachusetts, matched to cases by week of conception • Information on co-variables obtained from birth records or census data • Mean HAA5 level = 22.44 µg/L (±15.02 µg/L) • Exposure period: 1st trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Luben et al. (2008)	CM (hypospadias)	Arkansas 1998-2002	Case-control	320 cases and 614 controls	HAAs (total and individual)	Publically available monitoring data for quarterly THM and HAA concentrations collected from 263 water utilities throughout the state. Questionnaire data on showering, bathing, water use available for a subset of subjects.	HAA5: Elevated ORs in the middle (OR = 2.43 (95% CI: 0.94-6.28) for concentrations of >0-20.5 µg/day vs. "no exposure") but not upper exposure categories Individual HAAs: no clear associations or dose-response patterns after adjustments ORs based on monitoring data (all subjects) near 1.0	BMI, race, BW, and plurality	<ul style="list-style-type: none"> Exposure period: 6-16 weeks of pregnancy Selection: cases ascertained from a reproductive health monitoring system for the state; controls randomly selected from Arkansas birth records Subset of subjects (n=40 cases, 242 controls) selected from the National Birth Defects Prevention Study (NBDPS) Data on showering and bathing, hot beverages, and water consumption available on the NBDPS subjects Authors note that there were fewer cases than expected and note the possibility that some cases may have been diagnosed outside the state
Klotz and Pynch (1999)	CM (NTDs)	New Jersey 1993-1994	Case-control	62 cases and 114 controls	HAAs	Tap water samples collected beginning in the "11 th month of the 2 years of fieldwork"	Unadjusted PORs = 1.0 (ref), 0.9 (95% CI: 0.4-2.0), and 1.2 (95% CI: 0.5-2.6) for concentrations of <3, 3-<35, and ≥35 ppb	"Adjusting for maternal age, ethnicity, education, and onset of prenatal care did not alter ORs by ≥10%"	<ul style="list-style-type: none"> Selection: NTDs ascertained from the New Jersey Birth Defects Registry Co-variate data from subject interviews Exposure period: unclear, possibly 4 months after birth
MacLehose et al. (2008)	Other (time to pregnancy)	US (three sites) 2000-2004	Prospective cohort	236 women prior to pregnancy	HAAs (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal questionnaire data on water intake and residence in "early gestation" (average of 9 th week of pregnancy).	Some ORs for cycle specific probability of conception are above 1.0 (indicating decreased time to pregnancy) No clear pattern of ORs below 1.0	Age, race, ethnicity, marital status, smoking, and BMI; employment, education, and other factors also assessed	<ul style="list-style-type: none"> Separate testing showed HAA concentrations were spatially uniform in all 3 water systems Differences seen across the 3 sites in terms of age, race/ethnicity, education, and income Exposure period: unclear, but probably the 9th week of pregnancy on average

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Savitz et al. (2006)	Pregnancy loss	US (three sites) 2000-2004	Prospective cohort	2,409 pregnant women; 258 pregnancy losses	HAAs (total)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on water intake.	Some ORs for water concentrations or ingested amount above 1.0 but inconsistent dose-response patterns	Age, race, ethnicity, education, marital status, age at menarche, alcohol, and vitamin use	<ul style="list-style-type: none"> • Selection: subjects who were trying to become pregnant or were <12 weeks pregnant were recruited from prenatal care practices • Authors state that, "...THM and HAA concentrations were spatially uniform through the distribution system" • Pregnancy losses identified by self-report • Co-variate data from subject interviews • Differences seen across the 3 sites in race/ethnicity and education • Pregnancy loss not well defined • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011 • Exposure period: weeks 3-8 of pregnancy
King et al. (2005)	Pregnancy loss (stillbirth)	Nova Scotia, Eastern Ontario, Canada 1999-2001	Case-control	112 cases and 398 controls	HAAs (total and individual)	Residential tap water samples collected from all subjects approximately 1 year after 4-5th month of pregnancy. THM measurements were linked to questionnaire data on water consumption, filter use, showering and other factors.	ORs = 1.00 (ref), 1.15 (95% CI: 0.59-2.23), 1.43 (95% CI: 0.77-2.66), and 1.60 (95% CI: 0.88-2.89) for brominated HAA water concentrations of 0, and three tertiles above 0 (cut-off points not given), although all decreased after adjustment for THMs; ORs for total HAA, DCA, and TCA near 1.0 or with λ -shaped dose-response patterns	Age, province, income, occupation, smoking, and THMs (in some analyses)	<ul style="list-style-type: none"> • Selection: stillbirths and controls (randomly selected) identified through a population-based perinatal database • HAA and THM levels highly correlated (R = 0.81) • Note adjustments for THMs • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hoffman et al. (2008b)	Preterm	US (three study sites) 2000-2004	Prospective cohort	2,039 pregnant women; 185 preterm	HAA5 (total and individual)	Weekly or biweekly water samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on residence and water intake.	All ORs near or below 1.0 for 2nd trimester exposures; authors reported that results were similar for 1st trimester exposures although actual results not provided	Age, race, ethnicity, education, income, employment, marital status, BMI, parity, and caffeine use	<ul style="list-style-type: none"> • Selection: subjects recruited from prenatal clinics, advertisements, and targeted mailings • Differences seen across the 3 sites in terms of age, race/ethnicity, education, and income • Exposure period: 1st and 2nd trimester • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011
Horton et al. (2011)	Preterm	US (two study sites) 2000-2004	Prospective cohort	31,008 births; 1,543 SGA and 2,075 preterm	HAA5	Weekly samples collected from a single representative location in the water distribution systems during 2000-2004. Further details on exposure assessment provided in Savitz et al., 2006.	Chlorinated site: HAA5: all ORs near 1.0 Brominated site: HAA5: all ORs near 1.0	Age, race, education, smoking, marital status, alcohol, and parity	<ul style="list-style-type: none"> • Data on outcomes and co-variables from birth records • Includes 2 of the 3 sites in Hoffman et al., 2008a and related publications • Exposure period: 2nd trimester • No personal interviews • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011
(Rivera-Nunez and Wright, 2013)	Preterm	Massachusetts (whole state) 1996-2004	Retro-spective cohort	37,136 preterm births	HAA5 (total and individual)	Quarterly town averages linked to addresses.	<i>For 2nd trimester exposures:</i> Many unadjusted ORs near 1.2-1.4 and statistically significant but are close to 1.0 and not statistically significant after adjustments, including adjustments for THM) <i>For 1st trimester exposures:</i> Similar results	Source, disinfection, maternal age, race, education, marital status, payment method, and income (census tract)	<ul style="list-style-type: none"> • Exposure period: 1st and 2nd trimester • Adjustments for “source” and “disinfection” are unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Wright et al. (2004)	Preterm	Massachusetts (109 towns) 1995-1998	Retro-spective cohort	194,827 births; 11,580 preterm	HAA (total and individual)	Weekly to quarterly HAA monitoring data from 1995-1998. Town averages calculated and linked to subject residences.	All ORs near 1.0	Income, prenatal care, race, education, smoking, age, parity, and maternal medical history	<ul style="list-style-type: none"> • Exposure period: 3rd trimester • Includes towns with populations of >10,000 people • Birth outcomes and co-variables from birth certificates • Gestational age based on clinician estimate • Residence based on maternal zip code
(Luben et al., 2007)	Sperm quality	US (three sites) years unknown	Prospective cohort	228 presumed fertile males	HAA (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average concentrations linked to telephone interview data on residences and water intake.	<p>Clear associations not seen for the main indicators of sperm concentration or morphology ORs similar when using 10-, 30-, and 90-day lag periods</p> <p>Slight positive association seen for percent abnormal cytoplasmic drop and HAA9 concentrations</p>	Age, abstinence, and education; also assessed smoking, alcohol, illness, race, and other factors	<ul style="list-style-type: none"> • Male partners of women in the 2000-2004 Savitz et al., 2005 study • Co-variate data from subject interviews • Differences in ethnicity, education, income, and alcohol use seen across the three sites
Xie et al. (2011)	Sperm quality	Wuhan, China 2008	Cross-sectional	418 men	HAA: urinary TCA	Urinary TCA collected at the time of semen collection. Questionnaire information on tap water use, bathing, showering also collected.	Clear associations not seen with sperm concentration, count, motility, morphology or other parameters	Age, abstinence, and smoking	<ul style="list-style-type: none"> • Recruited from sub fertile couples seeking care for infertility • Creatinine adjusted ($\mu\text{g TCA/g creatinine}$) • Personal interviews
Zeng et al. (2014a)	Sperm quality	Wuhan, China 2011-2012	Cross-sectional	2,009 men	HAA: urinary TCA	Urine collection at the time of semen collection.	<p><u>Sperm concentration, motility, count, or sperm motion parameters:</u></p> <p>Some increased ORs or statistically significant regression coefficients but no clear dose-response patterns Percent abnormal head = -2.04 (95% CI: -3.08 to -0.99) comparing urine TCA >10.96 to $\leq 6.01 \mu\text{g/L}$</p>	Urine creatinine, age, education, abstinence, income, and smoking	<ul style="list-style-type: none"> • Men presenting to medical center "to seek semen analysis" • Men with occupational exposures excluded • Single spot morning urine sample • Questionnaire on water intake, bathing, showering, and swimming

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes																								
Zeng et al. (2016)	Sperm quality	Wuhan, China 2011-2012	Cross-sectional	337 men	HAA: urinary TCA	Urine collection appears to have been at the time of semen collection. Blood THMs also measured.	<p><i>ORs for sperm concentration <20 million/ml</i></p> <table border="1"> <thead> <tr> <th>TTHM</th> <th>TCA</th> <th>OR (95% CI)</th> </tr> </thead> <tbody> <tr> <td>Low</td> <td>Low</td> <td>1.00 (ref)</td> </tr> <tr> <td></td> <td></td> <td>2.97</td> </tr> <tr> <td>High</td> <td>Low</td> <td>(0.81-10.87)</td> </tr> <tr> <td></td> <td></td> <td>3.59</td> </tr> <tr> <td>Low</td> <td>High</td> <td>(0.96-13.42)</td> </tr> <tr> <td></td> <td></td> <td>6.35</td> </tr> <tr> <td>High</td> <td>High</td> <td>(1.83-22.06)</td> </tr> </tbody> </table> <p>Somewhat similar findings though less strong for individual THMs; Somewhat similar findings for sperm count <40 million/ml; No statistically significant interactions between THMs and TCA</p>	TTHM	TCA	OR (95% CI)	Low	Low	1.00 (ref)			2.97	High	Low	(0.81-10.87)			3.59	Low	High	(0.96-13.42)			6.35	High	High	(1.83-22.06)	Age, BMI, smoking, alcohol, income, and abstinence	<ul style="list-style-type: none"> Includes subjects in Zeng et al. 2014a and Zeng et al. 2013 (fertility clinic) Men with occupational exposures excluded Questionnaire on water intake, bathing, showering, and swimming High and low THM and TCA concentrations based on medians
TTHM	TCA	OR (95% CI)																															
Low	Low	1.00 (ref)																															
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Table B3. Epidemiologic Studies of Trihalomethanes and Fetal Growth (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Aggazzotti et al. (2004)	SGA	Italy (nine towns) 1999-2000	Case-control	239 SGA and 612 controls	THMs (total and individual)	Questionnaire information on water sources, cooking, swimming, linked to THM measurements collected from subject's home water source at the time of delivery.	Weak associations with THMs OR = 1.70 (95% CI: 0.97–3.00) for chlorites >200 µg/L and higher inhalation exposure	Sex, education, mother's smoking, water-based beverages, and type of water consumed	<ul style="list-style-type: none"> • Selection: controls selected from the same hospitals and matched to cases on date of birth • Exposure period: time of delivery
Bove et al. (1995)	BW, SGA	New Jersey 1985-1988	Retro-spective cohort	80,938 live births; 4,082 SGA	TTHM	Results of quarterly water testing of at least four samples from all water companies were used to estimate monthly concentrations. These were then linked to the mother's residence at birth.	<p><u>BW:</u> Reduction of 70.4 grams (90% CI: -40.6 to -100.2 grams) for concentrations of >100 ppb vs. ≤20 ppb Non-monotonic dose-response relationship</p> <p><u>SGA:</u> OR = 1.50 (90% CI: 1.19-1.86) for concentrations of >100 ppb vs. ≤20 ppb Non-monotonic dose-response relationship</p>	Maternal age, race, education, parity, previous stillbirth or miscarriage, sex, and prenatal care	<ul style="list-style-type: none"> • Data on outcomes and co-variates were obtained from vital records (birth and death certificates) and from the New Jersey Birth Defects Registry • Includes all live births and fetal deaths identified by birth or death certificates in 75 of the 146 towns in the area that were mostly served by public water supplies • Exposure assessment was blinded • Exposure period: averaged over the entire pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Cao et al. (2016)	BW, SGA	Wuhan and Xiaogan Cities, China 2011-2012	Retrospective cohort	1,184 pregnant women	THMs (total and individual)	Maternal serum collected in late pregnancy (≥ 35 weeks gestation, on the first day of hospital admittance for delivery)	<p><u>BW:</u> Highest tertile vs. lowest tertile of TTHMs associated with lower BW (-60.9 grams; 95% CI: -116.2 to -5.6 grams) Smaller BW changes seen for individual THMs</p> <p><u>SGA:</u> ORs = 1.00 (ref), 2.91 (95% CI: 1.32-6.42) and 2.25 (95% CI: 1.01-5.03) for TTHM concentrations of <44.2, 44.2-74.4, and >74.4 ng/L (p-trend=0.08) Non-monotonic dose-response relationship</p> <p><u>Birth length (in centimeters):</u> BDCM and DBCM associated with smaller birth length (p-trends of 0.04 and 0.02, respectively)</p>	Sex, maternal age, BMI, weight gain, education, income, parity, and study city	<ul style="list-style-type: none"> • Selection: methods not clear, and possibly not a randomly selected sample • Exposure period: late 3rd trimester
Costet et al. (2012)	IUGR	France (PELAGIE cohort) 2002-2006	Nested case-control	3,421 pregnant women	THMs (total and individual)	THM concentrations from municipal records linked to self-administered questionnaire data on residence, drinking water intake, showering and bathing, and swimming pool use. Urinary TCA at study inclusion (<19 weeks gestation).	<p>ORs = 1.0 (ref), 2.4 (95% CI: 1.0-5.7), 2.1 (95% CI: 0.9-5.1), and 2.0 (95% CI: 0.8-5.1) for TTHM intakes of <0.351, 0.351-<0.578, 0.578-<0.940, and ≥ 0.940 $\mu\text{g}/\text{day}$; highest ORs are for swimming pool use and brominated THMs; ORs for water ingestion near 1.0; OR = 1.8 (95% CI: 0.9-3.7) for urinary TCA concentrations above detection (≥ 0.01 mg/L)</p> <p>Λ-shaped dose-response relationships</p>	Smoking, alcohol, hypertension, and marital status; urine TCA also adjusted for urine creatinine	<ul style="list-style-type: none"> • Gestational age based on last menstrual period and ultrasound • Pregnancy outcomes from midwives, pediatricians, and medical records • Selection: nested case-control study; controls randomly selected from the underlying cohort; participation rates estimated at 80% and 99.4% followed through the end of pregnancy • Exposure period: water results are for the 3rd trimester, urinary TCA results are for the 1st trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Costet et al. (2012)	LBW and VLBW	Nova Scotia, Canada 1988-1995	Retro-spective cohort	50,755 pregnant women	TTHM	THMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models were used to model data and were linked to mother's residence at birth.	All ORs near 1.0 Similar findings for VLBW	Income and smoking	<ul style="list-style-type: none"> Information on residence, outcome and co-variables obtained from the Nova Scotia Atlee Perinatal Database, which contains information on all live and stillborn infants ≥ 500 g; information on income from the 1991 census Restricted to municipalities where $>90\%$ of households were served by a public water supply and to subjects receiving surface water Exposure period: last 3 months of pregnancy Highest exposure category ≥ 100 $\mu\text{g/L}$
Gallagher et al. (1998)	LBW	Colorado 1990-1993	Retro-spective cohort	1,893 births; 29 LBW	TTHM	THM concentrations measured quarterly at 4 different locations. These measurements and the hydraulic characteristics of each drinking water system were modeled using EPA-NET to estimate quarterly THM concentrations for each census block group. Exposure "scores" were then assigned to the 3rd trimester of pregnancy.	ORs = 1.0 (ref), 1.0 (95% CI: 0.6-1.8), 0.8 (95% CI: 0.3-1.7), and 2.1 (95% CI: 1.0-4.8) for concentrations of ≤ 20 , 21-40, 41-60, and ≥ 61 ppb OR for term LBW of 5.9 (95% CI: 2.0-17.0) for ≥ 61 ppb vs. ≤ 20 ppb	Prenatal care and maternal education	<ul style="list-style-type: none"> Exposure period: 3rd trimester Exposures across all trimesters were highly correlated Selection: includes birth records for mothers residing in census blocks served by two municipal water districts near Denver Only includes Whites Only includes census blocks with THM sampling data No personal interviews

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Grazuleviciene et al. (2011)	LBW, SGA	Kaunas, Lithuania (HiWate Study) 2007-2009	Prospective cohort	4,161 pregnant women; 156 LBW, and 270 SGA	THMs (total and individual)	THM concentrations measured 4 times per year in municipal supplies linked to personal interview data on residence, drinking water intake, showering and bathing, and swimming pool use. These factors are linked to "estimated uptake factors" to derive an "integrated index of blood concentration" expressed in mg of THM/day.	<p><u>LBW:</u> ORs = 1.00 (ref), 1.77 (95% CI: 0.95-3.30), and 2.13 (95% CI: 1.17-3.87) for estimated TTHM uptakes of 0.0025-0.0386, 0.0386-0.3545, and 0.3545-2.4040 mg/day; OR = 1.08 (95% CI: 1.01-1.16) for each 0.1 µg/day increase in TTHM uptake, all trimesters combined Similar results across the three trimesters separately Similar results for chloroform, BDCM, and DBCM</p> <p><u>SGA:</u> ORs = 1.00 (ref), 1.18 (95% CI: 0.86-1.82), and 1.34 (95% CI: 0.98-1.84) for TTHM uptakes of 0.0025-0.0386, 0.0386-0.3545, and 0.3545-2.4040 mg/day; OR = 1.03 (95% CI: 0.99-1.07) for each 0.1 µg/day increase in TTHM uptake, all trimesters combined Similar results across the three trimesters separately Similar results for chloroform and BDCM; most ORs for DBCM below 1.0</p>	Age, marital status, education, chronic disease, BMI, blood pressure, smoking, alcohol, preterm, sex, and birth year	<ul style="list-style-type: none"> • Gestational age determined by ultrasound • Outcomes from medical records • Some evidence of interaction between GSTM1 SNPs and TTHMs seen for LBW (Danileviciute et al., 2012). For example, OR = 4.37 (95% CI: 1.36-14.08) for TTHM uptakes above the median and <i>GSTM1-0</i> genotype vs. TTHM uptakes below the median and <i>GSTM1-1</i> genotype • Selection: on first visit to a general practitioner, all pregnant women in Kaunas were invited to participate; 79% agreed to participate • Further details on uptake factors and calculations of indices of blood concentrations are provided by the authors. • Exposure period: each trimester and all trimesters combined
Hinckley et al. (2005)	LBW, IUGR	Arizona (24 zip codes) 1998-2003	Retro-spective cohort	48,119 pregnant women; 3,760 IUGR and 859 LBW	THMs (total and individual)	THM concentrations in 3 water facilities measured quarterly in 1998-2002, averaged over the 3rd trimester and assigned to zip code of residence listed in birth records.	<p><u>IUGR:</u> All ORs near 1.0</p> <p><u>LBW:</u> All ORs near 1.0</p>	Age, parity, education, race, ethnicity, smoking, and prenatal care	<ul style="list-style-type: none"> • No personal interviews • Data on birth outcomes and co-variables from birth records • Only term births included • Exposure period: 3rd trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hoffman et al. (2008a)	BW, SGA	US (three study sites) 2000-2004	Prospective cohort	1,958 live births; 113 SGA	TTHMs (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on residence, water intake, showering and bathing, and uptake factors.	<p><u>SGA:</u> OR = 2.0 (95% CI: 1.1-3.6) for residential TTHM concentrations of ≥ 80 vs. < 80 $\mu\text{g/L}$ in the 3rd trimester (other categorical analyses with mixed results); ORs for other trimesters below 1.0; OR = 1.6 (1.0-2.7) for intakes of 1.6-27.1 vs. 0.02-0.09 μg absorbed/day from showering or bathing in 3rd trimester; ORs for other trimesters slightly lower; U-shaped dose-response relationships</p> <p><u>BW:</u> No clear associations</p>	Age, race, ethnicity, education, income, employment, marital status, BMI, parity, and caffeine use	<ul style="list-style-type: none"> • Selection: few details provided • Possible differences in sociodemographic characteristics seen across the 3 sites (see Hoffman et al., 2008a) • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011 • Exposure period: each trimester
Horton et al. (2011)	SGA	US (two study sites) 2000-2004	Prospective cohort	31,008 births; 1,543 SGA	TTHM	Weekly samples collected from a single representative location in the water distribution systems during 2000-2004. Further details provided in Savitz et al., 2006.	<p>Chlorinated site: THMs: all ORs near 1.0</p> <p>Brominated site: THMs: all ORs near 1.0</p>	Age, race, education, smoking, marital status, alcohol, and parity	<ul style="list-style-type: none"> • Data on outcomes and co-variables from birth records • Includes 2 of the 3 sites in Hoffman et al., 2008a and related publications • Exposure period: 3rd trimester • Only term births used in the SGA analysis • No personal interviews • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Infante-Rivard (2004)	IUGR	Montreal, Quebec, Canada 1998-2000	Case-control	458 cases and 426 controls	THMs (total and individual)	Monitoring data from municipalities and provincial government linked to personal questionnaire data on residential history, drinking water sources, bottled water and filter use, water intake, and showering habits. These data were used to estimate average and cumulative exposure over "the pregnancy period."	TTHM: No clear associations comparing >90 th percentile (29.4 µg/L) to ≤90 th percentile Bromoform: OR = 2.44 (95% CI: 0.19-31.10) comparing >90 th percentile (1.22 µg/L) to ≤90 th percentile	Age, sex, race, maternal weight gain, BMI, smoking, parity, pre-eclampsia, and previous IUGR	<ul style="list-style-type: none"> • Selection: cases ascertained from the largest university based hospital in Montreal; controls were infants from the same hospital with BWs ≥10th percentile matched to cases by gestational age, sex, and race • Participation rates: cases (97.6%), controls (98.3%) • Mean TTHM level = 18.74 µg/L in cases and 18.26 µg/L in controls • Some evidence of association with CYP2E1 SNP (OR = 13.20, 95% CI: 1.19-146.72); no interaction with MTHFR C677T SNP • Exposure period: whole pregnancy average
Iszatt et al. (2014)	LBW	Northwest England 2000-2007	Retro-spective cohort:	429,599 live births	THMs (total and individual)	Public water measurements, 4 per year in each water zone, were linked to residential address on birth certificates. Enhanced coagulation water treatment was introduced in 2003-4 and changes in chloroform levels in each water zone from before to after this time were assessed. Study area included 258 water zones.	Areas with the highest decrease in chloroform (30-65 µg/L) had the greatest percentage decreases in LBW (-9%; 95% CI: -12 to -5%) and very low BW (-16%; 95% CI: -24 to -8)	Deprivation index	<ul style="list-style-type: none"> • Limited data available from birth certificates • Census data on socioeconomic variables • Adjustments are unclear • Exposure period: annual averages based on year of birth

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Kogevinas et al. (2016)	LBW	Multiple sites in Europe (HiWate study) 2002-2010	Prospective cohort	14,005 mothers; 704 LBW	THMs (total and individual)	THM concentrations collected for regulatory purposes and for the study combined with data on water quality parameters and treatment methods and modeled to obtain monthly predictions. These were then linked to personal questionnaire data on water intake, water sources, showering, bathing, and swimming pool use.	THMs, chloroform, brominated THMs: no clear associations No clear associations by trimester, or with ingestion, showering or bathing	Sex, gestational age, ethnicity, parity, and site; also assessed maternal age, education, and smoking.	<ul style="list-style-type: none"> No interaction with CYP2E1 or GSTT1 SNPs Outcome data from birth records Exposure period: whole pregnancy average and each trimester
Kramer et al. (1992)	LBW, IUGR	Iowa (towns with populations of 1,000-5,000) 1989-1990	Case-control	LBW: 159 cases and 795 controls; SGA: 187 cases and 935 controls	THMs (individual)	1987 municipal water survey data linked to maternal residence at the time of birth.	<u>IUGR:</u> Chloroform: ORs = 1.0 (ref), 1.3 (95% CI: 0.9-1.8), and 1.8 (95% CI: 1.1-2.9) for concentrations of non-detect, 1-9, and ≥ 10 $\mu\text{g/L}$ BDCM: ORs = 1.0 (ref), 1.2 (95% CI: 0.8-1.7), and 1.7 (95% CI: 0.9-2.9) for concentrations of non-detect, 1-9, and ≥ 10 $\mu\text{g/L}$ <u>LBW:</u> No clear associations	Marital status, age, parity, prenatal care, smoking, and education	<ul style="list-style-type: none"> Only includes non-Hispanic White women Each town derived public water from a single source Selection: cases selected using Iowa birth certificate data; 5 randomly selected controls "from the same study population"; little other information provided on control selection Outcome and co-variate data from birth certificates Exposure period: unclear
Kumar et al. (2014)	LBW, SGA	New York State 1998-2003	Retro-spective cohort	1,528,681 births; 47,264 LBW and 71,909 SGA	TTHM	Time weighted average THM concentrations in each public water system (generally monitored once per quarter) linked to residential address on birth certificates.	<u>For all outcomes:</u> Some statistically significant ORs in the middle exposure categories but other ORs are close to 1.0	Race, ethnicity, age, education, employment, smoking, prenatal care, utilization index, and sex	<ul style="list-style-type: none"> Outcome and co-variate data from birth records Utilization index is the adequacy of prenatal care utilization (inadequate, intermediate, adequate, and adequate plus) based on the Kotelchuck Adequacy of Prenatal Care Utilization Index Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Levallois et al. (2012)	SGA	Quebec City, Canada 2006-2008	Case-control	571 cases and 1,925 controls	THMs (total and individual)	Data from monthly sampling in 46 sites during 2006-2008 used to develop spatio-temporal models linked to personal questionnaire data on water consumption, filter use, boiling water, showering habits, and other factors.	<u>TTHM:</u> OR = 1.5 (95% CI: 1.1-1.9) for >80 vs. <80 µg/L, p-trend = 0.07 Strongest association with ingestion vs. inhalation or dermal exposure <u>Individual THMs:</u> Some ORs up to 1.2, but not statistically significant and most ORs near 1.0	Age, calendar week, education, income, BMI, parity, history of LBW, smoking, passive smoking, coffee, alcohol, chronic disease, and preeclampsia	<ul style="list-style-type: none"> • Selection: cases and controls selected from birth records; controls randomly selected, matched to cases on period of birth • Exposure period: 3rd trimester • Possible interactions were seen with CYP2E1 SNPs although not statistically significant (Levallois et al., 2016) • No clear interaction with haloacetaldehydes or haloacetonitriles (Ileka-Priouzeau et al., 2015) • Some evidence of interaction with CYP17A1 polymorphisms (Bonou et al., 2017)
Lewis et al. (2006)	LBW	Massachusetts (27 communities) 1999-2001	Retrospective cohort	36,529 births; 780 LBW	TTHM	Weekly samples collected and measured for THMs. Measurements linked to residences in birth records. All 27 communities received water from a single supplier.	OR = 1.50 (95% CI: 1.07-2.10) for ≥70 vs. <40 µg/L in the 2nd trimester OR = 1.08 (95% CI: 1.00-1.17) for each 10 µg/L increase ORs for other trimesters or overall pregnancy average near 1.0	Sex, gestational age, marital status, prenatal care, maternal age, race/ethnicity, education, parity, smoking, payment method, conception and birth season, income, previous adverse birth outcomes, previous trimester exposure, and maternal diseases	<ul style="list-style-type: none"> • Outcome data from birth records • All water supplied by a single utility • Appears that most variability in exposure is due to time (i.e., season) rather than location • Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Patelarou et al. (2011)	LBW, SGA	Crete 2007-2008	Prospective cohort	1,359 live births; 76 LBW and 73 SGA	THMs (brominated)	THM concentrations in tap water samples collected from a representative sample of subjects' homes were linked to personal interview data on water intake, showering and bathing, dishwashing, and swimming pool use.	<u>SGA:</u> OR = 1.5 (95% CI: 0.6-3.7) for 1st trimester exposures Other ORs 1.3 or lower and not statistically significant <u>LBW:</u> All ORs near 1.0 for all trimesters	Country of origin, parity, marital status, education, age, and sex	<ul style="list-style-type: none"> • Co-variate data from subject interviews • Exposure period: whole pregnancy average and each trimester • Gestational age assessed by LMP and ultrasound • Analyses focused on brominated THMs since chloroform concentrations were low
(Porter et al., 2005)	IUGR	Maryland 1998-2002	Retrospective cohort	15,315 births; 1,114 IUGR	THMs (total and individual)	Monthly THM concentrations from 1997-2000 collected at 4 sampling points from a local water utility in the study county.	All ORs near 1.0 or inconsistent non-monotonic dose-response patterns	Prenatal care, marital status, age, and smoking	<ul style="list-style-type: none"> • Outcome and co-variate data from birth certificates • Large seasonal variations seen in THM concentrations • Exposure period: whole pregnancy average and each trimester
(Rivera-Nunez and Wright, 2013)	BW, SGA	Massachusetts (whole state) 1996-2004	Retrospective cohort	68,409 SGA	THMs (total and individual)	Quarterly town averages linked to addresses.	<p><i>For 3rd trimester exposure:</i> <u>SGA:</u> Most elevated ORs below 1.10 (some remain statistically significant) following statistical adjustments; ORs of about 1.0 when adjusted for HAAs</p> <p><u>BW:</u> Decreased BWs with TTHM, chloroform, BDCM, and THMBr but some with unclear dose-response relationships and marked reduction in associations with statistical adjustments</p> <p><i>For 2nd trimester exposure:</i> Similar results</p>	Source, disinfection, maternal age, race, education, marital status, payment method, and income (census tract)	<ul style="list-style-type: none"> • Exposure period: 2nd and 3rd trimesters • Mean TTHM = 37.5 µg/L • Adjustments for "source" and "disinfection" are unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Savitz et al. (1995)	LBW	Alamance, Durham, and Orange Counties, North Carolina 1988-1991	Case-control	178 cases and 333 controls	TTHM	Quarterly THM concentrations from water suppliers linked to mother's residence at birth. Concentrations for 28 th week used for the LBW analysis. Questionnaire data on water sources, and water consumption also collected.	All ORs near 1.0 or λ-shaped dose-response patterns for both THM concentration and dose (ppb × glasses of water per day)	Age, race, hospital, education, marital status, poverty, smoking, alcohol, employment, and nausea	<ul style="list-style-type: none"> • Selection: LBW infants identified from 6 area hospitals covering "virtually all births to area residents"; controls were deliveries immediately following preterm and LBW cases of the same race and from the same hospital but restricted to normal birthweight infants • >50-60% non-participation due to refusals, untraceable, or missing THM data • Exposure period: 28th week of pregnancy
(Smith et al., 2016)	BW	Northern England (Bradford cohort) 2007-2010	Prospective cohort	12,453 women	THMs (total and individual)	Routine water monitoring data linked to personal questionnaire information on water consumption, hot and cold beverage consumption, consumption of other fluids, filter use, and showering and bathing. This information was then used to develop exposure models for time weighted average water concentrations and estimated blood THM concentrations.	<u>TTHM and THMBr:</u> Decreased BW with increasing exposures but only in Pakistani-origin newborns (p-trend=0.009) for exposures averaged over whole pregnancy; similar findings for individual trimesters	Caffeine intake, SES, education, glucose levels, ethnicity, parity, age, BMI, gestational age, and sex	<ul style="list-style-type: none"> • BWs obtained from clinical records • Mean TTHM = 45.6 µg/L • In some analyses, major changes in results are seen after statistical adjustments • Positive results seem mostly related to showering, bathing, and swimming rather than drinking water consumption • Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Summerhayes et al. (2012)	SGA	New South Wales, Australia 1998-2004	Retrospective cohort	314,982 births; SGA 10%	THMs (total and individual)	Used data from the Sydney/llawarra water utility monthly THM monitoring program which rotated through 3-6 sites in each distribution system on a 3-6 month cycle. THM exposure assigned at the distribution system level.	<p><i>RRs for interquartile increases in water concentrations during the 3rd trimester:</i></p> <p>Chloroform (25 µg/L): 1.04 (95% CI: 1.02-1.06) BDCM (5 µg/L): 1.02 (95% CI: 1.01-1.04) DBCM (1 µg/L): 1.00 (95% CI: 0.99-1.01) TTHM (25 µg/L): 1.03 (95% CI: 1.01-1.05)</p> <p>RRs are somewhat higher in non-smokers; similar results for other trimesters and whole pregnancy average (numbers in parentheses after each chemical or chemical group name are the interquartile ranges)</p>	Age, indigenous, country of birth, sex, smoking, hypertension, diabetes, preeclampsia, antenatal visit, year of birth, season, and SES	<ul style="list-style-type: none"> • Birth outcomes and covariate data from a population based mandatory birth surveillance system • Gestational age based on LMP and ultrasound • Increases in RR are small • Exposure period: whole pregnancy average and each trimester
Toledano et al. (2005)	LBW	England (three regions) 1992-1998	Retrospective cohort	Approximately 1 million births	TTHM	Records of routine measurements of public utilities (generally 4 samples per year) modeled and linked to registry data on birth address.	ORs for LBW and very LBW were above 1.00 but below 1.10 and not statistically significant	Age, Carstairs quintile, sex, and year	<ul style="list-style-type: none"> • Data on outcomes obtained from national birth and stillbirth registers; registration of stillbirths is a national requirement • No individual data on exposure or most potential confounders • Exposure period: 3rd trimester
Villanueva et al. (2011)	BW, LBW, SGA	Spain (four regions) 2000-2008	Prospective cohort	2,074 births	THMs (chloroform and brominated)	THM measurements from a sampling campaign and from municipal records linked to personal interview data on water intake, showering and bathing, and swimming.	<p><u>BW:</u> Consistent associations not seen</p> <p><u>LBW:</u> ORs near 1.0 for each trimester and pregnancy overall average</p> <p><u>SGA:</u> ORs near 1.0 for each trimester and pregnancy overall average</p>	Sex, weeks of gestation, parity, BMI, weight gain, and smoking; maternal education also examined	<ul style="list-style-type: none"> • BW was recorded by midwives at delivery • Gestational age based on LMP and ultrasound • Increases in ORs are small • Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Wright et al. (2003)	BW, LBW, SGA	Massachusetts 1990	Retrospective cohort	56,513 singleton births; 1,325 LBW and 5,310 SGA	TTHM	Routine monitoring data collected quarterly to annually were used to calculate city or town specific quarterly averages. These were linked to city of residence reported on birth certificates.	<p><u>BW:</u> 2.8 grams (95% CI: 5.5 to 0.2 gram) decrease for each 20 µg/L increase in TTHM for overall pregnancy average (p = 0.03) Somewhat similar decrease seen for each trimester separately</p> <p><u>LBW:</u> No clear association, all ORs near 1.0</p> <p><u>SGA:</u> ORs of 1.00 (ref), 1.00 (95% CI: 0.92-1.09), and 1.14 (95% CI: 1.02-1.26) for average pregnancy concentrations of 0-60, >60-80, and >80 µg/L. Highest OR for the 2nd trimester (1.13, 95% CI: 1.03-1.24)</p>	Gestational age, sex, prenatal care, race, education, smoking, maternal age, parity, income, previous LBW or preterm birth, and medical history	<ul style="list-style-type: none"> Data were obtained from birth records and "hospital worksheets" Median TTHM concentrations were approximately 25-34 µg/L Exposure period: whole pregnancy average and each trimester
Wright et al. (2004)	SGA	Massachusetts (109 towns) 1995-1998	Retrospective cohort	194,827 births; 17,359 SGA	THMs (total and individual)	Weekly to quarterly THM monitoring data from 1995-1998. Town averages calculated and linked to subject residences.	TTHM: ORs = 1.00 (ref), 1.06 (95% CI: 1.02-1.10), and 1.13 (95% CI: 1.07-1.20) for water concentrations of 0-33, >33-74, and >74-163 µg/L Similar ORs for chloroform and BDCM	Income, prenatal care, race, education, smoking, age, parity, and maternal medical history	<ul style="list-style-type: none"> Exposure period: 3rd trimester Includes towns with populations of >10,000 people Birth outcomes and covariates from birth certificates Gestational age based on clinician estimate Residence based on maternal zip code Increases in ORs are small

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Yang et al. (2016)	LBW, SGA	Taiwan (65 municipalities) 2000-2002	Ecologic	90,848 pregnant women; 8,938 SGA and 2,766 LBW	TTHM	TTHM concentrations collected quarterly in the years 2000 and 2002. Randomly selected 96 municipalities from 361 total in Taiwan. Excluded 31 due to overlapping water supplies. Concentrations averaged over the two years. Exposure based on municipality of residence at birth.	<u>Term LBW:</u> All ORs near 1.0 <u>SGA:</u> All ORs near 1.0	Age, marital status, education, and sex	<ul style="list-style-type: none"> • Outcome and other data collected from birth registries • Ecologic exposure assessment • High exposure category (TTHM) >13.11 µg/L • Exposure period: unclear

Table B4. Epidemiologic Studies of Trihalomethanes and Preterm Birth or Gestational Age (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Aggazzotti et al. (2004)	Preterm	Italy (nine towns) 1999-2000	Case-control	343 cases and 612 controls	THMs (total and individual)	Questionnaire information on water sources, cooking, and swimming linked to THM measurements collected from subject's home water source at the time of delivery.	No clear associations	Sex, education, mothers smoking, water-based beverages, and type of water consumed	<ul style="list-style-type: none"> • Selection: controls selected from the same hospitals and matched to cases on date of birth • Exposure period: time of delivery
Cao et al. (2016)	Gestational age	Wuhan and Xiaogan Cities, China 2011-2012	Cross-sectional	1,184 pregnant women	THMs (total and individual)	Maternal serum collected in late pregnancy (≥ 35 weeks gestation)	No clear associations	Sex, maternal age, BMI, weight gain, education, income, parity, and study city	<ul style="list-style-type: none"> • Selection: methods not clear • Exposure period: late 3rd trimester
Costet et al. (2012)	Preterm	France (PELAGIE cohort) 2002-2006	Prospective cohort and nested case-control	3,421 pregnant women	THMs (total and individual)	THM concentrations from municipal records linked to self-administered questionnaire data on residence, drinking water intake, showering and bathing, and swimming pool use. Urinary TCA at study inclusion (<19 weeks gestation).	All ORs near 1.0	Smoking, alcohol, hypertension, and marital status; urine TCA also adjusted for urine creatinine	<ul style="list-style-type: none"> • Gestational age based on LMP and ultrasound • Pregnancy outcomes from midwives, pediatricians, and medical records • Selection: the urinary TCA portion of this study was a nested case-control study; controls randomly selected from the underlying cohort; participation rates estimated at 80% and 99.4% followed through the end of pregnancy • Exposure period: water results are for the 3rd trimester, urinary TCA results are for the 1st trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Dodds et al. (1999)	Preterm	Nova Scotia, Canada 1988-1995	Retro-spective cohort	50,755 pregnant women	TTHM	THMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models used to model data and were linked to mother's residence at birth.	All ORs near 1.0	Income and smoking	<ul style="list-style-type: none"> Information on residence, outcome and co-variables obtained from the Nova Scotia Atlee Perinatal Database, which contains information on all live and stillborn infants ≥ 500 g; information on income from the 1991 census Restricted to municipalities where $>90\%$ of households were served by a public water supply and to subjects receiving surface water Exposure periods: last 3 months of pregnancy Highest exposure category ≥ 100 $\mu\text{g/L}$
Gallagher et al. (1998)	Preterm	Colorado 1990-1993	Retro-spective cohort	1,893 births; 68 preterm	TTHM	THM concentrations measured quarterly at 4 different locations. These measurements and the hydraulic characteristics of each drinking water system were modeled using EPA-NET to estimate quarterly THM concentrations for each census block group. Exposure "scores" were then assigned to the 3rd trimester of pregnancy.	All ORs near 1.0	Prenatal care and maternal education	<ul style="list-style-type: none"> Exposure period: 3rd trimester Exposures across all trimesters were highly correlated Selection: includes birth records for mothers residing in census blocks served by two municipal water districts near Denver Only includes Whites Only includes census blocks with THM sampling data No personal interviews

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hoffman et al. (2008b)	Preterm	US (three study sites) 2000-2004	Prospective cohort	2,039 pregnant women; 185 preterm	THMs (total and individual)	Weekly or biweekly water samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on residence, water intake, showering and bathing, and uptake factors.	All ORs near or below 1.0 for 2nd trimester exposures; authors reported that findings were similar for the 1st trimester although specific results not provided	Age, race, ethnicity, education, income, employment, marital status, BMI, parity, and caffeine use	<ul style="list-style-type: none"> • Selection: subjects recruited from prenatal clinics, advertisements, and targeted mailings • Differences seen across the 3 sites in terms of age, race/ethnicity, education, and income • Exposure periods: 1st and 2nd trimesters • THM concentrations highly correlated (e.g., R = 0.9) • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011
Horton et al. (2011)	Preterm	US (two study sites) 2000-2004	Prospective cohort	31,008 births; 2,075 preterm	TTHM	Weekly samples collected from a single representative location in the water distribution systems during 2000-2004. Further details on exposure assessment provided in Savitz et al., 2006.	Chlorinated site: THMs: all ORs near 1.0 Brominated site: THMs: all ORs near 1.0	Age, race, education, smoking, marital status, alcohol, and parity	<ul style="list-style-type: none"> • Data on outcomes and co-variables from birth records • Includes 2 of the 3 sites in Hoffman et al., 2008a and related publications • Exposure period: 2nd trimester • No personal interviews • Some overlap between Savitz et al., 2006, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011
Kramer et al. (1992)	Preterm	Iowa (towns with populations of 1,000-5,000) 1989-1990	Case-control	342 cases and 1,710 controls	THMs (individual)	1987 municipal water survey data linked to maternal residence at birth.	No clear associations	Marital status, age, parity, prenatal care, smoking, and education	<ul style="list-style-type: none"> • Only includes non-Hispanic White women • Each town derived public water from a single source • Selection: cases selected using Iowa birth certificate data; 5 randomly selected controls "from the same study population"; little other information provided on control selection • Outcome and co-variate data from birth certificates • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Kumar et al. (2014)	Preterm	New York State 1998-2003	Retro-spective cohort	1,528,681 births; 62,004 preterm	TTHM	Time weighted average THM concentrations in each public water system (generally monitored once per quarter) linked to residential address on birth certificates.	Some statistically significant ORs in the middle exposure categories but other ORs are close to 1.0	Race, ethnicity, age, education, employment, smoking, prenatal care, utilization index, and sex	<ul style="list-style-type: none"> • Outcome and co-variate data from birth certificates • Exposure period: unclear
Lewis et al. (2007)	Preterm	Massachusetts (27 Communities) 1999-2001	Nested case-control	37,498 births, 2,813 preterm	TTHM	Weekly samples collected and measured for THMs. Measurements linked to residences in birth records. All 27 communities received water from a single supplier.	<p><u>All women:</u> <i>Second trimester exposures:</i> OR = 0.82 (95% CI: 0.71-0.94) for ≥ 60 vs. < 40 $\mu\text{g/L}$; OR = 0.95 (95% CI: 0.92-0.99) per 10 $\mu\text{g/L}$ increase in TTHMs; ORs near or below 1.0 at other time periods</p> <p><u>Government payment for prenatal care:</u> OR = 1.39 (95% CI: 1.06-1.81) for ≥ 60 vs. < 40 $\mu\text{g/L}$ at four weeks before birth; ORs near 1.0 for 2nd trimester exposure</p>	Sex, marital status, gestational age, prenatal care, maternal age, race/ethnicity, education, parity, smoking, payment method, conception and birth season, income, previous adverse birth outcomes, previous trimester exposure, and maternal diseases	<ul style="list-style-type: none"> • Outcome and co-variate data from birth records • All water supplied by a single utility • Appears that most variability in exposure is due to time (i.e., season) rather than location • Some evidence of an association in women with lower SES • Comparison of different methods for selecting controls gives mostly similar results (Lewis et al., 2011) • Exposure periods: whole pregnancy average, each trimester, and 4 weeks before birth
Patelarou et al. (2011)	Preterm	Crete 2007-2008	Prospective cohort	1,359 live births; 11.5% preterm	THMs (brominated)	THM concentrations in tap water samples collected from a representative sample of subjects homes were linked to personal interview data on water intake, showering and bathing, dishwashing, and swimming pool use.	All ORs near 1.0 for all trimesters	Country of origin, parity, marital status, education, age, and sex; also assessed smoking and ethnicity	<ul style="list-style-type: none"> • Co-variate data from subject interviews • Exposure period: whole pregnancy average and each trimester • Gestational age assessed by LMP and ultrasound • Analyses focused on brominated THMs since chloroform concentrations were low
Righi et al. (2003)	Preterm	Modena, Italy 1999-2000	Case-control	93 cases and 166 controls	THMs	Drinking water sampling at subject's home and personal questionnaire data.	No clear associations	Unclear	<ul style="list-style-type: none"> • Only 5.2% subjects reported "usually" drinking tap water • Mean THM = 0.73 $\mu\text{g/L}$ • Full study not reviewed; study description here is from the English abstract of an article written in Italian • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Rivera-Nunez and Wright (2013)	Preterm	Massachusetts (whole state) 1996-2004	Retro-spective cohort	37,136 preterm births	THMs (total and individual)	Quarterly town averages linked to addresses. Towns with only annual measurements were assigned the same concentration for each quarter.	<i>For second trimester exposures:</i> OR = 1.04 (95% CI: 0.91-1.19) for TTHM >62 µg/L vs. ≤10 µg/L <i>For 1st trimester exposures:</i> Similar results	Source, disinfection, maternal age, race, education, marital status, payment method, and income (census tract)	<ul style="list-style-type: none"> •Exposure period: 1st and 2nd trimesters •Mean TTHM = 37.5 µg/L •Adjustments for “source” and “disinfection” are unclear
Savitz et al. (1995)	Preterm	Alamance, Durham, and Orange Counties, North Carolina 1988-1991	Case-control	244 cases and 333 controls	TTHM	Quarterly THM concentrations from water suppliers linked to mother's residence at birth. Concentrations for 28 th week used for the preterm analysis. Questionnaire data on water sources, and water consumption also collected.	All ORs near 1.0 for both THM concentration and dose (ppb × glasses of water per day)	Age, race, hospital, education, marital status, poverty, smoking, alcohol, employment, and nausea	<ul style="list-style-type: none"> •Selection: preterm deliveries identified from 6 area hospitals covering “virtually all births to area residents”; controls were deliveries immediately following preterm and LBW cases of the same race and from the same hospital but restricted to normal birthweight infants •>50-60% non-participation due to refusals, untraceable, or missing THM data •Exposure period: 28th week of pregnancy
Villanueva et al. (2011)	Preterm	Spain (four regions) 2000-2008	Prospective cohort	2,074 births	THMs (chloroform and brominated)	THM measurements from a sampling campaign and from municipal records linked to personal interview data on water intake, showering, bathing, and swimming. These were then multiplied by estimated “uptake factors” for each exposure source derived from the literature to estimate daily THM concentration in the bloodstream.	Overall ORs near or below 1.0 for residential ingestion, showering and bathing, and for swimming pool use OR = 1.115 (95% CI: 1.007-1.235) for each 10% increase in estimated daily concentration of brominated THMs in the bloodstream from residential sources (showering, bathing, drinking) during the 3rd trimester ORs near 1.0 for other trimesters and whole pregnancy average	Sex, weeks of gestation, parity, BMI, weight gain, and smoking; maternal education also examined	<ul style="list-style-type: none"> •Gestational age based on LMP and ultrasound •Increases in ORs are small •Exposure period: whole pregnancy average and each trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Wright et al. (2003)	Preterm	Massachusetts 1990	Retro-spective cohort	56,513 singleton births; 3,173 preterm	TTHM	Routine monitoring data collected quarterly to annually were used to calculate city or town specific quarterly averages. These were linked to city of residence reported on birth certificates.	All ORs near 1.0	Gestational age, sex, prenatal care, race, education, smoking, maternal age, parity, income, previous LBW and preterm births, and medical history	<ul style="list-style-type: none"> Data were obtained from birth records and "hospital worksheets" Median TTHM concentrations were approximately 25-34 µg/L Exposure period: whole pregnancy average and each trimester
Wright et al. (2004)	Preterm	Massachusetts (109 towns) 1995-1998	Retro-spective cohort	194,827 births; 11,580 preterm	THMs (total and individual)	Weekly to quarterly THM monitoring data from 1995-1998. Town averages calculated and linked to subject residences.	All ORs near 1.0	Income, prenatal care, race, education, smoking, age, parity, and maternal medical history	<ul style="list-style-type: none"> Exposure period: 3rd trimester Includes towns with populations of >10,000 people Birth outcomes and co-variates from birth certificates Gestational age based on clinician estimate Residence based on maternal zip code
Yang et al. (2007)	Preterm	Taiwan (65 municipalities) 2000-2002	Ecologic	90,848 pregnant women; 2,818 preterm	TTHM	TTHM concentrations collected quarterly in the years 2000 and 2002. Randomly selected 96 municipalities from 361 total in Taiwan. Excluded 31 due to overlapping water supplies. Concentrations averaged over the two years. Exposure based on municipality of residence at birth.	All ORs near 1.0	Age, marital status, education, and sex	<ul style="list-style-type: none"> Outcome and other data collected from birth registries Ecologic exposure assessment High exposure category (TTHM) >13.11 µg/L Exposure period: unclear

Table B5. Epidemiologic Studies of Trihalomethanes and Pregnancy Loss (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Dodds et al. (2004)	Stillbirth	Nova Scotia, Canada 1999-2001	Case-control	112 cases and 398 controls	THMs (total and individual)	Residential tap water samples collected from all subjects approximately 1 year after the 4 th -5 th month of pregnancy. THM measurements were linked to questionnaire data on water consumption, filter use, showering and other factors.	OR = 2.2 (95% CI: 1.1-4.4) for >80 vs. 0 µg/L TTHM but with non-linear dose-response pattern Similar results for chloroform and BDCM	Age, province, and income	<ul style="list-style-type: none"> • Selection: cases and controls obtained through population based perinatal databases; controls randomly selected and matched on 3-month birth period • Exposure period: unclear
Hwang and Jaakkola (2012)	Stillbirth	Taiwan 2001-2003	Unclear	Random sample of 32,890 from 396,049 births; 3,289 stillbirths	TTHM	Weighted average of quarterly THM measurements done by each water treatment plant during the date of conception and the date of birth. Exposure assignments appear to be based on municipality.	OR = 1.06 (95% CI: 0.96-1.17) for ≥20 vs. 0-4 µg/L	Sex, maternal age, plurality, season, and population density	<ul style="list-style-type: none"> • Birth data from the Birth Registry of Taiwan, reporting is compulsory • Smoking mothers excluded • Exposure period: appears to be averaged values for the date of conception and the date of birth
Iszatt et al. (2014)	Stillbirth	Northwest England 2000-2007	Retrospective cohort	429,599 live births; 2,279 stillbirths	THMs (total and individual)	Public water measurements, 4 per year in each water zone, were linked to residential address on birth certificates. Enhanced coagulation water treatment was introduced in 2003-4 and changes in chloroform levels in each water zone from before to after this time were assessed. Study area included 258 water zones.	No clear associations	Deprivation index	<ul style="list-style-type: none"> • Rates of stillbirths assessed before and after enhanced coagulation was introduced to some parts of the study area • Limited data available from birth certificates • Census data on SES variables • Adjustments unclear • Exposure period: annual averages based on year of birth

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
King et al. (2000)	Stillbirth	Nova Scotia, Canada 1988-1995	Retro-spective cohort	49,756 deliveries; 214 stillbirths	THMs (total and individual)	THMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models used to model data and were linked to mother's residence at birth. Average values throughout pregnancy used.	<p><u>TTHM:</u> ORs = 1.00 (ref), 1.27 (95% CI: 0.88-1.85), 1.28 (95% CI: 0.81-2.03), and 1.66 (95% CI: 1.09-2.54) for concentrations of 0-49, 50-74, 75-99, and ≥ 100 $\mu\text{g/L}$; OR = 1.05 (95% CI: 1.01-1.09) for each 10 $\mu\text{g/L}$ increase</p> <p><u>Chloroform:</u> ORs = 1.00 (ref), 1.20 (95% CI: 0.85-1.68), 1.35 (95% CI: 0.87-2.08), and 1.56 (95% CI: 1.04-2.34) for concentrations of 0-49, 50-74, 75-99, and ≥ 100 $\mu\text{g/L}$; OR = 1.04 (95% CI: 1.00-1.09) for each 10 $\mu\text{g/L}$ increase</p> <p><u>BDCM:</u> ORs = 1.00, 1.07 (95% CI: 0.77-3.19), 1.44 (95% CI: 0.90-2.27), and 1.98 (95% CI: 1.23-3.49) for concentrations of < 5, 5-9, 10-19, and ≥ 20 $\mu\text{g/L}$; OR = 1.29 (95% CI: 1.10-1.53) for each 10 $\mu\text{g/L}$ increase</p> <p>Higher ORs when cause of death confined to "asphyxia"</p>	Maternal age and smoking; parity, sex, and family income also assessed	<ul style="list-style-type: none"> Information on residence, outcome and co-variables obtained from the Nova Scotia Atlee Perinatal Database, which contains information on all live and stillborn infants ≥ 500 g; information on terminated pregnancies obtained from the Fetal Anomaly Database; information on income from the 1991 census Restricted to municipalities where $>90\%$ of households served by a public water supply and to subjects served by surface water Highest exposure category: ≥ 100 $\mu\text{g/L}$ Bromoform and DBCM concentrations were low and not assessed Autopsies were performed on 75% of known stillbirths Same study reported in Dodds et al., 1999 Mother's residence at birth assumed to be residence through pregnancy Exposure period: throughout pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Savitz et al. (1995)	SAB	Alamance, Durham, and Orange Counties, North Carolina 1988-1991	Case-control	126 cases and 122 controls	TTHM	Quarterly TTHM concentrations from water suppliers linked to mother's residence at birth. Concentrations for the 4 th week of pregnancy used for SAB analysis. Questionnaire data on water sources, and water consumption also collected.	ORs = 1.0 (ref), 1.0 (95% CI: 0.5-2.0), and 1.2 (95% CI: 0.6-2.4) for concentrations of 40.8-59.9, 60.0-81.0, and 81.1-168.8 ppb OR = 1.7 (95% CI: 1.1-2.7) per 50 ppb increase ORs for TTHM dose (ppb × glasses of water per day) all near 1.0	Age, race, hospital, education, marital status, poverty, smoking, alcohol, employment, and nausea	<ul style="list-style-type: none"> • Selection: SABs identified through medical care providers; controls were deliveries immediately following preterm and LBW cases of the same race and from the same hospital but restricted to normal birthweight infants • >50-60% non-participation due to refusals, untraceable, or missing TTHM data • Inconsistency between THM concentration (ppb) categories, per ppb change, and dose (ppb × glasses per day) results • Exposure period: 4th week of pregnancy
Savitz et al. (2006)	Pregnancy loss	US (three sites)	Prospective cohort	2,409 pregnant women; 258 pregnancy losses	THMs (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal interview data on water intake and hot and cold beverage consumption, showering and bathing, and uptake factors.	<u>TTHM:</u> OR = 1.0 (95% CI: 0.7-1.4) for water concentrations ≥75 vs. <75 µg/L; similar result for ingested amount (µg/day); OR = 1.3 (95% CI: 0.9-1.9) for intake >1.9 µ/day from bathing and showering <u>BDCM:</u> OR = 1.6 (95% CI: 1.0-2.4) comparing the upper to lower quartiles ORs near 1.0 when using other exposure category cut-off points <u>Other THMs:</u> ORs closer to 1.0	Age, race, ethnicity, education, marital status, age at menarche, alcohol, and vitamin use	<ul style="list-style-type: none"> • Selection: subjects who were trying to become pregnant or were <12 weeks pregnant were recruited from prenatal care practices • Authors state that, "...THM and HAA concentrations were spatially uniform through the distribution system" • Pregnancy losses identified by self-report • Co-variate data from subject interviews • Differences seen across the 3 sites in race/ethnicity and education • Pregnancy loss not well defined • Some overlap between Savitz et al., 2005, Hoffman et al., 2008a, Hoffman et al., 2008b, and Horton et al., 2011 • Exposure period: weeks 3-8 of pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Toledano et al. (2005)	Stillbirth	England (three regions) 1992-1998	Retro-spective cohort	Approximately 1 million births	TTHM	Records of routine measurements of public utilities (generally 4 samples per year) modeled and linked to registry data on birth address.	ORs = 1.00 (ref), 1.06 (95% CI: 0.99-1.15), and 1.11 (95% CI: 1.00-1.23) for TTHM concentrations of <30, 30-59, and ≥60 µg/L	Age, Carstairs quintile, sex, and year	<ul style="list-style-type: none"> •Data on outcomes obtained from national birth and stillbirth registers; registration of stillbirths is a national requirement •No individual data on exposure or most potential confounders •Increases in ORs are small •Exposure period: 3rd trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Waller et al. (1998)	SAB	Northern California (Pregnancy Outcome Study) 1989-1991	Prospective cohort	5,144 pregnant women; approximately 10% SABs	THMs (total and individual)	Average of quarterly THM measurements done by the utilities (85 in the study area) linked to subject's residence in the 1st trimester. Telephone interview data collected on tap and bottled water consumption.	<p><u>TTHM:</u> Increase in SAB percentage from 7.8 to 15.6% for TTHM concentrations <1 µg/L (non-detectable) to ≥120 µg/L, respectively, with somewhat monotonic dose-response increase (p = 0.16) (unadjusted) OR = 1.2 (95% CI: 1.0-1.5) for ≥75 vs. <75 µg/L OR = 2.0 (95% CI: 1.1-3.6) for ≥75 vs. <75 µg/L among those drinking at least 5 glasses of cold tap water per day</p> <p><u>Individual THMs:</u> Percentages of SAB increased slightly with increasing water concentrations of brominated THMs, but increases were mostly small (e.g., 9.1 to 10.4% for BDCM water concentrations of 0-2 to ≥18 µg/L; p = 0.74)</p> <p>BDCM: OR = 3.0 (95% CI: 1.4-6.6) for ≥5 glasses of cold tap water per day and TTHM water concentration ≥75 µg/L vs. <5 glasses of tap water per day, or a TTHM water concentration <75 µg/L, or receiving water from a utility that provided ≥95% groundwater, in analyses adjusted for other THMs</p>	Age, smoking, prior SAB, race, and employment; some analyses of individual THMs adjusted for other THMs	<ul style="list-style-type: none"> Members of Kaiser Permanente Medical Care Program Exposure period: 1st trimester Selection: women calling to make first prenatal care check. 5,144 of 7,881 participated Outcomes based on hospital records, follow-up interviews or the California Birth Registry For 7% of subjects the annual average THM concentrations reported in the utility's annual water report was used TTHM measured in 53 samples of bottled water, 72% below detection and a mean of 10 µg/L in the remainder Similar findings seen in the exposure re-analysis by Waller et al. (2001)

Table B6. Epidemiologic Studies of Trihalomethanes and Congenital Malformations (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Bove et al. (1995)	CM (multiple)	New Jersey 1985-1988	Retro-spective cohort	80,938 live births; 669 CMs	TTHM	Results of quarterly water testing of at least four samples from all water companies were used to estimate monthly concentrations. These were then linked to the mother's residence at birth (from birth certificates) and used to estimate exposures during the 1st trimester of birth.	ORs comparing >80 to ≤20 ppb (except oral cleft defects, where high exposure group is >100 ppb) CNS: 2.59 (90% CI: 1.53-4.30) NTD: 2.96 (90% CI: 1.26-6.62) Cleft defects: 3.17 (90% CI: 1.18-7.26) Cardiac defects: 1.83 (90% CI: 0.97-3.29) Mostly non-monotonic dose-response relationships	Maternal age, race, education, parity, previous stillbirth or miscarriage, sex, and prenatal care	<ul style="list-style-type: none"> • Data on outcomes and co-variables were obtained from vital records (birth and death certificates) and from the New Jersey Birth Defects Registry • Includes all live births and fetal deaths identified by birth or death certificates in 75 of the 146 towns in the area that were mostly served by public water supplies • Mothers residence at birth was assumed to be the residence for the entire pregnancy • Exposure assessment was blinded • Exposure period: 1st trimester
Cedergren et al. (2002)	CM (cardiac)	Ostergotland County, Sweden 1982-1996	Unclear	58,669 women; 753 cardiac defect	TTHM	Data on physical and chemical properties of potable water were requested from all waterworks supplying the county for the years 1983-1994. Data on chlorination procedures were obtained from published reports. TTHM concentrations were obtained for the years 1994-1995 from the National Food Administration Board.	OR = 1.30 (95% CI: 1.08-1.56) for all cardiac defects for TTHM concentrations >10 vs. <10 µg/L	Age, parity, smoking, and education	<ul style="list-style-type: none"> • Infants were identified from the Swedish Medical Birth Registry, the Hospital Discharge Registry, and the Registry of CM • TTHM exposure data only available for 1994-1995 • Little information provided on exposure assessment • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Chisholm et al. (2008)	CM (multiple)	Perth, Western Australia 2000-2004	Cross-sectional	20,870 births; 1,097 CMs	TTHM	Water samples collected from 47 locations in the greater Perth area from 2005-2006 linked to maternal residential postcodes in birth records	<p>For TTHM levels of <60, 60-130, and >130 µg/L</p> <p><u>Any birth defect:</u> ORs of 1.00 (ref), 0.98 (95% CI: 0.75-1.28), and 1.22 (95% CI: 1.01-1.48)</p> <p><u>Cardiovascular:</u> ORs of 1.00 (ref), 1.00 (95% CI: 0.55-1.81), and 1.62 (95% CI: 1.04-2.51)</p> <p><u>Musculoskeletal:</u> ORs of 1.00 (ref), 1.05 (95% CI: 0.60-1.83), and 1.48 (95% CI: 0.99-1.21)</p> <p><u>Urogenital:</u> ORs of 1.00 (ref), 1.09 (95% CI: 0.68-1.77), and 1.40 (0.98-1.99)</p> <p><u>Other sites (gastrointestinal, nervous system, respiratory system, and skin):</u> ORs near 1.0 or highest ORs in the middle exposure category</p>	Age and SES	<ul style="list-style-type: none"> • Data on outcomes and covariates from the statutory Western Australia Midwives' Notification System and birth defects registry • SES from census data • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Dodds and King (2001)	CM (multiple)	Nova Scotia, Canada 1988-1995	Retro-spective cohort	49,842 births; 77 NTDs, 430 cardiovascular anomalies, and 82 cleft defects	THMs (individual)	Routine monitoring results for THMs obtained from the Nova Scotia Department of Environment. THMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models were used to model data and were linked to mother's residence at birth.	<p><u>BDCM:</u> NTDs: ORs = 1.0 (ref), 1.4 (95% CI: 0.8-2.3), 0.6 (95% CI: 0.2-1.5), and 2.5 (95% CI: 1.2-5.1) for concentrations of <5, 5-9, 10-19, and ≥20 µg/L Cardiac defects: OR = 0.3 (95% CI: 0.2-0.7) for ≥20 µg/L vs. <5 µg/L Other: clear associations or dose-response relationships not seen for cleft defects</p> <p><u>Chloroform:</u> Cleft defects: ORs = 1.0 (ref), 1.2 (95% CI: 0.7-2.0), 0.9 (95% CI: 0.4-2.0), and 1.5 (95% CI: 0.8-2.8) for concentrations of <50, 50-74, 75-99, and ≥100 µg/L Other: clear associations or dose-response relationships not seen for neural tube and cardiac defects</p>	Maternal age and income	<ul style="list-style-type: none"> •Outcome information from the Nova Scotia Atlee Perinatal Database •Little information provided on exposure assessment •Only BDCM and chloroform assessed; concentrations of other THMs were too low to be evaluated •Selection: included all births in study area during 1988-1995 •Exposure periods: cleft and cardiac defects: first 2 months; NTDs: 3 months before pregnancy •Mother's residence at birth was assumed to be the residence for the entire pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Dodds et al. (1999)	CM (multiple)	Nova Scotia, Canada 1988-1995	Retro-spective cohort	50,755 pregnant women; 77 NTDs, 430 cardiovascular anomalies, and 82 cleft defects	TTHM	Routine monitoring results for THMs obtained from the Nova Scotia Department of Environment. THMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models were used to model data and were linked to mother's residence at birth.	<p><u>NTDs:</u> ORs = 1.00 (ref), 0.67 (95% CI: 0.38-1.17), 0.42 (95% CI: 0.17-1.01), and 1.18 (95% CI: 0.67-2.10) for concentrations of 0-49, 50-74, 75-99, and ≥ 100 $\mu\text{g/L}$</p> <p><u>Other:</u> ORs near or below 1.0 for cleft and cardiac defects</p>	Income and smoking	<ul style="list-style-type: none"> Information on residence, outcome and co-variables obtained from the Nova Scotia Atlee Perinatal Database, which contains information on all live and stillborn infants ≥ 500 g; information on terminated pregnancies obtained from the Fetal Anomaly Database. Information on income from the 1991 census Restricted to municipalities where >90% of households served by a public water supply and to subjects receiving surface water Exposure periods: cleft and cardiac defects: first 2 months; NTDs: 1 month before and after conception Mother's residence at birth was assumed to be the residence for the entire pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Grazuleviciene et al. (2013)	CM (multiple)	Kaunas, Lithuania (HiWate Study) 2007-2009	Prospective cohort	3,341 pregnant women; 57 cardiac, 37 musculoskeletal, and 23 urogenital abnormalities	THMs (total and individual)	THM concentrations measured 4 times per year in municipal supplies linked to personal interview data on residence, blood uptake factors, drinking water intake, showering and bathing, and swimming pool use. Exposure levels expressed as estimated daily uptake in blood.	<p><u>Heart abnormalities</u> For exposures in the 1st trimester TTHM: OR = 1.88 (95% CI: 0.96-3.69) for each 1 µg/day average increase in TTHM Chloroform: OR = 1.97 (95% CI: 0.90-4.35) for each 1 µg/day average increase in chloroform BDCM: OR = 1.70 (95% CI: 1.09-2.66) for each 0.1 µg/day average increase in BDCM DBCM: OR = 1.25 (95% CI: 1.01-1.54) for each 0.01 µg/day average increase in DBCM</p> <p><u>Musculoskeletal abnormalities</u> For exposures in the first month of pregnancy DBCM: ORs = 1.00 (ref), 1.41, and 2.56 (p = 0.024) for uptakes of 0.000-0.002, 0.002-0.006, 0.006-0.093 µg/day in blood Other species and TTHM: no clear associations For other exposure periods Findings for months 2 and 3 are similar but ORs for average DBCM exposure in the 1st trimester are markedly lower</p> <p><u>Urogenital abnormalities</u> For exposures in the 1st trimester TTHM: OR = 2.00 (95% CI: 0.72-5.56) for each 1 µg/day average increase in TTHM Chloroform: OR = 2.22 (95% CI: 0.69-7.17) for each 1 µg/day average increase in chloroform BDCM: OR = 1.57 (95% CI: 0.74-3.37) for each 0.1 µg/day average increase in BDCM DBCM: OR = 1.17 (95% CI: 0.80-1.72) each 0.01 µg/day average increase in DBCM Also: TTHM: OR = 3.01 (95% CI 1.11-8.16) for high vs. low areas (21.9 vs. 1.3 µg/L)</p>	Age, chronic disease, alcohol, and fetus number	<ul style="list-style-type: none"> •Pregnancy outcomes from medical and registry records •Selection: on first visit to a general practitioner, all pregnant women in Kaunas were invited to participate; 79% agreed •Exposure period: 1st trimester

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Hwang et al. (2008)	CM (multiple)	Taiwan 2001-2003	Unclear	396,049 births, 2,148 CMs	TTHM	Weighted average of quarterly THM measurements collected from each water treatment plant during the date of conception and the date of birth. Exposure assignments appear to be based on municipality.	ORs for TTHMs ≥ 20 $\mu\text{g/L}$ vs. 0-4 $\mu\text{g/L}$: Anencephaly: OR = 1.96 (95% CI: 0.94-4.07) Ventricular septal defect: OR = 1.81 (95% CI: 0.98-3.35) Cleft palate: OR = 1.56 (95% CI: 1.00-2.41)	Age, plurality, and population density	<ul style="list-style-type: none"> • Birth defects, residential, and co-variate data from birth registry records • Highest exposure category is ≥ 20 $\mu\text{g/L}$ • Exposure period: appears to be averaged values for the date of conception and the date of birth
Iszatt et al. (2011)	CM (hypo-spadias)	Southwest England 2000-2003	Case-control	468 cases and 485 controls	THMs (total and individual)	Quarterly data from 1998 on THM concentrations from six water companies and 140 water zones modeled to create annual average concentrations. These were linked to personal interview data on maternal water consumption, cold and hot tap water use, bottled water use, and duration of dishwashing, showering, bathing, and swimming.	<p><u>THM water concentrations:</u> All ORs near 1.0</p> <p><u>Estimated THM intakes at home:</u> ORs = 1.00, 1.23 (95% CI: 0.73-2.11), 1.31 (95% CI: 0.77-2.24), and 1.55 (95% CI: 0.91-2.66) for TTHM intakes of 0.0, >0.0-8.4, 8.5-21.0, and 22-190 $\mu\text{g/d}$ (p-trend = 0.11) Similar findings for individual THMs</p> <p>No clear associations with dishwashing, bathing, showering, or swimming</p>	Income, BW, folate supplement use, smoking, phthalate exposure, and swimming	<ul style="list-style-type: none"> • Selection: cases ascertained from 40 of the 41 surgeons in the study area. Controls randomly selected from all male births in the register of the Office of National Statistics • Association seen with maternal water consumption • For cases born between 1997-1998 • Participation rates: cases (64%), controls (33%) • Exposure data not available from 1997 but THM data from later years showed greater spatial vs. temporal variation • Exposure period: annual average water concentrations (which appear to be based on year of conception in some subjects and year of birth in others) and 1st trimester water intake

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Kaufman et al. (2017)	CM (cranio-facial)	Massachusetts (113 towns) 1999-2004	Case-control	366 cases and 3,660 controls	THMs (total and individual)	Quarterly water measurements and disinfection treatment information from 1999-2004 linked to town of residence and data on month of birth. Averaged concentrations for the 1st trimester used.	<p><u>Cleft palate:</u> OR = 3.08 (95% CI: 1.01-9.39) for TTHM >63.86 vs. ≤20.27 µg/L OR = 2.17 (95% CI: 0.71-6.69) for chloroform >54.53 vs. ≤10.88 µg/L Non-monotonic dose-response patterns ORs decrease somewhat with additional adjustment for haloacetic acids</p> <p>ORs for BDCM, DBCM, and bromoform near or below 1.0.</p> <p><u>Cleft lip:</u> ORs near 1.0</p> <p><u>Eye defects:</u> ORs above 1.0 for TTHM and chloroform but small numbers of cases, ORs not statistically significant, and highest ORs in the middle exposure categories</p> <p><u>Ear defects:</u> Some ORs above 1.0 but small numbers of cases and ORs not statistically significant</p>	Water source and treatment type, income (zip code), race, and prenatal care	<ul style="list-style-type: none"> • Selection: cases obtained from the state birth defects monitoring program; controls randomly selected from all live births in Massachusetts, matched to cases by week of conception • Information on co-variables obtained from birth records or census data (e.g., income) • Mean TTHM level = 41.66 µg/L (±24.22 µg/L) • Exposure period: 1st trimester
Klotz and Pynch (1999)	CM (NTDs)	New Jersey 1993-1994	Case-control	112 cases and 248 controls	TTHM	Municipal records on THM concentrations linked to residence during the first month of pregnancy. Also, tap water samples collected 4 months after the birth date from subjects' residences.	<p><u>For water records:</u> PORs = 1.0 (ref), 0.9 (95% CI: 0.4-1.9), and 2.1 (95% CI: 1.1-4.0) for concentrations of <5, 5-<40, and ≥40 ppb</p> <p><u>For tap water sampling:</u> PORs = 1.0 (ref), 1.4 (95% CI: 0.6-3.3), and 2.0 (95% CI: 0.9-4.9) for concentrations of <5, 5-<40, and ≥40 ppb</p>	"adjusting by demographic and pregnancy data... did not alter PORs by 10% or more"	<ul style="list-style-type: none"> • Selection: NTDs ascertained from the New Jersey Birth Defects Registry • Co-variate data from subject interviews • Exposure periods: 1st month of pregnancy (water records) and 4 months after birth (tap water sampling)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Luben et al. (2008)	CM (hypo-spadias)	Arkansas 1998-2002	Case-control	320 cases and 614 controls	TTHM	Publically available monitoring data for quarterly THM and HAA concentrations collected from 263 water utilities throughout the state. Questionnaire data on showering, bathing, water use available for a subset of subjects.	ORs = 1.00 (ref), 1.59 (95% CI: 0.50-4.89), 1.96 (95% CI: 0.65-6.42) for TTHM intakes of >0-289.2, >289.2-549.9, and >549.9 µg/d ORs based on water concentrations alone near 1.0	BMI, race, BW, and plurality	<ul style="list-style-type: none"> • Selection: cases ascertained from a reproductive health monitoring system for the state; controls randomly selected from Arkansas birth records • Subset of subjects (40 cases, 242 controls) selected from the National Birth Defects Prevention Study (NBDPS) • Data on showering and bathing, hot beverages, and water consumption available on the NBDPS subjects • Authors note that there were fewer cases than expected and note the possibility that some cases may have been diagnosed outside the state • Exposure period: 6-16 weeks of pregnancy
Nieuwenh uijzen et al. (2008)	CM (multiple)	England and Wales (12 water companies) 1993-2001	Retro-spective cohort	2,605,226 live births; 22,828 CMs	THMs (total and brominated)	Water samples routinely collected from each water zone using random samples at consumers' taps, collected typically on a quarterly basis. These data were modeled to create weighted averages and estimate missing data.	<p><u>TTHM:</u> All ORs near 1.0</p> <p><u>Brominated THMs:</u> ORs for major cardiac defects of 1.00 (ref), 1.12 (95% CI: 1.01-1.23), and 1.13 (95% CI: 0.93-1.37) for levels of <10, 10-20, and > 20 µg/L All other ORs near 1.0</p> <p><u>Bromoform:</u> All ORs near 1.0</p>	Age, SES (census tract), year of birth, and registry	<ul style="list-style-type: none"> • Data on outcomes from the National Congenital Anomalies System • Exposure period: first 93 days of pregnancy • Gestational age not available in all infants • Correlations between specific THMs range from - 0.44 to 0.93 • Highest exposure group: TTHMs >60 µg/L; brominated THMs >20 µg/L; bromoform >4 µg/L

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Righi et al. (2012)	CM (multiple)	Emilia-Romagna, Italy 2002-2005	Case-control	1,150 cases and 4,984 controls	THMs	Mother's address at birth or pregnancy termination linked to average concentrations from routine monitoring data (at least 1 sample per year).	No clear associations	Maternal age, SES, previous abortion, twin, and consanguinity	<ul style="list-style-type: none"> • Selection: cases from the regional malformations registry; controls randomly selected from the regional birth registry • Exposure period: 1st trimester • Mother's residence at birth or pregnancy termination was assumed to be the residence for the entire pregnancy • Mean TTHM = 3.8 µg/L • Co-variate data from the malformations and birth registries
Shaw et al. (2003)	CM (multiple)	California 1987-1991	Case-control	Study 1: 538 cases (NTD) and 539 controls; Study 2: 881 (multiple types) and 481 controls	THMs (total and individual)	Residential data from personal interviews linked to municipal water source and THM concentrations estimated by water company personnel.	ORs >1.5 but not statistically significant for NTDs and cleft lip/palate in study 2 for TTHMs ≥75 vs. <1 µg/L. No other clear associations. ORs below 1.0 in some analyses	BMI, race, education, and vitamin use	<ul style="list-style-type: none"> • Selection: unclear case and control selection methods • Unclear quality of the exposure data • Exposure period: 3-4 months before conception to 3 months after conception
Wright et al. (2017)	CM (cardiac)	Massachusetts (68 towns) 1999-2004	Case-control	904 cases and 9,040 controls	THMs (total and individual)	Quarterly water measurements and disinfection treatment information from 1999-2004 linked to town of residence and data on month of birth. Averaged concentrations for the 1st trimester used.	<u>THMs (total and individual):</u> No clear associations or dose-response relationships for all cardiovascular defects combined, conotruncal heart defects, transposition of the great arteries, or Tetralogy of Fallot	Water source and treatment, BW, income, prenatal care, maternal health and reproductive health risk factors	<ul style="list-style-type: none"> • Selection: cases obtained from the state birth defects monitoring program; controls randomly selected from all live births in Massachusetts, matched to cases by week of conception • Information on co-variables obtained from birth records or Census data (e.g., income) • Mean TTHM level = 42.67 µg/L (±24.05 µg/L) • Exposure period: 1st trimester

Table B7. Epidemiologic Studies of Trihalomethanes and Sperm Quality (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Fenster et al. (2003)	Sperm quality	Santa Clara County, California (California Men's Reproductive Health Study) 1990-1991	Prospective cohort	157 healthy men	TTHM	Measurements collected in public water supplies for subject's residence within 90 days prior to semen collection. TTHM in public water and estimated TTHM intakes (calculated by multiplying TTHM concentration by questionnaire data on water intake) were used.	No clear associations with TTHM concentrations Decreased % normal morphology using World Health Organization morphology criteria (7.1% difference, 95% CI: -12.7 to -1.6) but not when using "strict" morphology criteria	Age, race, smoking, heat at work, education, income, and abstinence	<ul style="list-style-type: none"> • Selection: husbands of women participating in the California Women's Health Study; 324 men contacted regarding participation • Approximately 50% participation rate • Utility wide average TTHM concentrations • Men were ages 18-39 years old
Iszatt et al. (2013)	Sperm quality	England and Wales (Chap-UK study) 1999-2002	Case-control	642 cases (low motile sperm concentration) and 926 controls	THMs (total and individual)	Quarterly water records in 1,568 water zones in the UK modeled and linked to addresses 90 days prior to sperm collection.	No clear associations, most ORs near 1.0	Surgery, alcohol, and glycol ether exposure; social class and smoking also assessed	<ul style="list-style-type: none"> • Selection: all men recruited from fertility clinics
(Luben et al., 2007)	Sperm quality	US (three sites) Years unknown	Prospective cohort	228 presumed fertile men	THMs (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average concentrations linked to telephone interview data on water intake, boiling water, showering and bathing, and uptake factors.	Slight positive association seen for percent abnormal cytoplasmic drop and TTHM concentration e.g. correlation coefficient of 0.15 ($p < 0.05$) between TTHM concentrations and percent abnormal cytoplasmic drop No other clear associations seen for the main indicators of sperm concentration or morphology ORs similar when using 10, 30, and 90 day lag periods	Age, abstinence, and education; smoking, alcohol use, and other factors also assessed	<ul style="list-style-type: none"> • Male partners of women in the Savitz et al. (2005) study • Co-variate data from subject interviews • Differences in ethnicity, education, income, and alcohol use seen across the three sites

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Yang et al. (2016) and Zeng et al. (2013)	Sperm quality	Wuhan, China 2011-2012	Cross-sectional	401 men	THMs (chloroform and brominated)	Blood levels at the time of semen collection.	<p><u>Sperm concentration, count, or motility:</u> No clear associations; for example, sperm concentration difference of -0.08 (95% CI: -0.16-0.01) million/mL comparing chloroform levels >66.35 ng/L to <35.87 ng/L (p-trend = 0.07)</p> <p><u>Difference in straight line velocity (VSL, a sperm motion parameter) between exposure groups:</u> Chloroform: 1.95 µm/s (95% CI: 0.46-3.44) comparing chloroform >66.35 ng/L to <35.87 ng/L (p-trend=0.01) TTHM: 1.94 µm/s (95% CI: 0.44-3.44) comparing TTHM levels >72.48 ng/L to <40.09 ng/L (p-trend=0.01) Clear associations not seen for other sperm motion parameters or for other THMs; p-trends are for trends in increasing VSL by increasing exposure categories of chloroform or TTHM</p> <p><u>Testosterone levels:</u> No clear associations</p>	Age, BMI, abstinence, alcohol, and smoking; other demographic data also collected.	<ul style="list-style-type: none"> Men presenting to medical center "to seek semen examination" Some indication of interaction with GSTT1 SNP and brominated THMs (Yang et al., 2016) Questionnaire on water intake, boiled water use, bathing, showering, and swimming

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes															
Zeng et al. (2014b)	Sperm quality	Wuhan, China 2011-2012	Prospective cohort	324 men	THMs (total and individual)	Tap water concentrations measured at 3 sites in the water distribution system 3 months before semen collection. These were linked to personal questionnaire on water intake, boiled water consumption, bathing, showering, and swimming.	<p><u>THM ingestion:</u> Association with decreases in sperm concentration ($p = 0.01$) and count ($p = 0.02$), with a λ-shaped dose-response pattern in the latter</p> <p><u>Chloroform ingestion:</u> Association with decreases in sperm concentration ($p = 0.03$) and sperm count ($p = 0.05$), with a λ-shaped dose-response relationship in the latter</p> <p><u>Brominated THMs ingestion:</u> Borderline association with decreases in sperm concentration ($p = 0.05$) with a U-shaped dose-response relationship</p> <p><u>Showering/bathing:</u> No clear associations with any agent</p> <p><u>Sperm motion parameters (VSL, VCL, and LIN):</u> Some associations seen with all agents and exposure routes (ingestion and showering/bathing)</p>	Age, smoking, alcohol, education, and abstinence	<ul style="list-style-type: none"> • Male partners of sub fertile couples • Wide variability in THM concentrations seen by season • Few people with exposure from swimming • Sperm count was assessed by multiplying sperm concentration by sperm volume 															
Zeng et al. (2016)	Sperm quality	Wuhan, China 2011-2012	Cross-sectional	337 men	THMs (total and individual)	Blood THMs collection appears to have been at the time of semen collection. Urine TCA also measured.	<p><i>ORs for sperm concentration <20 million/ml</i></p> <table border="1"> <thead> <tr> <th>TTHM</th> <th>TCA</th> <th>OR (95% CI)</th> </tr> </thead> <tbody> <tr> <td>Low</td> <td>Low</td> <td>1.00 (ref)</td> </tr> <tr> <td>High</td> <td>Low</td> <td>2.97 (0.81-10.87)</td> </tr> <tr> <td>Low</td> <td>High</td> <td>3.59 (0.96-13.42)</td> </tr> <tr> <td>High</td> <td>High</td> <td>6.35 (1.83-22.06)</td> </tr> </tbody> </table> <p>Somewhat similar findings though less strong for individual THMs Somewhat similar findings for sperm count <40 million No statistically significant interactions between THMs and urine TCA</p>	TTHM	TCA	OR (95% CI)	Low	Low	1.00 (ref)	High	Low	2.97 (0.81-10.87)	Low	High	3.59 (0.96-13.42)	High	High	6.35 (1.83-22.06)	Age, BMI, smoking, alcohol, income, and abstinence	<ul style="list-style-type: none"> • Includes subjects in Zeng et al. 2014b and Zeng et al. 2013 • Men with occupational exposures excluded • Questionnaire on water intake, bathing, showering, and swimming • High and low THM and TCA concentrations based on medians
TTHM	TCA	OR (95% CI)																						
Low	Low	1.00 (ref)																						
High	Low	2.97 (0.81-10.87)																						
Low	High	3.59 (0.96-13.42)																						
High	High	6.35 (1.83-22.06)																						

Table B8. Epidemiologic Studies of Trihalomethanes and Other Reproductive Outcomes (studies sorted by author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical	Exposure assessment	Results	Adjustments	Notes
Joyce et al. (2008)	Term pre-labor rupture of membranes	Western Australia 2002-2004	Unclear	16,229 women; 686 with pre-labor rupture of membranes	TTHM	Routine monitoring of THMs in the 24 water distribution zones for the years 2002-2004 linked to residences listed in the midwives' notification system	No clear associations	Age, smoking, and economic status	<ul style="list-style-type: none"> • Outcome and co-variate data from the Western Australia Midwives' Notification System, includes all home and hospital births in the area • Variability within a sample of 6 zones found to be "limited" with "several outliers" in two zones but specific results not provided • Exposure period: unclear
MacLehose et al. (2008)	Time to pregnancy	US (three sites) 2000-2004	Prospective cohort	236 women prior to pregnancy	THMs (total and individual)	Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to residence at baseline (average 9 th week of gestation), personal questionnaire data on water intake, hot and cold beverage consumption, showering and bathing, and uptake factors.	Some ORs for cycle specific probability of conception are above 1.0 (indicating decreased time to pregnancy) No clear pattern of ORs below 1.0 (indicative of increased time to pregnancy)	Age, race, ethnicity, marital status, smoking, and BMI; education and occupation also assessed	<ul style="list-style-type: none"> • Separate testing showed THM concentrations were spatially uniform in all 3 water systems • Differences seen across the 3 sites in terms of age, race/ethnicity, education, and income • Exposure period: unclear, but probably the 9th week of pregnancy on average
Windham et al. (2003)	Menstrual cycle length	Northern California 1990-1991	Prospective cohort	403 premenopausal women	THMs (total and individual)	Quarterly THM concentrations from the utilities collected at 4-20 points in each distribution system used to create utility wide averages. These were then linked to telephone interview data on hot and cold tap water consumption, bottled water use, and showering to create 90-day exposure estimates for each cycle (during each cycle plus the 60 days before).	Decrease in cycle length of -1.1 days (95% CI: -1.8 to -0.40 days) for >60 µg/L TTHM vs. ≤40 µg/L, with evidence of a dose-response pattern Strongest associations seen for brominated THMs	Age, race, BMI, income, pregnancy history, smoking, alcohol, and caffeine consumption	<ul style="list-style-type: none"> • Women's Reproductive Health Study • Members of Kaiser Permanente Medical Care Program • 6,500 women screened to identify those most likely to become pregnant and willing to collect daily urine samples • Daily urine samples collected for 2-9 menstrual cycles (average of 5.6) • Daily diaries used to collect menstrual information

Table B9. Epidemiologic Studies of Other Disinfection Byproduct Exposure Metrics and Reproductive Outcomes (studies sorted by outcome then author)

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Wennborg et al. (2000)	BW	Sweden 1990-1994	Retro-spective cohort	419 female laboratory workers and 278 controls; 856 pregnancies	Chloroform: occupation	Mailed questionnaire data about use of specific chemicals and their month of use. Exposure variable was any reported chloroform use.	No clear associations	Age and previous SAB; chronic disease and smoking also assessed	<ul style="list-style-type: none"> •Subjects identified from the Swedish Employee Salaries and Pension Board records •Selection: women working >1 year from 1990 to 1994 at a biomedical research lab or a non-lab department at one of two Universities, and had given birth during the study period •Data on reproductive history, specific chemical exposures (and their month of use), and co-variables collected via mailed questionnaire •Response rate: 73% •Most outcomes were self-reported; BW validated against the Swedish Medical Birth Register •Other chemical exposures possible •Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Shaw et al. (1991)	CM (cardiac)	Santa Clara County, California 1981-1983	Case-control	138 cases and 168 controls	Chlorination	Information collected from all water companies in the county about whether maternal residences received chlorinated water during time corresponding to the first three months of pregnancy. Data on cold water consumption, showering and bathing at home during the first three months of pregnancy collected in telephone interviews.	Using a chlorinated water supply: OR = 1.0 (95% CI: 0.65-1.6) Using a chlorinated water supply and drinking cold tap water at home: OR = 1.0 (95% CI: 0.64-1.6)	Age, race, and education	<ul style="list-style-type: none"> • Selection: cases ascertained from the California Birth Defects Monitoring Program ("nearly complete ascertainment"); controls randomly selected from all live births in the study county for the same time period as the case births • Association seen with maternal tap water consumption, with differences in results based on differential reporting by subjects • Telephone interviews were 3-7 years after birth • TTHMs assessed but CM ORs not reported here • Exposure period: 1st trimester
Aschengrau et al. (1993)	CM (multiple)	Massachusetts (Brigham and Women's Hospital) 1977-1980	Nested case-control	1,039 cases and 1,177 controls	Chlorination	Data from routine monitoring of public water supplies and water treatment practices linked to residence listed in medical records for the time of pregnancy or the 1st trimester, if available. Sample collected closest to conception date was used (3.3 months on average). Frequency of testing or number of sites tested not reported.	OR = 1.0 (unadjusted) Adjusted OR not provided	Race, age, payment method, prior CM, alcohol, and water source	<ul style="list-style-type: none"> • Selection: this study was nested in a cohort of 14,130 obstetric patients representing 82.5% of all delivery patients during the study period • CM diagnoses based on newborn examinations by pediatric residents • Eligible controls were 1,490 women randomly selected from all women whose children had no CMs • Data on co-variables based on medical records review (86.6% of the total) and interviews • No information collected during interviews on water source or drinking habits • Exposure period: for most women, the residence during the 1st trimester was used

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Hwang et al. (2002)	CM (multiple)	Norway 1993-1998	Ecologic	184,676 live births; 5,764 CMs	Chlorination	Municipalities with waterworks that routinely chlorinated their water compared to those that did not. Routine monitoring data on water color collected but few details provided.	<p><u>ORs for high color and chlorination vs. low color and no chlorination:</u> Any birth defect: OR = 1.18 (95% CI: 1.02-1.36) Hydrocephalus: OR = 2.70 (95% CI: 0.77-9.51) Ventricular septal: OR = 1.81 (95% CI: 1.05-3.09) Respiratory: OR = 1.96 (95% CI: 0.89-4.34) Cleft lip: OR = 2.01 (95% CI: 0.63-6.46)</p> <p>Other ORs near 1.0 including NTD and urinary deficits (OR = 1.35)</p>	Age, parity, centrality (urbanicity), and population density	<ul style="list-style-type: none"> • Outcome data from the Norwegian Birth Registry • Includes all eligible births for the study years • Color: an indicator of natural organic matter; water color found to be highly correlated with concentration of dissolved solids (R = 0.817) • Co-variate data from birth registry records. • Partial overlap with Magnus et al., 1999 • Exposure period: unclear
Kallen and Robert (2000)	CM (multiple)	Sweden 1985-1994	Ecologic	Approximately 75,000 births	Chlorine dioxide, sodium hypochlorite	Based on published reports on municipality drinking water treatment and composition for the years 1985, 1989 and 1994. Only includes municipalities where drinking water disinfection was the same before and after delivery and was the same throughout the municipality.	<p><u>Chlorine dioxide use:</u> Hydrocephaly: OR = 1.5 (95% CI: 0.3-7.3; 10 exposed cases) Anal atresia: OR = 1.5 (95% CI: 0.6-3.6; 6 exposed cases) ORs for other CM sites including cardiac defects near 1.0</p> <p><u>Sodium hypochlorite use:</u> Anal atresia: OR = 1.8 (95% CI: 0.7-4.3, 16 exposed cases) Spine malformation: OR = 3.2 (95% CI: 1.0-10.0; 11 exposed cases) Limb reduction deficit: OR = 1.6 (95% CI: 0.9-3.0; 26 exposed cases) Diaphragmatic hernia: OR = 2.0 (95% CI: 0.8-5.1) ORs for other CM sites including cardiac defects near 1.0; urogenital anomalies not assessed</p>	Year of birth and maternal age	<ul style="list-style-type: none"> • Outcome data obtained from the Swedish Medical Birth Registry • Exposure period: throughout pregnancy

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Magnus et al. (1999)	CM (multiple)	Norway 1993-1995	Ecologic	141,077 births; 2,608 CMs	Chlorination	Municipalities at the time of birth with waterworks that routinely chlorinated their water were compared to those that did not.	<p><u>All birth defects:</u> OR = 1.14 (95% CI: 0.99-1.31) for chlorination and high color vs. no chlorination and low color</p> <p><u>Specific defects:</u> OR = 1.99 (95% CI: 1.10-3.57) for urinary tract defects ORs near 1.0 for neural tube, cardiac, respiratory, and cleft defects</p>	Age, parity, place of birth, centrality, population density, and industry profile	<ul style="list-style-type: none"> • Outcome data from the Norwegian Birth Registry • Includes all eligible births for the study years • Color: an indicator of natural organic matter • Exposure period: unclear
Righi et al. (2012)	CM (multiple)	Emilia-Romagna, Italy 2002-2005	Case-control	1,150 cases and 4,984 controls	Chlorite Chlorate	Mother's address at birth linked to average concentrations from routine monitoring data (at least 1 sample per year).	<p><u>Chlorite:</u> <i>For concentrations >700 vs. ≤200 µg/L</i> Urinary tract defects: OR = 2.00 (95% CI: 1.05-3.82) Renal defects: OR = 3.30 (95% CI: 1.35-8.09) Abdominal wall defects: OR = 6.88 (95% CI: 1.67-28.33)</p> <p><u>Chlorate:</u> <i>For concentrations >200 vs. ≤200 µg/L</i> Urinary tract defects: OR = 2.07 (95% CI: 1.04-4.13) Obstructive urinary defects: OR = 2.88 (95% CI: 1.09-7.63) Cleft palate: OR = 9.60 (95% CI: 1.04-88.92) Spina bifida: OR = 4.94 (95% CI: 1.10-22.27) Most other ORs near 1.0</p>	Maternal age, SES, previous abortion, twin, and consanguinity	<ul style="list-style-type: none"> • Selection: cases from the regional malformations registry; controls randomly selected from the regional birth registry • Mothers residence at birth was assumed to be the residence for the entire pregnancy • Exposure period: 1st trimester • Co-variate data from the malformations and birth registries

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Aschengrau et al. (1989)	SAB	Massachusetts (Brigham and Women's Hospital) 1976-1978	Case-control	286 cases and 1,391 controls	Chloride	Data from routine monitoring of public water supplies and water treatment practices linked to residence listed in medical records for the time of pregnancy. Sample collected closest to conception date was used (median = 65 days). Frequency of testing or number of sites tested not reported.	No evidence of an association; all ORs near or below 1.0	Water source, maternal age, education, and prior SAB; data on smoking and medical history also collected.	<ul style="list-style-type: none"> • Selection: inclusion based on availability of the subject and interviewer; of the 1,238 subjects with SAB during the study period, 399 were asked to participate and 96.0% agreed; controls randomly sampled from all women delivering within one week of each case's pregnancy loss; 1,981 potential controls approached; according to authors the included women were similar to all admitted women in terms of residence, age, race, and method of payment • Information on co-variables obtained from personal interviews • Exposure period: near conception
Dahl et al. (1999)	Fecundability ratio	Norway 1991	Retrospective cohort	558 female dental surgeons and 450 female high school teachers; 1,408 pregnancies	Chloroform: occupational	Exposure based on use of chloroform-based root canal sealers using information gathered from a postal questionnaire.	Fecundability ratio = 1.06 (95% CI: 0.95-1.10)	Age, smoking, and history of reduced fecundability	<ul style="list-style-type: none"> • Other exposures include benzene, ethanol, and x-rays • Selection: female dental surgeons selected from among all of those registered by the Norwegian Dental Association • The reference group included female high school teachers randomly selected from the Norwegian Educational Association (70% participation rate) • Exposure period: "The respondents reported their exposure emphasizing the 6 months prior to pregnancy"

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Kanitz et al. (1996)	LBW	Liguria, Italy 1988-1989	Cross-sectional	676 births; 20 LBW	Chlorination	Births at one of two hospitals: Galliera Hospital ("where it was known whether mothers were drinking water treated with sodium (Na+) hypochlorite, chlorine dioxide, or both"), and Chiavari Hospital (where "drinking water is not disinfected"). Residence linked to type of disinfection chemical used in that area.	<p><u>Agent:</u> <u>OR (95% CI)</u></p> None 1.0 (ref) Chlorine dioxide 5.9 (0.8-14.9) Na+ hypochlorite 6.0 (0.6-12.6) Both 6.6 (0.9-14.6) <p>Elevated ORs also seen for shorter body length and smaller cranial circumference</p>	Education, income, mothers age, smoking, and sex	<ul style="list-style-type: none"> • Includes births at a single hospital; unclear if all births are included • THM concentrations are relatively low, 1-16 µg/L • Outcome and co-variate data from hospital or municipal records • Small numbers of LBW cases • Exposure period: unclear
Righi et al. (2003)	LBW	Modena, Italy 1999-2000	Case-control	73 cases and 166 controls	Chlorite Chlorate	Drinking water sampling at subject's home and personal questionnaire data.	OR = 4.7 (95% CI: 1.15-19.72) for chlorite >200 µg/L	Unclear	<ul style="list-style-type: none"> • Only 5.2% subjects reported "usually" drinking tap water • Mean chlorites = 217.8 µg/L • Mean chlorates = 95.2 µg/L • Full study not reviewed; study description here is from the English abstract of an article written in Italian • Exposure period: unclear
Yang et al. (2000a)	LBW	Taiwan (28 municipalities) 1994-1996	Ecologic	18,025 births; 456 LBW	Chlorination	Compared 14 municipalities with mostly chlorinated water (i.e., in >90% of the population) to 14 municipalities with mostly non-chlorinated drinking water (i.e., in <5% of the population) matched on urbanization index. Other information on measurements not provided.	2.49% in chlorinated areas vs. 2.81% in non-chlorinated areas (p = 0.148)	Age, marital status, education, and sex	<ul style="list-style-type: none"> • Outcome and other data collected from birth registries • Only includes term LBW • Ecologic exposure assessment with little information on methods • Small difference in education levels between exposure groups • Most chlorinated water is from surface water and most non-chlorinated water is from ground water • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Jaakkola et al. (2001)	LBW, SGA	Norway 1993-1995	Ecologic	123,747 births	Chlorination	Municipalities with waterworks that routinely chlorinated their water compared to those that did not.	<p><u>LBW:</u> No association, OR near 1.0</p> <p><u>SGA:</u> No association, OR near 1.0</p>	Age, parity, place of birth, centrality, population density, and industry profile	<ul style="list-style-type: none"> • Outcome data from the Norwegian Birth Registry • Includes all eligible births for the study years with data on gestational age (90.2%) • Considered both chlorination and amount of organic matter ("color") • Exposure period: unclear
Kallen and Robert (2000)	LBW, SGA	Sweden 1985-1994	Ecologic	Approximately 75,000 births	Chlorine dioxide, sodium hypochlorite	Based on published reports on municipality drinking water treatment and composition for the years 1985, 1989 and 1994. Only includes municipalities where drinking water disinfection was the same before and after delivery and was the same throughout the municipality.	<p><u>Chlorine dioxide use:</u> No clear associations for LBW or SGA</p> <p><u>Sodium hypochlorite use:</u> LBW: OR = 1.15 (95% CI: 1.05-1.26) SGA: OR = 1.07 (95% CI: 0.96-1.19)</p>	Year of birth, age, parity, education, and smoking	<ul style="list-style-type: none"> • Outcome data obtained from the Swedish Medical Birth Registry • Exposure period: throughout pregnancy
Aschengrau et al. (1993)	Neonatal death	Massachusetts (Brigham and Women's Hospital) 1977-1980	Case-control	55 cases and 1,177 controls	Chlorination	Data from routine monitoring of public water supplies and water treatment practices linked to residence listed in medical records for the time of pregnancy or the 1st trimester, if available. Sample collected closest to conception date was used (3.3 months on average). Frequency of testing or number of sites tested not reported.	<p>OR = 1.1 (unadjusted); confidence interval includes 1.0</p> <p>Adjusted OR not provided</p>	Race, age, payment method, prior CM, alcohol, and water source	<ul style="list-style-type: none"> • Selection: this study was nested in a cohort of 14,130 obstetric patients representing 82.5% of all delivery patients during the study period • Eligible controls were 1,490 women randomly selected from all women whose children had no CMs • Data on co-variables based on medical records review (86.6% of the total) and interviews • No information collected during interviews on water source or drinking habits • Exposure period: for most women, the residence during the 1st trimester was used

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes										
Kallen and Robert (2000)	Preterm	Sweden 1985-1994	Ecologic	Approximately 75,000 births	Chlorine dioxide, sodium hypochlorite	Based on published reports on municipality drinking water treatment and composition for the years 1985, 1989 and 1994. Only includes municipalities where drinking water disinfection was the same before and after delivery and was the same throughout the municipality.	<p><u>Chlorine dioxide use:</u> No clear associations</p> <p><u>Sodium hypochlorite use:</u> OR = 1.09 (95% CI: 1.01-1.17)</p>	Year of birth, age, parity, education, and smoking except for CM analysis. For CMs: year of birth and maternal age	<ul style="list-style-type: none"> • Outcome data obtained from the Swedish Medical Birth Registry • Increase in OR is small • Exposure period: throughout pregnancy 										
Kanitz et al. (1996)	Preterm	Liguria, Italy 1988-1989	Cross-sectional	676 births; 50 preterm	Chlorination	Births at one of two hospitals: Galliera Hospital ("where it was known whether mothers were drinking water treated with sodium (Na+) hypochlorite, chlorine dioxide, or both), and Chiavari Hospital (where "drinking water is not disinfected"). Residence linked to type of disinfection chemical used in that area.	<table border="0"> <tr> <td><u>Agent:</u></td> <td><u>OR (95% CI)</u></td> </tr> <tr> <td>None</td> <td>1.0 (ref)</td> </tr> <tr> <td>Chlorine dioxide</td> <td>1.8 (0.7-4.7)</td> </tr> <tr> <td>Na+ hypochlorite</td> <td>1.1 (0.3-3.7)</td> </tr> <tr> <td>Both</td> <td>1.8 (0.6-5.0)</td> </tr> </table>	<u>Agent:</u>	<u>OR (95% CI)</u>	None	1.0 (ref)	Chlorine dioxide	1.8 (0.7-4.7)	Na+ hypochlorite	1.1 (0.3-3.7)	Both	1.8 (0.6-5.0)	Education, income, mother's age, smoking, and sex	<ul style="list-style-type: none"> • Includes births at a single hospital; unclear if all births are included • THM concentrations are relatively low, 1-16 µg/L • Outcome and co-variate data from hospital or municipal records • Exposure period: unclear
<u>Agent:</u>	<u>OR (95% CI)</u>																		
None	1.0 (ref)																		
Chlorine dioxide	1.8 (0.7-4.7)																		
Na+ hypochlorite	1.1 (0.3-3.7)																		
Both	1.8 (0.6-5.0)																		
Righi et al. (2003)	Preterm	Modena, Italy 1999-2000	Case-control	93 cases and 166 controls	Chlorite Chlorate	Drinking water sampling at subject's home and personal questionnaire data.	No clear association	Unclear	<ul style="list-style-type: none"> • Only 5.2% subjects reported "usually" drinking tap water • Mean chlorites = 217.8 µg/L • Mean chlorates = 95.2 µg/L • Full study not reviewed; study description here is from the English abstract of an article written in Italian • Exposure period: unclear 										

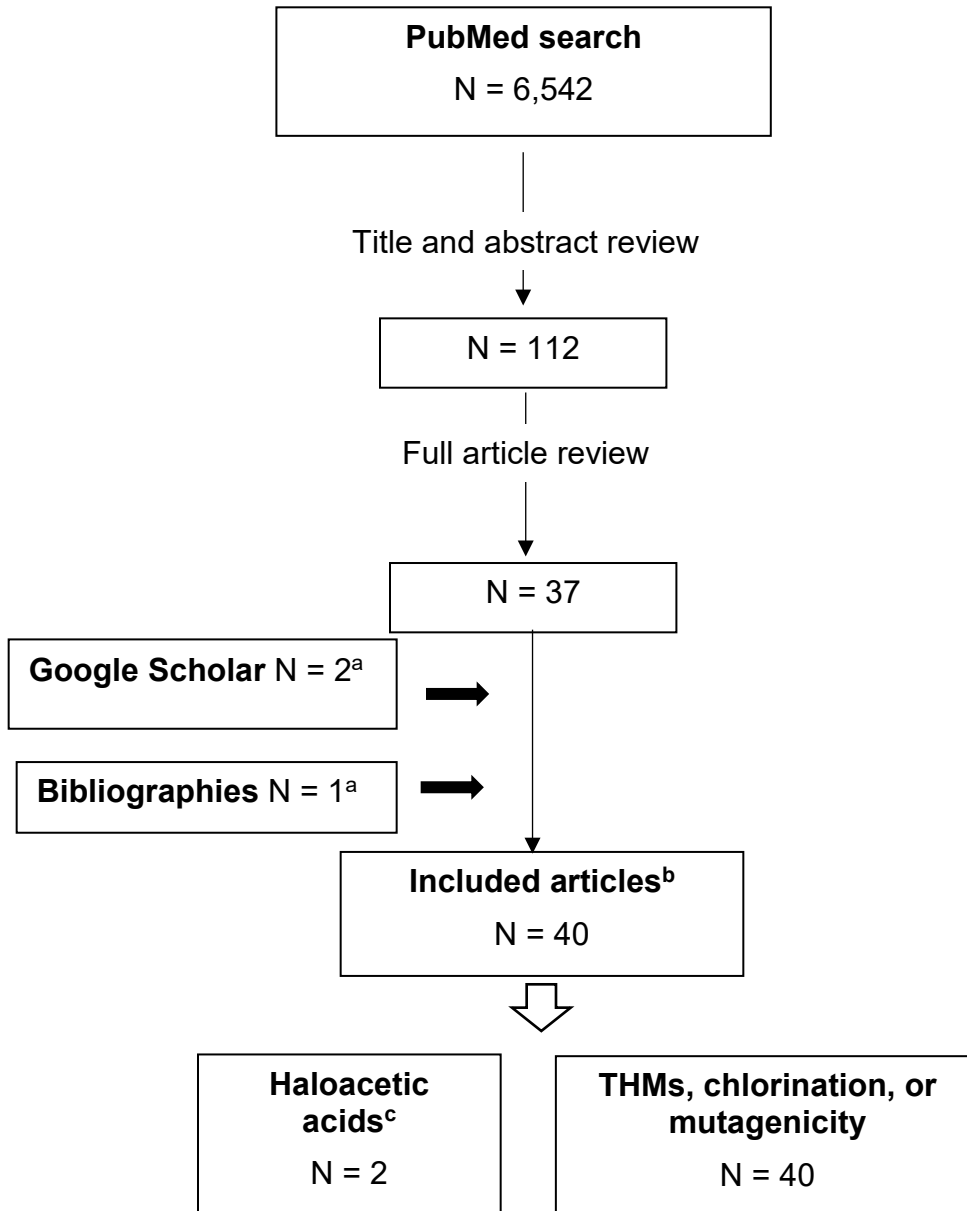
Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Yang et al. (2000a)	Preterm	Taiwan (28 municipalities) 1994-1996	Ecologic	18,025 births; 719 preterm	Chlorination	Compared 14 municipalities with mostly chlorinated water (i.e., in >90% of the population) to 14 municipalities with mostly non-chlorinated drinking water (i.e., in <5% of the population) matched on urbanization index. Other information on measurements not provided.	OR = 1.34 (95% CI: 1.15-1.56) comparing mostly chlorinated to mostly non-chlorinated areas	Age, marital status, education, and sex	<ul style="list-style-type: none"> • Outcome and other data collected from birth registries • Ecologic exposure assessment with little information on methods • Small difference in education levels between exposure groups • Most chlorinated water is from surface water and most non-chlorinated water is from ground water • Preterm results overlap with Yang et al., 2004 • Exposure period: unclear
Yang (2004)	Preterm	Taiwan (128 municipalities) 1994-1996	Ecologic	182,796 pregnant women; 8,250 preterm	Chlorination	Compared 113 municipalities with mostly chlorinated water (i.e., in >90% of the population) to 15 municipalities with mostly non-chlorinated drinking water (i.e., in <5% of the population). Other information on measurements not provided.	OR = 1.37 (95% CI: 1.20-1.56) comparing mostly chlorinated to mostly non-chlorinated areas	Age, marital status, education, urbanization, and sex	<ul style="list-style-type: none"> • Outcome and other data collected from birth registries • Ecologic exposure assessment with little information on methods • Most chlorinated water is from surface water and most non-chlorinated water is from ground water • Differences seen between exposure areas in maternal age, education and urbanization • Population overlaps with Yang et al., 2000a • Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Wennborg et al. (2000)	SAB	Sweden 1990-1994	Retro-spective cohort	419 female laboratory workers and 278 controls; 856 pregnancies; 73 SABs	Chloroform: occupational	Mailed questionnaire data about use of specific chemicals and their month of use. Exposure variable was any reported chloroform use.	OR = 2.3 (95% CI: 0.9-5.9)	Age and previous SAB; chronic disease and smoking also assessed	<ul style="list-style-type: none"> •Subjects identified from the Swedish Employee Salaries and Pension Board records. •Women worked >1 year from 1990 to 1994 at a biomedical research lab or a non-lab department at one of two Universities, and had given birth during the study period •Data on reproductive history, specific chemical exposures (and their month of use), and co-variates collected via mailed questionnaire •Response rate: 73% •Most outcomes were self-reported •Exposure period: unclear
Yang et al. (2000b)	Sex ratio	Taiwan (28 municipalities) 1994-1996	Ecologic	18,025 births	Chlorination	Compared 14 municipalities with mostly chlorinated water (i.e., in >90% of the population) to 14 municipalities with mostly non-chlorinated drinking water (i.e., in <5% of the population) matched on urbanization index. Other information on measurements not provided.	No clear associations	None	<ul style="list-style-type: none"> •Outcome data collected from birth registries •Ecologic exposure assessment with little information on methods •Most chlorinated water is from surface water and most non-chlorinated water is from ground water •Exposure period: unclear

Author, year	Outcome	Location, years	Design	Subjects	Chemical/ Process	Exposure assessment	Results	Adjustments	Notes
Aschengrau et al. (1993)	Stillbirth	Massachusetts (Brigham and Women's Hospital) 1977-1980	Case-control	77 cases and 1,177 controls	Chlorination	Data from routine monitoring of public water supplies and water treatment practices linked to residence listed in medical records for the time of pregnancy or the 1st trimester, if available. Sample collected closest to conception date was used (3.3 months on average). Frequency of testing or number of sites tested not reported.	OR = 2.6 Confidence interval not provided	Race, age, payment method, prior CM, alcohol, and water source	<ul style="list-style-type: none"> • Selection: this study was nested in a cohort of 14,130 obstetric patients representing 82.5% of all delivery patients during the study period • Eligible controls were 1,490 women randomly selected from all women whose children had no CMs • Data on co-variables based on medical records review (86.6% of the total) and interviews • No information collected during interviews on water source or drinking habits • Exposure period: for most women, the residence during the 1st trimester was used

APPENDIX C. EPIDEMIOLOGIC STUDIES OF CANCER OUTCOMES

Literature search results for epidemiologic studies of THMs and HAAs and cancer



^a Additional studies identified through Google Scholar or from the bibliographies of the included articles or relevant reviews

^b Studies meeting the inclusion criteria discussed in Section 4

^c Both studies also provided data for THMs

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 (2016)	All cancers combined (mortality)	United States 1994-2011	Retro-spective 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Only 23 cancer cases

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Beane 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<p><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)</p> <p><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)</p> <p>Women: no clear associations</p>	Age, study period, education, occupation, and smoking	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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.	<p><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</p> <p><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</p>	Age, gender, smoking, education, and total calories	<ul style="list-style-type: none"> 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
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.	<p>ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity:</p> <p>Women: OR=1.48 (95% CI: 1.01-2.18)</p> <p>Men: OR=1.03 (95% CI: 0.82-1.28)</p>	Age, urbanization, and SES	<ul style="list-style-type: none"> 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 <i>Salmonella typhimurium</i> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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.	<p><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)</p> <p><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)</p> <p>Similar results by sex and smoking</p>	Sex, coffee, smoking, water intake, family history, and other urinary conditions	<ul style="list-style-type: none"> 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. (2007b); 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)	<p>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 $\mu\text{g/L}$ average residential TTHM exposure (p-trend<0.01)</p> <p>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 $\mu\text{g/L}$ (p-trend<0.61)</p> <p>Duration of chlorinated surface water at residence: Men: similar to women except p-trend=0.20</p> <p>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)</p> <p>Individual THMs: inconsistent dose-response relationships, ORs near 1.0, or low power (Salas et al., 2013)</p> <p>OR for swimming pool use: 1.57 (95% CI: 1.18-2.09)</p> <p>ORs by TTHM concentration generally higher when daily water intake is lower (Michaud et al., 2007)</p>	Age, gender, smoking, education, urbanicity, interview quality, and geographic area	<ul style="list-style-type: none"> Cases ascertained from 18 hospitals from five areas in Spain Controls ascertained from the same hospitals with noncancer 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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)
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	<u>Chlorination:</u> 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) <u>TTHM:</u> 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	<ul style="list-style-type: none"> • 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> • 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 (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	<ul style="list-style-type: none"> • 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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 $\mu\text{g/L}$ (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.	<ul style="list-style-type: none"> 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)
Koivusalo et al. (1997)	Breast (incident cases)	Finland (56 towns) 1970-93	Retrospective 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	<ul style="list-style-type: none"> 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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 \geq 80 vs. $<$ 40 $\mu\text{g/L}$ Similar results in White and Black women	Age, income, education, urban status, and race from Census data	<ul style="list-style-type: none"> 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
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 $\mu\text{g/L}$) 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> • 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 (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	<ul style="list-style-type: none"> • 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.	<p><u>Chlorination:</u> No association (p-trend=0.13)</p> <p><u>TTHM:</u> No association (p-trends=0.54 to 0.85)</p> <p>Similar results in men and women</p>	Age and sex. Further adjustment for smoking, diet, and other factors did not alter results.	<ul style="list-style-type: none"> 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.	<p>Only presents age adjusted risk ratios for nickel and dichloroethane in analyses stratified by chlorination status (yes or no)</p> <p>No clear increases seen by chlorination strata in men or women</p>	Age adjusted, sex specific rate ratios	<ul style="list-style-type: none"> 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.	<p><u>Chlorination:</u> 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</p> <p><u>TTHM:</u> 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 $\mu\text{g/L}$-years of cumulative exposure. OR of 1.17 (95% CI: 1.06-1.29) for each 1,000 $\mu\text{g/L}$-years increase in exposure</p> <p>Women: no clear associations</p>	Age, sex, education, BMI, total calories, cholesterol, calcium, alcohol, and coffee	<ul style="list-style-type: none"> 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.	<p>ORs (95% CI) for each 3,000 net rev/L increase in mutagenicity:</p> <p>ORs near 1.0 in men and women</p>	Age, urbanization, and SES	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<p><u>Bromoform</u>: Incidence rate ratios in men of 1.035 (95% CI: 1.017-1.053) for each interquartile increase in exposure (2 µg/L)</p> <p>ORs for other THMs near 1.0</p>	Area level data on SES, alcohol, smoking, water source, and year of diagnosis	<ul style="list-style-type: none"> 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. (2016)	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)	<p><u>Total THMs</u>: no association</p> <p><u>Chloroform</u>: OR of 0.31 (95% CI: 0.24-0.41) comparing the highest to lowest quartile (p-trend <0.001)</p> <p>Similar results in men and women</p>	Age, sex, area, education, non-steroidal anti-inflammatory drugs, smoking, physical activity, and family history. Adjustment for diet had little impact on results	<ul style="list-style-type: none"> 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	Retrospective 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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
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).	<u>Chlorination:</u> Some ORs > 1.2 depending on source of controls and latency but no clear or consistent patterns <u>TTHMs:</u> ORs near 1.0	Age, sex, and population size	<ul style="list-style-type: none"> • 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo et al. (1997)	Esophageal (incident cases)	Finland (56 towns) 1970-93	Retrospective 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	<ul style="list-style-type: none"> 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
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 \geq 4.9 μ g/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	<ul style="list-style-type: none"> Information on deaths obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health All other noncancer 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> 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
Flaten (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	<ul style="list-style-type: none"> 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
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 $\geq 95^{\text{th}}$ percentile is 1.63 (95% CI: 0.84-3.19) ($>102 \mu\text{g/L}$). OR of 0.91 (95% CI: 0.59-1.41) for $>75^{\text{th}}$ percentile (44 $\mu\text{g/L}$) vs. $\leq 24^{\text{th}}$ percentile (12.9 $\mu\text{g/L}$). <u>TTHMs:</u> OR of 1.54 (95% CI: 0.78-3.03) for cumulative total TTHM $>95^{\text{th}}$ percentile (level not clear) vs. $\leq 95^{\text{th}}$ 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.	<ul style="list-style-type: none"> 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
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 bromodichloromethane)	Lifetime residential history for the 40 years prior to interview linked to multiple sources of THM survey data from municipalities	<u>TTHM:</u> No associations for all leukemia types combined. OR of 1.70 (95% CI: 1.00-3.03) for >31 years $>20 \mu\text{g/L}$ vs. no exposure for CML. Dose-response pattern seen but not statistically significant ($p\text{-trend}=0.11$). OR of 0.47 (95% CI: 0.32-0.68) for >31 years $>20 \mu\text{g/L}$ vs. no exposure for chronic lymphocytic leukemia <u>BDCM:</u> Elevated OR for CML (1.63, 95% CI: 1.00-3.10, $p\text{-trend}=0.12$) for >24 years $>5 \mu\text{g/L}$. No association for all leukemias and other subtypes	Age, gender, occupation, benzene, and radiation	<ul style="list-style-type: none"> 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
Koivusalo et al. (1997)	Hematopoietic (incident cases)	Finland (56 towns) 1970-93	Retrospective 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	<ul style="list-style-type: none"> 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
Vinceti et al. (2004)	Hematopoietic (mortality)	Guastalla and Reggio Emilia, Italy 1965-99	Retrospective 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> 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 <i>Salmonella typhimurium</i> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> 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)
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.	<p>Only presents age adjusted risk ratios for nickel and dichloroethane in analyses stratified by chlorination status (yes or no).</p> <p>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</p> <p>Risk ratios across strata appear similar in women</p>	Age adjusted, sex specific rate ratios	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> 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)
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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> • Cancer incidence data from the Cancer Registry of Norway • No information on residential history • Limited data on other cancer risk factors
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, oophorectomy, and other factors	<ul style="list-style-type: none"> • 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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)

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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	<ul style="list-style-type: none"> 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
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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> 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	Prospective 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: <ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	Retrospective 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 (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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> 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 (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	<ul style="list-style-type: none"> 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)
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.	<p><u>Chlorination:</u> 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)</p> <p><u>TTHM:</u> 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)</p> <p>Similar results in men and women</p>	Age and sex. Further adjustment for smoking, diet, and other factors did not alter results.	<ul style="list-style-type: none"> 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

Author, year	Cancer	Location, years	Design	Subjects	Chemical or surrogate	Exposure assessment	Results	Adjustments	Notes
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 dichloroethane 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 (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	<ul style="list-style-type: none"> • 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.	<p>ORs for each 3,000 net rev/L increase in mutagenicity:</p> <p>ORs in men and women both near 1.0</p>	Age, urbanization, and SES	<ul style="list-style-type: none"> • 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	Retrospective 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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
			RR >1.2	Statistically sig.	Dose-response	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1								
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/ND	≥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	Chlorof orm	≥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+	M S	-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	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
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	+	+	+	+	+	+	+	u	0	+	+	+	+	-	0	+		+	+	0	None	Chlorin/TTHM	≥35 yrs/1957 µg/L-yrs	4+	C	-1
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+	M N	-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

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	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
Cantor et al. (1999)	B R	CC	+	+	+	-	+	+	+	+	0	+	+	+	-	0			+	+	-	Subgroup: men; 74% proxy interviews	Chlorin/TTHM	≥40 yrs/≥32.6	3+	M	-2	
Koivusalo et al. (1997)	B R	CO	+	-	+	-	+	+	+	u	0	+	+	+	u	-	-			-	+	0	Glioma; subgroup: women; mutagenicity; exposure 1955-60	Mutagenesis	NA	2+	W	-5
Vinceti et al. (2004)	B R	CO	-	-	-	-	+	+	+	+	0	+	+	+	u	-	0			+	+	0	No interviews	TTHM	70.7	-	B	-2
Yang et al. (1998)	B R	E	-	-	-	-	+	+	+	+	0	+	-	-	-	-	0			+	+	0	Cross-sectional; ecologic	Chlorination	Yes/no	-	B	-4
Doyle et al. (1997)	B T	CO	-	-	-	-	+	+	-	+	0	+	+	-	-	-	0	+		+	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	-	W	-4
Flaten (1992)	B T	E	-	+	-	+	+	+	+	u	0	+	-	-	-	-	0	-		-	+	0	Ecologic, no residential history; confounding; low RR	Chlorination	Yes/no	2+	W	-7
Font-Ribera et al. (2018)	B T	CC	+	+	+	+	+	+	-	+	0	+	+	+	+	+	0	+		+	+	0	Low participation	Chloroform	>24.3	4+	W	-1
Koivusalo et al. (1997)	B T	CO	-	+	+	+	+	+	+	u	0	+	+	+	u	-	-			-	+	0	Mutagenicity; exposure 1955-60; confounding; low RR	Mutagenesis	NA	3+	W	-6
Marcus et al. (1998)	B T	E	-	-	-	-	+	+	+	-	0	+	-	-	-	-	0	-		+	-	0	Cross-sectional; ecologic; White and Black women only	TTHM	≥80	-	W	-7
Vinceti et al. (2004)	B T	CO	+	-	-	+	+	+	+	+	0	+	+	+	u	-	0	-		-	+	0	No interviews; confounding; 1 male case	TTHM	70.7	2+	W	-4

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	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
Yang et al. (1998)	B T	E	+	-	-	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	2+	W	-6	
Doyle et al. (1997)	C O	CO	+	+	+	+	+	-	+	0	+	+	-	-	-	0	+	+	-	+	0	Women only; water source 1989; follow-up to 1993; low participation	Chloroform	≥14	4+	W	-5	
Flaten (1992)	C O	E	+	+	-	+	+	+	u	0	+	-	-	-	-	0	-	-	-	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	3+	B	-8	
Hildesheim et al. (1998)	C O	CC	-	-	-	-	+	+	+	u	0	+	+	+	-	0	+	+	+	+	0	None	Chlorin/TTHM	≥60 yrs/≥46.4	-	B	-2	
Isacson et al. (1985)	C O	E	-	-	-	-	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	-	Demographics and exposure unclear; stratified results; confounding	Chlorination	Yes/no	-	B	-8
King et al. (2000)	C O	CC	+	+	u	-	+	+	+	+	0	+	+	+	-	0	-	+	+	+	0	Subgroup: men; inconsistent dose-response; no smoking data	Chlorin/TTHM	≥35 yrs/≥195 7 µg/L-yrs	2+	M	-2	
Koivusalo et al. (1997)	C O	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-	-	-	+	+	0	Mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	-	B	-6
Kuo et al. (2010b)	C O	CC	-	+	-	+	+	+	+	u	0	+	u	-	-	-	0	-	-	+	+	0	Cross-sectional; municipality; confounding; low RR	TTHM	≥4.9	2+	C	-6
Rahman et al. (2014)	C O	E	-	+	+	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Subgroup: men; ecologic; low RR; confounding	Bromoform	Per 2 µg/L	2+	M	-7
Villanueva et al. (2016)	C O	CC	-	-	-	-	u	u	-	+	0	+	+	+	+	+	0	+	+	+	+	0	Low participation in some areas	Chloroform	>23.4	-	B	-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	ethy	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
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	

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	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
Koivusalo et al. (1998)	K	CC	+	+	+	-	+	+	+	+	0	+	+	+	-	-	+	-	+	+	0	Subgroup: men; mutagenicity; confounding	Mutagenesis	NA	3+	M	-3	
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

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	only	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources	Other	#1									
Flaten (1992)	LH	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
Infante-Rivard et al. (2001)	LH	CC	+	-	-	+	+	+	+	+	0	+	+	+	-	-	+	+	+	+	+	0	ALL; limited usable exposure data	TTHM/Chloroform	Unc/>102	2+	C	-2
Kasim et al. (2006)	LH	CC	+	+	+	+	+	+	-	+	0	+	+	+	-	0	+	+	+	+	+	0	CML; BDCM; low participation	TTHM/BDCM	>20/>5	4+	C	-2
Koivusalo et al. (1997)	LH	CO	+	-	+	-	+	+	+	u	0	+	+	+	u	-	-	-	+	-	+	0	NHL; subgroup: women; mutagenicity; exposure 1955-60; confounding	Mutagenesis	NA	2+	W	-6
Vinceti et al. (2004)	LH	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

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)	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/ND	>50	-	B	-1
Flaten (1992)	P N	E	-	-	-	-	+	+	+	u	0	+	-	-	-	-	0	-	-	+	+	0	Ecologic, no residential history; confounding	Chlorination	Yes/no	-	B	-7
IJsseldiën 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
Yang et al. (1998)	P N	E	+	-	-	+	+	+	+	+	0	+	-	-	-	-	0	-	-	-	+	0	Cross-sectional; ecologic; confounding	Chlorination	Yes/no	2+	B	-7
Flaten (1992)	P R	E	-	+	-	+	+	+	+	u	0	+	-	-	-	-	0			-	+	0	Ecologic, no residential history; low RR	Chlorination	Yes/no	2+	M	-6
Koivusalo et al. (1997)	P R	CO	-	-	-	-	+	+	+	u	0	+	+	+	u	-	-			+	+	0	Mutagenicity; exposure 1955-60	Mutagenesis	NA	-	M	-4

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	ethy	No subgroup	Cases	Controls	Participation	Similar demo		Other	Individual	Past	Long-term	Other sources		Other	#1								
Vinceti et al. (2004)	P R	CO	+	-	-	+	+	+	+	0	+	+	+	u	-	0			-	+	0	No interviewers	TTHM	70.7	2+	M	-3	
Yang et al. (1998)	P R	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	only	No subgroup	Cases	Controls	Participation		Similar demo	Other	Individual	Past	Long-term	Other sources	Other										#1
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	

* A detailed description of the contents of this table is provided in the section below.

Abbreviations: ALL, acute lymphocytic leukemia; BDCM, bromodichloromethane; Ca, calcium; Chlorin., chlorination; HAA5, haloacetic acid 5 (sum of monochloro-, dichloro-, trichloro-, monobromo-, and dibromoacetic acids); IND, individual trihalomethanes or haloacetic acids; Mg, magnesium; NA, not applicable; RR, relative risk estimate; TTHM, total trihalomethanes

Table C2: 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.

Cancer:

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 given 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 noncancer 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). <i>Environ Sci Pollut Res Int.</i> 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. <i>J Environ Pathol Toxicol Oncol.</i> 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. <i>J Environ Sci Eng.</i> 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. <i>J Water Health.</i> 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. <i>J Environ Public Health.</i> 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, <i>Critical Reviews in Environmental Control</i> , 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? <i>Fundam Appl Toxicol.</i> 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. <i>Fundam Appl Toxicol.</i> 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. <i>Environ Health.</i> 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. <i>Int J Epidemiol</i> 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. <i>Eur J Public Health.</i> 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]. <i>Ai Zheng.</i> 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]. <i>Zhonghua Liu Xing Bing Xue Za Zhi.</i> 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]. <i>Gig Sanit.</i> 2009 May-Jun;(3):28-33.	Review
Chowdhury S, Rodriguez MJ, Sadiq R. Disinfection byproducts in Canadian provinces: associated cancer risks and medical expenses. <i>J Hazard Mater.</i> 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. <i>J Natl Cancer Inst.</i> 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, <i>International Journal of Hygiene and Environmental Health</i> , Volume 214, Issue 6, 2011, Pages 461-469,	Review
Goebell, P.J., Villanueva, C.M., Rettenmeier, A.W. et al. <i>World J Urol</i> (2004) 21: 424.	Review
Hang C, Zhang B, Gong T, Xian Q. Occurrence and health risk assessment of halogenated disinfection byproducts in indoor swimming pool water. <i>Sci Total Environ.</i> 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]. <i>Tidsskr Nor Laegeforen.</i> 1999 Jun 30;119(17):2528-30.	Review

Article Not Used	Reason
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. <i>Environ Res</i> . 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. <i>Occup Environ Med</i> . 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. <i>Environ Health Perspect</i> . 2010 Nov;118(11):1531-7.	Genotoxicity
Koivusalo M, & Vartiainen T. (2011). Drinking Water Chlorination By-Products And Cancer. <i>Reviews on Environmental Health</i> , 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. <i>Sci Total Environ</i> . 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. <i>Asian Pac J Cancer Prev</i> . 2003 Jan-Mar;4(1):31-8.	No cancer data
Lee H-K, Yir-Yarn Yeh Email author Wei-Ming Chen. Cancer risk analysis and assessment of trihalomethanes in drinking water. <i>Stochastic Environmental Research and Risk Assessment</i> . 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. <i>Environ Monit Assess</i> . 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. <i>J Environ Manage</i> . 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. <i>J Toxicol Environ Health A</i> . 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. <i>J Toxicol Environ Health A</i> . 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. <i>Environ Sci Technol</i> . 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. <i>J Environ Sci (China)</i> . 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. <i>Environ Int</i> . 2016 Jul-Aug;92-93:486-93.	Not cancer
McDonald TA, Komulainen H. Carcinogenicity of the chlorination disinfection by-product MX. <i>J Environ Sci Health C Environ Carcinog Ecotoxicol Rev</i> . 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. <i>J Expo Sci Environ Epidemiol</i> . 2007 Mar;17(2):207-14.	Atrazine
Melnick RL, Dunnick JK, Sandler DP, Elwell MR, Barrett JC. Trihalomethanes and Other Environmental Factors That Contribute to Colorectal Cancer. <i>Environ Health Perspect</i> . 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. <i>Science</i> . 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. <i>Philos Trans A Math Phys Eng Sci</i> . 2009 Oct 13;367(1904):4043-76.	Review

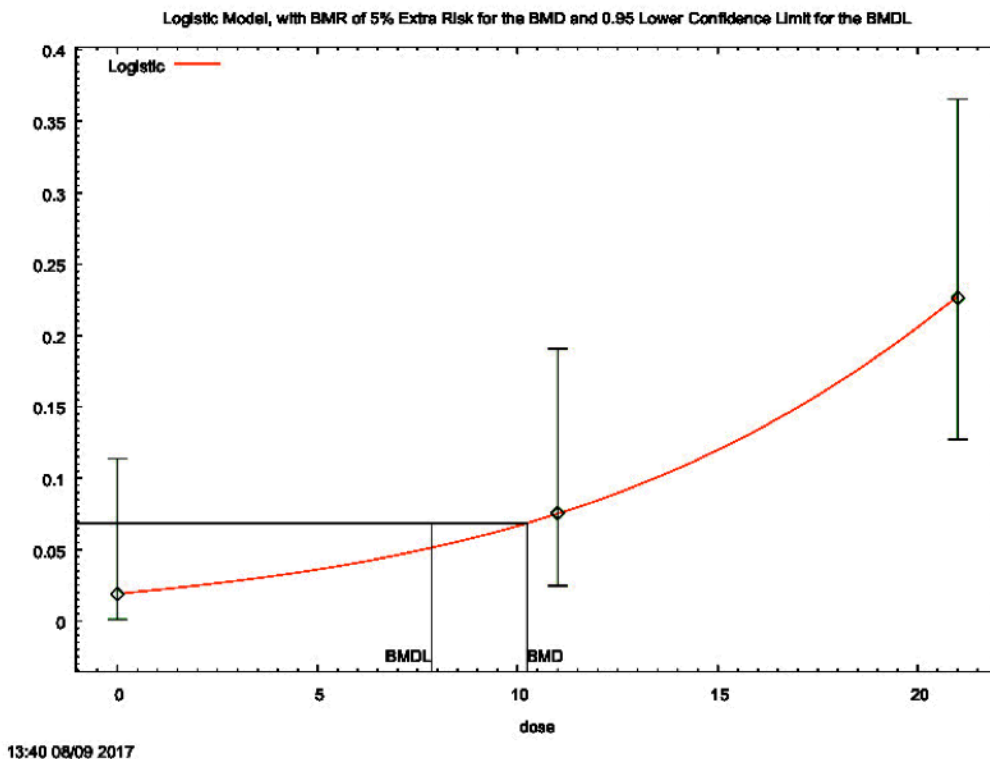
Article Not Used	Reason
Nieuwenhuijsen MJ, Smith R, Golfinopoulos S, Best N, Bennett J, Aggazzotti G, Righi E, Fantuzzi G, Bucchini L, Cordier S, Villanueva CM, Moreno V, La Vecchia C, Bosetti C, Vartiainen T, Rautiu R, Toledano M, Iszatt N, Grazuleviciene R, Kogevinas M. Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE. <i>J Water Health</i> . 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. <i>J Hazard Mater</i> . 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. <i>Environ Sci Technol</i> . 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. <i>Epigenetics</i> . 2015;10(7):650-61.	DNA methylation, not cancer
Salas LA, Font-Ribera L, Bustamante M, Sumoy L, Grimalt JO, Bonnín 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. <i>J Environ Sci (China)</i> . 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. <i>Am J Epidemiol</i> . 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. <i>Occup Environ Med</i> . 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. <i>Environ Res</i> . 2004 Nov;96(3):345-52.	Risk assessment
Trihalomethanes and colorectal cancer. <i>Environ Health Perspect</i> . 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. <i>Occup Environ Med</i> . 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. <i>Curr Environ Health Rep</i> . 2015 Mar;2(1):107-15.	Review
Villanueva CM, Kogevinas M, Grimalt JO. [Chlorination of drinking water in Spain and bladder cancer]. <i>Gac Sanit</i> . 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. <i>Environ Health Perspect</i> 69: 275-279.	Compared chlorination to chloramine

APPENDIX D. BENCHMARK DOSE ANALYSIS RESULTS FOR NONCANCER ENDPOINTS

This appendix provides the BMD modeling outputs for the HAA5 where data were amenable to dose-response modeling. All models were run with default parameters and a benchmark response of 5% or 1 standard deviation from the control mean. Model selection criteria when comparing outputs of different models for the same endpoint/dataset were: the lowest Akaike's information criterion (AIC), goodness of fit p-value ≥ 0.05 , scaled residual \leq the absolute value of 2, and visual inspection of the dose-response curve. When using BMD modeling, the BMDL, which is the lower limit of the 95% confidence interval of the BMD resulting in the benchmark response, is selected as the POD.

Monochloroacetic Acid

Figure D1. BMD modeling of increased mortality in male rats from NTP, 1992



=====
Logistic Model. (Version: 2.14; Date: 2/28/2013)
Input Data File: K:/HAA/NTP 1992/log_NTP1992_male_mortality_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 1992/log_NTP1992_male_mortality_Opt.plt
Wed Aug 09 13:40:29 2017
=====

BMDS_Model_Run

~~~~~  
The form of the probability function is:

$P[\text{response}] = 1/[1+\text{EXP}(-\text{intercept}-\text{slope}*\text{dose})]$   
 Dependent variable = Effect  
 Independent variable = Dose  
 Slope parameter is not restricted

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 Parameter Values

background = 0 Specified  
 intercept = -3.57956  
 slope = 0.112046

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 )

|           | intercept | slope |
|-----------|-----------|-------|
| intercept | 1         | -0.94 |
| slope     | -0.94     | 1     |

Parameter Estimates

| Variable  | Estimate | Std. Err. | 95.0% Wald Confidence Interval |                   |
|-----------|----------|-----------|--------------------------------|-------------------|
|           |          |           | Lower Conf.Limit               | Upper Conf. Limit |
| Intercept | -3.93344 | 0.756294  | -5.41575                       | -2.45113          |
| Slope     | 0.128898 | 0.0419254 | 0.046726                       | 0.211071          |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance   | Test d.f. | P-value  |
|---------------|-----------------|-----------|------------|-----------|----------|
| Full model    | -47.492         | 3         |            |           |          |
| Fitted model  | -47.4924        | 2         | 0.00072512 | 1         | 0.9785   |
| Reduced model | -54.0637        | 1         | 13.1433    | 2         | 0.001399 |

AIC: 98.9848

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0.0192     | 1.018    | 1        | 53   | -0.018          |
| 11   | 0.0748     | 3.963    | 4        | 53   | 0.019           |
| 21   | 0.2268     | 12.019   | 12       | 53   | -0.006          |

Chi^2 = 0.00

d.f. = 1

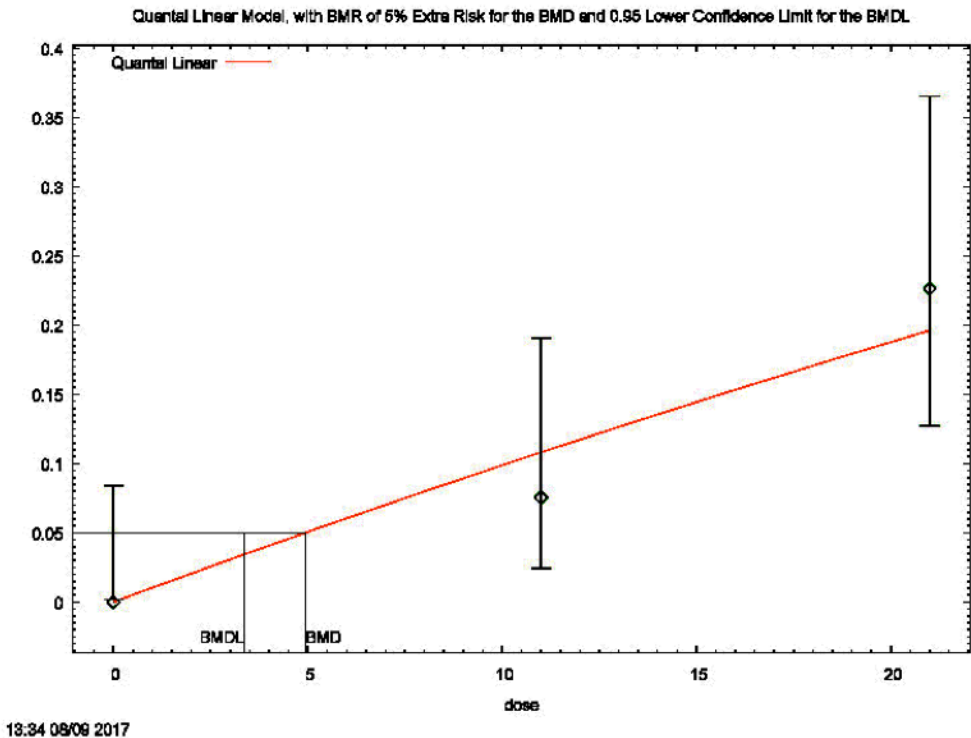
P-value = 0.9785

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk  
Confidence level = 0.95  
BMD = 10.236  
BMDL = 7.84716

**Figure D2. BMD modeling of increased mortality in female rats from NTP, 1992**



Quantal Linear Model using Weibull Model (Version: 2.16; Date: 2/28/2013)  
Input Data File: K:/HAA/NTP 1992/qIn\_NTP1992\_female\_mortality\_Opt.(d)  
Gnuplot Plotting File: K:/HAA/NTP 1992/qIn\_NTP1992\_female\_mortality\_Opt.plt  
Wed Aug 09 13:34:48 2017

BMDS\_Model\_Run

The form of the probability function is:  
 $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose})]$

Dependent variable = Effect  
Independent variable = Dose

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 = 0.0181818  
 Slope = 0.0119674  
 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      | 0.0103693 | 0.00259653 | 0.00528023                     | 0.0154584         |

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    | -42.5312        | 3         |          |           |         |
| Fitted model  | -43.0025        | 1         | 0.942429 | 2         | 0.6242  |
| Reduced model | -51.9076        | 1         | 18.7527  | 2         | <.0001  |

AIC: 88.0049

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0          | 0        | 0        | 53   | 0               |
| 11   | 0.1078     | 5.713    | 4        | 53   | -0.759          |
| 21   | 0.1957     | 10.371   | 12       | 53   | 0.564           |

Chi^2 = 0.89

d.f. = 2

P-value = 0.6395

Benchmark Dose Computation

Specified effect = 0.05

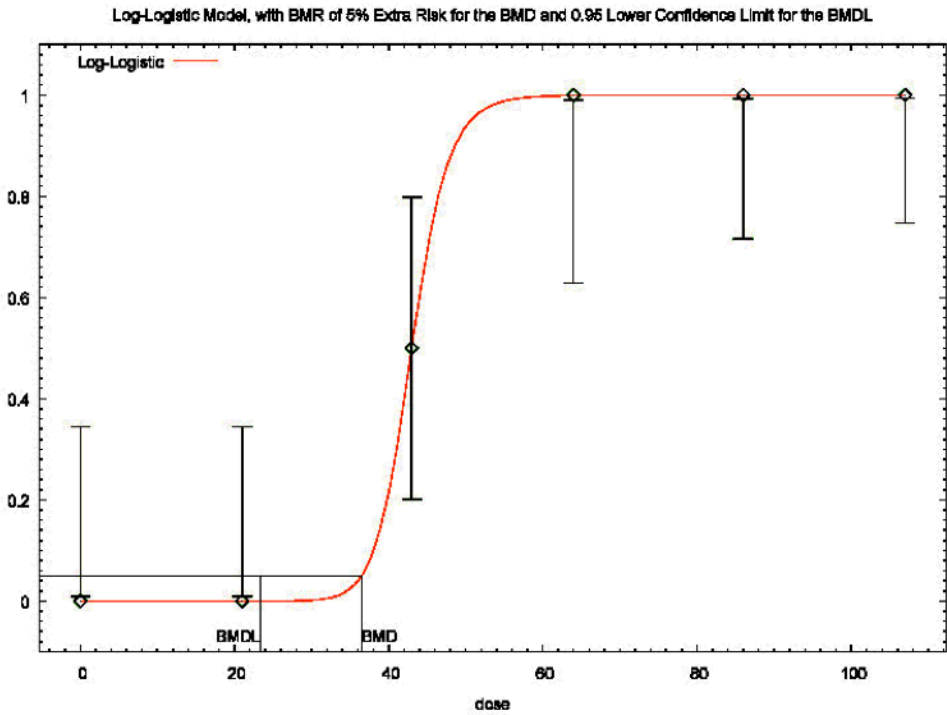
Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.94664

BMDL = 3.36386

**Figure D3. BMD modeling of cardiomyopathy in male rats from NTP, 1992**



```

=====
Logistic Model. (Version: 2.14; Date: 2/28/2013)
Input Data File: K:/HAA/NTP 1992/lnl_NTP1992_male_cardiomyopathy_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 1992/lnl_NTP1992_male_cardiomyopathy_Opt.plt
Wed Aug 23 09:13:09 2017
=====

```

BMDS\_Model\_Run

```

~~~~~
The form of the probability function is:
P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1
Total number of observations = 6
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

```

```

User has chosen the log transformed model
Default Initial Parameter Values
background = 0
intercept = -15.8475
slope = 4.27376
Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,

```

and do not appear in the correlation matrix )

```

intercept
intercept 1

```

Parameter Estimates

| Variable   | Estimate  | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|-----------|-----------|--------------------------------|-------------------|
|            |           |           | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0         | NA        |                                |                   |
| Intercept  | -67.69888 | 0.631566  | -68.9366                       | -66.461           |
| Slope      | 18        | 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    | -6.9315         | 6         |          |           |         |
| Fitted model  | -6.9385         | 1         | 0.01414  | 5         | 1       |
| Reduced model | -44.26          | 1         | 74.6578  | 5         | <.0001  |

AIC: 15.8771

Goodness of Fit

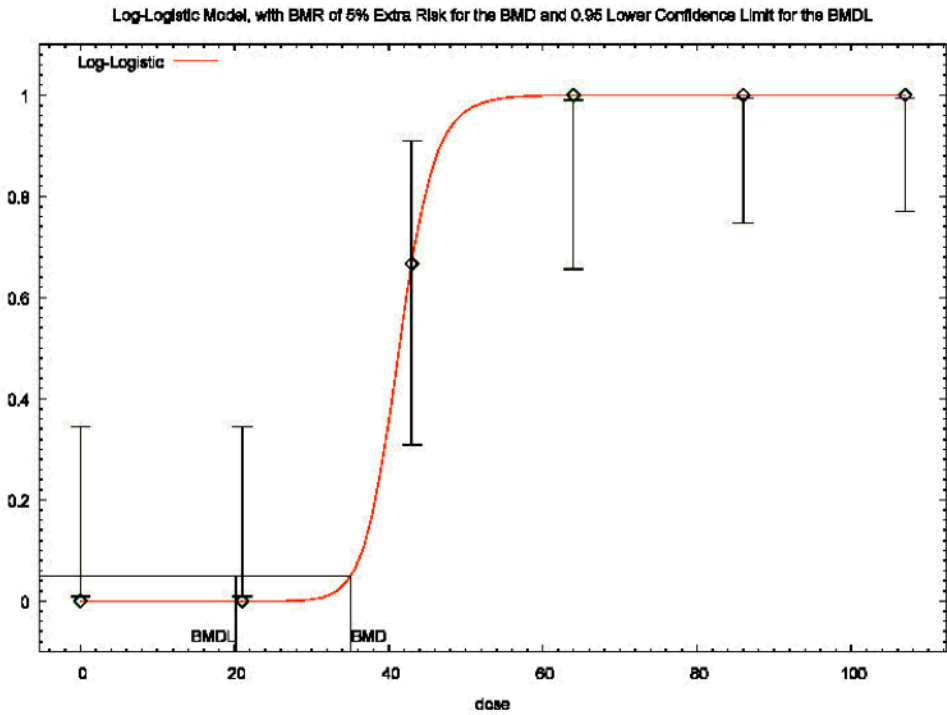
| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0          | 0        | 0        | 10   | 0               |
| 21   | 0          | 0        | 0        | 10   | -0.005          |
| 43   | 0.5007     | 5.007    | 5        | 10   | -0.004          |
| 64   | 0.9992     | 8.993    | 9        | 9    | 0.084           |
| 86   | 1          | 13       | 13       | 13   | 0.007           |
| 107  | 1          | 15       | 15       | 15   | 0.001           |

Chi^2 = 0.01  
d.f. = 5  
P-value = 1.0000

Benchmark Dose Computation  
Specified effect = 0.05  
Risk Type = Extra risk

Confidence level = 0.95  
BMD = 36.5056  
BMDL = 23.3608

**Figure D4. BMD modeling of cardiomyopathy in female rats from NTP, 1992**



```

=====
Logistic Model. (Version: 2.14; Date: 2/28/2013)
Input Data File: K:/HAA/NTP 1992/lnl_NTP1992_female_cardiomyopathy_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 1992/lnl_NTP1992_female_cardiomyopathy_Opt.plt
Wed Aug 23 09:15:38 2017
=====

```

BMDS\_Model\_Run

```

~~~~~
The form of the probability function is:
P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1
Total number of observations = 6
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

```

```

User has chosen the log transformed model
Default Initial Parameter Values
background = 0
intercept = -15.5774
slope = 4.25892

```

```

Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s) -background -slope

```

have been estimated at a boundary point, or have been specified by the user,

```

intercept
intercept      1

```

Parameter Estimates

| Variable   | Estimate | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|----------|-----------|--------------------------------|-------------------|
|            |          |           | Lower Conf. Limit              | Upper Conf. Limit |
| background | 0        | NA        |                                |                   |
| intercept  | -67.0065 | 0.706635  | -68.3915                       | -65.6215          |
| slope      | 18       | 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   | -5.7286         | 6         |          |           |         |
| Fitted model | -5.7326         | 1         | 0.00793  | 5         | 1       |
| Reduced mode | -44.716         | 1         | 77.9753  | 5         | <.0001  |

AIC: 13.4652



Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0          | 0        | 0        | 10   | 0               |
| 21   | 0          | 0        | 0        | 10   | -0.007          |
| 43   | 0.6671     | 6.004    | 6        | 9    | -0.003          |
| 64   | 0.9996     | 9.996    | 10       | 10   | 0.062           |
| 86   | 1          | 15       | 15       | 15   | 0.005           |
| 107  | 1          | 17       | 17       | 17   | 0.001           |

Chi<sup>2</sup> = 0.00

d.f. = 5

P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

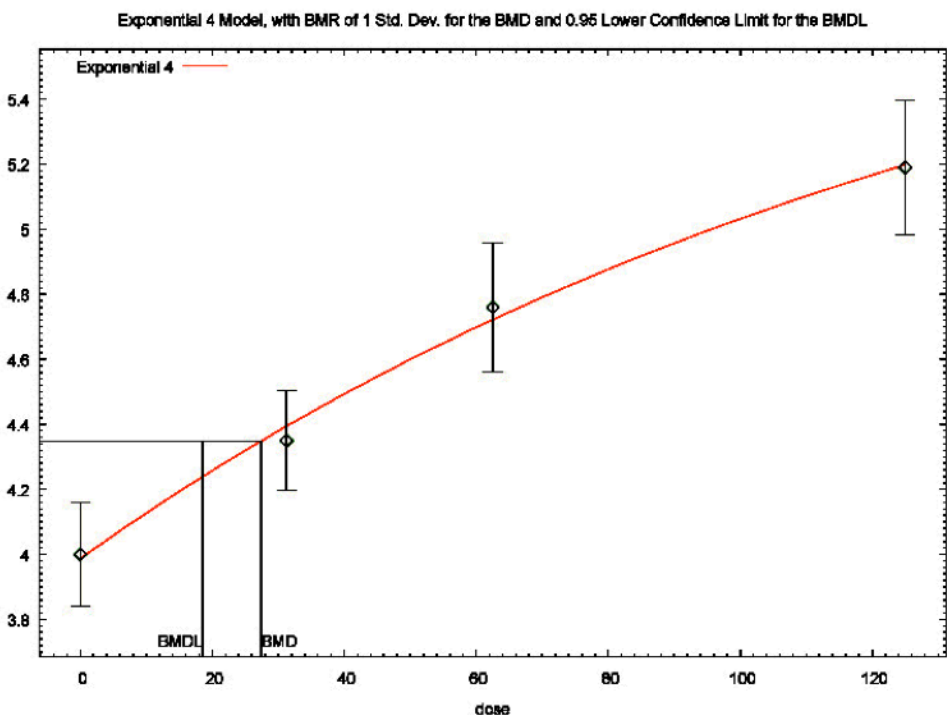
Confidence level = 0.95

BMD = 35.1282

BMDL = 20.1149

Dichloroacetic Acid

Figure D5. BMD modeling of increased relative liver weight to body weight in male Long-Evans rats from Toth et al. (1992)



14:43 08/09 2017

=====  
 Exponential Model. (Version: 1.10; Date: 01/12/2015)

Input Data File: K:/HAA/Toth 1992/exp\_Toht\_male\_rel\_liver\_Opt.(d)  
 Gnuplot Plotting File:  
 Wed Aug 09 14:43:57 2017

=====

BMDS Model Run

~~~~~

The form of the response function by Model:
 Model 2: $Y[\text{dose}] = a * \exp\{\text{sign} * b * \text{dose}\}$
 Model 3: $Y[\text{dose}] = a * \exp\{\text{sign} * (b * \text{dose})^d\}$
 Model 4: $Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$
 Model 5: $Y[\text{dose}] = a * [c - (c - 1) * \exp\{-(b * \text{dose})^d\}]$

Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
 Model 3 is nested within Model 5.
 Model 4 is nested within Model 5.

Dependent variable = Mean
 Independent variable = Dose
 Data are assumed to be distributed: normally
 Variance Model: $\exp(\ln\alpha + \rho * \ln(Y[\text{dose}]))$
 rho is set to 0.
 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
 MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-2.03793	NC
rho	NC	NC	0 *	NC
a	NC	NC	3.8	NC
b	NC	NC	0.0145494	NC
c	NC	NC	1.43408	NC
d	NC	NC	1 *	NC

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-2.03114	NC
rho	NC	NC	0 *	NC
a	NC	NC	3.98657	NC
b	NC	NC	0.00695216	NC
c	NC	NC	1.52333	NC
d	NC	NC	--	NC

NC = No Convergence

-- Indicates that this parameter does not appear in model

* Indicates that this parameter has been specified

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	NA	NC
rho	NC	NC	NA	NC
a	NC	NC	NA	NC
b	NC	NC	NA	NC
c	NC	NC	NA	NC
d	NC	NC	NA	NC

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	19	4	0.33
31.25	18	4.35	0.31
62.5	18	4.76	0.4
125	19	5.19	0.43

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
4	0	3.987	0.3622	0.1616
	31.25	4.394	0.3622	-0.5151
	62.5	4.722	0.3622	0.4473
	125	5.198	0.3622	-0.09563

Other models for which likelihoods are calculated:

Model A1: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\alpha + \log(\text{mean}(i)) * \rho)$

Model R: $Y_{ij} = \mu + e(i)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.40327	5	-66.80654
A2	39.73068	8	-63.46135
A3	38.40327	5	-66.80654
R	3.692388	2	-3.384776
4	38.15211	4	-68.30423

Additive constant for all log-likelihoods = -68. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	$-2 * \log(\text{Likelihood Ratio})$	D. F.	p-value
Test 1	72.08	6	< 0.0001
Test 2	2.655	3	0.448
Test 3	2.655	3	0.448
Test 6a	0.5023	1	0.4785

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.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

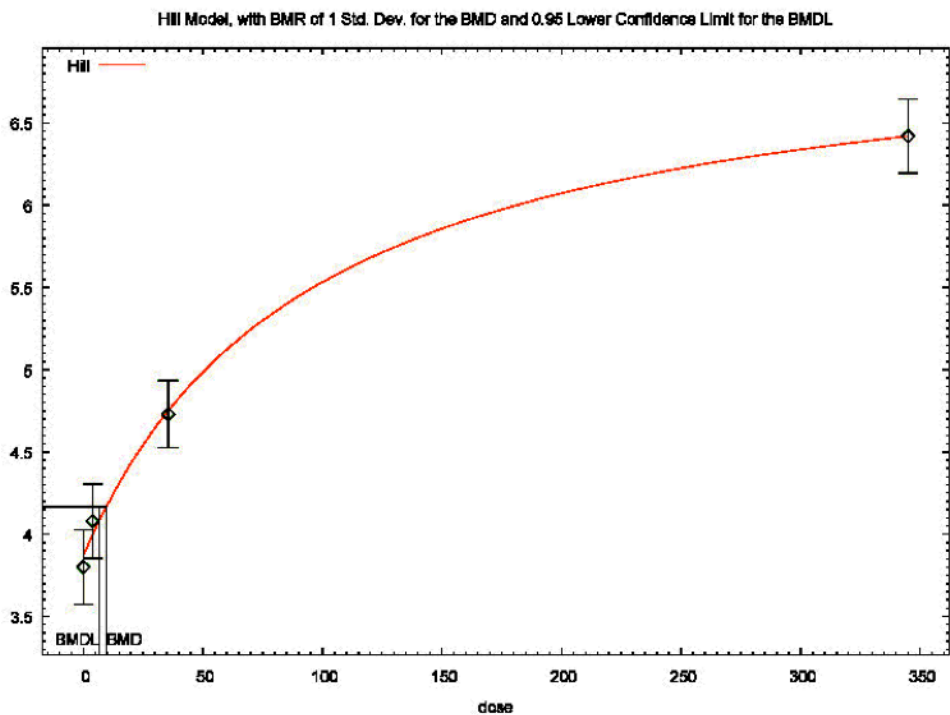
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	0	0 Not computed
3	0	0 Not computed

Model	BMD	BMDL
4	27.4284	18.522
5	0	0 Not computed

Figure D6. BMD modeling of increased relative liver weight to body weight in male Sprague-Dawley rats from Mather et al. (1990)



```

=====
Hill Model. (Version: 2.17; Date: 01/28/2013)
Input Data File: K:/HAA/Mather 1990/hil_Mather1990_rel_liver_Opt.(d)
Gnuplot Plotting File: K:/HAA/Mather 1990/hil_Mather1990_rel_liver_Opt.plt
Wed Aug 09 14:49:09 2017
=====

```

BMDS Model Run

~~~~~

The form of the response function is:

$$Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$$

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 = 0.0951982

rho = 0 Specified

intercept = 3.8

v = 2.62

n = 0.318455

k = 584.908

Asymptotic Correlation Matrix of Parameter Estimates

(\*\*\* The model parameter(s) -rho -n 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        | k         |
| alpha     | 1         | -1.20E-06 | 6.30E-07 | -2.30E-07 |
| intercept | -1.20E-06 | 1         | 0.025    | 0.53      |
| v         | 6.30E-07  | 0.025     | 1        | 0.74      |
| k         | -2.30E-07 | 0.53      | 0.74     | 1         |

Parameter Estimates

| Variable  | Estimate  | Std. Err. | 95.0% Wald Confidence Interval |                   |
|-----------|-----------|-----------|--------------------------------|-------------------|
|           |           |           | Lower Conf. Limit              | Upper Conf. Limit |
| alpha     | 0.0887117 | 0.0198366 | 0.0498327                      | 0.127591          |
| intercept | 3.86856   | 0.0740008 | 3.72352                        | 4.0136            |
| v         | 3.26006   | 0.226142  | 2.81683                        | 3.70329           |
| n         | 1         | NA        |                                |                   |
| k         | 96.1449   | 24.8764   | 47.3881                        | 144.902           |

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

| Dose | N  | Obs Mean | Est Mean | Obs Std Dev | Est Std Dev | Scaled Res. |
|------|----|----------|----------|-------------|-------------|-------------|
| 0    | 10 | 3.8      | 3.87     | 0.316       | 0.298       | -0.728      |

| Dose | N  | Obs Mean | Est Mean | Obs Std Dev | Est Std Dev | Scaled Res. |
|------|----|----------|----------|-------------|-------------|-------------|
| 3.9  | 10 | 4.08     | 4        | 0.316       | 0.298       | 0.896       |
| 35.5 | 10 | 4.73     | 4.75     | 0.285       | 0.298       | -0.188      |
| 345  | 10 | 6.42     | 6.42     | 0.316       | 0.298       | 0.0201      |

Model Descriptions for likelihoods calculated

Model A1:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A2:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A3 uses any fixed variance parameters that were specified by the user

Model R:  $Y_i = \mu + e(i)$

$\text{Var}\{e(i)\} = \sigma^2$

Likelihoods of Interest

| Model  | Log(likelihood) | # Param's | AIC        |
|--------|-----------------|-----------|------------|
| A1     | 29.143085       | 5         | -48.286169 |
| A2     | 29.220263       | 8         | -42.440527 |
| A3     | 29.143085       | 5         | -48.286169 |
| fitted | 28.447305       | 4         | -48.894610 |
| R      | -22.280804      | 2         | 48.561608  |

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   | $-2 \cdot \log(\text{Likelihood Ratio})$ | Test df | p-value |
|--------|------------------------------------------|---------|---------|
| Test 1 | 103.002                                  | 6       | <.0001  |
| Test 2 | 0.154357                                 | 3       | 0.9846  |
| Test 3 | 0.154357                                 | 3       | 0.9846  |
| Test 4 | 1.39156                                  | 1       | 0.2381  |

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

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

### Benchmark Dose Computation

Specified effect = 1

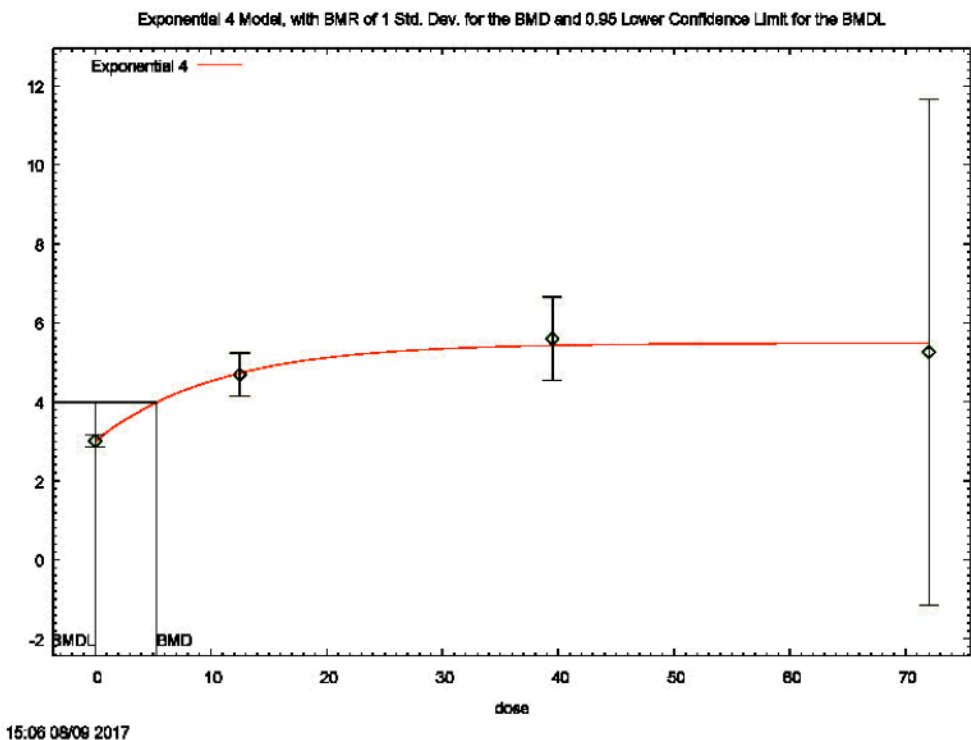
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 9.6672

BMDL = 6.74981

**Figure D7. BMD modeling of increased relative liver weight to body weight in male beagle dogs from Cicmanec et al. (1991)**



=====  
Exponential Model. (Version: 1.10; Date: 01/12/2015)  
Input Data File: K:/HAA/Cicmanec 1991/exp\_Cicmanec\_male\_rel\_liver\_Opt.(d)  
Gnuplot Plotting File:  
Wed Aug 09 15:07:31 2017  
=====

### BMDS Model Run

~~~~~  
The form of the response function by Model:

Model 2: $Y[\text{dose}] = a * \exp\{\text{sign} * b * \text{dose}\}$

Model 3: $Y[\text{dose}] = a * \exp\{\text{sign} * (b * \text{dose})^d\}$

Model 4: $Y[\text{dose}] = a * [c - (c - 1) * \exp\{-b * \text{dose}\}]$

Model 5: $Y[\text{dose}] = a * [c - (c - 1) * \exp\{-(b * \text{dose})^d\}]$

Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.

Model 3 is nested within Model 5.

Model 4 is nested within Model 5.

Dependent variable = Mean

Independent variable = Dose

Data are assumed to be distributed: normally

Variance Model: $\exp(\ln\alpha + \rho * \ln(Y[\text{dose}]))$

The variance is to be modeled as $\text{Var}(i) = \exp(\ln\alpha + \log(\text{mean}(i)) * \rho)$

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

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-13.0416	NC
rho	NC	NC	7.93493	NC
a	NC	NC	2.8595	NC
b	NC	NC	0.0318236	NC
c	NC	NC	2.0563	NC
d	NC	NC	1 *	NC

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-13.268	NC
rho	NC	NC	7.85573	NC
a	NC	NC	3.0131	NC
b	NC	NC	0.0570347	NC
c	NC	NC	1.9997	NC
d	NC	NC	--	NC

NC = No Convergence

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	7.46E-152	NC
rho	NC	NC	1.33699	NC

Variable	Model 2	Model 3	Model 4	Model 5
a	NC	NC	0.044819	NC
b	NC	NC	0.0201195	NC
c	NC	NC	0.217295	NC
d	NC	NC	NA	NC

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	5	3.01	0.121
12.5	5	4.69	0.436
39.5	5	5.6	0.849
72	3	5.26	2.579

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
4	0	3.013	0.1001	-0.06931
	12.5	4.549	0.5046	0.6261
	39.5	5.709	1.232	-0.1974
	72	5.976	1.474	-0.8411

Other models for which likelihoods are calculated:

Model A1: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \exp(\alpha + \log(\text{mean}(i)) * \rho)$

Model R: $Y_{ij} = \mu + e(i)$

$\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-8.488027	5	26.97605
A2	5.968438	8	4.063123
A3	4.620703	6	2.758594
R	-15.23268	2	34.46535
4	3.722844	5	2.554311

Additive constant for all log-likelihoods = -16.54. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	42.4	6	< 0.0001
Test 2	28.91	3	< 0.0001
Test 3	2.695	2	0.2598
Test 6a	1.796	1	0.1802

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 less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seem to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

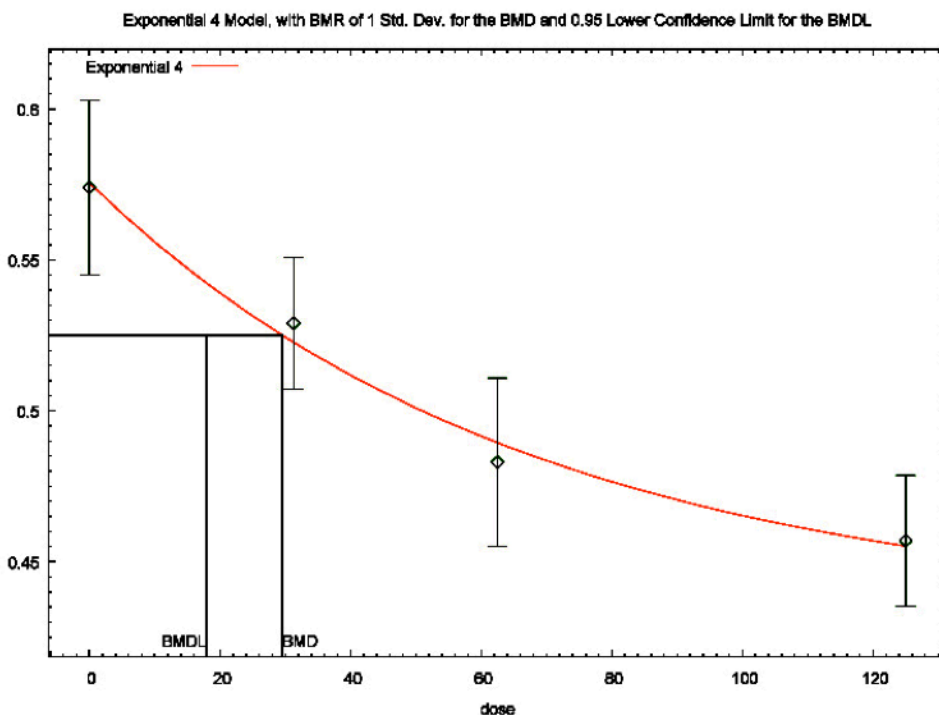
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	
2	0	0	Not computed
3	0	0	Not computed
4	0.5925	0.32888	
5	0	0	Not computed

Figure D8. BMD modeling of decreased absolute epididymis weight in male Long-Evans rats from Toth et al. (1992)



14:46 09/09 2017

```
=====
Exponential Model. (Version: 1.10; Date: 01/12/2015)
Input Data File: K:/HAA/Toth 1992/exp_Toth_male_abs_right_epididymis_Opt.(d)
Gnuplot Plotting File:
Wed Aug 09 14:46:33 2017
=====
```

BMDS Model Run

```
~~~~~
The form of the response function by Model:
Model 2:  Y[dose] = a * exp{sign * b * dose}
Model 3:  Y[dose] = a * exp{sign * (b * dose)^d}
Model 4:  Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5:  Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
```

Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
 Model 3 is nested within Model 5.
 Model 4 is nested within Model 5.

Dependent variable = Mean
 Independent variable = Dose
 Data are assumed to be distributed: normally
 Variance Model: $\exp(\ln(\alpha + \rho * \ln(Y[\text{dose}])))$
 rho is set to 0.
 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
 MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-5.97818	NC
rho	NC	NC	0 *	NC
a	NC	NC	0.6027	NC
b	NC	NC	0.017145	NC
c	NC	NC	0.722147	NC
d	NC	NC	1 *	NC

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	-5.9699	NC
rho	NC	NC	0 *	NC
a	NC	NC	0.575588	NC
b	NC	NC	0.0147743	NC
c	NC	NC	0.7517736	NC
d	NC	NC	--	NC

NC = No Convergence

-- Indicates that this parameter does not appear in model

* Indicates that this parameter has been specified

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
Inalpha	NC	NC	4.73E-152	NC
rho	NC	NC	NA	NC
a	NC	NC	0.0113281	NC
b	NC	NC	0.00727965	NC
c	NC	NC	0.0555462	NC
d	NC	NC	NA	NC

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	19	0.574	0.06
31.25	18	0.529	0.044
62.5	18	0.483	0.056
125	19	0.457	0.045

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
4	0	0.5756	0.05054	-0.1369
	31.25	0.5227	0.05054	0.525
	62.5	0.4894	0.05054	-0.541
	125	0.4552	0.05054	0.1525

Other models for which likelihoods are calculated:

Model A1: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Model A2: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3: $Y_{ij} = \mu(i) + e(ij)$
 $\text{Var}\{e(ij)\} = \exp(\alpha + \log(\text{mean}(i)) * \rho)$

Model R: $Y_{ij} = \mu + e(i)$
 $\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	184.1927	5	-358.3853
A2	185.5391	8	-355.0783
A3	184.1927	5	-358.3853
R	162.3593	2	-320.7186
4	183.8863	4	-359.7725

Additive constant for all log-likelihoods = -68. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	46.36	6	< 0.0001
Test 2	2.693	3	0.4414
Test 3	2.693	3	0.4414
Test 6a	0.6128	1	0.4337

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.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

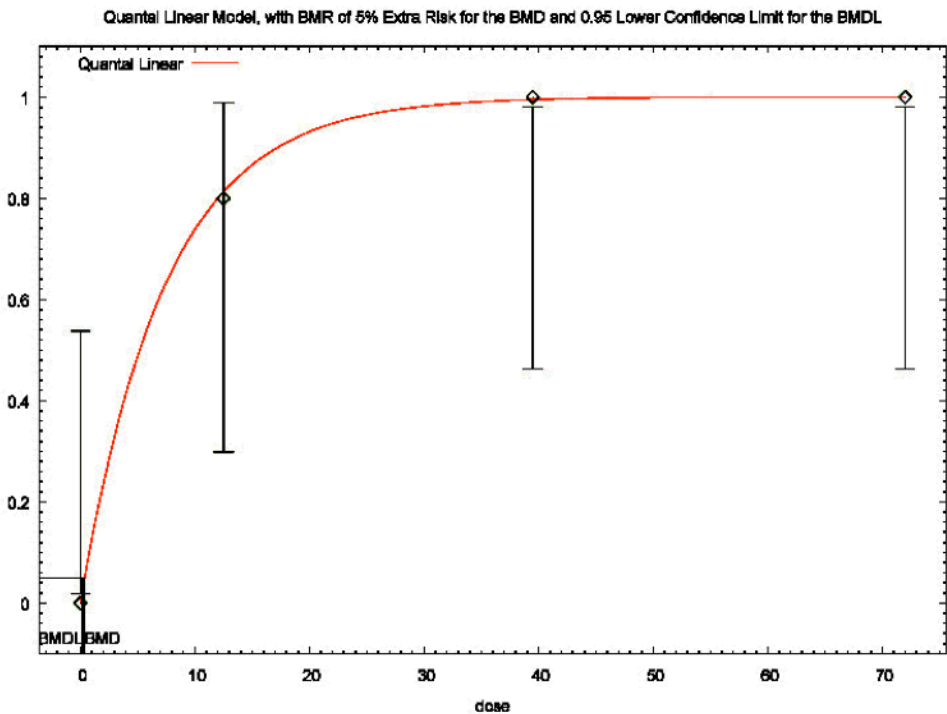
Risk Type = Estimated standard deviations from control

Confidence Level = 0.95000

BMD and BMDL by Model

Model	BMD	BMDL	
2	0	0	Not computed
3	0	0	Not computed
4	29.5433	17.8665	
5	0	0	Not computed

Figure D9. BMD modeling of testicular degeneration in male beagle dogs from Cicmanec et al. (1991)



15:13 09/09 2017

```

=====
Quantal Linear Model using Weibull Model (Version: 2.16; Date: 2/28/2013)
Input Data File: K:/HAA/Cicmanec 1991/qln_Cicmanec_male_testicular_degeneration_Opt.(d)
Gnuplot Plotting File: K:/HAA/Cicmanec
1991/qln_Cicmanec_male_testicular_degeneration_Opt.plt
Wed Aug 09 15:13:07 2017
=====

```

BMDS_Model_Run

The form of the probability function is:
 $P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(-\text{slope} * \text{dose})]$

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 = 0.142857
 Slope = 0.0248855

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	0.134206	0.0678033	0.0013141	0.267098

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Debynce Table

Model	Log(likelihood)	# Param's	Debynce	Test d.f.	P-value
Full model	-2.50201	4			
Fitted model	-2.53013	1	0.0562319	3	0.9965
Reduced model	-12.2173	1	19.4305	3	0.000227

AIC: 7.06026

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0	5	0
12.5	0.8132	4.066	4	5	-0.076
39.5	0.995	4.975	5	5	0.158
72	0.9999	5	5	5	0.018

Chi² = 0.03

d.f. = 3

P-value = 0.9986

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

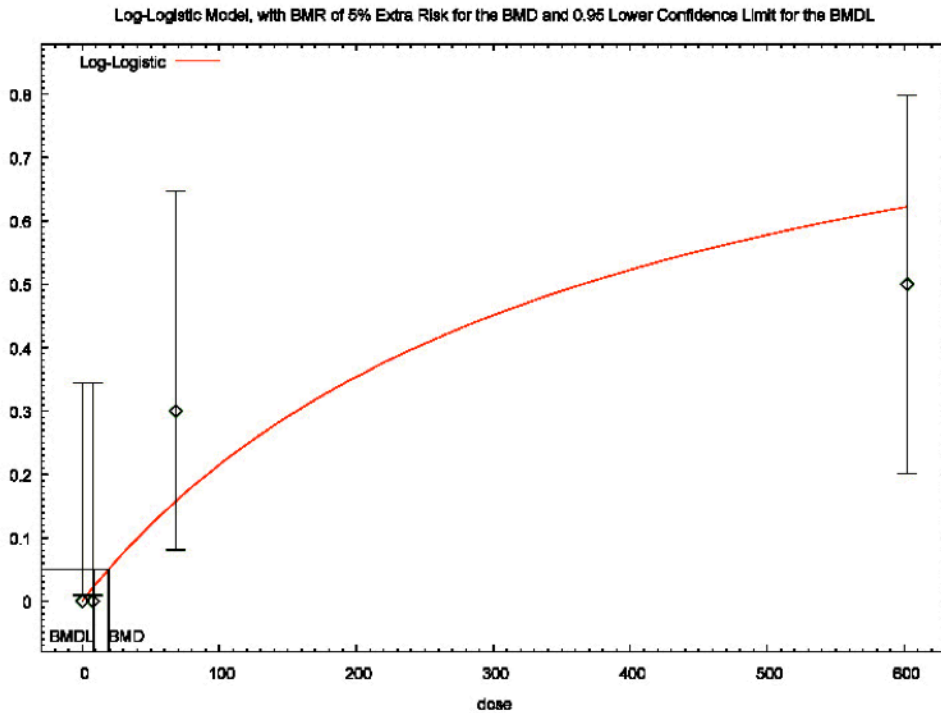
Confidence level = 0.95

BMD = 0.382198

BMDL = 0.169102

Trichloroacetic Acid

Figure D10. BMD modeling of hepatic necrosis (transient) in male mice from DeAngelo et al. (2008)



15:16 08/09 2017

=====
Logistic Model. (Version: 2.14; Date: 2/28/2013)
Input Data File: K:/HAA/DeAngelo 2008/Inl_DeAngelo2008_60w_hepatic_necrosis_Opt.(d)
Gnuplot Plotting File: K:/HAA/DeAngelo
2008/Inl_DeAngelo2008_60w_hepatic_necrosis_Opt.plt
Wed Aug 09 15:16:25 2017
=====

BMDS_Model_Run
~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$$

Dependent variable = Effect

Independent variable = Dose

Slope parameter is restricted as slope  $\geq 1$

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

User has chosen the log transformed model

Default Initial Parameter Values

background = 0  
intercept = -5.96666  
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

( \*\*\* The model parameter(s) -background -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix )

|           |           |
|-----------|-----------|
|           | intercept |
| intercept | 1         |

Parameter Estimates

| Variable   | Estimate | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|----------|-----------|--------------------------------|-------------------|
|            |          |           | Lower Conf. Limit              | Upper Conf. Limit |
| background | 0        | NA        |                                |                   |
| intercept  | -5.90164 | 0.507828  | -6.89697                       | -4.90632          |
| slope      | 1        | 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    | -13.0401        | 4         |          |           |          |
| Fitted model  | -14.1956        | 1         | 2.31088  | 3         | 0.5104   |
| Reduced model | -20.0161        | 1         | 13.952   | 3         | 0.002971 |

AIC: 30.3911

Goodness of Fit

| Dose  | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|-------|------------|----------|----------|------|-----------------|
| 0     | 0          | 0        | 0        | 10   | 0               |
| 7.7   | 0.0206     | 0.206    | 0        | 10   | -0.459          |
| 68.2  | 0.1572     | 1.572    | 3        | 10   | 1.241           |
| 602.1 | 0.6222     | 6.222    | 5        | 10   | -0.797          |

Chi<sup>2</sup> = 2.38

d.f. = 3

P-value = 0.4965

Benchmark Dose Computation

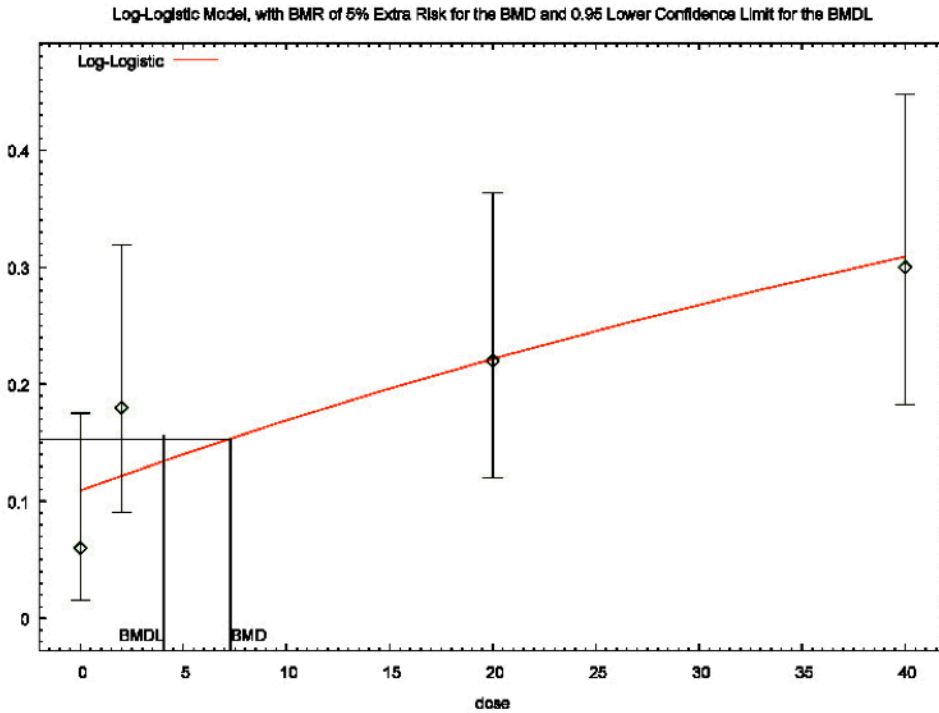
Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95  
BMD = 19.244  
BMDL = 8.45432

### Dibromoacetic Acid

Figure D11. BMD modeling of hepatic cystic degeneration in male F344/N rats from NTP (2007)



=====  
Logistic Model. (Version: 2.14; Date: 2/28/2013)  
Input Data File: K:/HAA/NTP  
2007/Rats/Inl\_NTP2007\_rats\_male\_hepaticcysticdegeneration\_Opt.(d)  
Gnuplot Plotting File: K:/HAA/NTP  
2007/Rats/Inl\_NTP2007\_rats\_male\_hepaticcysticdegeneration\_Opt.plt  
Wed Aug 09 15:27:58 2017  
=====

#### BMDS\_Model\_Run

~~~~~  
The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$$

Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1

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

User has chosen the log transformed model
 Default Initial Parameter Values
 background = 0.06
 intercept = -4.38726
 slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.58
intercept	-0.58	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.108807	0.0338664	0.0424299	0.175184
intercept	-4.9311	0.42657	-5.76717	-4.0951
slope	1		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	-91.8067	4			
Fitted model	-93.2479	2	2.88253	2	0.2366
Reduced model	-97.2446	1	10.8759	3	0.01242

AIC: 190.496

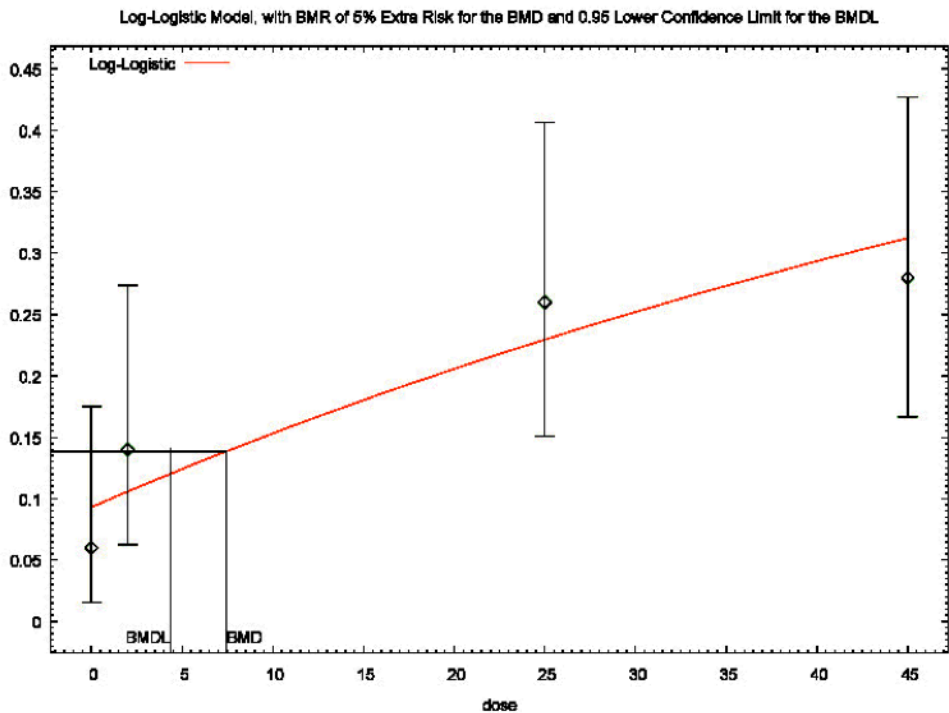
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.1088	5.44	3	50	-1.108
2	0.1215	6.074	9	50	1.266
20	0.2212	11.062	11	50	-0.021
40	0.3085	15.424	15	50	-0.13

Chi^2 = 2.85
 d.f. = 2
 P-value = 0.2406

Benchmark Dose Computation
 Specified effect = 0.05
 Risk Type = Extra risk
 Confidence level = 0.95
 BMD = 7.29122
 BMDL = 4.03523

Figure D12. BMD modeling of alveolar epithelial hyperplasia in female F344/N rats from NTP (2007)



```

=====
Logistic Model. (Version: 2.15; Date: 3/20/2017)
Input Data File: K:/HAA/NTP 2007/Rats/Inl_NTP2007_rats_
female_lung_alveolarepitheliumhyperplasia_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 2007/Rats/Inl_NTP2007_rats_
female_lung_alveolarepitheliumhyperplasia_Opt.plt
Thu Feb 15 12:37:06 2018
=====
  
```

BMD5_Model_Run

```

~~~~~
The form of the probability function is:
P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
  
```

Dependent variable = Effect
 Independent variable = Dose
 Slope parameter is restricted as slope >= 1

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

User has chosen the log transformed model

Default Initial Parameter Values
 background = 0.06
 intercept = -4.52347
 slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.56
intercept	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.093128	0.0320584	0.0302948	0.155961
intercept	-4.95274	0.380781	-5.69906	-4.20642
slope	1	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	-89.897	4			
Fitted model	-90.8	2	1.80497	2	0.4056
Reduced model	-95.778	1	11.7623	3	0.00824

AIC: 185.599

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0	0.0931	4.656	3	50	-0.806
2	0.1058	5.288	7	50	0.787
25	0.2292	11.462	13	50	0.517
45	0.3119	15.594	14	50	-0.486

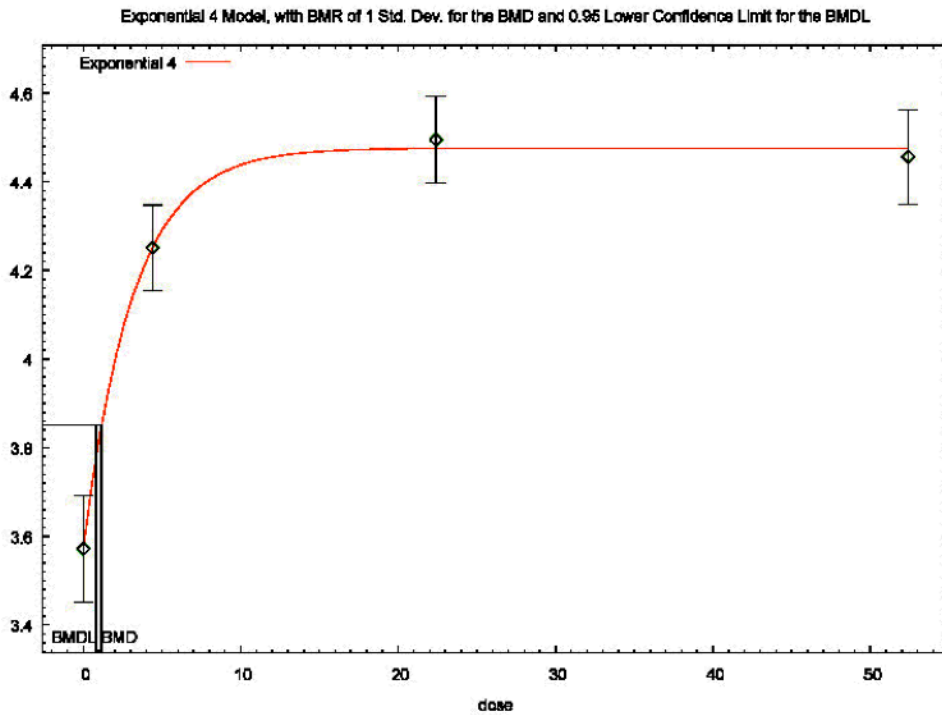
Chi^2 = 1.77

d.f. = 2
P-value = 0.4119

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95

BMD = 7.45066
BMDL = 4.34252
BMDU = 22.7427

Figure D13. BMD modeling of increased relative liver weight to body weight in male Crl Sprague-Dawley rats from Christian et al. (2002)



=====
Exponential Model. (Version: 1.10; Date: 01/12/2015)
Input Data File: K:/HAA/Christian2002/exp_Christian2002_male_rel_liver_Opt.(d)
Gnuplot Plotting File:
Wed Aug 09 15:56:47 2017
=====

BMDS Model Run

~~~~~  
The form of the response function by Model:  
Model 2:  $Y[\text{dose}] = a * \exp\{\text{sign} * b * \text{dose}\}$   
Model 3:  $Y[\text{dose}] = a * \exp\{\text{sign} * (b * \text{dose})^d\}$   
Model 4:  $Y[\text{dose}] = a * [c - (c-1) * \exp\{-b * \text{dose}\}]$



Model 5:  $Y[\text{dose}] = a * [c - (c - 1) * \exp\{-(b * \text{dose})^d\}]$

Note: Y[dose] is the median response for exposure = dose;  
 sign = +1 for increasing trend in data;  
 sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.

Model 3 is nested within Model 5.

Model 4 is nested within Model 5.

Dependent variable = Mean

Independent variable = Dose

Data are assumed to be distributed: normally

Variance Model:  $\exp(\ln(\alpha + \rho * \ln(Y[\text{dose}])))$

rho is set to 0.

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

MLE solution provided: Exact

Initial Parameter Values

| Variable | Model 2 | Model 3 | Model 4   | Model 5 |
|----------|---------|---------|-----------|---------|
| Inalpha  | NC      | NC      | -2.56166  | NC      |
| rho      | NC      | NC      | 0 *       | NC      |
| a        | NC      | NC      | 3.3934    | NC      |
| b        | NC      | NC      | 0.0394801 | NC      |
| c        | NC      | NC      | 1.39086   | NC      |
| d        | NC      | NC      | 1 *       | NC      |

\* Indicates that this parameter has been specified

Parameter Estimates by Model

| Variable | Model 2 | Model 3 | Model 4  | Model 5 |
|----------|---------|---------|----------|---------|
| Inalpha  | NC      | NC      | -2.55912 | NC      |
| rho      | NC      | NC      | 0 *      | NC      |
| a        | NC      | NC      | 3.57193  | NC      |
| b        | NC      | NC      | 0.316373 | NC      |
| c        | NC      | NC      | 1.25312  | NC      |
| d        | NC      | NC      | --       | NC      |

NC = No Convergence

-- Indicates that this parameter does not appear in model

\* Indicates that this parameter has been specified

Std. Err. Estimates by Model

| Variable | Model 2 | Model 3 | Model 4   | Model 5 |
|----------|---------|---------|-----------|---------|
| Inalpha  | NC      | NC      | 5.49E-152 | NC      |
| rho      | NC      | NC      | NA        | NC      |
| a        | NC      | NC      | 0.050775  | NC      |
| b        | NC      | NC      | 0.059786  | NC      |
| c        | NC      | NC      | 0.020513  | NC      |
| d        | NC      | NC      | NA        | NC      |

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

| Dose | N  | Obs Mean | Obs Std Dev |
|------|----|----------|-------------|
| 0    | 30 | 3.572    | 0.322       |
| 4.4  | 30 | 4.251    | 0.26        |
| 22.4 | 30 | 4.495    | 0.263       |
| 52.4 | 29 | 4.456    | 0.281       |

Estimated Values of Interest

| Model | Dose | Est Mean | Est Std | Scaled Residual |
|-------|------|----------|---------|-----------------|
| 4     | 0    | 3.572    | 0.2782  | 0.001324        |
|       | 4.4  | 4.251    | 0.2782  | -0.006635       |
|       | 22.4 | 4.475    | 0.2782  | 0.3875          |
|       | 52.4 | 4.476    | 0.2782  | -0.3887         |

Other models for which likelihoods are calculated:

Model A1:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma^2$

Model A2:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \sigma(i)^2$

Model A3:  $Y_{ij} = \mu(i) + e(ij)$

$\text{Var}\{e(ij)\} = \exp(\alpha + \log(\text{mean}(i)) * \rho)$

Model R:  $Y_{ij} = \mu + e(i)$

$\text{Var}\{e(ij)\} = \sigma^2$

Likelihoods of Interest

| Model | Log(Likelihood) | DF | AIC       |
|-------|-----------------|----|-----------|
| A1    | 92.91852        | 5  | -175.837  |
| A2    | 93.82337        | 8  | -171.6467 |
| A3    | 92.91852        | 5  | -175.837  |
| R     | 31.9332         | 2  | -59.86641 |

| Model | Log(likelihood) | DF | AIC       |
|-------|-----------------|----|-----------|
| 4     | 92.76769        | 4  | -177.5354 |

Additive constant for all log-likelihoods = -109.4. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

#### Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

#### Tests of Interest

| Test    | -2*log(Likelihood Ratio) | D. F. | p-value  |
|---------|--------------------------|-------|----------|
| Test 1  | 123.8                    | 6     | < 0.0001 |
| Test 2  | 1.81                     | 3     | 0.6128   |
| Test 3  | 1.81                     | 3     | 0.6128   |
| Test 6a | 0.3017                   | 1     | 0.5828   |

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.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

#### Benchmark Dose Computations:

Specified Effect = 1.000000

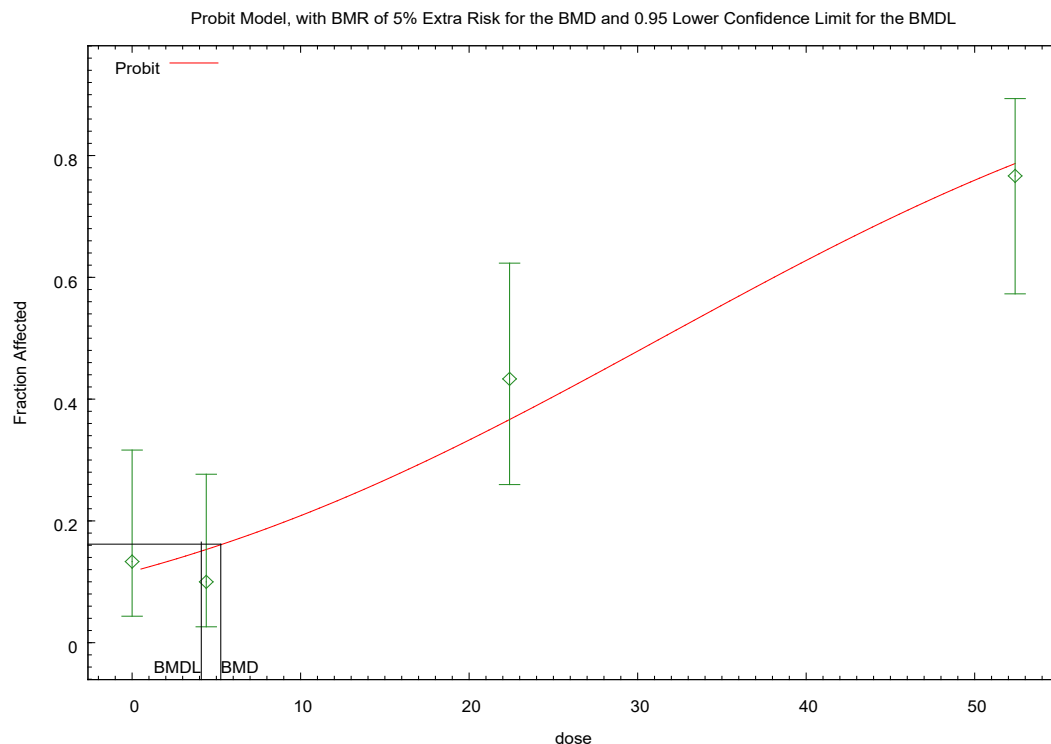
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

#### BMD and BMDL by Model

| Model | BMD     | BMDL    |              |
|-------|---------|---------|--------------|
| 2     | 0       | 0       | Not computed |
| 3     | 0       | 0       | Not computed |
| 4     | 1.16212 | 0.81603 |              |
| 5     | 0       | 0       | Not computed |

**Figure D14. BMD modeling of retention of step 19 spermatids in male Crl Sprague-Dawley rats from Christian et al. (2002)**



12:03 12/21 2022

```

=====
Probit Model. (Version: 3.4; Date: 5/21/2017)
Input Data File: K:/BMDS files/pro_DBA_Christian2002_mP_step19 retention_Opt.(d)
Gnuplot Plotting File: K:/BMDS files/pro_DBA_Christian2002_mP_step19 retention_Opt.plt
Fri Dec 23 09:42:30 2022
=====
  
```

**BMDS\_Model\_Run**

The form of the probability function is:

$$P[\text{response}] = \text{CumNorm}(\text{Intercept} + \text{Slope} * \text{Dose}),$$

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Effect

Independent variable = Dose

Slope parameter is not restricted

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 Specified  
 intercept = -1.19784  
 slope = 0.0376585

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 )

|           | intercept | slope |
|-----------|-----------|-------|
| intercept | 1         | -0.74 |
| slope     | -0.74     | 1     |

Parameter Estimates

| Variable  | Estimate  | Std. Err.  | 95.0% Wald Confidence Interval |                   |
|-----------|-----------|------------|--------------------------------|-------------------|
|           |           |            | Lower Conf. Limit              | Upper Conf. Limit |
| intercept | -1.18601  | 0.195128   | -1.56846                       | -0.80357          |
| slope     | 0.0378912 | 0.00652284 | 0.0251066                      | 0.0506757         |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -58.3579        | 4         |          |           |         |
| Fitted model  | -59.0768        | 2         | 1.43788  | 2         | 0.4873  |
| Reduced model | -78.2944        | 1         | 39.8731  | 3         | <.0001  |

AIC: 122.154

Goodness of Fit

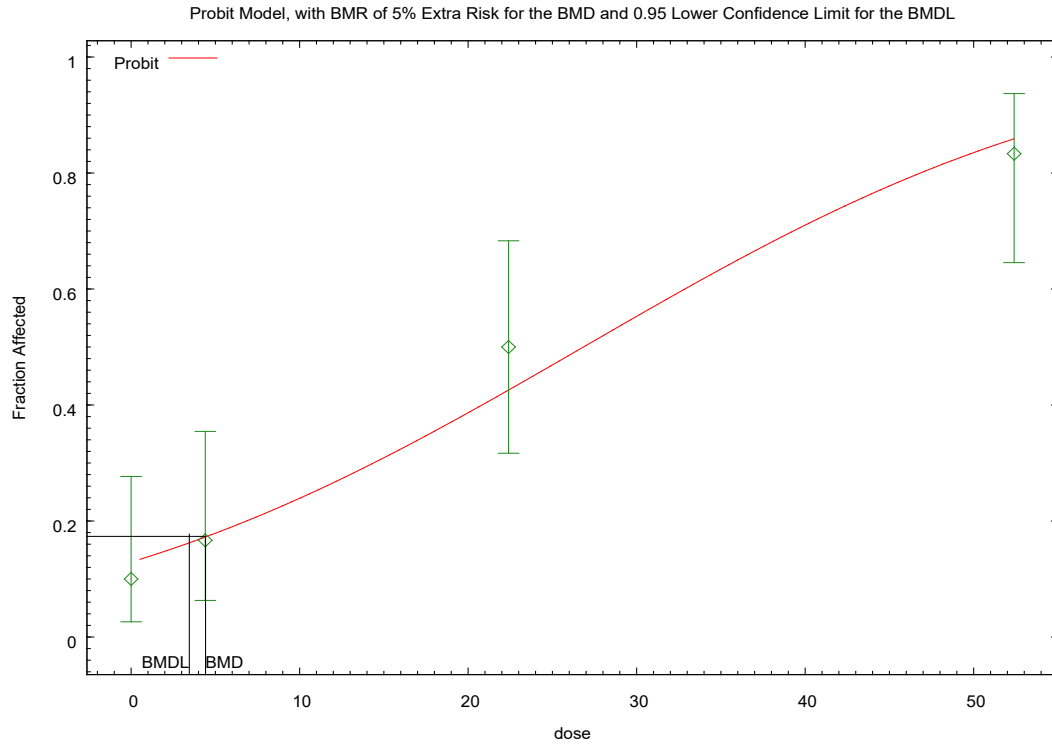
| Dose    | Est._Prob. | Expected | Observed | Size   | Scaled Residual |
|---------|------------|----------|----------|--------|-----------------|
| 0.0000  | 0.1178     | 3.534    | 4.000    | 30.000 | 0.264           |
| 4.4000  | 0.1540     | 4.621    | 3.000    | 30.000 | -0.820          |
| 22.4000 | 0.3680     | 11.039   | 13.000   | 30.000 | 0.742           |
| 52.4000 | 0.7880     | 23.640   | 23.000   | 30.000 | -0.286          |

Chi^2 = 1.37 d.f. = 2 P-value = 0.5029

Benchmark Dose Computation  
 Specified effect = 0.05  
 Risk Type = Extra risk  
 Confidence level = 0.95  
 BMD = 5.26265

BMDL = 4.1061  
BMDU = 6.84917

**Figure D15. BMD modeling of increased residual bodies in male Crl Sprague-Dawley rats from Christian et al. (2002)**



12:08 12/21 2022

=====  
Probit Model. (Version: 3.4; Date: 5/21/2017)  
Input Data File: K:/BMDS files/pro\_DBA\_Christian2002\_mP\_residual bodies\_Opt.(d)  
Gnuplot Plotting File: K:/BMDS files/pro\_DBA\_Christian2002\_mP\_residual bodies\_Opt.plt  
Fri Dec 23 09:39:47 2022  
=====

BMDS\_Model\_Run  
~~~~~

The form of the probability function is:
 $P[\text{response}] = \text{CumNorm}(\text{Intercept} + \text{Slope} * \text{Dose}),$
where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Effect
Independent variable = Dose
Slope parameter is not restricted

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 Specified
 intercept = -1.1397
 slope = 0.0415219

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)

	intercept	slope
intercept	1	-0.72
slope	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
intercept	-1.12659	0.191961	-1.50283	-0.750354
slope	0.0420993	0.00684265	0.0286879	0.0555106

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-57.5806	4			
Fitted model	-58.1177	2	1.07419	2	0.5844
Reduced model	-80.7614	1	46.3616	3	<.0001

AIC: 120.235
 Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1300	3.899	3.000	30.000	-0.488
4.4000	0.1733	5.198	5.000	30.000	-0.095
22.4000	0.4272	12.815	15.000	30.000	0.806
52.4000	0.8598	25.794	25.000	30.000	-0.417

Chi^2 = 1.07 d.f. = 2 P-value = 0.5852

Benchmark Dose Computation
 Specified effect = 0.05
 Risk Type = Extra risk
 Confidence level = 0.95

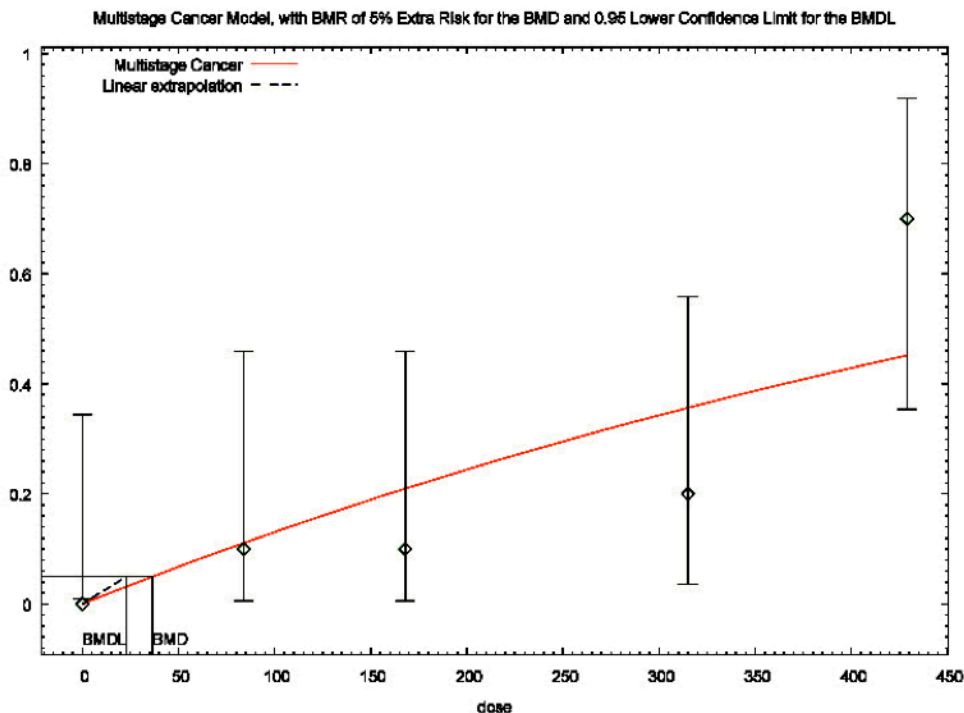
BMD = 4.41836
 BMDL = 3.45426
 BMDU = 5.74407

APPENDIX E. BENCHMARK DOSE ANALYSIS RESULTS FOR CANCER ENDPOINTS

This appendix provides the BMD modeling outputs for each HHA5. 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 \leq the absolute value of 2, and visual inspection of the dose-response curve prior to selection for use in the PHG calculation.

Dichloroacetic Acid

Figure E1. BMD modeling of hepatic adenomas or carcinomas in male B6C3F1 mice at 52 weeks from DeAngelo et al. (1999)



```

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/HAA/DeAngelo 1999/msc_DeAngelo1999_52 weeks_A_C_Opt.(d)
Gnuplot Plotting File: K:/HAA/DeAngelo 1999/msc_DeAngelo1999_52 weeks_A_C_Opt.plt
Thu Aug 10 11:03:02 2017
=====

```

BMDS_Model_Run

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose}^1)]$$

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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1
 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) = 0.00238956

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(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Beta(1)	0.00140296	0.000427812	0.000564469	0.00224146

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	-17.6143	5			
Fitted model	-19.895	1	4.56036	4	0.3354
Reduced model	-26.3454	1	17.4621	4	0.001571

AIC: 41.789

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0	10	0
84	0.1112	1.112	1	10	-0.112
168	0.21	2.1	1	10	-0.854
315	0.3572	3.572	2	10	-1.037

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
429	0.4522	4.522	7	10	1.574

Chi² = 4.30

d.f. = 4

P-value = 0.3673

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 36.5606

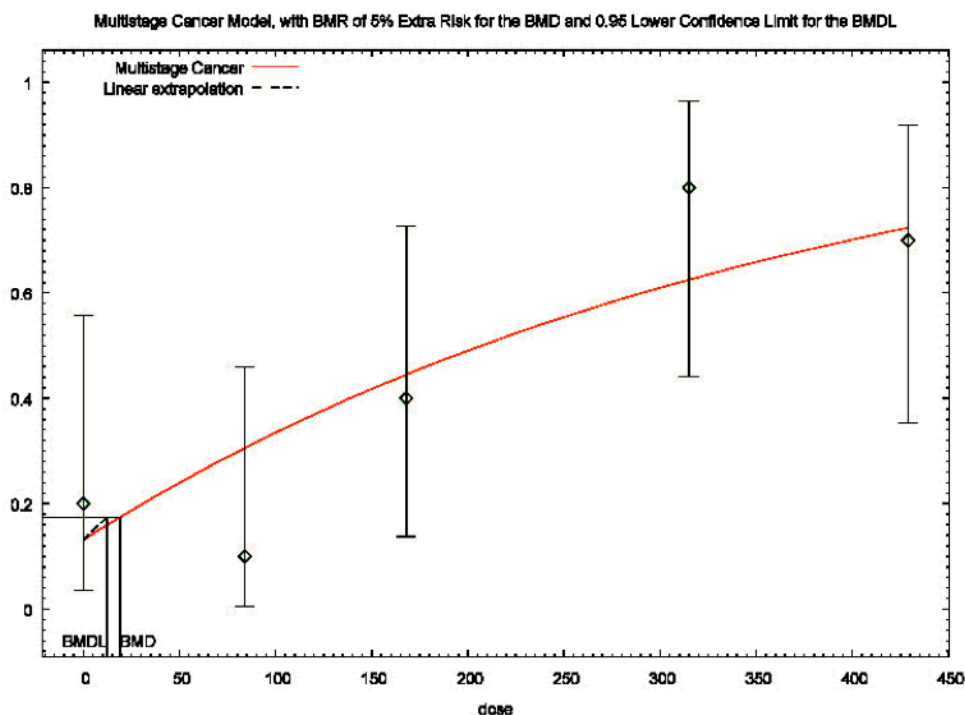
BMDL = 22.9711

BMDU = 63.9131

Taken together, (22.9711, 63.9131) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00217664

Figure E2. BMD modeling of hepatic adenomas or carcinomas in male B6C3F1 mice at 78 weeks in male B6C3F1 mice from DeAngelo et al. (1999)



12:53 08/10 2017

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)

Input Data File: K:/HAA/DeAngelo 1999/msc_DeAngelo1999_78 weeks_A_C_Opt.(d)

Gnuplot Plotting File: K:/HAA/DeAngelo 1999/msc_DeAngelo1999_78 weeks_A_C_Opt.plt

Thu Aug 10 12:53:20 2017

=====
BMDS_Model_Run
~~~~~

The form of the probability function is:

P[response] = background + (1-background)\*[1-EXP(-beta1\*dose^1)]

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 = 2

Total number of specified parameters = 0

Degree of polynomial = 1

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 = 0.0796993

Beta(1) = 0.00325047

Asymptotic Correlation Matrix of Parameter Estimates

Table with 3 columns: Parameter, Background, Beta(1). Rows: Background, Beta(1). Values: 1, -0.42, -0.42, 1.

Parameter Estimates

Table with 5 columns: Variable, Estimate, Std. Err., Lower Conf. Limit, Upper Conf. Limit. Rows: Background, Beta(1). Includes 95.0% Wald Confidence Interval.

Analysis of Deviance Table

Table with 6 columns: Model, Log(likelihood), # Param's, Deviance, Test d.f., P-value. Rows: Full model, Fitted model, Reduced model.

AIC: 60.54

Goodness of Fit

| Dose | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0.1308     | 1.308    | 2        | 10   | 0.649           |
| 84   | 0.3057     | 3.057    | 1        | 10   | -1.412          |
| 168  | 0.4453     | 4.453    | 4        | 10   | -0.288          |
| 315  | 0.6256     | 6.256    | 8        | 10   | 1.14            |
| 429  | 0.724      | 7.24     | 7        | 10   | -0.17           |

Chi^2 = 3.82  
d.f. = 3  
P-value = 0.2811

**Benchmark Dose Computation**

Specified effect = 0.05  
Risk Type = Extra risk  
Confidence level = 0.95  
BMD = 19.1843  
BMDL = 12.3157  
BMDU = 36.5473

Taken together, (12.3157, 36.5473) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00405986

**Multistage Weibull modeling of hepatic adenomas or carcinomas in male B6C3F1 mice at 100 weeks in male B6C3F1 mice from DeAngelo et al. (1999)**

=====

Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)  
Solutions are obtained using donlp2-intv, (c) by P. Spellucci  
Input Data File: DCA\_MSW\_wo26\_1.(d)  
Mon Apr 03 14:40:09 2017

=====

DeAngelo et al. (1999) omit 26 weeks, To estimated, BMD for Risk Type = Incidental Risk

~~~~~

The form of the probability function is:
 $P[\text{response}] = 1 - \text{EXP}\{-(t - t_0)^c * (\beta_0 + \beta_1 * \text{dose}^1 + \beta_2 * \text{dose}^2)\}$

The parameter betas are restricted to be positive

Dependent variable = CLASS
Independent variables = DOSE, TIME

Total number of observations = 275
Total number of records with missing values = 0
Total number of parameters in model = 5
Total number of specified parameters = 1
Degree of polynomial = 2

User specifies the following parameters:

t_0 = 0

Maximum number of iterations = 36

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

c = 3.66667

t_0 = 0 Specified

beta_0 = 2.01746e-008

beta_1 = 2.0767e-011

beta_2 = 1.94223e-012

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -t_0 -beta_1

have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	c	beta_0	beta_2
c	1	-1	-1
beta_0	-1	1	0.99
beta_2	-1	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	4.0305	0.81777	2.42771	5.63329
beta_0	3.81028E-09	1.44028E-08	-2.44187E-08	3.20392E-08
beta_1	0	NA		
beta_2	4.17609E-13	1.49147E-12	-2.50562E-12	3.34084E-12

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

	Log(likelihood)	# Param	AIC
Fitted Model	-130.76	4	269.524

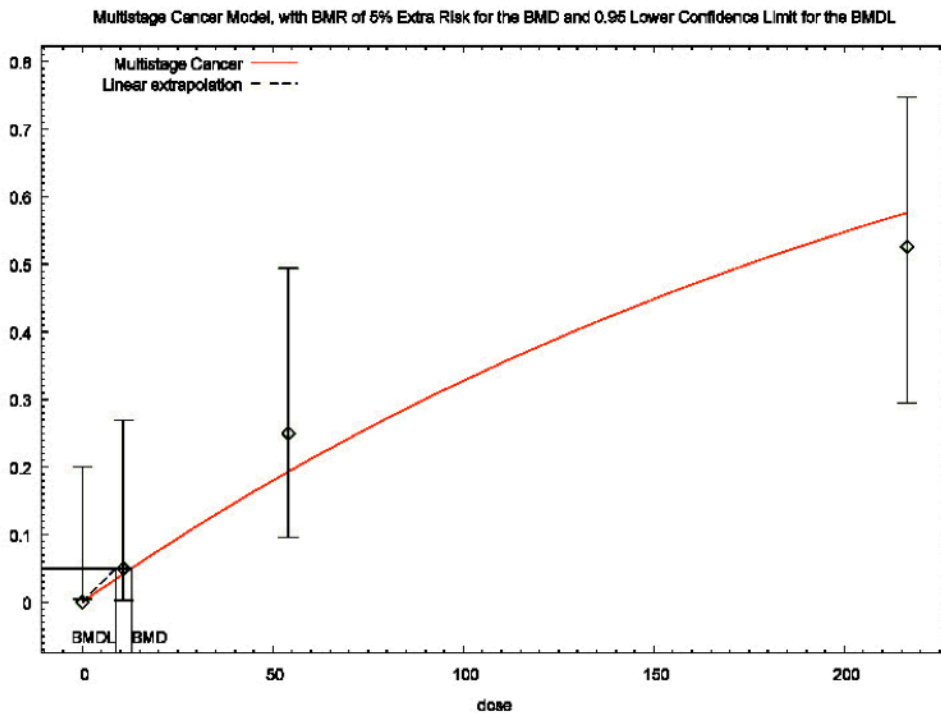
Data Summary

DOSE	CLASS					Total
	C	F	I	U		
0	50	0	20	0	70	
8	22	0	11	0	33	
84	29	0	16	0	45	
1.70E+02	20	0	35	0	55	
3.20E+02	10	0	31	0	41	

DOSE	CLASS				Total
	C	F	I	U	
4.30E+02	4	0	27	0	31

Benchmark Dose Computation
 Risk Response = Incidental
 Risk Type = Extra
 Specified effect = 0.05
 Confidence level = 0.95
 Time = 100
 BMD = 32.6696
 BMDL = 7.86286
 BMDU = 40.2138

Figure E3. BMD modeling of hepatic adenomas and carcinomas in male B6C3F1 mice at 52 weeks in male B6C3F1 mice from Bull et al. (2002)



11:14 11/14 2017

=====
 Multistage Model. (Version: 3.4; Date: 05/02/2014)
 Input Data File: K:/BMDS analysis/msc_DCA_Bull2002_m-mice_A+C_Opt.(d)
 Gnuplot Plotting File: K:/BMDS analysis/msc_DCA_Bull2002_m-mice_A+C_Opt.plt
 Tue Nov 14 11:14:32 2017
 =====

BMDS_Model_Run

~~~~~  
 The form of the probability function is:  
 $P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(\dots)]$

-beta1\*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect

Independent variable = Dose

Total number of observations = 4

Total number of records with missing values = 0

Total number of parameters in model = 2

Total number of specified parameters = 0

Degree of polynomial = 1

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 = 0.0339387

Beta(1) = 0.00336915

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(1) | 1       |

Parameter Estimates

95.0% Wald Confidence Interval

| Variable   | Estimate   | Std. Err.  | 95.0% Wald Confidence Interval |                   |
|------------|------------|------------|--------------------------------|-------------------|
|            |            |            | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0          | NA         |                                |                   |
| Beta(1)    | 0.00396994 | 0.00101206 | 0.00198635                     | 0.00595354        |

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    | -28.361         | 4         |          |           |         |
| Fitted model  | -28.666         | 1         | 0.61171  | 3         | 0.8937  |
| Reduced model | -39.808         | 1         | 22.894   | 3         | <.0001  |

AIC: 59.3327

Goodness of Fit

| Dose  | Est._Prob. | Expected | Observed | Size | Scaled Residual |
|-------|------------|----------|----------|------|-----------------|
| 0     | 0          | 0        | 0        | 20   | 0               |
| 10.8  | 0.042      | 0.839    | 1        | 20   | 0.179           |
| 54.1  | 0.1933     | 3.866    | 5        | 20   | 0.642           |
| 216.5 | 0.5766     | 10.956   | 10       | 19   | -0.444          |

Chi<sup>2</sup> = 0.64

d.f. = 3

P-value = 0.8868

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 12.9204

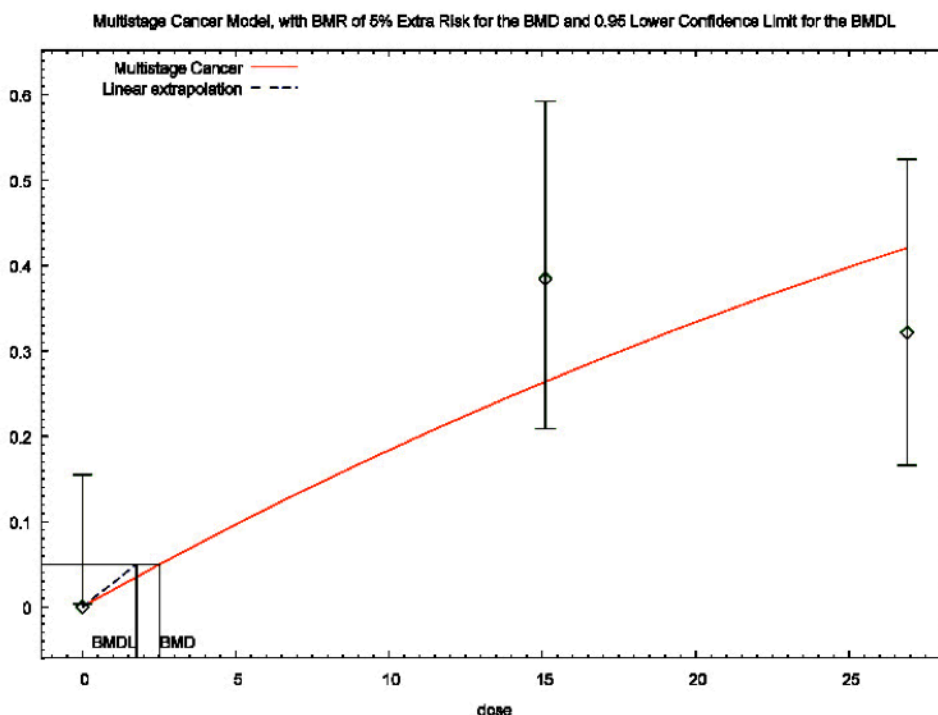
BMDL = 8.71182

BMDU = 21.0802

Taken together, (8.71182, 21.0802) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00573933

**Figure E4. BMD modeling of hepatic adenomas, carcinomas or hepatoblastomas in female B6C3F1 mice (averaged doses) from Wood et al. (2015)**



-----  
 Multistage Model. (Version: 3.4; Date: 05/02/2014)



Input Data File: K:/BMDS analysis/msc\_DCA\_Wood2015\_f-mice\_A+C+H\_ArmDoll\_Opt.(d)  
 Gnuplot Plotting File: K:/BMDS analysis/msc\_DCA\_Wood2015\_f-  
 mice\_A+C+H\_ArmDoll\_Opt.plt  
 Nov 27 10:42:47 2017

=====  
 BMDS\_Model\_Run  
 ~~~~~

The form of the probability function is:
 $P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$

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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

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 = 0.0749769
 Beta(1) = 0.0152253

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(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Beta(1)	0.0203336	0.00470203	0.0111178	0.0295495

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	-34.906	3			
Fitted model	-36.389	1	2.9659	2	0.227
Reduced model	-44.124	1	18.4362	2	<.0001

AIC: 74.7771

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0	27	0
15.1	0.2644	6.874	10	26	1.39
26.9	0.4213	11.796	9	28	-1.07

Chi² = 3.08

d.f. = 2

P-value = 0.2146

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.52258

BMDL = 1.76269

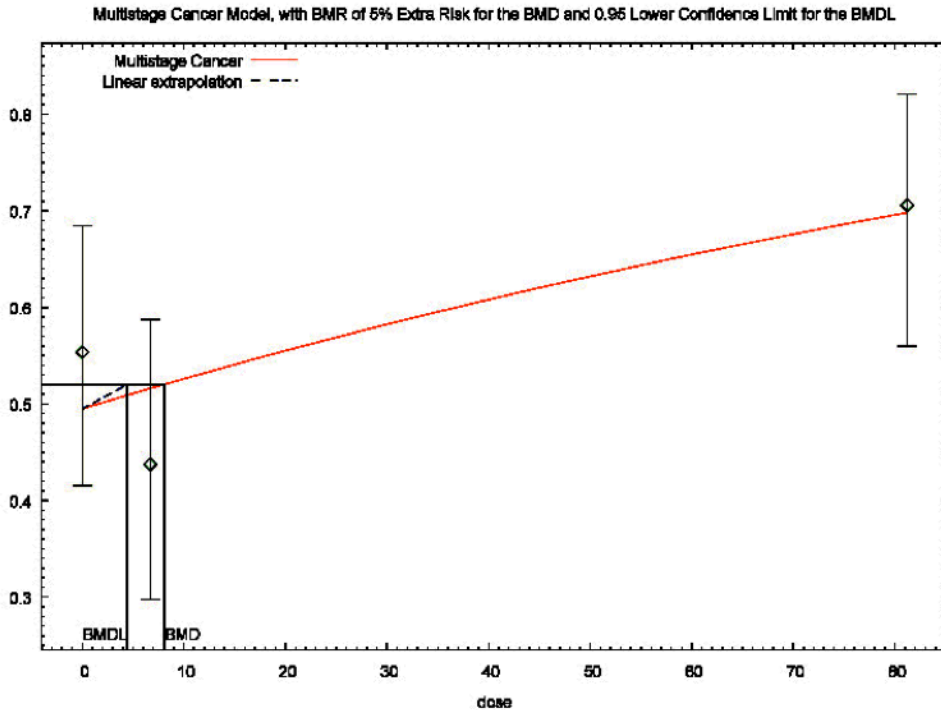
BMDU = 3.79006

Taken together, (1.76269, 3.79006) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.0283658

Trichloroacetic Acid

Figure E5. BMD modeling of Study 3 hepatocellular adenoma and/ or carcinoma in male B6C3F1 mice from DeAngelo et al. (2008)



```

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/BMDS analysis/msc_TCA_DeAngelo2008_mice_liver-
ad+carc_checkPHG_Opt.(d)
Gnuplot Plotting File: K:/BMDS analysis/msc_TCA_DeAngelo2008_mice_liver-
ad+carc_checkPHG_Opt.plt
Thu Dec 28 13:42:14 2017
=====

```

BMDS_Model_Run

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

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 = 0.490682
 Beta(1) = 0.00661627

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.48
Beta(1)	-0.48	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.494639	0.0516139	0.393478	0.595801
Beta(1)	0.00632717	0.00295261	0.00054016	0.0121142

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-102.285	3			
Fitted model	-103.27	2	1.96925	1	0.1605
Reduced model	-106.011	1	7.4518	2	0.02409

AIC: 210.539

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.4946	27.7	31	56	0.882
6.7	0.5156	24.75	21	48	-1.083
81.2	0.6977	35.581	36	51	0.128

Chi^2 = 1.97
 d.f. = 1
 P-value = 0.1608

Benchmark Dose Computation

Specified effect = 0.05
 Risk Type = Extra risk

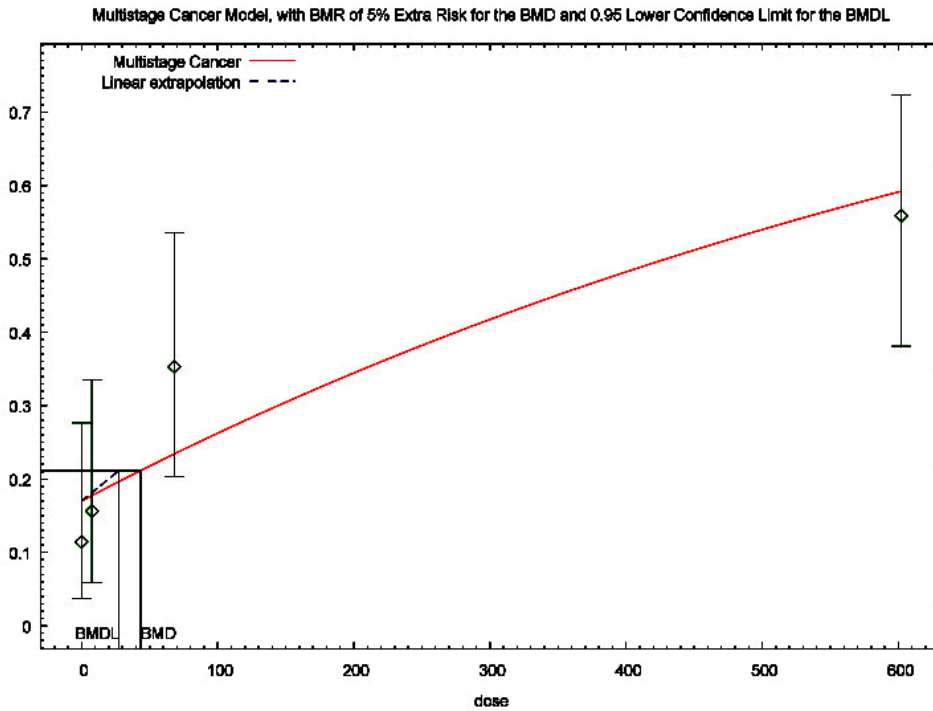
Confidence level = 0.95

BMD = 8.10682
BMDL = 4.43166
BMDU = 28.8358

Taken together, (4.43166, 28.8358) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.0112825

Figure E6. BMD modeling of Study 1 hepatocellular adenoma and/or carcinoma in male B6C3F1 mice from DeAngelo et al. (2008)



=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/HAA/DeAngelo 2008/msc_DeAngelo2008_60_weeks_A_C_Opt.(d)
Gnuplot Plotting File: K:/HAA/DeAngelo 2008/msc_DeAngelo2008_60_weeks_A_C_Opt.plt
Mon Aug 14 09:59:55 2017
=====

BMDS_Model_Run

~~~~~  
The form of the probability function is:  
$$P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive  
Dependent variable = Effect  
Independent variable = Dose

Total number of observations = 4  
Total number of records with missing values = 0

Total number of parameters in model = 2  
 Total number of specified parameters = 0  
 Degree of polynomial = 1

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 = 0.189705  
 Beta(1) = 0.00103763

Asymptotic Correlation Matrix of Parameter Estimates

|            |            |         |
|------------|------------|---------|
|            | Background | Beta(1) |
| Background | 1          | -0.42   |
| Beta(1)    | -0.42      | 1       |

Parameter Estimates

| Variable   | Estimate   | Std. Err.   | 95.0% Wald Confidence Interval |                   |
|------------|------------|-------------|--------------------------------|-------------------|
|            |            |             | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.170136   | 0.0437678   | 0.0843528                      | 0.25592           |
| Beta(1)    | 0.00117888 | 0.000370512 | 0.000452693                    | 0.00190507        |

Analysis of Deviance Table

| Model         | Log(Likelihood) | # Param's | Deviance | Test d.f. | P-value   |
|---------------|-----------------|-----------|----------|-----------|-----------|
| Full model    | -71.7128        | 4         |          |           |           |
| Fitted model  | -73.4831        | 2         | 3.54069  | 2         | 0.1703    |
| Reduced model | -82.0386        | 1         | 20.6517  | 3         | 0.0001243 |

AIC: 150.966

Goodness of Fit

| Dose     | Est. Prob. | Expected | Observed | Size   | Scaled Residual |
|----------|------------|----------|----------|--------|-----------------|
| 0.0000   | 0.1701     | 5.955    | 4.000    | 35.000 | -0.879          |
| 7.6500   | 0.1776     | 5.683    | 5.000    | 32.000 | -0.316          |
| 68.1600  | 0.2342     | 7.963    | 12.000   | 34.000 | 1.635           |
| 602.1400 | 0.5919     | 20.126   | 19.000   | 34.000 | -0.393          |

Chi<sup>2</sup> = 3.70

d.f. = 2

P-value = 0.1573

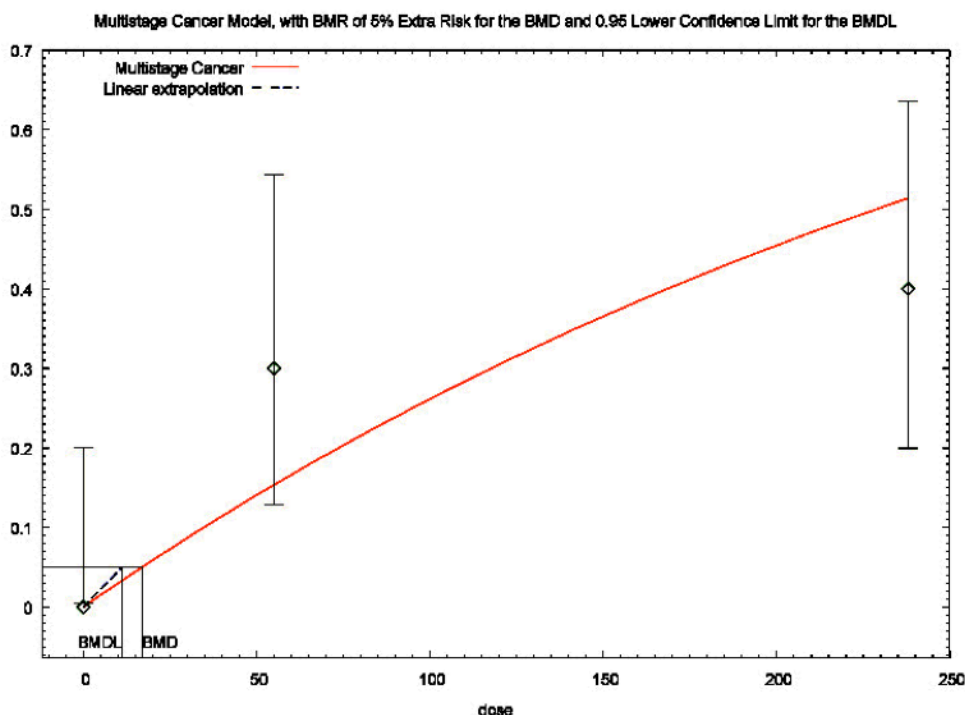
Benchmark Dose Computation

Specified effect = 0.05  
 Risk Type = Extra risk  
 Confidence level = 0.95  
 BMD = 43.5101  
 BMDL = 27.5868  
 BMDU = 80.9731

Taken together, (27.5868, 80.9731) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00181246

**Figure E7. BMD modeling of hepatocellular adenoma and/or carcinoma in male B6C3F1 mice from Bull et al. (2002)**



14:53 12/28 2017

=====  
Multistage Model. (Version: 3.4; Date: 05/02/2014)  
Input Data File: K:/BMDS analysis/msc\_TCA\_Bull2002\_Liver\_Ad+Carc\_RCHAB dosing\_Opt.(d)  
Gnuplot Plotting File: K:/BMDS analysis/msc\_TCA\_Bull2002\_Liver\_Ad+Carc\_RCHAB dosing\_Opt.plt  
Thu Dec 28 14:53:59 2017  
=====

BMDS\_Model\_Run

~~~~~  
The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

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 = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1

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 = 0.105567
 Beta(1) = 0.00181845

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(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Beta(1)	0.00302697	0.000819328	0.00142111	0.00463282

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	-25.6775	3			
Fitted model	-27.5583	1	3.76151	2	0.1525
Reduced model	-32.5964	1	13.8377	2	0.000989

AIC: 57.1165

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0	20	0
55	0.1534	3.067	6	20	1.82
238	0.5135	10.269	8	20	-1.015

Chi^2 = 4.34

d.f. = 2

P-value = 0.1140

Benchmark Dose Computation
 Specified effect = 0.05

Risk Type = Extra risk

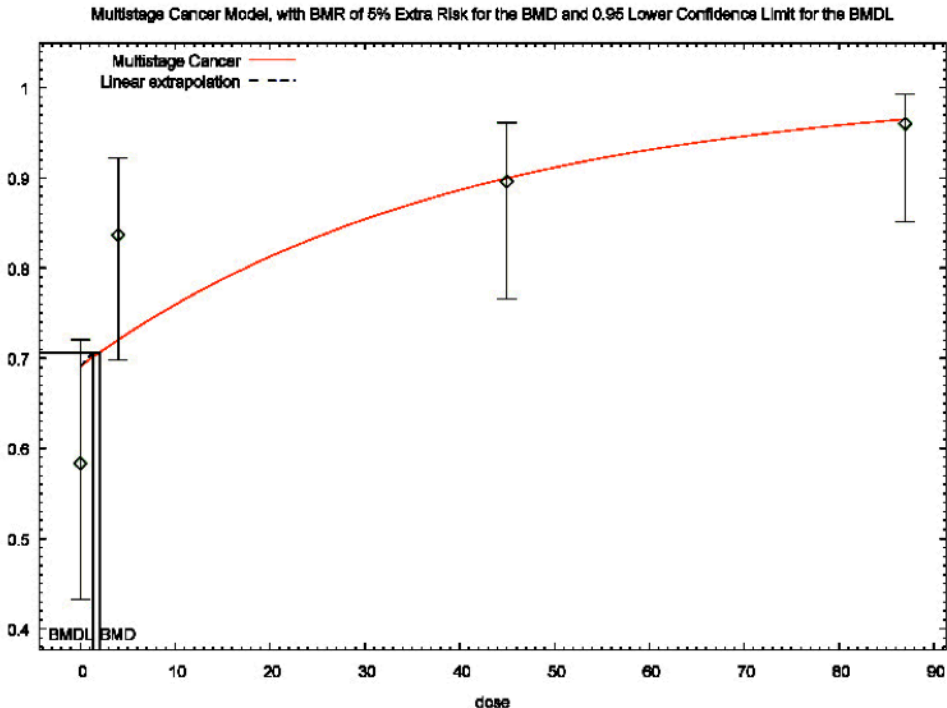
Confidence level = 0.95
BMD = 16.9454
BMDL = 11.1775
BMDU = 35.9817

Taken together, (11.1775, 35.9817) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00447325

Dibromoacetic Acid

Figure E8. BMD modeling of hepatocellular adenoma, carcinoma and/or hepatoblastoma in male B6C3F1 mice from NTP (2007)



13:59 08/11 2017

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Male_Liver_A_C_H_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Male_Liver_A_C_H_Opt.plt
Fri Aug 11 13:59:42 2017
=====
BMDS_Model_Run
~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect  
Independent variable = Dose

Total number of observations = 4  
Total number of records with missing values = 0  
Total number of parameters in model = 2  
Total number of specified parameters = 0  
Degree of polynomial = 1

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 = 0.722759  
Beta(1) = 0.022331

Asymptotic Correlation Matrix of Parameter Estimates

|            |            |         |
|------------|------------|---------|
|            | Background | Beta(1) |
| Background | 1          | -0.45   |
| Beta(1)    | -0.45      | 1       |

Parameter Estimates

| Variable   | Estimate  | Std. Err.  | 95.0% Wald Confidence Interval |                   |
|------------|-----------|------------|--------------------------------|-------------------|
|            |           |            | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.690737  | 0.0502308  | 0.592286                       | 0.789187          |
| Beta(1)    | 0.0250304 | 0.00709902 | 0.0111166                      | 0.0389442         |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -78.8445        | 4         |          |           |         |
| Fitted model  | -81.9353        | 2         | 6.18152  | 2         | 0.04547 |
| Reduced model | -91.7699        | 1         | 25.8508  | 3         | <.0001  |

AIC: 167.871

Goodness of Fit

| Dose | Est._Prob | Expected | Observed | Size | Scaled Residual |
|------|-----------|----------|----------|------|-----------------|
| 0    | 0.6907    | 33.155   | 28       | 48   | -1.61           |

| Dose | Est._Prob | Expected | Observed | Size | Scaled Residual |
|------|-----------|----------|----------|------|-----------------|
| 4    | 0.7202    | 35.29    | 41       | 49   | 1.817           |
| 45   | 0.8997    | 43.187   | 43       | 48   | -0.09           |
| 87   | 0.965     | 48.248   | 48       | 50   | -0.191          |

Chi<sup>2</sup> = 5.94  
d.f. = 2  
P-value = 0.0513

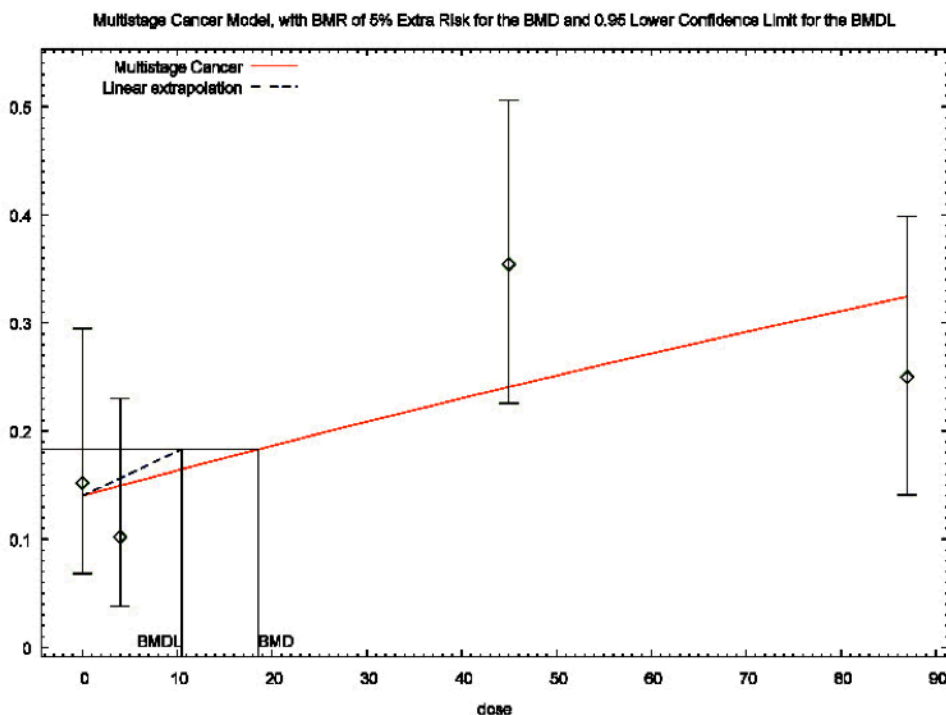
Benchmark Dose Computation  
Specified effect = 0.05  
Risk Type = Extra risk

Confidence level = 0.95  
BMD = 2.04924  
BMDL = 1.34485  
BMDU = 3.54636

Taken together, (1.34485, 3.54636) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.0371788

**Figure E9. BMD modeling of alveolar/bronchiolar adenoma in male B6C3F1 mice from NTP (2007)**



14:01 08/11 2017

-----  
Multistage Model. (Version: 3.4; Date: 05/02/2014)

Input Data File: K:/HAA/NTP 2007/Mice/msc\_NTP 2007\_Male\_Lung\_A\_Opt.(d)  
 Gnuplot Plotting File: K:/HAA/NTP 2007/Mice/msc\_NTP 2007\_Male\_Lung\_A\_Opt.plt  
 Fri Aug 11 14:01:24 2017

=====

BMDS\_Model\_Run

~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect

Independent variable = Dose

Total number of observations = 4

Total number of records with missing values = 0

Total number of parameters in model = 2

Total number of specified parameters = 0

Degree of polynomial = 1

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 = 0.1588

Beta(1) = 0.00224927

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.61
Beta(1)	-0.61	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.140136	0.0383961	0.0648809	0.215391
Beta(1)	0.00276677	0.00123662	0.000343035	0.0051905

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-93.9564	4			
Fitted model	-96.6398	2	5.36683	2	0.06833
Reduced model	-99.3325	1	10.7522	3	0.01314

AIC: 197.28

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.1401	6.446	7	46	0.235
4	0.1496	7.33	5	49	-0.933
45	0.2408	11.558	17	48	1.837
87	0.3241	15.556	12	48	-1.097

Chi^2 = 5.50

d.f. = 2

P-value = 0.0638

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 18.5391

BMDL = 10.4764

BMDU = 64.8264

Taken together, (10.4764, 64.8264) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00477261

BMD modeling of multisite tumors: hepatocellular adenoma, carcinoma, and/or hepatoblastoma and alveolar/bronchiolar adenoma in male B6C3F1 mice from NTP (2007)

**** Start of combined BMD and BMDL Calculations. ****

Combined Log-Likelihood -178.57511608625254

Combined Log-likelihood Constant 158.1398155487546

Obs	Poly	nRest	nParms
4	1	1	2
4	1	1	2

In Multistage_ComboBMD, Before zeroin() Line 2423, printing variables xa=0.553885, xb=0.95935, cxmax=87

Printing values in cp:

cp[1]=1.32454

cp[2]=0.0277972

Computed Combined Log-Likelihood -6013.1293400927416

Combined BMD 1.845269759

Combined Risk at BMD -2.22

In BMDL_combofunc, Tumor 1 data

DOSE	Inc	N
0	28	48

DOSE	Inc	N
4	41	49
45	43	48
87	48	50

In BMDL_combofunc, Tumor 2 data

DOSE	Inc	N
0	7	46
4	5	49
45	17	48
87	12	48

In BMDL_combofunc, aParmList[j+1].pdParms[j+1](MLEs)

Tumor 1=> 0.69074 0.02503
Tumor 2=> 0.14014 0.0027668

In BMDL_combofunc, pdParms Values (MLEs, k=3, nParms=4)

Tumor 1	Tumor 2
0.14014	0.69074
0.00277	0.02503

In BMDL_combofunc, Tumor Starting Values

Tumor 1 => 0.14014
Tumor 2 => 0.69074

Maximum Dose = 87

Scale = 87

Combined Loglikelihood -178.57511608625254

Target -179.9278878132852

Values BEFORE call 1 to getclmt_()BMR= 0.05 target= -179.93

bmdl= 0 optite=-5

Tumor 1		Tumor 2	
Scaled	Unscaled	Scaled	Unscaled
0.15098	0.14014	1.1736	0.69074
0.24071	0.0027668	2.1776	0.02503

Values AFTER call 1 to getclmt_()BMR= 0.05 target= -179.93

bmdl= 0.014363 optite=0

Tumor 1	Tumor 2
0.15098	1.1736
0.24071	2.1776

***** pdParms2 Values *****

Tumor 1	Tumor 2
0.14501	1.074

Tumor 1 Tumor 2
0.26539 3.3059

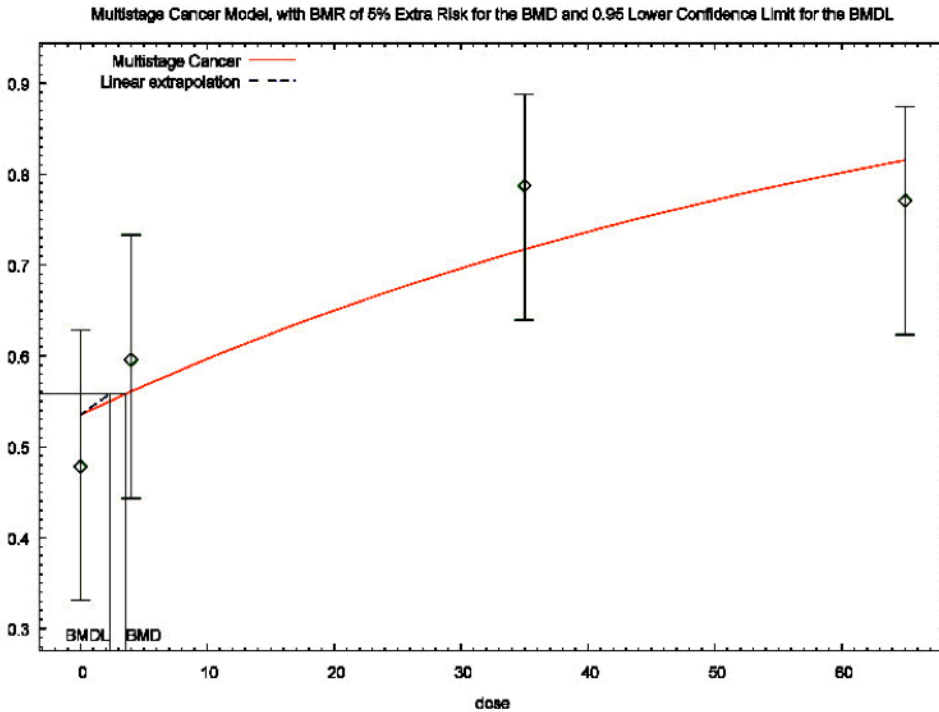
Combined Log-Likelihood at BMDL (getcl) 0
Combined Log-Likelihood at BMDL (combomaxlike) -1.#IND

Combined BMDL 1.249568
Combined Risk at BMDL 1

Tumor 1
Observation = 4
pdYp -> {28, 41, 43, 48, }
pdYn -> {20, 8, 5, 2, }
pdXi -> {0, 4, 45, 87, }

Tumor 2
Observation = 4
pdYp -> {7, 5, 17, 12, }
pdYn -> {39, 44, 31, 36, }
pdXi -> {0, 4, 45, 87, }

Figure E10. BMD modeling of hepatocellular adenoma, carcinoma and/or hepatoblastoma in female B6C3F1 mice from NTP (2007)



=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)

Input Data File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Female_Liver_A_C_Opt.(d)
 Gnuplot Plotting File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Female_Liver_A_C_Opt.plt
 Fri Aug 11 14:09:36 2017

=====
 BMDS_Model_Run
 ~~~~~

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect  
 Independent variable = Dose

Total number of observations = 4  
 Total number of records with missing values = 0  
 Total number of parameters in model = 2  
 Total number of specified parameters = 0  
 Degree of polynomial = 1

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 = 0.559168  
 Beta(1) = 0.0125076

Asymptotic Correlation Matrix of Parameter Estimates

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.57   |
| Beta(1)    | -0.57      | 1       |

Parameter Estimates

| Variable   | Estimate  | Std. Err.  | 95.0% Wald Confidence Interval |                   |
|------------|-----------|------------|--------------------------------|-------------------|
|            |           |            | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.53489   | 0.0550901  | 0.426915                       | 0.642864          |
| Beta(1)    | 0.0142436 | 0.00463741 | 0.00515447                     | 0.0233328         |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value  |
|---------------|-----------------|-----------|----------|-----------|----------|
| Full model    | -113.716        | 4         |          |           |          |
| Fitted model  | -115.031        | 2         | 2.62991  | 2         | 0.2685   |
| Reduced model | -120.568        | 1         | 13.7031  | 3         | 0.003338 |



AIC: 234.062

Goodness of Fit

| Dose | Est. Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0.5349     | 24.605   | 22       | 46   | -0.77           |
| 4    | 0.5606     | 26.35    | 28       | 47   | 0.485           |
| 35   | 0.7175     | 33.722   | 37       | 47   | 1.062           |
| 65   | 0.8157     | 39.155   | 37       | 48   | -0.802          |

Chi<sup>2</sup> = 2.60

d.f. = 2

P-value = 0.2726

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 3.60114

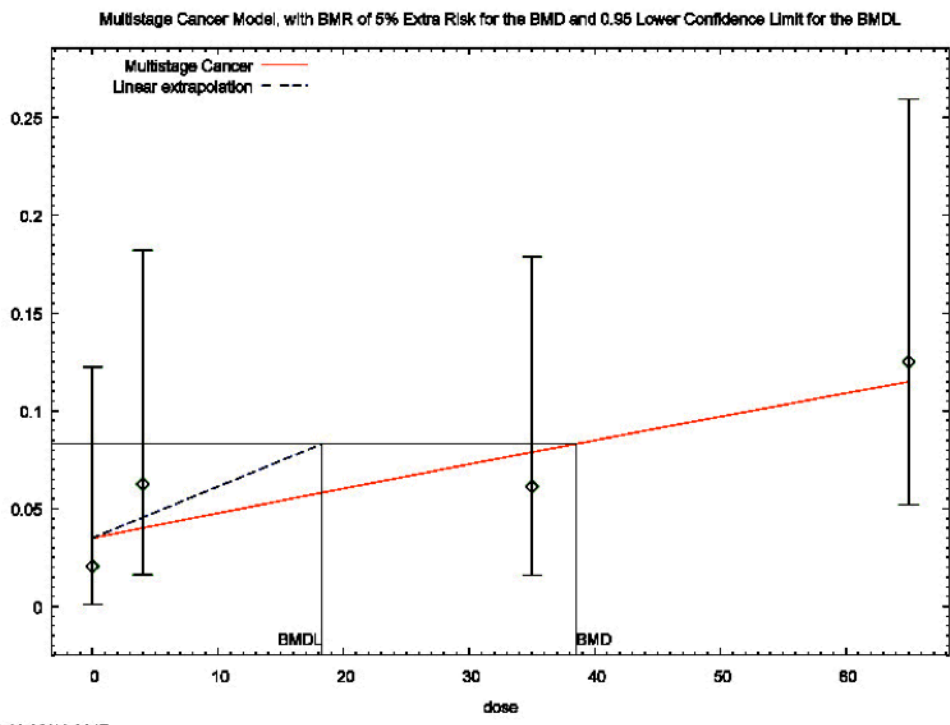
BMDL = 2.30113

BMDU = 7.38136

Taken together, (2.30113, 7.38136) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.0217284

**Figure E11. BMD modeling of alveolar/bronchiolar adenoma in female B6C3F1 mice from NTP (2007)**



14:10 08/11 2017

```

=====
Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Female_Lung_A_Opt.(d)
Gnuplot Plotting File: K:/HAA/NTP 2007/Mice/msc_NTP 2007_Female_Lung_A_Opt.plt
Fri Aug 11 14:10:53 2017
=====
BMDS_Model_Run
~~~~~

```

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = Effect  
 Independent variable = Dose

Total number of observations = 4  
 Total number of records with missing values = 0  
 Total number of parameters in model = 2  
 Total number of specified parameters = 0  
 Degree of polynomial = 1

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 = 0.0339155  
 Beta(1) = 0.0013832

Asymptotic Correlation Matrix of Parameter Estimates

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.55   |
| Beta(1)    | -0.55      | 1       |

Parameter Estimates

| Variable   | Estimate   | Std. Err.   | 95.0% Wald Confidence Interval |                   |
|------------|------------|-------------|--------------------------------|-------------------|
|            |            |             | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.0348601  | 0.0197432   | -0.00383587                    | 0.073556          |
| Beta(1)    | 0.00133167 | 0.000795975 | -0.000228409                   | 0.00289175        |

Analysis of Deviance Table

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -45.4744        | 4         |          |           |         |
| Fitted model  | 46.0603         | 2         | 1.17186  | 2         | 0.5566  |
| Reduced model | -47.6922        | 1         | 4.43562  | 3         | 0.2181  |

AIC: 96.1206

Goodness of Fit

| Dose | Est. Prob. | Expected | Observed | Size | Scaled Residual |
|------|------------|----------|----------|------|-----------------|
| 0    | 0.0349     | 1.708    | 1        | 49   | -0.552          |
| 4    | 0.04       | 1.919    | 3        | 48   | 0.796           |
| 35   | 0.0788     | 3.862    | 3        | 49   | -0.457          |
| 65   | 0.1149     | 5.515    | 6        | 48   | 0.22            |

Chi<sup>2</sup> = 1.19  
 d.f. = 2  
 P-value = 0.5502

Benchmark Dose Computation

Specified effect = 0.05  
 Risk Type = Extra risk  
 Confidence level = 0.95  
 BMD = 38.5179  
 BMDL = 18.301  
 BMDU = 445.998

Taken together, (18.301 , 445.998) is a 90 % two-sided confidence

interval for the BMD

Cancer Slope Factor = 0.00273209

**BMD modeling of multisite tumors: hepatocellular adenoma, carcinoma, and/or hepatoblastoma and alveolar/bronchiolar adenoma in female B6C3F1 mice from NTP (2007)**

\*\*\*\* Start of combined BMD and BMDL Calculations. \*\*\*\*

Combined Log-Likelihood -161.09136310212719

Combined Log-likelihood Constant 145.27271187162017

| Obs | Poly | nRest | nParms |
|-----|------|-------|--------|
| 4   | 1    | 1     | 2      |
| 4   | 1    | 1     | 2      |

In Multistage\_ComboBMD, Before zeroin() Line 2423, printing variables  
xa=1.17865, xb=1.36098, cxmax=65

Printing values in cp:

cp[1]=0.800963

cp[2]=0.0155753

Computed Combined Log-Likelihood -8398.4179775259163

Combined BMD 3.293247736

Combined Risk at BMD -1.14

In BMDL\_combofunc, Tumor 1 data

| DOSE | Inc | N  |
|------|-----|----|
| 0    | 22  | 46 |
| 4    | 28  | 47 |
| 35   | 37  | 47 |
| 65   | 37  | 48 |

In BMDL\_combofunc, Tumor 2 data

| DOSE | Inc | N  |
|------|-----|----|
| 0    | 1   | 49 |
| 4    | 3   | 48 |
| 35   | 3   | 49 |
| 65   | 6   | 48 |

In BMDL\_combofunc, aParmList[j+1].pdParms[j+1](MLEs)

Tumor 1=> 0.53489 0.014244

Tumor 2=> 0.03486 0.0013317

In BMDL\_combofunc, pdParms Values (MLEs, k=3, nParms=4)

|         |          |
|---------|----------|
| Tumor 1 | Tumor 2  |
| 0.03486 | 0.53489  |
| 0.00133 | 0.014244 |

In BMDL\_combofunc, Tumor Starting Values

Tumor 1 => 0.03486  
Tumor 2 => 0.53489

Maximum Dose = 65

Scale = 65

Combined Loglikelihood -161.09136310212719

Target -162.44413482915985

Values BEFORE call 1 to getclmt\_()BMR= 0.05 target= -162.44  
bmdl= 0 optite=-5

| Tumor 1 |           | Tumor 2 |          |
|---------|-----------|---------|----------|
| Scaled  | Unscaled  | Scaled  | Unscaled |
| 0.03548 | 0.03486   | 0.76548 | 0.53489  |
| 0.08656 | 0.0013317 | 0.92584 | 0.014244 |

Values AFTER call 1 to getclmt\_()BMR= 0.05 target= -162.44  
bmdl= 0.033261 optite=0

|         |         |
|---------|---------|
| Tumor 1 | Tumor 2 |
| 0.03548 | 0.76548 |
| 0.08656 | 0.92584 |

\*\*\*\*\* pdParms2 Values \*\*\*\*\*

|         |         |
|---------|---------|
| Tumor 1 | Tumor 2 |
| 0.03274 | 0.67173 |
| 0.10018 | 1.442   |

Combined Log-Likelihood at BMDL (getcl) 0

Combined Log-Likelihood at BMDL (combomaxlike) -1.#IND

Combined BMDL 2.161933

Combined Risk at BMDL 1

Tumor 1

Observation = 4

pdYp -> {22, 28, 37, 37, }

pdYn -> {24, 19, 10, 11, }

pdXi -> {0, 4, 35, 65, }

Tumor 2

Observation = 4

pdYp -> {1, 3, 3, 6, }

pdYn -> {48, 45, 46, 42, }  
pdXi -> {0, 4, 35, 65, }

## **APPENDIX F. AN EVALUATION OF CONFOUNDING IN EPIDEMIOLOGIC STUDIES OF DISINFECTION BYPRODUCT EXPOSURE AND ADVERSE REPRODUCTIVE OUTCOMES**

### **1. Background and Objectives**

In order for a factor to cause important confounding the factor must be associated with both the exposure of interest and outcome of interest, and in general the magnitude of these associations must be fairly strong (Axelson, 1978; Schlesselman, 1978). A relatively large number of the studies OEHHA reviewed presented data showing that factors like smoking, alcohol use, or body mass index were associated with adverse birth outcomes, and that in some studies the magnitude of these associations were strong (e.g. relative risk estimates >2.0). However, many fewer of the studies reviewed presented data on whether these factors were also associated with disinfection byproduct (DBP) exposure, and if they were, the magnitude of these associations.

To evaluate the likelihood that confounding may have caused some of the associations identified in the DBP-adverse reproductive outcome studies reviewed, OEHHA selected two factors of concern (alcohol and smoking) and: 1) evaluated whether these might be associated with DBP exposure, and if they were, 2) evaluated the potential magnitude of the confounding they might cause. Alcohol use and smoking were selected because both can be relatively prevalent in pregnancy (Substance Abuse and Mental Health Services Administration, 2014; Drake et al., 2018), both are established causes of adverse reproductive outcomes, and both were commonly referred to as potential causes of confounding by the authors of the DBP-reproductive outcome studies OEHHA reviewed.

### **2. Assessing Possible Associations between Smoking or Alcohol and DBP Exposure**

#### ***Methods***

OEHHA assessed whether or not smoking or alcohol use might be associated with DBP exposure by searching for epidemiologic literature on these associations from the following three sources:

- a. All articles in OEHHA's review of the epidemiologic data on DBPs and adverse reproductive outcomes,
- b. All articles in OEHHA's review of the epidemiologic data on DBPs and cancer,
- c. A separate literature search on any other information on whether smoking or alcohol use might be associated with DBP exposure. This separate literature search was done in PubMed using the following search string:

(disinfection byproducts OR haloacetic acid OR trihalomethane) AND (drinking water OR tap water OR showering OR bathing OR swimming) AND (smoking OR smoker OR cigarettes OR alcohol).

Data on the numbers and percentages of smokers and non-smokers, or alcohol users and non-users, in categories of DBP exposure, were abstracted when available. Because there were only a small number of studies on this topic, and in order to be more inclusive than exclusive, studies using

a broad range of methods to classify DBP exposure were included. These methods included metrics based on residential total (TTHM) or individual trihalomethane (THM) water concentrations, urinary trichloroacetic acid, mean years exposed to chlorinated water, or others. For the same reason, no limits were put on study population characteristics like age or sex, and studies in pregnant women, non-pregnant women, and men were included. Only a few of the identified studies provided statistical tests of association (e.g., odds ratios) or chance (i.e., p-values or confidence intervals). As such, odds ratios were calculated using 2 × 2 table data when available. When confidence intervals were not provided for odds ratios, they were calculated using the online calculator provided by hutchon.net (<http://www.hutchon.net/confidor.htm>) (Bland and Altman, 2000).

## **Results and Discussion**

A summary of the literature search is shown in Figure F1. Summaries of the results of the studies OEHHA identified in the literature search are provided in Table F1. With regards to smoking, the results of the studies identified are mixed, with some studies suggesting that smoking may be associated with higher DBP exposure and others suggesting there is either no association or that smoking is associated with lower DBP exposure. In those studies suggesting a positive association between smoking and DBP exposure, most (although not all) reported effect sizes that are relatively small and not statistically significant.

The inconsistency of findings in the studies OEHHA identified suggests that that links between smoking and DBP exposure vary from study to study and could depend on where the study was done, what type of participants were involved in the study (e.g., males vs. females, pregnant vs. not pregnant), or how information on smoking was ascertained. It's also possible at least some of this variation is due to chance. Regardless, given the inconsistency of these findings, OEHHA cannot make any broad generalizations on whether smoking is or is not associated with higher DBP exposures. Importantly though, it should be noted that some studies did identify associations between smoking and increased DBP exposures. This highlights the need to consider the potential impact of confounding by smoking in any study of DBPs and adverse reproductive outcomes that hadn't thoroughly evaluated or controlled for this potential confounder.

Fewer studies reported information on the association between alcohol use and DBP exposure. Like smoking, the results of these studies are also mixed. Based on these mixed results, OEHHA's conclusions regarding alcohol are similar to those for smoking: broad generalizations cannot be made with regards to this association, and the potential for confounding by alcohol should be considered when evaluating studies of DBP exposure and adverse reproductive outcomes, especially in studies of outcomes known to be associated with alcohol.

### **3. Evaluating the Potential Magnitude of the Confounding**

#### **Methods**

As mentioned above, the relationship between smoking or alcohol and DBP exposure is likely to vary from study to study. As such, the potential for these factors to cause important confounding may also vary from study to study. In some studies, OEHHA found that smoking or alcohol use were unrelated or only very weakly related to DBP exposure. In these studies smoking or alcohol use are



unlikely to cause major confounding (Axelson, 1978; Schlesselman, 1978). In other studies, at least some evidence of an inverse association between smoking and DBP exposure was identified. In these studies, confounding by smoking is most likely to bias relative risk estimates downwards. OEHHA also identified a few studies where there was evidence that smoking or higher alcohol use was at least moderately associated with increased DBP exposure. In these studies, smoking would most likely increase relative risk estimates for reproductive outcomes related to smoking (e.g., cause a false association between increasing DBP exposure and an adverse reproductive outcome). Data from these later studies were used to estimate the magnitude of the effect smoking might have as a confounder.

This evaluation was done using the methods presented by Axelson (1978). Here, the potential impact that a factor may have as a confounder can be estimated by using information on the magnitude of the association between the main outcome variable of interest (an adverse reproductive outcome) and the confounder, and information on any differences in the prevalence of the confounder between people with higher and lower levels of the main exposure of interest (DBP exposure). For this evaluation, small for gestational age (SGA) was chosen as the main outcome variable since a number of studies reported possible associations between this outcome and DBP exposure. OEHHA focused on smoking because in the evaluations described above, much more information was available on smoking than on alcohol consumption.

Data from Wright et al. (2004) were used to estimate the magnitude of the association between maternal smoking and SGA. This study was chosen because, with 196,000 total births, it was the largest prospective cohort study that provided information on this relationship. Data from this study are shown in Table F2. Wright et al. (2004) did not report an odds ratio so one was calculated using the 2 × 2 table data they provided.

As seen in Table F1, information on the prevalence of smoking in pregnant women ranged greatly from study to study. In order to evaluate how the impact of confounding might vary depending on smoking prevalence, OEHHA chose to do one set of calculations using data from a study where maternal smoking prevalence was high (30% in the low DBP exposure group) (Dodds et al., 1999) and one set of calculations where the maternal smoking prevalence was fairly low (5% in the low DBP exposure group) (MacLehose et al., 2008). This range in prevalence (5% to 30%) is similar to that reported for the US (Tong et al., 2013). Smoking data by low and high DBP exposure groups from Dodds et al. (1999) and MacLehose et al. (2008) are presented in Table F1.

### ***Results and Discussion***

Using data from Wright et al. (2004) (Table F2), OEHHA calculated an odds ratio between maternal smoking and SGA of 2.43 (95% CI, 2.34-2.51). This is similar to the odds ratios reported in other studies (Cnattingius, 2004). The odds ratio from Wright et al. (2004) and the prevalence of smoking in high and low DBP exposure groups were entered into the Axelson equations in order to produce estimates of the potential impact confounding by smoking might have on SGA-DBP odds ratios. The prevalence of smoking in the low and high DBP exposure groups were 30% and 37%, respectively, in Dodds et al. (1999) and 5% and 10%, respectively, in MacLehose et al. (2008). Figure F2A (for the high smoking prevalence study of Dodds et al. (1999)) and Figure F2B (for the low smoking

prevalence of study of MacLehose et al. (2008)) show the odds ratios that might be caused by confounding by smoking if there is no true association between SGA and DBP exposure. In both Figures, the horizontal gray line represents the prevalence of smoking in the low DBP group, and the circles and blue line show the odds ratios due to confounding by smoking as the prevalence of smoking varies in the higher DBP exposure group.

As seen in Figure F2A, the smoking prevalence difference of 7% seen in Dodds et al. (1999) (30% vs. 37% in the low and high DBP groups, respectively) would lead to an estimated odds ratio due to confounding of about 1.07. Similarly, the smoking prevalence difference of 5% seen in MacLehose et al. (2008) (5% vs. 10% in the low and high DBP groups, respectively) would lead to an estimated odds ratio due to confounding of also about 1.07 (Figure F2B).

### 3. Summary

Overall, OEHHA's analyses suggest that the impact of confounding from smoking, both the magnitude and the direction, is likely to vary from study to study. However, these evaluations also suggest that where confounding by smoking exists it is likely to be small and that confounding by smoking is unlikely to have a major impact on reported odds ratios between DBP exposure and SGA that are greater than about 1.10 to 1.15. Importantly though, because information on possible associations between smoking and DBP exposure were fairly scarce, OEHHA cannot rule out the possibility that more significant confounding might have occurred in some studies, especially in those that have reported small effect sizes.

OEHHA's evaluation of the magnitude of confounding only considered smoking and SGA, and it is unknown exactly how the findings presented here might relate to other confounders or other outcomes. However, SGA was more commonly linked to DBP exposures than to any of the other adverse reproductive outcomes reviewed. In addition, smoking is one of the most prevalent and important known risk factors for SGA and a number of other important adverse reproductive outcomes. For example, for the year 2002, the US Centers for Disease Control and Prevention estimated that 5.3% to 7.7% of preterm births, 13.1% to 19.0% of term low birth weight births, 23.2% to 33.6% of sudden infant death syndrome cases, and 5.0% to 7.3% of preterm-related deaths were attributable to prenatal smoking (Dietz et al., 2010). Finally, in this review, the relationship between smoking and DBP varied greatly, from no associations, to inverse associations, to positive associations. OEHHA's evaluation focused on the data from those studies that reported the greatest positive associations between smoking and DBP exposure, and as such may represent a "worst-case scenario" (i.e., the scenario in which the impact of confounding is to have the greatest potential for causing a false positive result), at least with regard to the available data. As a whole, because of its prevalence and strong links to reproductive health, maternal smoking is likely among the most important confounders of concern in the studies OEHHA reviewed.

**Table F1. Review of the Epidemiologic Literature on Associations between Smoking or Alcohol Use and Disinfection Byproduct Exposure<sup>1, 2</sup>**

| First author, year          | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Alcohol       | Exposure assessment | Summary      |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------------|--------------|--|--|------|--------|-------|-------------|----------|-------------|-------------|--------------|-------------|-------------|---------|-------------|-------------|-------------|-------------|-----------------|---------------|---------------|--------------|---------------------|-----------------|-----------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|---------------------|-----|-----------|-----------|-----------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|-----------|-----------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Dodds et al. (1999)         | <p><b>Smoking and average TTHM levels in water (µg/L)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="4">TTHM level</th> </tr> <tr> <th></th> <th>0-49</th> <th>50-74</th> <th>75-99</th> <th>100+</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>9,198 (70%)</td> <td>13,436 (71%)</td> <td>5,096 (68%)</td> <td>4,987 (63%)</td> </tr> <tr> <td>Smokers</td> <td>3,866 (30%)</td> <td>5,550 (29%)</td> <td>2,355 (32%)</td> <td>2,914 (37%)</td> </tr> <tr> <td>Total</td> <td>13,064 (100%)</td> <td>18,986 (100%)</td> <td>7,451 (100%)</td> <td>7,901 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>0.98</td> <td>1.1</td> <td>1.39</td> </tr> <tr> <td>95% CI<sup>3</sup></td> <td>Ref</td> <td>0.94-1.03</td> <td>1.03-1.17</td> <td>1.31-1.47</td> </tr> </tbody> </table> |               | TTHM level          |              |  |  |      | 0-49   | 50-74 | 75-99       | 100+     | Non-smokers | 9,198 (70%) | 13,436 (71%) | 5,096 (68%) | 4,987 (63%) | Smokers | 3,866 (30%) | 5,550 (29%) | 2,355 (32%) | 2,914 (37%) | Total           | 13,064 (100%) | 18,986 (100%) | 7,451 (100%) | 7,901 (100%)        | OR <sup>3</sup> | 1.00      | 0.98      | 1.1                                                                                                                                                                                                                                                                                                                                                                                                    | 1.39 | 95% CI <sup>3</sup> | Ref | 0.94-1.03 | 1.03-1.17 | 1.31-1.47 | Not assessed | TTHMs were measured in water samples from an average of 3 locations 4 times per year within the distribution system of each public water facility in the study area for the years 1987-1995. Linear regression models were used to model data and were linked to mother's residence at birth. | Smoking: associated with higher TTHM levels |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|                             | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |               |                     |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|                             | 0-49                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 50-74         | 75-99               | 100+         |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Non-smokers                 | 9,198 (70%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 13,436 (71%)  | 5,096 (68%)         | 4,987 (63%)  |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Smokers                     | 3,866 (30%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 5,550 (29%)   | 2,355 (32%)         | 2,914 (37%)  |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Total                       | 13,064 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 18,986 (100%) | 7,451 (100%)        | 7,901 (100%) |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| OR <sup>3</sup>             | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 0.98          | 1.1                 | 1.39         |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| 95% CI <sup>3</sup>         | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 0.94-1.03     | 1.03-1.17           | 1.31-1.47    |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Fenster et al. (2003)       | <p><b>Smoking and TTHM levels in water (µg/L)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM level</th> </tr> <tr> <th></th> <th>0-40</th> <th>&gt;40-80</th> <th>&gt;80</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>59 (77%)</td> <td>59 (77%)</td> <td>12 (100%)</td> </tr> <tr> <td>Smokers</td> <td>18 (23%)</td> <td>16 (24%)</td> <td>0 (0%)</td> </tr> <tr> <td>Total</td> <td>77 (100%)</td> <td>67 (100%)</td> <td>12 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>1.03</td> <td>0.13</td> </tr> <tr> <td>95% CI<sup>3</sup></td> <td>Ref</td> <td>0.48-2.22</td> <td>0.01-2.28</td> </tr> </tbody> </table>                                                                                                                                                 |               | TTHM level          |              |  |  | 0-40 | >40-80 | >80   | Non-smokers | 59 (77%) | 59 (77%)    | 12 (100%)   | Smokers      | 18 (23%)    | 16 (24%)    | 0 (0%)  | Total       | 77 (100%)   | 67 (100%)   | 12 (100%)   | OR <sup>3</sup> | 1.00          | 1.03          | 0.13         | 95% CI <sup>3</sup> | Ref             | 0.48-2.22 | 0.01-2.28 | <p><b>Alcoholic drinks per week and TTHM levels in water (µg/L)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM level</th> </tr> <tr> <th></th> <th>0-40</th> <th>&gt;40-80</th> <th>&gt;80</th> </tr> </thead> <tbody> <tr> <td>Drinks per week (mean ± SE)</td> <td>2.6 ± 0.4</td> <td>3.9 ± 0.8</td> <td>2.5 ± 0.8</td> </tr> </tbody> </table> <p>p-values not provided</p> |      | TTHM level          |     |           |           | 0-40      | >40-80       | >80                                                                                                                                                                                                                                                                                           | Drinks per week (mean ± SE)                 | 2.6 ± 0.4 | 3.9 ± 0.8 | 2.5 ± 0.8 | Measurements collected in public water supplies for subject's residence within 90 days prior to semen collection. TTHM in public water and estimated TTHM intakes (calculated by multiplying TTHM concentration by questionnaire data on water intake) were used. | Smoking: associated with lower TTHM levels<br>Alcohol: unclear |
|                             | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |               |                     |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|                             | 0-40                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | >40-80        | >80                 |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Non-smokers                 | 59 (77%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 59 (77%)      | 12 (100%)           |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Smokers                     | 18 (23%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 16 (24%)      | 0 (0%)              |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Total                       | 77 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 67 (100%)     | 12 (100%)           |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| OR <sup>3</sup>             | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 1.03          | 0.13                |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| 95% CI <sup>3</sup>         | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 0.48-2.22     | 0.01-2.28           |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|                             | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |               |                     |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
|                             | 0-40                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | >40-80        | >80                 |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |
| Drinks per week (mean ± SE) | 2.6 ± 0.4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 3.9 ± 0.8     | 2.5 ± 0.8           |              |  |  |      |        |       |             |          |             |             |              |             |             |         |             |             |             |             |                 |               |               |              |                     |                 |           |           |                                                                                                                                                                                                                                                                                                                                                                                                        |      |                     |     |           |           |           |              |                                                                                                                                                                                                                                                                                               |                                             |           |           |           |                                                                                                                                                                                                                                                                   |                                                                |

| First author, year            | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Alcohol       | Exposure assessment | Summary |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------------|---------|-------------|-----------|---------------|---------------|---------------|-------------|---------------|---------------|---------------|---------|-----------|-----------|-----------|-------|--------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|------|------|------|---------------------|-----|-----------|-----------|--|--------------------|--|--|--|---------------|---------------|---------------|-------------|-------------|-------------|-------------|---------|-------------|-------------|-------------|-------|--------------|--------------|--------------|-----------------|------|------|------|---------------------|-----|-----------|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--------------------|--|--|--|---------------|---------------|---------------|----------|--|--|--|----|---------------|---------------|---------------|-----|-----------|-----------|-----------|-------|--------------|--------------|--------------|-----------------|------|------|------|--------|-----|-----------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gallagher et al. (1998)       | <p><b>Smoking and TTHM levels in water (µg/L)</b></p> <p style="text-align: center;">TTHM levels</p> <table border="1"> <thead> <tr> <th></th> <th>0-49</th> <th>≥50</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>890 (81%)</td> <td>114 (81%)</td> </tr> <tr> <td>Smokers</td> <td>203 (19%)</td> <td>26 (19%)</td> </tr> <tr> <td>Total</td> <td>1,093 (100%)</td> <td>140 (100%)</td> </tr> <tr> <td>OR</td> <td>1.00</td> <td>1.00</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.64-1.57</td> </tr> </tbody> </table> <p>Excludes those with missing data</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |               | 0-49                | ≥50     | Non-smokers | 890 (81%) | 114 (81%)     | Smokers       | 203 (19%)     | 26 (19%)    | Total         | 1,093 (100%)  | 140 (100%)    | OR      | 1.00      | 1.00      | 95% CI    | Ref   | 0.64-1.57    | Not assessed | TTHM concentrations measured quarterly at 4 different locations. These measurements and the hydraulic characteristics of each drinking water system were modeled using EPA-NET to estimate quarterly TTHM concentrations for each census block group. Exposure "scores" were then assigned to the 3rd trimester of pregnancy. | Smoking: no association with TTHM levels |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | 0-49                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | ≥50           |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Non-smokers                   | 890 (81%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 114 (81%)     |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Smokers                       | 203 (19%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 26 (19%)      |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Total                         | 1,093 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 140 (100%)    |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| OR                            | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 1.00          |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| 95% CI                        | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.64-1.57     |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Grazulevic iene et al. (2011) | <p><b>Maternal smoking and TTHM internal dose (mg/day)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM internal dose</th> </tr> <tr> <th></th> <th>0.0025-0.0386</th> <th>0.0025-0.0386</th> <th>0.3545-2.4040</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>1,003 (92.4%)</td> <td>1,076 (93.4%)</td> <td>1,031 (93.4%)</td> </tr> <tr> <td>Smokers</td> <td>82 (7.6%)</td> <td>76 (6.6%)</td> <td>73 (6.6%)</td> </tr> <tr> <td>Total</td> <td>1,085 (100%)</td> <td>1,152 (100%)</td> <td>1,104 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>0.85</td> <td>0.85</td> </tr> <tr> <td>95% CI<sup>3</sup></td> <td>Ref</td> <td>0.62-1.18</td> <td>0.62-1.19</td> </tr> </tbody> </table> <p><b>Paternal smoking and TTHM internal dose (mg/day)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM internal dose</th> </tr> <tr> <th></th> <th>0.0025-0.0386</th> <th>0.0386-0.3545</th> <th>0.3545-2.4040</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>574 (53.4%)</td> <td>629 (55.4%)</td> <td>545 (49.8%)</td> </tr> <tr> <td>Smokers</td> <td>501 (46.6%)</td> <td>507 (44.6%)</td> <td>550 (50.2%)</td> </tr> <tr> <td>Total</td> <td>1,075 (100%)</td> <td>1,136 (100%)</td> <td>1,095 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>0.92</td> <td>1.16</td> </tr> <tr> <td>95% CI<sup>3</sup></td> <td>Ref</td> <td>0.78-1.09</td> <td>0.98-1.37</td> </tr> </tbody> </table> |               | TTHM internal dose  |         |             |           | 0.0025-0.0386 | 0.0025-0.0386 | 0.3545-2.4040 | Non-smokers | 1,003 (92.4%) | 1,076 (93.4%) | 1,031 (93.4%) | Smokers | 82 (7.6%) | 76 (6.6%) | 73 (6.6%) | Total | 1,085 (100%) | 1,152 (100%) | 1,104 (100%)                                                                                                                                                                                                                                                                                                                  | OR <sup>3</sup>                          | 1.00 | 0.85 | 0.85 | 95% CI <sup>3</sup> | Ref | 0.62-1.18 | 0.62-1.19 |  | TTHM internal dose |  |  |  | 0.0025-0.0386 | 0.0386-0.3545 | 0.3545-2.4040 | Non-smokers | 574 (53.4%) | 629 (55.4%) | 545 (49.8%) | Smokers | 501 (46.6%) | 507 (44.6%) | 550 (50.2%) | Total | 1,075 (100%) | 1,136 (100%) | 1,095 (100%) | OR <sup>3</sup> | 1.00 | 0.92 | 1.16 | 95% CI <sup>3</sup> | Ref | 0.78-1.09 | 0.98-1.37 | <p><b>Alcohol use and TTHM internal dose (mg/day)</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM internal dose</th> </tr> <tr> <th></th> <th>0.0025-0.0386</th> <th>0.0386-0.3545</th> <th>0.3545-2.4040</th> </tr> </thead> <tbody> <tr> <td colspan="4">Alcohol:</td> </tr> <tr> <td>No</td> <td>1,000 (92.2%)</td> <td>1,094 (95.0%)</td> <td>1,048 (94.9%)</td> </tr> <tr> <td>Yes</td> <td>85 (7.8%)</td> <td>58 (5.0%)</td> <td>56 (5.1%)</td> </tr> <tr> <td>Total</td> <td>1,085 (100%)</td> <td>1,152 (100%)</td> <td>1,104 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>0.62</td> <td>0.63</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.44-0.88</td> <td>0.44-0.89</td> </tr> </tbody> </table> <p>Alcohol consumption ("Yes") defined as at least one drink per week during pregnancy</p> |  | TTHM internal dose |  |  |  | 0.0025-0.0386 | 0.0386-0.3545 | 0.3545-2.4040 | Alcohol: |  |  |  | No | 1,000 (92.2%) | 1,094 (95.0%) | 1,048 (94.9%) | Yes | 85 (7.8%) | 58 (5.0%) | 56 (5.1%) | Total | 1,085 (100%) | 1,152 (100%) | 1,104 (100%) | OR <sup>3</sup> | 1.00 | 0.62 | 0.63 | 95% CI | Ref | 0.44-0.88 | 0.44-0.89 | TTHM concentrations measured 4 times per year in municipal supplies linked to personal interview data on residence, drinking water intake, showering and bathing, and swimming pool use. These factors are linked to "estimated uptake factors" to derive an "integrated index of blood concentration" expressed in mg of TTHM/day. | Smoking: possible association with higher TTHM levels for paternal smoking and lower TTHM levels for maternal smoking<br><br>Alcohol: associated with lower TTHM internal doses |
|                               | TTHM internal dose                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |               |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | 0.0025-0.0386                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0.0025-0.0386 | 0.3545-2.4040       |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Non-smokers                   | 1,003 (92.4%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1,076 (93.4%) | 1,031 (93.4%)       |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Smokers                       | 82 (7.6%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 76 (6.6%)     | 73 (6.6%)           |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Total                         | 1,085 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1,152 (100%)  | 1,104 (100%)        |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| OR <sup>3</sup>               | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 0.85          | 0.85                |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| 95% CI <sup>3</sup>           | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.62-1.18     | 0.62-1.19           |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | TTHM internal dose                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |               |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | 0.0025-0.0386                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0.0386-0.3545 | 0.3545-2.4040       |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Non-smokers                   | 574 (53.4%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 629 (55.4%)   | 545 (49.8%)         |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Smokers                       | 501 (46.6%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 507 (44.6%)   | 550 (50.2%)         |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Total                         | 1,075 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1,136 (100%)  | 1,095 (100%)        |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| OR <sup>3</sup>               | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 0.92          | 1.16                |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| 95% CI <sup>3</sup>           | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.78-1.09     | 0.98-1.37           |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | TTHM internal dose                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |               |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
|                               | 0.0025-0.0386                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0.0386-0.3545 | 0.3545-2.4040       |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Alcohol:                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |               |                     |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| No                            | 1,000 (92.2%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1,094 (95.0%) | 1,048 (94.9%)       |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Yes                           | 85 (7.8%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 58 (5.0%)     | 56 (5.1%)           |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| Total                         | 1,085 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 1,152 (100%)  | 1,104 (100%)        |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| OR <sup>3</sup>               | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 0.62          | 0.63                |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |
| 95% CI                        | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.44-0.88     | 0.44-0.89           |         |             |           |               |               |               |             |               |               |               |         |           |           |           |       |              |              |                                                                                                                                                                                                                                                                                                                               |                                          |      |      |      |                     |     |           |           |  |                    |  |  |  |               |               |               |             |             |             |             |         |             |             |             |       |              |              |              |                 |      |      |      |                     |     |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                    |  |  |  |               |               |               |          |  |  |  |    |               |               |               |     |           |           |           |       |              |              |              |                 |      |      |      |        |     |           |           |                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                 |

| First author, year   | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Alcohol          | Exposure assessment | Summary          |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------------------|------------------|-----------------|-------------|----------|----------|----------|-------------|-----------|-----------|-----------|---------|-----------|-----------|-----------|-------|------------|------------|------------|-----------------|------|-----------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|------------|----------|----------|---------|----------|----------|----------|----------|-----------|-----------|-----------|----|----------|----------|----------|--------|-----------|-----------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|------------|------------|-----------------|------|------|------|--------|-----|-----------|-----------|----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Iszatt et al. (2013) | <p><b>Smoking and TTHM levels in water (µg/L) in males</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM level</th> </tr> <tr> <th></th> <th>&lt;30</th> <th>30-&lt;60</th> <th>≥60</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>283 (59%)</td> <td>506 (63%)</td> <td>168 (60%)</td> </tr> <tr> <td>Smokers</td> <td>198 (41%)</td> <td>301 (37%)</td> <td>112 (40%)</td> </tr> <tr> <td>Total</td> <td>481 (100%)</td> <td>807 (100%)</td> <td>280 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>0.85</td> <td>0.95</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.67-1.07</td> <td>0.71-1.29</td> </tr> </tbody> </table> |                  | TTHM level          |                  |                 |             | <30      | 30-<60   | ≥60      | Non-smokers | 283 (59%) | 506 (63%) | 168 (60%) | Smokers | 198 (41%) | 301 (37%) | 112 (40%) | Total | 481 (100%) | 807 (100%) | 280 (100%) | OR <sup>3</sup> | 1.00 | 0.85      | 0.95      | 95% CI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Ref | 0.67-1.07    | 0.71-1.29        | <p><b>Alcohol use and TTHM levels in water (µg/L) in males</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">TTHM level</th> </tr> <tr> <th></th> <th>&lt;30</th> <th>30-&lt;60</th> <th>≥60</th> </tr> </thead> <tbody> <tr> <td colspan="4">Alcohol:</td> </tr> <tr> <td>No</td> <td>94 (20%)</td> <td>94 (20%)</td> <td>69 (25%)</td> </tr> <tr> <td>Yes</td> <td>387 (80%)</td> <td>670 (83%)</td> <td>211 (75%)</td> </tr> <tr> <td>Total</td> <td>481 (100%)</td> <td>807 (100%)</td> <td>280 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1.00</td> <td>1.19</td> <td>0.74</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.89-1.59</td> <td>0.52-1.06</td> </tr> </tbody> </table> <p>Alcohol use ("Yes") defined as 1 drink per week for 1 month or more</p> |             | TTHM level |          |          |         | <30      | 30-<60   | ≥60      | Alcohol: |           |           |           | No | 94 (20%) | 94 (20%) | 69 (25%) | Yes    | 387 (80%) | 670 (83%) | 211 (75%) | Total                                                                                                                                                                                                                                                                        | 481 (100%)                                                                                                 | 807 (100%) | 280 (100%) | OR <sup>3</sup> | 1.00 | 1.19 | 0.74 | 95% CI | Ref | 0.89-1.59 | 0.52-1.06 | <p>Quarterly water records in 1,568 water zones in the UK modeled and linked to addresses 90 days prior to sperm collection.</p> | <p>Smoking: no association</p> <p>Alcohol: associated with lower TTHM levels</p> |
|                      | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                  |                     |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|                      | <30                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 30-<60           | ≥60                 |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Non-smokers          | 283 (59%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 506 (63%)        | 168 (60%)           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Smokers              | 198 (41%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 301 (37%)        | 112 (40%)           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Total                | 481 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 807 (100%)       | 280 (100%)          |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| OR <sup>3</sup>      | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.85             | 0.95                |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| 95% CI               | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.67-1.07        | 0.71-1.29           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|                      | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                  |                     |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|                      | <30                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 30-<60           | ≥60                 |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Alcohol:             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                  |                     |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| No                   | 94 (20%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 94 (20%)         | 69 (25%)            |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Yes                  | 387 (80%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 670 (83%)        | 211 (75%)           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Total                | 481 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 807 (100%)       | 280 (100%)          |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| OR <sup>3</sup>      | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.19             | 0.74                |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| 95% CI               | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.89-1.59        | 0.52-1.06           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Luben et al. (2007)  | <p><b>Smoking by DBP site in males</b></p> <table border="1"> <thead> <tr> <th></th> <th>Low DBP site</th> <th>Chlorinated site</th> <th>Brominated site</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>54 (59%)</td> <td>60 (65%)</td> <td>21 (47%)</td> </tr> <tr> <td>Smokers</td> <td>37 (41%)</td> <td>32 (35%)</td> <td>24 (53%)</td> </tr> <tr> <td>Total</td> <td>91 (100%)</td> <td>92 (100%)</td> <td>45 (100%)</td> </tr> <tr> <td>OR</td> <td>1.00</td> <td>0.77</td> <td>1.67</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.43-1.42</td> <td>0.81-3.43</td> </tr> </tbody> </table> <p>See Figure 1 in Luben et al., 2007 for DBP levels</p>             |                  | Low DBP site        | Chlorinated site | Brominated site | Non-smokers | 54 (59%) | 60 (65%) | 21 (47%) | Smokers     | 37 (41%)  | 32 (35%)  | 24 (53%)  | Total   | 91 (100%) | 92 (100%) | 45 (100%) | OR    | 1.00       | 0.77       | 1.67       | 95% CI          | Ref  | 0.43-1.42 | 0.81-3.43 | <p><b>Alcohol use by DBP site in males</b></p> <table border="1"> <thead> <tr> <th></th> <th>Low DBP site</th> <th>Chlorinated site</th> <th>Brominated site</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>54 (59%)</td> <td>60 (65%)</td> <td>21 (47%)</td> </tr> <tr> <td>Smokers</td> <td>37 (41%)</td> <td>32 (35%)</td> <td>24 (53%)</td> </tr> <tr> <td>Total</td> <td>91 (100%)</td> <td>92 (100%)</td> <td>45 (100%)</td> </tr> <tr> <td>OR</td> <td>1.00</td> <td>0.77</td> <td>1.67</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.43-1.42</td> <td>0.81-3.43</td> </tr> </tbody> </table> <p>See Figure 1 in Luben et al., 2007 for DBP levels<br/>Alcohol use not defined</p> |     | Low DBP site | Chlorinated site | Brominated site                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Non-smokers | 54 (59%)   | 60 (65%) | 21 (47%) | Smokers | 37 (41%) | 32 (35%) | 24 (53%) | Total    | 91 (100%) | 92 (100%) | 45 (100%) | OR | 1.00     | 0.77     | 1.67     | 95% CI | Ref       | 0.43-1.42 | 0.81-3.43 | <p>Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average concentrations linked to telephone interview data on water intake, boiling water, showering and bathing, and uptake factors.</p> | <p>Smoking: associated with higher brominated DBPs</p> <p>Alcohol: associated with higher DBP exposure</p> |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|                      | Low DBP site                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Chlorinated site | Brominated site     |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Non-smokers          | 54 (59%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 60 (65%)         | 21 (47%)            |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Smokers              | 37 (41%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 32 (35%)         | 24 (53%)            |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Total                | 91 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 92 (100%)        | 45 (100%)           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| OR                   | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.77             | 1.67                |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| 95% CI               | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.43-1.42        | 0.81-3.43           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
|                      | Low DBP site                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Chlorinated site | Brominated site     |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Non-smokers          | 54 (59%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 60 (65%)         | 21 (47%)            |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Smokers              | 37 (41%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 32 (35%)         | 24 (53%)            |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| Total                | 91 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 92 (100%)        | 45 (100%)           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| OR                   | 1.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.77             | 1.67                |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |
| 95% CI               | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.43-1.42        | 0.81-3.43           |                  |                 |             |          |          |          |             |           |           |           |         |           |           |           |       |            |            |            |                 |      |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |     |              |                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |             |            |          |          |         |          |          |          |          |           |           |           |    |          |          |          |        |           |           |           |                                                                                                                                                                                                                                                                              |                                                                                                            |            |            |                 |      |      |      |        |     |           |           |                                                                                                                                  |                                                                                  |

| First author, year           | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Alcohol              | Exposure assessment | Summary          |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------------|------------------|-------------------------|----------------------|--------------|-----------|-----------|------------------------------|----------------------|-----------|---------------------|------------------------------------------------------------------------------|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------|---|------|------|--------|--------|-----------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|--------------|------------------|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--|--|------|-----------|-----------|-----------|-----|---------|---------|--------|-------|------------|------------|------------|-----------------|---|------|------|--------|-----|-----------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| MacLehose et al. (2008)      | <p><b>Smoking by DBP site</b></p> <table border="1"> <thead> <tr> <th></th> <th>Low DBP site</th> <th>Chlorinated site</th> <th>Brominated site</th> </tr> </thead> <tbody> <tr> <td>Non-smokers</td> <td>465 (95%)</td> <td>600 (97%)</td> <td>188 (90%)</td> </tr> <tr> <td>Smokers</td> <td>23 (5%)</td> <td>19 (3%)</td> <td>20 (10%)</td> </tr> <tr> <td>Total</td> <td>488 (100%)</td> <td>619 (100%)</td> <td>208 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1</td> <td>0.64</td> <td>2.15</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.34-1.19</td> <td>1.15-4.01</td> </tr> </tbody> </table> <p>Mean THM and HAA concentrations at each site are provided in Table 2 of MacLehose et al., 2008</p> |                      | Low DBP site        | Chlorinated site | Brominated site         | Non-smokers          | 465 (95%)    | 600 (97%) | 188 (90%) | Smokers                      | 23 (5%)              | 19 (3%)   | 20 (10%)            | Total                                                                        | 488 (100%)                                   | 619 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 208 (100%) | OR <sup>3</sup> | 1 | 0.64 | 2.15 | 95% CI | Ref    | 0.34-1.19 | 1.15-4.01 | <p><b>Alcohol use by DBP site</b></p> <table border="1"> <thead> <tr> <th></th> <th>Low DBP site</th> <th>Chlorinated site</th> <th>Brominated site</th> </tr> </thead> <tbody> <tr> <td>Alcohol</td> <td></td> <td></td> <td></td> </tr> <tr> <td>None</td> <td>478 (98%)</td> <td>602 (98%)</td> <td>202 (97%)</td> </tr> <tr> <td>Any</td> <td>10 (2%)</td> <td>15 (2%)</td> <td>6 (3%)</td> </tr> <tr> <td>Total</td> <td>488 (100%)</td> <td>617 (100%)</td> <td>208 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1</td> <td>1.19</td> <td>1.41</td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.53-2.64</td> <td>0.51-3.96</td> </tr> </tbody> </table> <p>Mean THM and HAA concentrations at each site are provided in Table 2 of MacLehose et al., 2008</p> |           | Low DBP site | Chlorinated site | Brominated site | Alcohol                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                             |  |  | None | 478 (98%) | 602 (98%) | 202 (97%) | Any | 10 (2%) | 15 (2%) | 6 (3%) | Total | 488 (100%) | 617 (100%) | 208 (100%) | OR <sup>3</sup> | 1 | 1.19 | 1.41 | 95% CI | Ref | 0.53-2.64 | 0.51-3.96 | <p>Weekly or biweekly samples collected from a single representative location in the water distribution systems. System wide weekly average linked to personal questionnaire data on water intake and residence in "early gestation" (average of 9th week of pregnancy).</p> | <p>Smoking: associated with higher brominated DBPs</p> <p>Alcohol: no clear association</p> |
|                              | Low DBP site                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Chlorinated site     | Brominated site     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Non-smokers                  | 465 (95%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 600 (97%)            | 188 (90%)           |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Smokers                      | 23 (5%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 19 (3%)              | 20 (10%)            |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Total                        | 488 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 619 (100%)           | 208 (100%)          |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| OR <sup>3</sup>              | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 0.64                 | 2.15                |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| 95% CI                       | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.34-1.19            | 1.15-4.01           |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | Low DBP site                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Chlorinated site     | Brominated site     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Alcohol                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                      |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| None                         | 478 (98%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 602 (98%)            | 202 (97%)           |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Any                          | 10 (2%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 15 (2%)              | 6 (3%)              |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Total                        | 488 (100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 617 (100%)           | 208 (100%)          |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| OR <sup>3</sup>              | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 1.19                 | 1.41                |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| 95% CI                       | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.53-2.64            | 0.51-3.96           |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Windham et al. (2003)        | <p><b>Cigarettes smoked per day and TTHM level in water (µg/L)</b></p> <table border="1"> <thead> <tr> <th rowspan="2"></th> <th colspan="4">TTHM level</th> </tr> <tr> <th>0-40</th> <th>&gt;40-60</th> <th>&gt;60-80</th> <th>&gt;80</th> </tr> </thead> <tbody> <tr> <td>Cigs/day (mean ± SD)</td> <td>1.4 ± 5.0</td> <td>1.3 ± 4.2</td> <td>0.4 ± 2.9</td> <td>2.7 ± 6.1</td> </tr> </tbody> </table> <p>A p-value of 0.12 is provided by the authors for these data but it is unclear exactly what it represents</p>                                                                                                                                                                                                  |                      | TTHM level          |                  |                         |                      | 0-40         | >40-60    | >60-80    | >80                          | Cigs/day (mean ± SD) | 1.4 ± 5.0 | 1.3 ± 4.2           | 0.4 ± 2.9                                                                    | 2.7 ± 6.1                                    | <p><b>Alcoholic drinks per week and TTHM level in water (µg/L)</b></p> <table border="1"> <thead> <tr> <th rowspan="2"></th> <th colspan="4">TTHM level</th> </tr> <tr> <th>0-40</th> <th>&gt;40-60</th> <th>&gt;60-80</th> <th>&gt;80</th> </tr> </thead> <tbody> <tr> <td>Drinks/week (mean ± SD)</td> <td>1.3 ± 2.5</td> <td>2.5 ± 5.4</td> <td>1.3 ± 2.5</td> <td>2.2 ± 3.4</td> </tr> </tbody> </table> <p>A p-value of 0.04 is provided by the authors for these data but it is unclear exactly what it represents</p> |            | TTHM level      |   |      |      | 0-40   | >40-60 | >60-80    | >80       | Drinks/week (mean ± SD)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1.3 ± 2.5 | 2.5 ± 5.4    | 1.3 ± 2.5        | 2.2 ± 3.4       | <p>Quarterly TTHM concentrations from the utilities collected at 4-20 points in each distribution system used to create utility wide averages. These were then linked to telephone interview data on hot and cold tap water consumption, bottled water use, and showering to create 90-day exposure estimates for each cycle (during each cycle plus the 60 days before).</p> | <p>Smoking: possibly associated with higher TTHMs</p> <p>Alcohol: possibly associated with higher TTHMs</p> |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                      |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | 0-40                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | >40-60               | >60-80              | >80              |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Cigs/day (mean ± SD)         | 1.4 ± 5.0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.3 ± 4.2            | 0.4 ± 2.9           | 2.7 ± 6.1        |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | TTHM level                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                      |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | 0-40                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | >40-60               | >60-80              | >80              |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Drinks/week (mean ± SD)      | 1.3 ± 2.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2.5 ± 5.4            | 1.3 ± 2.5           | 2.2 ± 3.4        |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Cantor et al. (1987)         | <p><b>Smoking and mean years exposed to ground (not chlorinated) vs. surface (chlorinated) water</b></p> <table border="1"> <thead> <tr> <th rowspan="2"></th> <th colspan="2">Water type</th> </tr> <tr> <th>Ground, not chlorinated</th> <th>Surface, chlorinated</th> </tr> </thead> <tbody> <tr> <td>Never smoker</td> <td>17.3</td> <td>19.8</td> </tr> <tr> <td>Heavy smokers (≥40 cigs/day)</td> <td>10.3</td> <td>22.4</td> </tr> </tbody> </table> <p>Ranges or p-values not provided<br/>Data are based on 5,258 male and female controls in a bladder cancer case-control study</p>                                                                                                                             |                      | Water type          |                  | Ground, not chlorinated | Surface, chlorinated | Never smoker | 17.3      | 19.8      | Heavy smokers (≥40 cigs/day) | 10.3                 | 22.4      | <p>Not assessed</p> | <p>Lifetime residential history linked to historical water utility data.</p> | <p>Smoking: associated with chlorination</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | Water type                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                      |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
|                              | Ground, not chlorinated                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Surface, chlorinated |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Never smoker                 | 17.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 19.8                 |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |
| Heavy smokers (≥40 cigs/day) | 10.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 22.4                 |                     |                  |                         |                      |              |           |           |                              |                      |           |                     |                                                                              |                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |            |                 |   |      |      |        |        |           |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |           |              |                  |                 |                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                             |  |  |      |           |           |           |     |         |         |        |       |            |            |            |                 |   |      |      |        |     |           |           |                                                                                                                                                                                                                                                                              |                                                                                             |

| First author, year             | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Alcohol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Exposure assessment | Summary                                                                                              |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------------------------------------------------------------------------------------------------------|--|--|---|--------------|---------------|--------------|--------|---------------|------------|--------------------------------|----------|---------------|-----------------|-----------------|----------|----------|-----------|-----------------|---|------|-----------|--------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|-----------|-----------|--|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Chevrier et al. (2004)         | <p><b>Smoking and mean years exposed to chlorinated surface water</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="4">Years</th> </tr> <tr> <th></th> <th>0</th> <th>1-28</th> <th>29-30</th> <th>Total</th> </tr> </thead> <tbody> <tr> <td>Never smokers</td> <td>45 (62%)</td> <td>16 (22%)</td> <td>12 (16%)</td> <td>73 (100%)</td> </tr> <tr> <td>Current smokers</td> <td>52 (58%)</td> <td>22 (24%)</td> <td>16 (18%)</td> <td>90 (100%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td>1</td> <td>1.19</td> <td>1.15</td> <td></td> </tr> <tr> <td>95% CI</td> <td>Ref</td> <td>0.56-2.54</td> <td>0.49-2.69</td> <td></td> </tr> </tbody> </table> <p>Data based on male and female controls from a bladder cancer case-control study</p>                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Years               |                                                                                                      |  |  |   | 0            | 1-28          | 29-30        | Total  | Never smokers | 45 (62%)   | 16 (22%)                       | 12 (16%) | 73 (100%)     | Current smokers | 52 (58%)        | 22 (24%) | 16 (18%) | 90 (100%) | OR <sup>3</sup> | 1 | 1.19 | 1.15      |              | 95% CI                                                                                                                         | Ref                                                      | 0.56-2.54 | 0.49-2.69 |  | Not assessed | 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. | Smoking: possibly associated with a greater number of years of exposure to chlorinated water |
|                                | Years                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                     |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
|                                | 0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 1-28                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 29-30               | Total                                                                                                |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Never smokers                  | 45 (62%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 16 (22%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 12 (16%)            | 73 (100%)                                                                                            |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Current smokers                | 52 (58%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 22 (24%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 16 (18%)            | 90 (100%)                                                                                            |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| OR <sup>3</sup>                | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 1.19                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 1.15                |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| 95% CI                         | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 0.56-2.54                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.49-2.69           |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Doyle et al. (1997)            | <p><b>Smoking and percentage of women using ground vs. surface water</b></p> <table border="1"> <thead> <tr> <th></th> <th colspan="3">Water source</th> </tr> <tr> <th></th> <th>N</th> <th>Ground water</th> <th>Surface water</th> </tr> </thead> <tbody> <tr> <td>Never smoker</td> <td>15,107</td> <td>8,535 (56.5%)</td> <td>967 (6.4%)</td> </tr> <tr> <td>Heavy smokers (≥40 pack-years)</td> <td>2,032</td> <td>1,213 (59.7%)</td> <td>185 (9.1%)</td> </tr> <tr> <td>OR<sup>3</sup></td> <td></td> <td>1</td> <td>1.34</td> </tr> <tr> <td>95% CI</td> <td></td> <td>Ref</td> <td>1.14-1.59</td> </tr> </tbody> </table> <p>Percentages and N are for all subjects. Percentages do not add up to 100% since subjects using other sources (private wells and mixed water) are not included here. Further information on these subjects is provided in Table 1 of Doyle et al., 1997. Data are from the Iowa Women's Health Study Cohort. Surface is water more likely to be chlorinated according to the authors.</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Water source        |                                                                                                      |  |  | N | Ground water | Surface water | Never smoker | 15,107 | 8,535 (56.5%) | 967 (6.4%) | Heavy smokers (≥40 pack-years) | 2,032    | 1,213 (59.7%) | 185 (9.1%)      | OR <sup>3</sup> |          | 1        | 1.34      | 95% CI          |   | Ref  | 1.14-1.59 | Not assessed | Water source in 1989 linked to state water treatment data; THM measurements in 856 public water supplies collected in 1986-87. | Smoking: associated with surface water (chlorinated) use |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
|                                | Water source                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                     |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
|                                | N                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Ground water                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Surface water       |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Never smoker                   | 15,107                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 8,535 (56.5%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 967 (6.4%)          |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Heavy smokers (≥40 pack-years) | 2,032                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 1,213 (59.7%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 185 (9.1%)          |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| OR <sup>3</sup>                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 1.34                |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| 95% CI                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Ref                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 1.14-1.59           |                                                                                                      |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |
| Riederer et al. (2014)         | <p>In linear multivariable regression analysis, current smoking (vs. never/former smoking) was not associated with natural log blood TTHM levels (<math>p&gt;0.10</math>, regression coefficient not provided) but was negatively associated with blood BDCM levels (regression coefficient=-0.13, 95% CI: -0.23 to -0.04, <math>p=0.01</math>, units unclear).</p> <p>Included US adults in the 1999-2006 National Health and Nutrition Examination Surveys.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <p>In linear multivariable regression analysis, alcohol use was not associated with natural log blood TTHM levels (<math>p&gt;0.10</math>, regression coefficient not provided) but was positively associated with blood BDCM levels (regression coefficient= 0.13, 95% CI: 0.05 to 0.21, <math>p&lt;0.01</math>, units unclear).</p> <p>Included US adults in the 1999-2006 National Health and Nutrition Examination Surveys. Alcohol use defined as any use in the 24 hours before blood collection.</p> | Blood TTHMs         | <p>Smoking: associated with lower BDCM levels</p> <p>Alcohol: associated with higher BDCM levels</p> |  |  |   |              |               |              |        |               |            |                                |          |               |                 |                 |          |          |           |                 |   |      |           |              |                                                                                                                                |                                                          |           |           |  |              |                                                                                                                                                                                                               |                                                                                              |

| First author, year  | Smoking                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Alcohol        | Exposure assessment | Summary |              |   |     |               |                       |      |                |                        |        |              |             |                                                   |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|---------------------|---------|--------------|---|-----|---------------|-----------------------|------|----------------|------------------------|--------|--------------|-------------|---------------------------------------------------|
| Zeng et al. (2014c) | <p><b>Multivariate model for predicting log 10 creatinine adjusted urinary TCA levels by smoking status</b></p> <table border="1"> <thead> <tr> <th>Smoking status</th> <th>Estimate (95% CI)</th> <th>p-value</th> </tr> </thead> <tbody> <tr> <td>Never smoker</td> <td>0</td> <td>Ref</td> </tr> <tr> <td>Former smoker</td> <td>-0.01 (-0.10 to 0.08)</td> <td>0.88</td> </tr> <tr> <td>Current smoker</td> <td>-0.14 (-0.22 to -0.06)</td> <td>&lt;0.001</td> </tr> </tbody> </table> <p>Estimate = <math>10^{\beta} - 1</math>, where <math>\beta</math> is the regression coefficient between smoking level and log creatinine adjusted urinary TCA levels.<br/>Study includes 2,144 Chinese men, mean age 31.6 (interquartile range = 28-35 years old).</p> | Smoking status | Estimate (95% CI)   | p-value | Never smoker | 0 | Ref | Former smoker | -0.01 (-0.10 to 0.08) | 0.88 | Current smoker | -0.14 (-0.22 to -0.06) | <0.001 | Not assessed | Urinary TCA | Smoking: associated with lower urinary TCA levels |
| Smoking status      | Estimate (95% CI)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | p-value        |                     |         |              |   |     |               |                       |      |                |                        |        |              |             |                                                   |
| Never smoker        | 0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Ref            |                     |         |              |   |     |               |                       |      |                |                        |        |              |             |                                                   |
| Former smoker       | -0.01 (-0.10 to 0.08)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.88           |                     |         |              |   |     |               |                       |      |                |                        |        |              |             |                                                   |
| Current smoker      | -0.14 (-0.22 to -0.06)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <0.001         |                     |         |              |   |     |               |                       |      |                |                        |        |              |             |                                                   |

a Abbreviations: CI, confidence interval; BDCM, bromodichloromethane; DBP, disinfection byproduct; HAA, haloacetic acid; N, sample size; Ref, reference group; SD, standard deviation; TCA, trichloroacetic acid; THM, trihalomethane; TTHM, total trihalomethanes, sum of BDCM, chloroform, DBCM, and bromoform

1. Maternal smoking or alcohol use during pregnancy unless otherwise noted
2. Studies are sorted by source (DBP and reproductive outcomes review, DBP and cancer review, and separate literature search) then alphabetically
3. Calculated using <http://www.hutchon.net/confidor.htm>

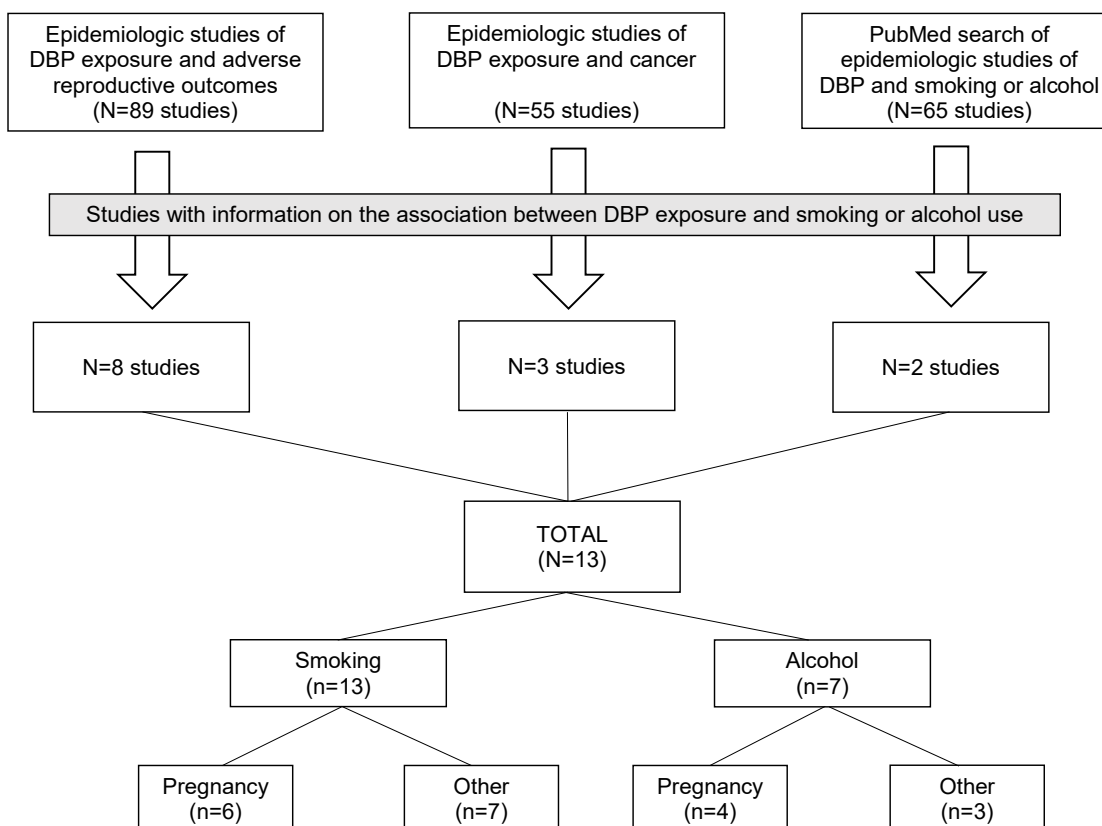


**Table F2. Data on maternal smoking and small for gestational age from Wright et al. (2004)**

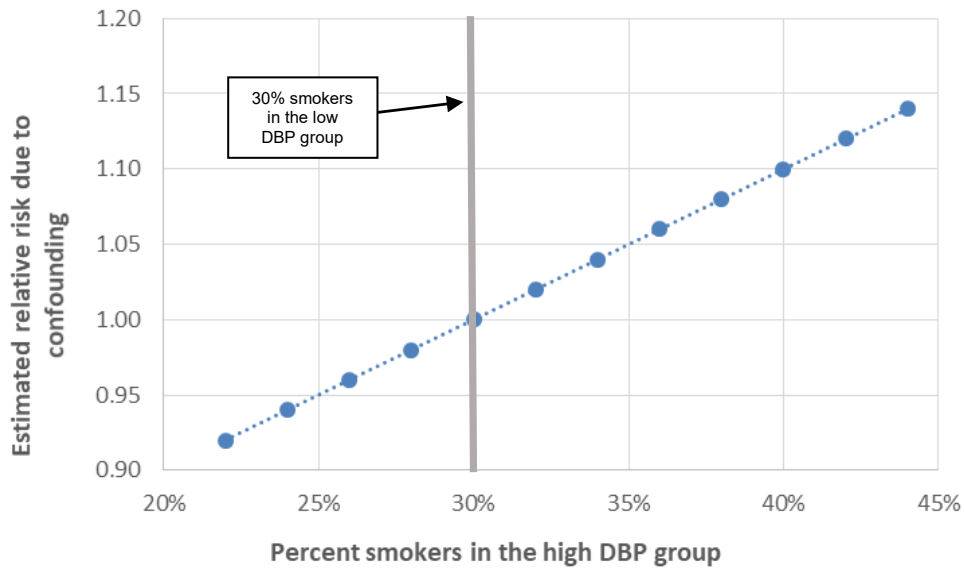
| Cigs/day    | Total   | SGA     |       |        |       |
|-------------|---------|---------|-------|--------|-------|
|             |         | No      |       | Yes    |       |
|             |         | N       | %     | N      | %     |
| 0           | 168,463 | 154,649 | 91.8% | 13,814 | 8.2%  |
| 1-5         | 8,711   | 7,396   | 84.9% | 1,315  | 15.1% |
| 6-10        | 10,134  | 8,320   | 82.1% | 1,814  | 17.9% |
| >10         | 7,888   | 6,255   | 79.3% | 1,633  | 20.7% |
| All smokers | 26,733  | 21,971  | 82.2% | 4,762  | 17.8% |

Abbreviations: Cigs, cigarettes; N, sample size; SGA, small for gestational age

**Figure F1. Results of the Literature Search for Studies of Disinfection Byproduct Exposure and Smoking or Alcohol Use**

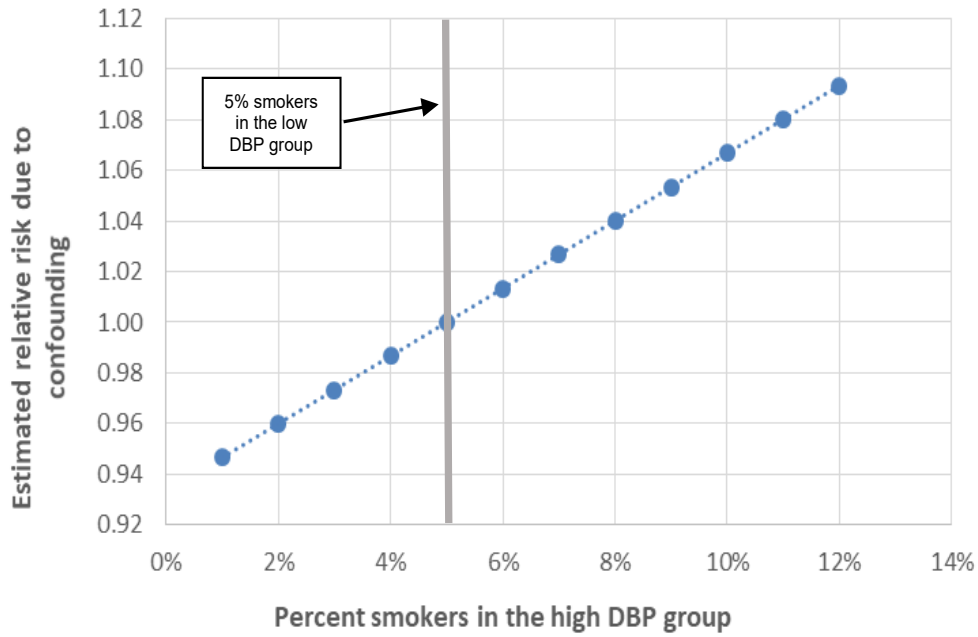


**Figure F2A. Estimated Impact of Confounding by Smoking in Studies of DBP Exposure and Small for Gestational Age with Higher Prevalence of Smoking**



The vertical gray line represents the prevalence of smoking in the low DBP group (30%). The circles and blue line show the odds ratios due to confounding by smoking with higher or lower prevalence of smoking in the higher DBP group.

**Figure F2B. Estimated Impact of Confounding by Smoking in Studies of DBP Exposure and Small for Gestational Age with Lower Prevalence of Smoking**



The vertical gray line represents the prevalence of smoking in the low DBP group (5%). The circles and blue line show the odds ratios due to confounding by smoking with higher or lower prevalence of smoking in the higher DBP group.