National Toxicology Program
U.S. Department of Health and Human Services

Draft Report on Carcinogens Monograph on Haloacetic Acids Found as Water Disinfection By-Products

Peer-Review Draft

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Office of the Report on Carcinogens
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
U.S. Department of Health and Human Services

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of identified substances (i) that either are *known to be human carcinogens* or are *reasonably anticipated to be human carcinogens* and (ii) to which a significant number of persons residing in the United States are exposed. The NTP, with assistance from other Federal health and regulatory agencies and nongovernmental institutions, prepares the report for the Secretary, Department of HHS. The most recent RoC, the 13th Edition (2014), is available at http://ntp.niehs.nih.gov/go/roc.

Nominations for (1) listing a new substance, (2) reclassifying the listing status for a substance already listed, or (3) removing a substance already listed in the RoC are evaluated in a scientific review process (http://ntp.niehs.nih.gov/go/rocprocess) with multiple opportunities for scientific and public input and using established listing criteria (http://ntp.niehs.nih.gov/go/15209). A list of candidate substances under consideration for listing in (or delisting from) the RoC can be obtained by accessing http://ntp.niehs.nih.gov/go/37893.

Background and Methods

Water disinfection is among the most important and beneficial public health advances of the 20th century and has substantially reduced United States incidence of cholera, typhoid, and amoebic dysentery caused by waterborne pathogens (Richardson *et al.* 2007). According to EPA, over 48,000 U.S. public water systems provide disinfected water to more than 250 million people, while 10% to 15% of the U.S. population uses private groundwater wells that are typically not disinfected (EPA 2005, 2015a, 2015b). In addition, swimming pools and spas use on-site chlorination or bromination of water for disinfection. A consequence of the water disinfection process is the formation of a large number of unintended compounds from chemicals and organic material in the water; these unintended chemicals are of potential public health concern (IPCS 2000). Reports have put the number at over 500 chemicals, and identification of more by-products is ongoing. Trihalomethanes make up the largest group by weight (58%) and haloacetic acids the second largest group by weight (36%) of total halogenated disinfection by-products found in public water supplies (Liang and Singer 2003). Two of four U.S. EPA-regulated trihalomethanes, chloroform and bromodichloromethane, are listed in the RoC as *reasonably anticipated to be a human carcinogen*. Over thirty different forms of haloacetic acids are chemically possible, including iodinated and fluorinated forms. Some of these halogen-substituted acetic acids have been identified in drinking water and five are regulated by U.S. EPA (2010).

Background

The Office of the Report on Carcinogens (ORoC) has evaluated mono-, di- and trihaloacetic acids identified in drinking water for possible listing in the RoC. The haloacetic acids evaluated consist of nine chlorine and bromine-containing mono-, di- or trihaloacetic acids either regulated by EPA or being considered for regulation and four iodine-containing acetic acids for a total of 13 haloacetic acids (see Properties section for a list of the HAAs evaluated.). As part of the evaluation, ORoC assessed whether some or all of these chemicals can be considered members of a class of carcinogens or if they should be considered separately. It is known that the type and proportion of haloacetic acids formed differ with different disinfection processes and water sources. In addition, some haloacetic acids in drinking water that are not monitored or regulated may have health consequences. It is important to review the haloacetic acid chemical group for carcinogenicity and identify chemicals that may be cancer hazards as this information can help to inform public health decisions on water regulations and on water disinfection processes.

As per the process for preparation of the Report on Carcinogens (RoC), the Office of the RoC released a draft concept document for "Haloacetic acids (HAAs) Found as Water Disinfection By-Products," which outlined the rationale and proposed the approach for their review, for public comment. ORoC also presented the draft to the NTP Board of Scientific Counselors (BSC) at the April 11, 2016 meeting, which provided opportunity for written and oral public comments. Subsequent to the meeting, the concept was finalized and HAAs were approved by the NTP Director as a candidate substance for review. The concept document is available on the RoC website (https://ntp.niehs.nih.gov/go/790113).

At an Information Group Meeting on HAAs at NIEHS on September 9, 2016, input from scientific experts on water disinfection by-products and cancer mechanisms was requested early

in the review process. The approach to evaluation of individual HAAs and data needs were discussed for evaluation of physicochemical, mechanistic, and cancer endpoints. A "read across" approach (see Section 7 for discussion of this approach) , based on available data for the HAAs (classified as the number of halogen substitutions (e.g., mono-, di- or tri-) or type of halogen substitution (e.g., chlorine, bromine, and iodine), to determine if haloacetic acids could be evaluated as a chemical class, or subclass(es) was considered. Technical advisors for the review of HAAs as Water Disinfection By-products are identified on the "CONTRIBUTORS" page of this monograph.

Public comments on scientific issues were requested at several times prior to the development of the RoC monograph, including the request for information on the nomination, and the request for comment on the draft concept document, which outlined the rationale and approach for conducting the scientific review. In addition, the NTP posted its protocol for preparing the draft RoC monograph on Haloacetic Acids Found as Water Disinfection By-products for public input on the ORoC webpage at (https://ntp.niehs.nih.gov/go/790113) prior to the release of the draft monograph.

Methods for developing the RoC monograph

This RoC monograph on HAAs Found as Water Disinfection By-Products evaluates the available, relevant scientific information and assesses its quality, for each individual HAA or for potential evaluation of the HAAs as a chemical class or subclass, applies the RoC listing criteria to the scientific information, and recommends an RoC listing status (see Figure 1). The monograph also contains draft profiles containing the NTP's preliminary listing recommendation, a summary of the scientific evidence considered key to reaching that recommendation, and data on properties, use, production, exposure, and Federal regulations and guidelines to reduce exposure to haloacetic acids in the public water supply and fromothre potential exposures.

Monograph contents

The process of applying the RoC listing criteria to the body of evidence includes an assessment of the level of evidence from cancer studies in humans and experimental animals on haloacetic acids. In addition, an assessment is made on the available, mechanistic and other relevant data (such as disposition and toxicokinetics) and the final listing recommendations are based on an integration of all the relevant information. A key question is whether the scientific information supports listing haloacetic acids as a class, as subclasses, or as individual haloacetic acids. Read across principles were used in this assessment based on discussions with information groups. In addition, listing in the RoC requires that a significant number of people residing in the United States are exposed to haloacetic acids and the monograph provides information on the relevant exposure information. This information is captured in different sections of the monograph as outlined below.

- Properties (Section 1)
- Human Exposure (Section 2)
- Disposition and Toxicokinetics (Section 3)

- Studies of Cancer in Experimental Animals (Section 4)
- Human Cancer Studies (Section 5)
- Mechanistic and Other Relevant Data (Section 6)
- Evaluation of HAAs as a Class or Subclass (Section 7)
- Overall Cancer Evaluation and Preliminary Listing Recommendation (Section 8).

The latter sections (Sections 7 and 8) of the monograph are informed by the information and assesments of the data reported in the earlier sections, especially Sections 1, 3, 4 and 6 (see Figure 1). The information must come from publicly available sources. The appendices in the RoC Monograph contain important supplementary information, including the literature search strategy, disposition data tables, tables with results of animal studies and/or study quality tables for cancer studies in experimental animals, and results from the mechanism studies (e.g., genotoxicity studies).

Key scientific questions for each type of evidence stream

The monograph provides information relevant to the following questions for each type of evidence stream or section topic. Only one human cancer study on exposure specific to haloacetic acids was identified. The study was summarized and reviewed; however, the data were inadequate to conduct a formal assessment.

Questions related to the evaluation of properties and human exposure information

- What are the physicochemical properties of HAAs and how do they differ with number and type of halogen substitutions?
- Are a significant number of people residing in the United States exposed to haloacetic acids found in disinfected drinking water?
- In what ways can the population be exposed to HAAs?
- What are the levels of HAAs in drinking water and what federal regulations and guidelines limit exposures?
- How is exposure controlled; what remediation methods are used or proposed?

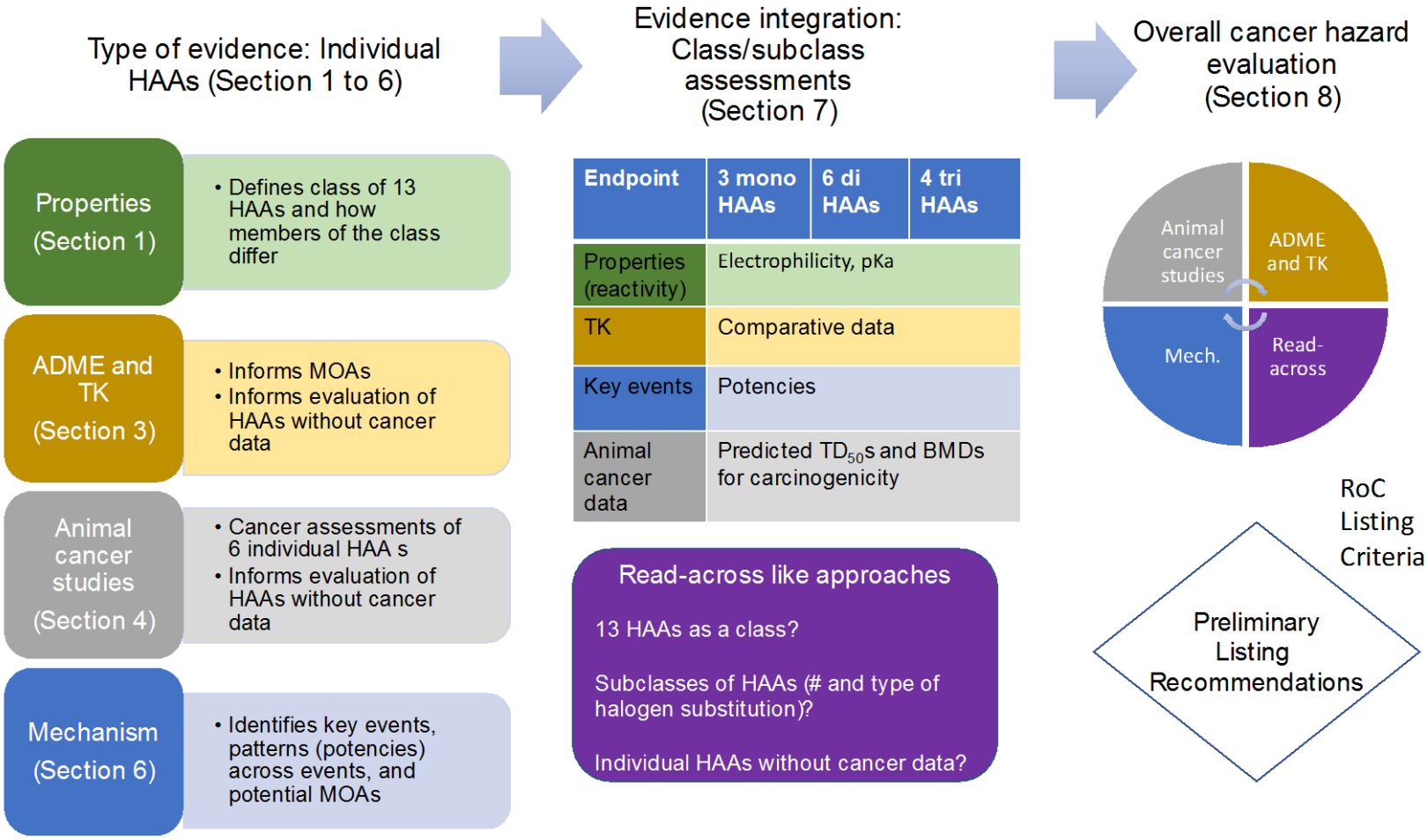

Figure 1. Organization of information in the RoC monograph for HAAs.

BMDs = benchmark doses; HAAs = haloacetic acids; MOAs = mechanisms of action; RoC = Report on Carcinogens; TD50s = chronic dose rate that would induce tumors in half the animals tested; TK = toxicokinetics.

Questions related to the evaluation of disposition and toxicokinetics

- How are HAAs absorbed, distributed, metabolized, and excreted (ADME)?
- What are the primary metabolites? What is their relative distribution in blood and/or urine? What parent compounds or metabolites may have a role in carcinogenesis?
- What are the differences/similarities between humans and experimental animals for ADME?
- Can existing data on ADME or toxicokinetics inform the potential outcomes for HAAs with insufficient data? For example, can information on chlorinated and brominated HAAs be used to predict outcomes for iodinated species?

Questions related to the evaluation of cancer studies in experimental animals

- What is the level of evidence (sufficient or not sufficient) of carcinogenicity of HAAs from animal studies?
- What are the methodological strengths and limitations of the studies?
- What are the tissue sites and are there any trends in tissue sites with number or type of halogen substitutions?

Questions related to the evaluation of mechanistic data and other relevant data

- What are the genotoxic effects due to exposure to HAAs? Does genotoxicity vary by individual HAA or by number or type of halogen substitutions? Do findings from *in vitro* studies correlate with those from *in vivo* studies?
- What are the cytotoxic or toxic effects of individual HAA exposure? Does cytotoxicity or toxicity vary by HAA, such as, by type or number of halogen substitutions?
- What are the major mechanistic modes of action for the carcinogenicity of HAAs?
    - What are the common key steps or potential molecular initiating events of toxicity or carcinogenicity across different HAAs?
    - What factors influence biological or carcinogenic effects?

Questions related to evaluation of HAAs as a class

- Is there evidence that supports grouping HAAs as a class or as a subclass in the assessment?

Methods for preparing the monograph

The methods for preparing the RoC monograph on HAAs are described in the "Haloacetic acids: RoC Protocol," which incorporated a systematic review approach for identification and selection of the literature (see Appendix A), using inclusion/exclusion criteria, extraction of data and evaluation of study quality using specific guidelines, and assessment of the level of evidence for

carcinogenicity using established criteria. Links are provided within the document to the appendices, and specific tables or sections can be selected from the table of contents.

General procedures (See the RoC Protocol for a detailed description of methods.)

Selection of the literature: The preparation of the RoC monograph on HAAs found as water disinfection by-products began with development of a literature search strategy to obtain information relevant to the topics listed above for Sections 1 through 7 using search terms outlined in the Protocol. The approximately 11,600 citations identified from these searches were uploaded to web-based systematic review software for evaluation by two separate reviewers using inclusion/exclusion criteria, and 305 references were selected for final inclusion in the monograph using these criteria. Literature searches were updated to May 2017.

Data extraction and quality assurance procedures: Information for the relevant cancer and mechanistic sections was systematically extracted in tabular format and/or summarized in the text from studies selected for inclusion in the monograph. All sections of the monograph underwent scientific review and quality assurance (QA, i.e., assuring that all the relevant data and factual information extracted from the publications have been reported accurately) by a separate reviewer. Any discrepancies between the writer and the reviewer were resolved by mutual discussion in reference to the original data source.

Evaluation of cancer studies in experimental animals: Evaluation of the potential for biases as well as other elements were assessed based on a series of *a priori* considerations (questions and guidelines for answering the questions), which are available in the protocol (available at https://ntp.niehs.nih.gov/go/790113). Two reviewers evaluated the quality of each study. Any disagreements between the two reviewers were resolved by mutual discussion or consultation with a third reviewer in reference to the original data source. The approach for synthesizing the evidence across studies and reaching a level of evidence conclusion is outlined in the protocol. Level of evidence conclusions were made by applying the RoC criteria (see above) to the body of evidence. Because one of the objectives of the monograph was to determine whether haloacetic acids could be evaluated as a class or subclasses, level of evidence conclusions for carcinogenicity (sufficient, not sufficient) were made after the evaluation of the mechanistic data and reported in the overall evaluation.

Evaluation of mechanistic and other relevant data and approaches for evaluating HAAs as a class or subclasses: As mentioned in the protocol, the mechanistic data were organized by characteristics of carcinogens (such as genotoxicity, oxidative stress, alters energy metabolism, and epigenetic alterations) to help inform the relevant modes of action, identify key events or molecular initiation events or adverse pathways. Mechanistic data and toxicokinetic data are discussed across the haloacetic acids to determine if there are any patterns between effects and the number and type of halogen substitutions. This information (e.g., key events and differences in potency for key events and toxicokinetics across haloacetic acids) as well as information on physical properties, and animal carcinogenicity data were used in a read-across like analysis to determine whether haloacetic acids could be evaluated as a class or subclass (see Figure 1). Details on these methods are reported in Section 7 of this monograph.

Overall evaluation and preliminary listing recommendation: The evidence from the cancer studies in experimental animals was integrated with the assessment of the mechanistic and other relevant data, as well as the conclusions of the assessments of HAAs as a class or subclass (see Figure 1). The RoC listing criteria were then applied to the body of knowledge to reach listing recommendation(s) regarding HAA exposures.

RoC Listing Criteria

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded, OR

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset, OR

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

Contributors

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1 Properties

Water disinfection is among the most important and beneficial public health advances of the 20th century in the United States and worldwide (Calderon 2000). Disinfection of water has substantially reduced the incidence of cholera, typhoid, and amoebic dysentery caused by waterborne pathogens and has contributed to decreases in infectious diseases and increases in life expectancy (Richardson *et al.* 2007). The use of chlorine-based disinfection methods for public water supplies began in the early 1900s in the United States (EPA 2000). Beginning in the 1970s, the formation of disinfection by-products (DBPs) in water due to the reaction between organic precursors in the source water and disinfection agents, primarily chlorine-based, was recognized as a concern (CDC 2016). Since that first discovery, more than 500 unique DBP molecules have been identified, including many different halogen-containing molecules such as haloacids. The two major classes of DBPs by weight are trihalomethanes and haloacetic acids.

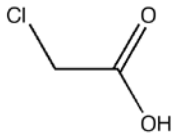
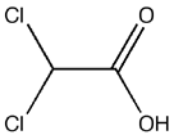
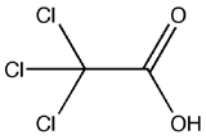
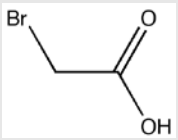
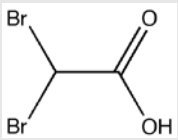
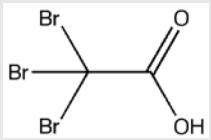
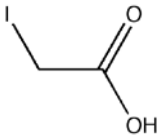
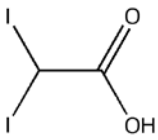
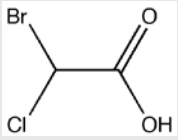
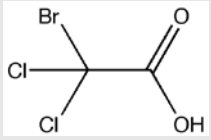
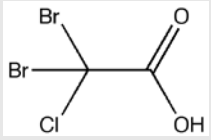
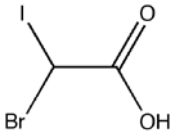
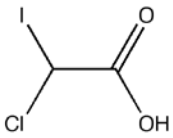
1.1 Haloacetic acids identified in disinfected water

Haloacetic acids all share a common structure with acetic acid as the parent compound, thus these molecules consist of two carbons, including a carboxylic acid and an alpha carbon. A total of 34 different mono- (4 molecules), di- (10 molecules), and trihaloacetic acids (20 molecules) can be formed by replacement of one or more of the 3 hydrogens on the alpha carbon with members of the halogens class, which include fluorine, chlorine, bromine, and iodine. No fluorine-containing haloacetic acids have been identified as water disinfection by-products, likely because the energy required to form these molecules is higher that the oxidation potential of the disinfectants in current use (chlorine, chloramine, ozone, and chlorine dioxide) (Richardson 2016, personal communication to NTP on September 7, 2016). Thirteen haloacetic acids containing chlorine, bromine, or iodine or a combination of these halogens have been identified in disinfected water and these are discussed in this document (Table 1-1).

In 1998, USEPA first regulated the sum of five haloacetic acids (HAA5) in drinking water (chloroacetic acid, bromoacetic acid, dichloroacetic acid, dibromoacetic acid, and trichloroacetic acid) (Federal Register 1998). In 2016, USEPA required monitoring for four additional haloacetic acids (bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, and tribromoacetic acid) in the Fourth Unregulated Contaminant Monitoring Rule (UCMR4) (Federal Register 2016) to encompass a group of haloacetic acids collectively referred to as HAA9.

Four HAAs containing one or more iodine atoms have been detected in treated water, but they are not currently regulated in the United States. Iodoacetic acid and bromoiodoacetic acid were identified in drinking water for the first time by Weinberg *et al.* (2002). In other laboratory studies of water treatment and uses of treated water, Smith *et al.* (2010b) showed that diiodoacetic acid was formed in water treated by point-of-use disinfection with iodine tincture, and Becalski *et al.* (2006) showed that chloroiodoacetic acid was formed from boiling chlorinated tap water with iodized table salt. The 13 HAAs identified in treated water (see Table 1-1) will be discussed in this monograph.

Table 1-1. Structures of 13 haloacetic acids (HAA) present in disinfected water

Halogen	Mono-HAA	Di-HAA	Tri-HAA
Chlorine	Monochloroacetic acid (MCA) 	Dichloroacetic acid (DCA) 	Trichloroacetic acid (TCA) 
Bromine	Monobromoacetic acid (MBA) 	Dibromoacetic acid (DBA) 	Tribromoacetic acid (TBA) 
Iodine	Monoiodoacetic acid (MIA) 	Diiodoacetic acid (DIA) 	
Chlorine and bromine		Bromochloroacetic acid (BCA) 	Bromodichloroacetic acid (BDCA)  Chlorodibromoacetic acid (CDBA) 
Iodine and chlorine or bromine		Bromoiodoacetic acid (BIA)  Chloroiodoacetic acid (CIA) 	

1.2 Physical and chemical properties

The halogens – fluorine, chlorine, bromine, and iodine – are reactive elements that form a family or group of elements in the periodic table; however, fluorine-containing haloacetic acids are not considered further in this monograph as noted in Section 1.1. The presence of halogen atoms affects the reactivity of a haloacetic acid, particularly the reactivity of the alpha carbon to which the halogens are attached and the ionizability of the carboxylic acid. Physical-chemical properties of the halogens that affect the reactivity of the haloacetic acids include electronegativity, polarizability, the physical size of these atoms, and related properties such as the strength of the bond between the halogen and a carbon atom and the potential of a halogen to act as a leaving group.

Electronegativity is defined as the tendency of an atom or functional group to attract electrons from other atoms in a molecule. The electronegativity of the halogens decreases with increasing atomic number moving down the periodic table from chlorine to bromine to iodine. The presence of one or more halogen atoms in a haloacetic acid will affect (1) the strength of the negative charge resulting from ionization of the carboxylic acid, (2) the magnitude of the pKa of the carboxylic acid, and (3) the reactivity of the alpha carbon in a substitution reaction. Thus, the physical-chemical characteristics of each haloacetic acid depends on the type and number of halogen atoms in the molecule.

The toxic potency of the monohaloacetic acids correlates highly with their electrophilic reactivity or alkylating potential and the quality of the halogen as a leaving group (Plewa *et al.* 2004a, Pals *et al.* 2011). The atomic size of the three halogens from smallest to largest is Cl < Br < I, and the length of the carbon-hydrogen bond increases in the same order while the bond dissociation energy decreases (Plewa *et al.* 2004a). The quality of each halogen as a leaving group also increases from chlorine to bromine to iodine and is related to the polarizability of the halogen atom (i.e., the ability of the electrons in the atom to distort or shift due to external influences) and delocalization of the electron cloud (i.e., the spatial distribution of electrons shared among the atoms in a molecule). Both polarizability and delocalization are highest for iodine among the halogens.

An important substitution reaction mechanism in organic chemistry consists of the breaking of one bond and the simultaneous formation of another between reacting molecules. This mechanism is described as “substitution, nucleophilic, with 2 molecules in the rate-determining step” and is commonly abbreviated as S_N2. The S_N2 reactivity of the monohaloacetic acids increases from chloride to bromide to iodide (Plewa *et al.* 2004a).

Three physical-chemical properties likely to be related to the toxicity of the HAAs because they describe the ability of the molecules to enter cells and their potential reactivity with other molecules within a cell are the octanol-water partition coefficient (log P), the negative log of the acid dissociation constant (pK_a), and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) (Table 1-2). At physiological pH, all of the haloacetic acids will exist in their ionized form, but the pK_a also indicates the strength of the acid form which increases as pK_a decreases. The relationship between these physical-chemical properties and the toxicity and potential mechanisms of carcinogenicity of haloacetic acids is discussed further in Section 5.

Table 1-2. Properties of haloacetic acids

Haloacetic acid	CAS No.	Formula	Molecular weight	Solubility in water (g/100 mL)[a]	Vapor pressure (mm Hg)[a, b]	Dipole moment (Debye units)[c]	Octanol-water partition coefficient (log P)[d]	Dissociation constant (pK_a)[d]	Energy of lowest unoccupied molecular orbital, E_{lumo} (eV)[d]
Chloroacetic acid	79-11-8	$C_2H_3ClO_2$	94.5	85.8	0.065	2.716	0.22	2.97	4.54
Bromoacetic acid	79-08-3	$C_2H_3BrO_2$	138.9	9.4	0.119	2.722	0.41	2.96	4.47
Iodoacetic acid	64-69-7	$C_2H_3IO_2$	185.9	–	0.03	1.794	0.85	2.95	2.88
Dichloroacetic acid	79-43-6	$C_2H_2Cl_2O_2$	128.9	100 (at 20°C)	0.179	1.284	0.92	1.41	3.07
Chlorobromoacetic acid	5589-96-8	$C_2H_2BrClO_2$	173.4	25	0.14	–	0.61	1.4	3.11
Dibromoacetic acid	631-64-1	$C_2H_2Br_2O_2$	217.8	211	0.023	1.048	0.7	1.39	2.76
Chloroiodoacetic acid	53715-09-6	$C_2H_2ClIO_2$	220.4	–	–	–	1.2	1.47	1.54
Bromoiodoacetic acid	71815-43-5	$C_2H_2BrIO_2$	264.8	–	–	–	1.4	1.67	1.6
Diiodoacetic acid	598-89-0	$C_2H_2I_2O_2$	311.8	–	–	2.163	1.3[e]	–	–
Trichloroacetic acid	76-03-9	$C_2HCl_3O_2$	163.4	4.4	0.06	1.564	1.33	0.66	2.79
Dichlorobromoacetic acid	71133-14-7	$C_2HBrCl_2O_2$	207.8	0.49	0.036	–	1.53	0.05	2.82
Dibromochloroacetic acid	5278-95-5	$C_2HBr_2ClO_2$	252.3	0.24	0.0052	–	1.62	0.04	2.47
Tribromoacetic acid	75-96-7	$C_2HBr_3O_2$	296.7	20	0.00028	1.552	1.71	0.03	2.42

[a]Reported at 25°C (298.15 K) unless noted otherwise.
[b]PubChem 2017, except chloroacetic acid, bromoacetic acid, and dichloroacetic acid from ChemIDplus 2017.
[c]Pérez-Garrido *et al.* 2008.
[d]Stalter *et al.* 2016 unless noted otherwise.
[e]PubChem 2016.

2 Human Exposure

A significant number of people living in the United States are exposed to haloacetic acids formed as water disinfection by-products because of the widespread use of chorine-containing disinfectants for water treatment. As noted in the previous section, water disinfection in the United States and worldwide has provided major public health benefits through decreases in infectious diseases and increases in life expectancy that result from providing safe, clean drinking water.

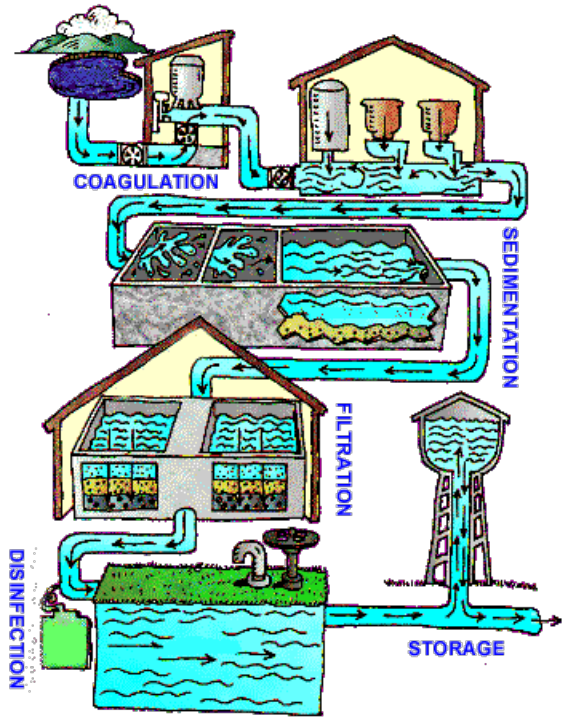
According to the U.S. Environmental Protection Agency (EPA), over 48,000 U.S. public water systems provide disinfected water to more than 250 million people, while 10% to 15% of the U.S. population uses private groundwater wells that are typically not disinfected (EPA 2005, 2015a, 2015b). Thus, the majority of the U.S. population is exposed to mono-, di- and trihaloacetic acids found as water disinfection by-products. Ingestion of chlorinated drinking water is the most common exposure route for haloacetic acids (HAAs) for the general public, but inhalation and dermal exposure also can occur. Other potential sources of exposure to HAAs for the general public include consumption of beverages prepared with treated water either in the home or commercially, consumption of food prepared with treated water, accidental ingestion of swimming pool or spa water by heavy swimmers or spa users, and in some limited circumstances from point-of-use disinfection. Information on potential occupational exposure to HAAs is limited, but exposure to swimming pool attendants at indoor and outdoor pools has been documented.

2.1 Water treatment and formation of disinfection by-products

The presence of HAAs in the United States is well established, but knowledge of the chemical and physical processes that lead to their formation is important to help control their levels as required by law. Source water, either groundwater or surface water, contains organic carbon that reacts with chlorine-based disinfectants. Elevated levels in source water of bromide and iodide from anthropogenic and natural sources will likely shift production of HAAs during water disinfection toward more brominated and iodinated species. Minimizing the content of HAAs in finished water provided to consumer is an important goal, and the best current strategy for remediation of HAAs is prevention or reduction of their production. This generally involves reducing the potential precursors present as organic carbon in the source water, but manipulation of conditions for disinfection, such as choice of disinfectant, dose, contact time with water, temperature, and pH can also be effective means to reduce formation. Research into the chemical reactions and intermediate molecules formed also contributes to greater understanding of the process of formation and how to control it.

Water disinfection is regulated by the U.S. EPA through Surface Water Treatment Rules (SWTRs) that established maximum contaminant level goals (MCLGs) for viruses, bacteria, such as *Legionella*, and other organisms such as the protozoa species *Giardia lamblia* and *Cryptosporidium*. The purpose of water treatment is to remove contaminants and disease-causing agents from drinking water (CDC 2015). The most common steps in conventional water treatment are (1) coagulation and flocculation, (2) sedimentation, (3) filtration, (4) disinfection, and (5) storage (see Figure 2-1) (CDC 2015, EPA 2016a). In disinfection, application of oxidants (chlorine, chloramine, chlorine dioxide, or ozone) or ultraviolet (UV) light kills disease-causing

microorganisms or renders them inactive. Reverse osmosis is another non-chemical water purification method, but its primary use is at the household level rather than by community water treatment facilities other than at desalination plants.



Coagulation and flocculation	Addition of chemicals to source water to allow particles to bind together and form larger particles called floc
Sedimentation	Transfer of floc particles to basins where they either settle to the bottom or are removed by skimming
Filtration	Passage of water through porous media to remove particles remaining from sedimentation
Disinfection	Application of oxidants
Storage	Holding of treated water in a tower or tank to allow time for disinfection to occur

Figure 2-1. Conventional water treatment flow diagram

Sources: CDC 2015, EPA 2016a

2.2 Factors that affect formation of disinfection by-products

The factors (see Figure 2-2) that determine the type and amount of disinfection by-products formed during water treatment include (1) the presence of organic matter and inorganic matter in the source water, (2) the disinfecting chemicals used, and (3) the length of time the organic matter is exposed to the disinfecting chemicals, the temperature at which the disinfection process takes place, and the pH of the water during the disinfection process. The organic molecules in source water are often extremely large, complex molecules and intermediate molecules will form as a result of exposure to disinfecting chemicals; further reaction with those chemicals during the disinfection process and storage will result in formation of halogenated by-products, including haloacetic acids.

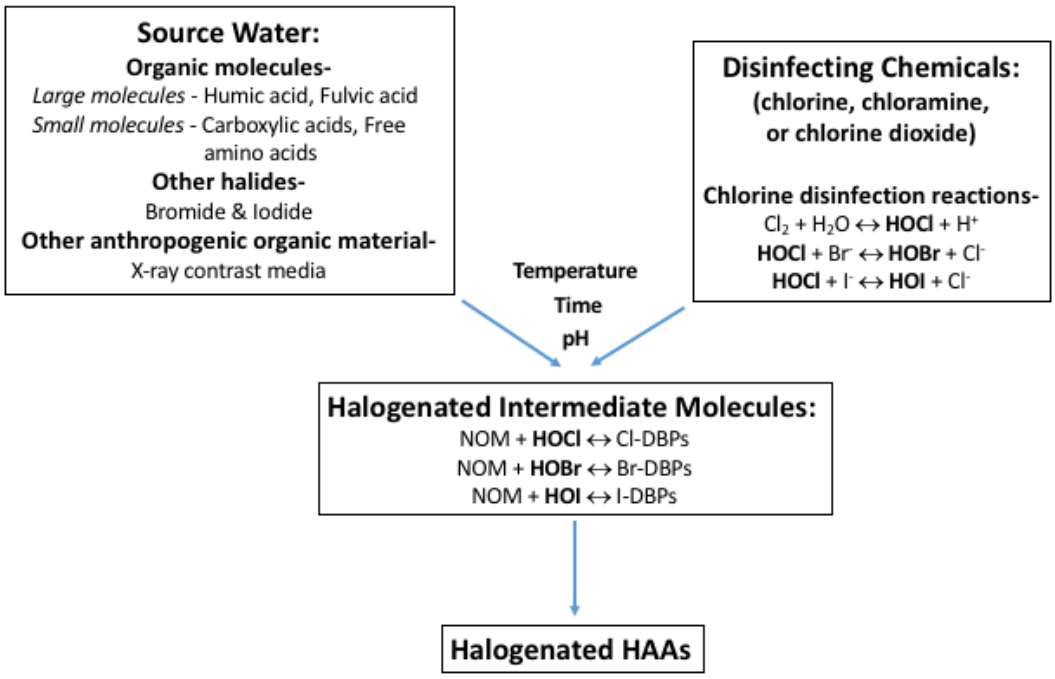


Figure 2-2. Major factors affecting the formation of halogenated disinfection by-products

Organic molecules in source water plus naturally occurring or anthropogenic bromide and iodide react with various chlorine-containing disinfecting chemicals to form halogenated intermediate molecules and ultimately the halogenated HAAs. HOBr = hypobromous acid; HOCl = hypochlorous acid; HOI = hypoiodous acid; NOM = natural organic matter.

2.2.1 Characteristics of source water

The major characteristic of the source water that determines the formation of disinfection by-products is the type and quantity of potentially reactive natural organic matter (NOM) and the inorganic halogen precursors, bromide and iodide. The organic matter found in either surface or groundwater consists of a mixture of organic compounds derived from sources such as terrestrial plants, microbially derived from algae and bacteria, or from anthropogenic sources. The latter class includes pesticides, pharmaceuticals, personal care products, and newer materials such as carbon nanotubes (Nelson 2015). The molecules of NOM in source water that react with chloride, bromide, and sometimes iodide to form HAAs range from very complex, e.g., humic and fulvic acids, to simple amino acids and dicarbonyl acids (Reckhow and Singer 1985, Reckhow *et al.* 2001, Zhai and Zhang 2011).

Anthropogenic and natural sources of bromide and iodide (see Table 2-1) can increase concentrations of these halide ions in source waters (e.g., due to incomplete removal or non-removal in wastewater treatment plants) and create brominated and iodinated HAAs and other disinfection by-products such as trihalomethanes and bromate (McTigue *et al.* 2014). Elevated levels of bromide and iodide in source water will likely shift production of HAAs during water disinfection toward brominated and iodinated species.

Table 2-1. Effects of sources of bromide and iodide in source water on HAA formation

Source	Effect on bromide or iodide ion concentration	Effect on disinfection by-product formation	Reference
Coal-fired electric power plants	Increased bromide ion concentration power plant wastewater discharges	Increased brominated HAA species[a]	McTigue *et al.* 2014
Oil and gas production	Bromide ion concentration increased from < 0.02 mg/L [< 20 µg/L] upstream of oil and gas CWT[b] to 75 mg/L [75,000 µg/L] downstream of CWT	Downstream concentration of DBCNM[c] ranging from 5.7–8.7 µg/L	Hladik *et al.* 2014[d]
	Increased bromide ion concentration in publicly owned treatment works and CWT discharges	Increased brominated disinfection by-product formation[a]	Hammer and VanBriesen 2012
Seawater intrusion	Bromide concentration increased from 38 µg/L to 974 µg/L as seawater content increased from 0% to 2%	HAA9 concentration increased from 39 µg/L to 75 µg/L and disinfection by-product formation shifted from chlorine-containing to bromine-containing species	Ged and Boyer 2014[e]
Seawater desalination	Saudi Arabia, Red Sea coast: Bromide concentration in seawater = 60 mg/L [60,000 µg/L]; iodide concentration = 0.05 mg/L [50 µg/L]	Reported HAA9 concentration range in chlorinated seawater = 5.35–6.86 µg/L	Kim *et al.* 2015
	United States, Tampa Bay: Bromide concentration range in seawater = 49 mg/L–56 mg/L [49,000 µg/L–56,000 µg/L]	Reported HAA5 concentration range in chlorinated seawater = 69–175 µg/L	
Iodinated x-ray contrast media	Various iodinated x-ray contrast media (iopamidol, iohexol, and iopromide) dissolved in raw river water at concentrations ranging from 3,880 µg/L–4,100 µg/L[f]	Iodoacetic acid concentrations ranged from 0.5 nM to 9.6 nM [0.09–1.8 µg/L]	Duirk *et al.* 2011[e]

[a]No quantitative data on increases in brominated HAA data were reported.
[b]CWT = commercial wastewater treatment plant.
[c]DBCNM = dibromochloronitromethane.
[d]No data on finished water haloacetic acids were reported.
[e]Laboratory chlorination study.
[f]Concentration reported as 5 µM for all contrast media.

2.2.2 Characteristics of disinfection methods

The choice of disinfection methods is generally based on their effectiveness, including their continued presence as a secondary disinfectant in the water distribution system, their overall cost, and their ease of use by water treatment facilities, but chlorine-containing chemical disinfection is by far the most widely used approach in the United States. The major disinfection methods, i.e., chlorine as either a gas (Cl_2), liquid (NaOCl), or solid ($Ca(OCl)_2$) form; chloramine; chlorine dioxide; ozone; and ultraviolet (UV) irradiation are generally effective as a primary disinfectant. A comparison of the major factors diffentiating these disinfection methods is provided in Table 2-2.

Table 2-2. Comparison of water disinfection methods

Disinfectant	Efficacy as primary disinfectant	Residual disinfection in distribution system	Total HAAs formation	Other DBPs formed	Ease of use; cost
Chlorine (Cl_2, HOCl, OCl^-)	High to intermediate	Yes	High	THMs, HANs[a]	Easiest method to use for HOCl/OCl^-; least expensive
Chloramine (NH_2Cl)	Intermediate	Yes (more effective that chlorine alone)	Intermediate (~1/3 level formed with chlorine)[b]	HANs[a]	Requires additional equipment to add ammonia; slightly more expensive than chlorine alone
Chlorine dioxide (ClO_2)	High to intermediate	No	Intermediate (less than 1/2 level formed with chlorine)[b]	Chlorite & chlorate (ClO_2 breakdown products)	Requires more technical skill & requires secondary disinfection; more expense for equipment and chemicals
Ozone (O_3)	High	No	Low (generally only in presence of Br^-)	Bromate	Requires more technical skill & requires secondary disinfection; more expense for equipment and chemicals
UV irradiation	High	No	No	No	Requires more technical skill and training & requires secondary disinfection; more expense for equipment and chemicals

HAN = haloacetonitrile; TOC = total organic carbon.
[a] IPCS 2000
[b] Zhang *et al.* 2000.

2.2.3 Effects of time, temperature, pH, and other factors on formation of HAAs

The initial reaction of chlorine with NOM results in rapid formation of HAAs during approximately the first 4 to 8 hours with approximately 90% of the final concentrations of TCA and DCA formed during the first 24 hours after chlorine is added to water. Formation of dibromoacetic acid may not level off until 18 to 20 hours after chlorination begins. In general,

the formation rates for HAAs increase with increasing temperature. Effects of pH on HAA formation can vary, depending on which chlorine species (i.e., hypochlorous acid or hypochlorite ion) predominates. For example, the more active form of chlorine, hypochlorous acid, is present at higher concentrations below pH 7.5 than above. Thus, increasing pH has been associated with decreasing concentrations of HAAs (IPCS 2000). The water disinfection process can be described by the product (CT) of chlorine concentration (C) times contact time (T) (SDWF 2009). Chlorine must be added until competing pathways with reducing compounds in the source water and the combination of chlorine with other molecules are saturated with chlorine and a free chlorine residual is present at what is referred to as the breakpoint for chlorination. Beyond the breakpoint, the remaining free chlorine will disinfect the water and provide a residual for secondary disinfection during storage and distribution.

2.2.4 Chemistry of formation of haloacetic acids during water disinfection with chlorine-containing disinfectants

Proof of the concept that interaction of chlorine-containing disinfectants with natural organic substances in source water can result in formation of HAAs has been achieved by laboratory experiments demonstrating formation of halogenated intermediate molecules that give rise to HAAs from samples of humic acid. Reaction pathways of the reactive forms of chloride with organic matter include oxidation, addition, and electrophilic substitution reactions (Deborde and von Gunten 2008), and most reactions between chlorine and humic acids within NOM result in oxidation of humic acids rather than chlorine substitution (Dickenson *et al.* 2008).

Research on formation of HAAs and other disinfection by-products supports the formation of a number of potential intermediate molecules from precursors in natural organic matter in source water (Reckhow and Singer 1985, Reckhow *et al.* 2001, Zhai and Zhang 2011). These smaller intermediate molecules, mostly organic acids (but also substituted phenolic compounds), can give rise to HAAs (Dickenson *et al.* 2008, Bond *et al.* 2012)

2.3 Remediation of HAAs

Remediation of haloacetic acid disinfection by-products can be divided into three general approaches: (1) removal of precursors (i.e., NOM) prior to disinfection, (2) modification of disinfection practices (e.g., altering disinfectant dose, type, or application point in the water treatment process), and (3) removal of disinfection by-products after formation. Table 2-3 summarizes potential methods for remediation of HAAs before, during, and after the water treatment process.

Table 2-3. Methods for remediation of HAAs

Remediation process	Method description	Effectiveness/Comments
Removal of precursors prior to disinfection		
Coagulation	Addition of metallic salts, e.g., alum, that neutralize negative charges on particles so they agglomerate and precipitate to remove precursors	15%–78% removal of precursors by alum coagulation; (typically15%–20%)
Ion exchange	Exchange of ions between aqueous solution and solid phase, e.g., resin, to remove precursors	52%–72% removal of precursors 52%–80% removal in combination with alum coagulation
Membrane filtration	Size exclusion, electrostatic repulsion, and differences in solute diffusion rates across membranes to remove precursors	67%–99% removal by nanofiltration, which is most effective for removal of hydrophilic, low-molecular weight precursors
Activated carbon filtration	Reversible physical adsorption by non-specific forces with preferential removal of hydrophobic NOM	60%–91% removal of precursors by granular activated carbon (GAC)
Biotreatment (Biologically Activated Carbon)	Enzyme-controlled microbial degradation and adsorption involving growth of a biofilm on sand or activated carbon filter media	Up to 62% removal by bioactive sand; TCA precursors more biodegradable than DCA precursors
Advanced oxidation processes (AOPs)	*In situ* generation of highly reactive hydroxyl radicals to degrade precursors through fast, non-selective reactions with organic compounds. May include ozone plus UV, ozone plus hydrogen peroxide, or UV plus hydrogen peroxide	Up to 83% removal by O_3/UV Up to 85% removal by O_3/H_2O_2 with biologically activated carbon
Ozone	Oxidation, bond cleavage, and hydroxyl radical reactions preferentially with aromatic compounds, alkenes, and amines	Relatively ineffective at low ozone concentrations At higher concentrations may increase HAA levels
Modified disinfection practices	Eliminating pre-oxidation or changing the pre-oxidation chemical, e.g., using potassium permanganate, hydrogen peroxide, or ozone as a pre-oxidant rather than pre-chlorination Alternative disinfectants, such as chloramines, chlorine dioxide, ozone, or UV irradiation	No data found on percent effectiveness Alternative methods, particularly ozone and UV, do not leave a disinfectant residual in the distribution system
Removal of HAAs after formation	Filtration using biologically active granular activated charcoal	Up to 99% at early stage of operation (via physical adsorption) but decreased over 3.5 month time period; removal again increased up to 99% after 6 months (via biodegradation) Removal of precursors is greatest when temperatures are high and residual chlorine concentration is low.

Source: Singer *et al.* 2002, Kim and Kang 2008, Bond *et al.* 2011.

2.4 Formation in swimming pools and spas

The disinfection of water for swimming pools and spas also results in formation of HAAs but often at higher levels than in disinfected tap water because of the use of a higher chlorine residual and higher temperatures than in typical water distribution systems (Parinet *et al.* 2012, Chowdhury *et al.* 2014). Dichloroacetic acid and trichloroacetic acid are the most abundant HAAs detected in swimming pools (Teo *et al.* 2015). For U.S. swimming pools disinfected with chlorine, dichloroacetic acid concentrations have been reported to range from 52 μg/L to 6,800 μg/L, and trichloroacetic acid concentrations have been reported to range from 76 μg/L to 1,900 μg/L (Kanan 2010). Additional human precursors (e.g., sweat, urine, hair, cosmetics) can affect the speciation of disinfection by-products formed in swimming pools (Richardson and Postigo 2015). The levels of HAA9 in seawater swimming pools treated with chlorine bleach as disinfectant ranged from 417 μg/L to 2,233 μg/L for different pools tested (Parinet *et al.* 2012). However, the levels of individual HAAs were generally highest for brominated HAAs, i.e., bromoacetic acid, bromochloroacetic acid, dibromoacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid, consistent with the presence of bromide in sea water.

2.5 Point-of-use disinfection

Most laboratory studies of disinfection by-product formation from point-of-use disinfection report trihalomethane occurrence data; only limited data were identified for HAA formation (Lantagne *et al.* 2008, Lantagne *et al.* 2010, Smith *et al.* 2010b, Werner *et al.* 2016). Iodoacetic acid levels of 9.7 nM to 22.9 nM [1.8 to 4.3 μg/L] have been reported from use of iodine tincture; bromoiodoacetic acid and diiodoacetic acid were also detected, but were not quantified because they were below method detection limits (Smith *et al.* 2010b).

2.6 Other uses of haloacetic acids

Several haloacetic acids have had limited uses medically or in research laboratories; the more extensive commercial uses are listed below. Dichloroacetic acid is used as a chemical manufacturing intermediate (e.g., for glyoxylic acid), in polyethylene terephthalate production, as a skin cauterizing agent, as a medicinal disinfectant (e.g., a substitute for formalin), as a treatment for congenital lactic acidosis, and it has been proposed as a targeted cancer therapeutic agent (IARC 2014a). The main use of trichloroacetic acid in the past was as an herbicide; however, all registrations for this use in the United States were voluntarily canceled by 1992 (some existing stock may have been used after that date). Trichloroacetic acid also has other industrial uses (e.g., surface treatment of metals), and is widely used as a laboratory reagent and as a treatment for dermatological diseases. Chloroacetic acid is used in the manufacture of organic chemicals including cellulose ethers (used mainly for drilling muds, detergents, food, and pharmaceuticals), glycine, thioglycolic acid, dyes, synthetic caffeine, and as a post-emergence contact herbicide and defoliant (PubChem 2017). Tribromoacetic acid has been used in organic synthesis (HSDB 2009). Diiodoacetic acid has been used as a chemical intermediate. Bromoacetic acid has been used for organic synthesis and abscission of citrus fruit (HSDB 2009). Iodoacetic acid has been used as a food additive and as an intermediate in pharmaceuticals, herbicides, antipyretic, anti-inflammatory, and analgesics (HSDB 2003). Dibromoacetic acid and bromochloroacetic acid were reported to be used only in research (IARC 2013c).

2.7 Exposure to HAAs

Exposure to HAAs as disinfection by-products affects almost all people living in the United States because the vast majority of water treatment facilities use chlorine-based disinfection methods due to their ease of use and low cost. In addition to ingesting HAAs by drinking plain tap water, humans can also be exposed to HAAs from other beverages prepared with treated water such as tea or coffee or fruit drinks and soft drinks or by ingesting food that came in contact with treated water. Foods, both canned and fresh, are generally washed with or soaked in treated water and are cooked in treated water. Dermal and inhalation exposure from swimming pools and spas where water is disinfected and from occupational exposure also can occur. Occupational exposures to some HAAs can also occur.

Eleven of the 13 HAAs discussed in this monograph have been identified in disinfected water (see Section 2.3.1); the remaining two are iodinated molecules formed under experimental conditions. Both HAAs and total trihalomethanes (TTHMs) are regulated by the USEPA and epidemiological studies have tended to use TTHMs as a surrogate for disinfection byproduct exposures and HAAs are generally correlated with TTHMs. Relevant epidemiological studies measuring internal exposure to HAAs are not available, in part because of the lack of a specific marker.

2.7.1 Occurrence of haloacetic acids in treated water

The highest levels of HAAs have been detected for the molecules chloroacetic acid, dichloroacetic acid, trichloroacetic acid, chlorobromoacetic acid, and bromodichloroacetic acid (Table 2-4). The presence of chlorine atoms in these molecules is expected since chlorination is overwhelmingly the most commonly used water disinfection method in the United States and estimates indicate that about 98% of U.S. water treatment systems use some type of chlorine disinfection process such as chlorine, chlorine dioxide, or chloramine (American Chemistry Council 2016). As described above, a number of sources (Table 2-1) for increased bromide concentrations in source water have been identified.

National occurrence data from the American Water Works Association (AWWA) for HAA5 (the sum of five HAAs (bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, and trichloroacetic acid) for 1997 through 2014 and representing 93% of U.S. systems serving populations greater than 100,000 people (collected to assess impacts of the USEPA Stage 2 Disinfectants and Disinfection By-products Rule [DBPR]) indicate that 95th percentile HAA5 concentrations have displayed a generally decreasing trend since 2000 (largely due to plants switching from chlorine to chloramines for disinfection) and have been at or below the USEPA maximum contaminant level (MCL) of 60 µg/L for HAA5 since 2004 (Seidel *et al.* 2017). Figure 2-3 presents this information. There is some evidence that smaller facilities have more difficulty in meeting the regulatory limits. Data from the USEPA collected under the Safe Drinking Water Act (SDWA) national compliance data for the third Six-Year Review (SYR3) for 2006 to 2011 indicated that systems serving fewer than 10,000 people had a 95th percentile HAA5 concentration that remained above the HAA5 60 µg/L MCL throughout the period studied whereas the 95% percentile was below the MCL for systems serving more than 100,000 people (see Figure 2-4). In addition, the 10 highest levels reported for HAA5 from data from the Environmental Working Group (EWG 2016) were facilities serving fewer than 4,000 persons.

Table 2-4. Concentration ranges for mono-, di-, and trihaloacetic acids in tap water, finished drinking water, and other similar sources

Mono, di-, or trihaloacetic acid	Range (µg/L)	Reference
Chloroacetic acid	3 (1–11)[a]	EPA 2016b[b]
Bromoacetic acid	1.6 (0.59–7.3)[a]	EPA 2016b[b]
Iodoacetic acid	Up to 1.7	Richardson *et al.* 2008
Dichloroacetic acid	10.4 (1.3–32)	EPA 2016b
Chlorobromoacetic acid	BDL–18	HSDB 2009a, IARC 2013c
Dibromoacetic acid	2.1 (0.63–12)	EPA 2016b
Chloroiodoacetic acid	–[d]	–
Bromoiodoacetic acid	Up to 1.4[b]	Richardson *et al.* 2008
Diiodoacetic acid	–[d]	–
Trichloroacetic acid	8 (1.1–32)	EPA 2016b
Bromodichloroacetic acid	5.28–12.2	HSDB 2009b
Chlorodibromoacetic acid	BDL–5.37	HSDB 2009d
Tribromoacetic acid	0–approx. 10[ej]	McGuire *et al.* 2002

[a]Median (5th percentile–95th percentile).
[b]Third Six-Year Review Information Collection Request dataset, https://www.epa.gov/dwsixyearreview/six-year-review-3-compliance-monitoring-data-2006-2011.
[c]Below detection limit; detection limit not specified.
[d]No data identified.
[e]One extreme value of ~ 20 µg/L was reported.

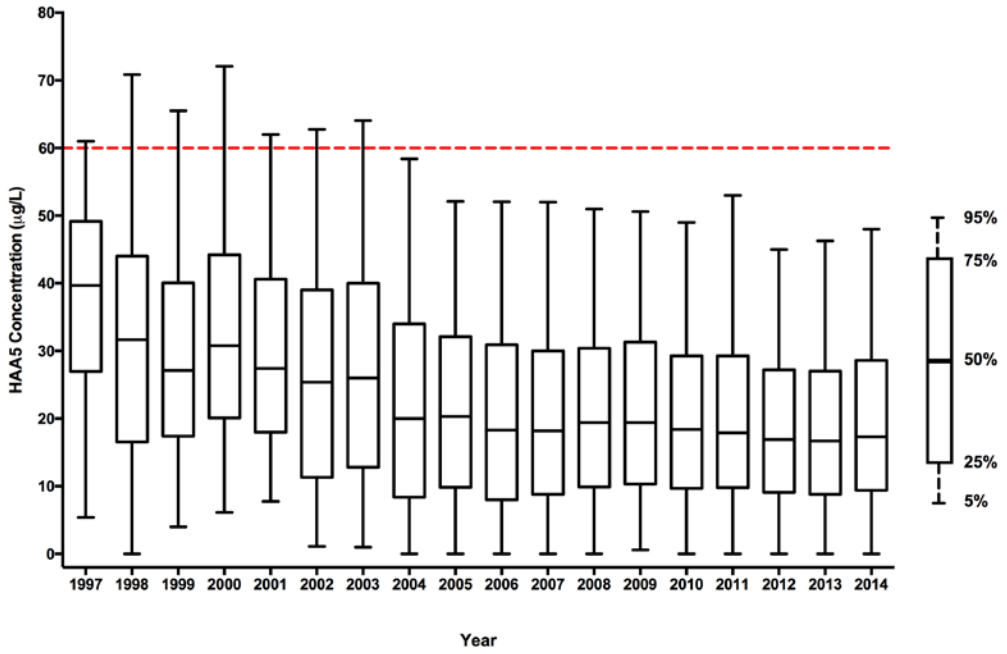


Figure 2-3. National HAA5 occurrence data for 1997 through 2014

Source: Seidel *et al.* 2017.
Data are presented for 5th, 25th, 50th, 75th and 95th percentiles for each year from 1997 to 2014 for U.S. water systems supplier more than 100,000 people.
Dashed horizontal line = HAA5 maximum contaminant (MCL) of 60 µg/L.

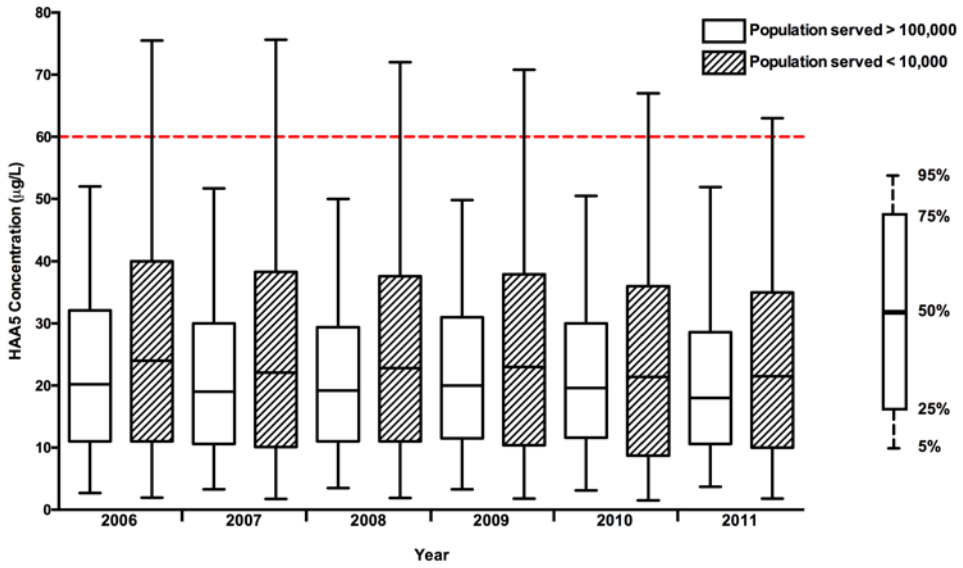


Figure 2-4. HAA5 occurrence data for 2006 through 2011 from USEPA Safe Drinking Water Act (SDWA) national compliance monitoring for the third Six-Year Review (SYR3)

Source: EPA 2016b.
Data are presented for 5th, 25th, 50th, 75th and 95th percentiles for each year from 1997 to 2014 for U.S. water systems supplier more than 100,000 people.
Dashed horizontal line = HAA5 maximum contaminant (MCL) of 60 µg/L.

2.7.2 Correlation of haloacetic acids and trihalomethanes in treated water

In the majority of reported studies, total HAAs (THAA) have been found to correlate with total trihalomethanes (TTHM); correlation coefficients ranging from ~0.6 to 0.92 in the majority of studies although a few studies found lower rates, mostly between 0.4 and 0.6 with one outlier of 0.1 (see Figure 2-5). While TTHMs and THAAs or HAA5 have been the primary surrogate measure used in epidemiological studies to estimate exposure to disinfection by-products, concern has been raised for how well these substances can represent the hundreds of disinfection by-products in treated water (Parvez *et al.* 2011, Plewa and Wagner 2015). No data were identified for correlations between either THMs or HAAs and other disinfection by-products. However, THMs and HAAs are expected to be inversely related to some emerging disinfection by-products (e.g., *N*-nitrosodimethylamine [NDMA] and iodinated disinfection by-products) because chloramination maximizes their formation and vastly reduces the formation of THMs and HAAs.

The ratio of HAAs to THMs is usually around 1:1 but can be higher or lower depending on source water characteristics and disinfection conditions (e.g., Singer *et al.* [2002] reported that lower chlorination pH tends to favor HAA9 formation while higher pH favors THM formation) (Roberts *et al.* 2002, Singer *et al.* 2002, Weinberg *et al.* 2002, Liang and Singer 2003, Villanueva *et al.* 2003, Krasner *et al.* 2006, Chang *et al.* 2010a, Roccaro *et al.* 2014). Individual studies with different ratios between HAAs and THMs suggest as much as a 4-fold spread for the

ratio (i.e., ranging from 2:1 for THAA to TTHM [Villanueva *et al.* 2003] to 1:2 for the same comparison [Roccaro *et al.* 2014]).

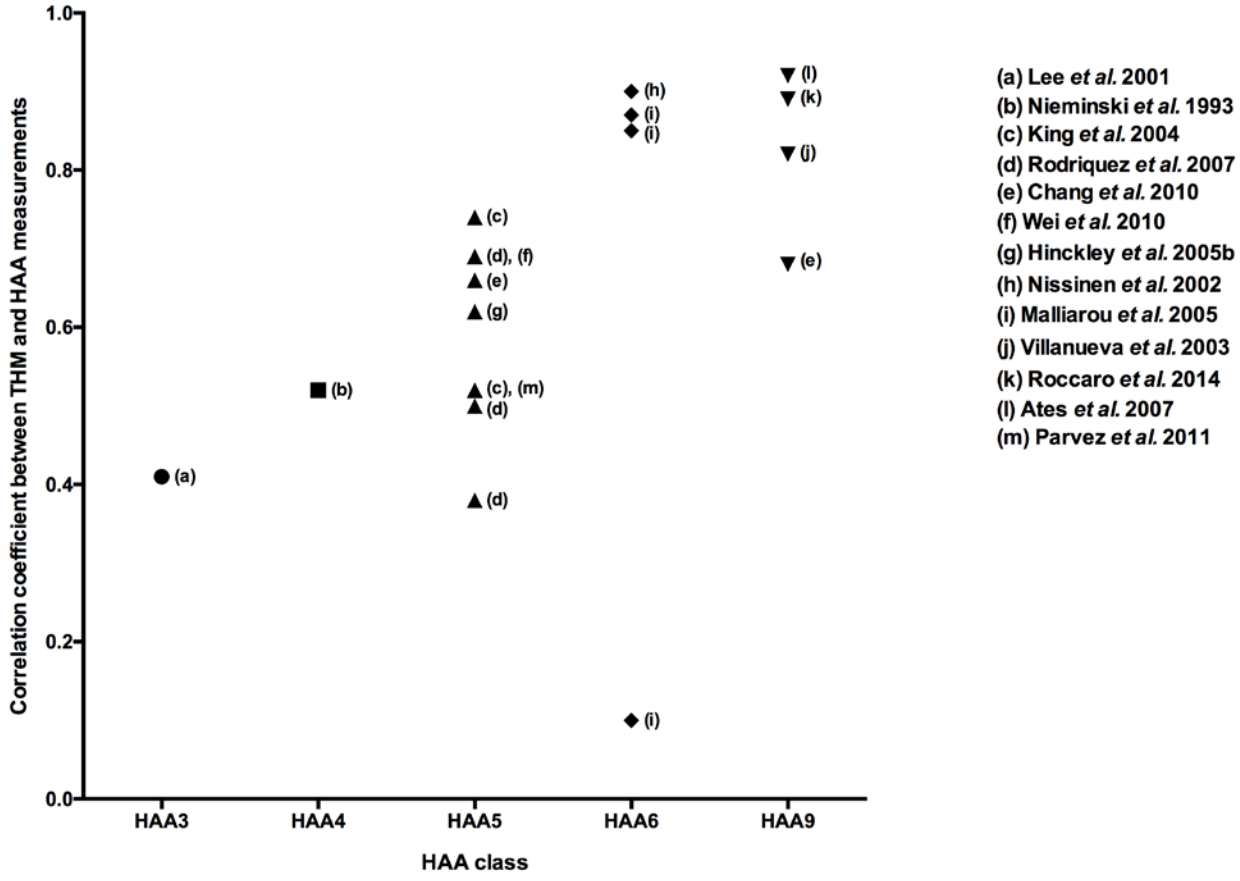


Figure 2-5. Correlation data for HAAs and trihalomethanes in treated water

2.7.3 Potential exposure from beverages prepared with treated water

Beverages that are prepared in the home, such as tea, coffee, or infant formula, or commercially, such as fruit juices and soft drinks, may be prepared with treated water. Several HAAs, primarily dichloroacetic acid, chloroacetic acid, dibromoacetic acid, and bromochloroacetic acid, are quite stable in boiling water with losses of less than 20% after 60 minutes (Raymer and Michael 2010), so home-prepared tea or coffee would have levels of HAAs similar to those in tap water. Cardador and Gallego (2015) measured levels of 10 HAAs in 2 100% juice products (0.07 to 0.08 μg/L), 37 reconstituted juices (mean = 4.5; 0.5 to 31 μg/L), 32 nectar juices (mean = 6.7; 0.2 to 22 μg/L), and 55 soft drinks (mean = 12; 0.3 to 73 μg/L) sold in Spain. The mean for soft drinks was increased by inclusion of tonic water (mean = 40.2 μg/L) and soda water (mean = 33.5 μg/L) which had higher levels than other drinks sampled.

2.7.4 Potential exposure from foods

HAAs may be present in natural foods in relatively low amounts, but preparation of food by rinsing before or after cooking or cooking in treated water may add to the levels. Another

potential source of HAAs is reaction of iodide in iodized table salt with chlorine in tap water to form hypoiodous acid (HOI), which can react with residual organic matter in tap water and organic matter from food to form iodinated disinfection by-products (Becalski *et al.* 2006, Pan *et al.* 2016).

Solid and liquid phases of canned vegetables can contain disinfection by-products from contact with treated water and chemicals used in the canning process (e.g., washing, sanitizing, blanching, and filling with sauces or brine solutions) (Cardador and Gallego 2016). HAAs have been found to be predominant in the liquid phase, which is consistent with their ionic and non-volatile characteristics.

A limited range of six foods — chicken, three vegetables, dried beans, and pasta — were cooked in purified water, which would not be expected to contain disinfection by-products, and analyzed for nine HAAs; all six foods contained dichloroacetic acid (31 to 100 ng/g) and trichloroacetic acid (19 to 81 ng/g) with one to five other HAAs also detected (Raymer and Michael 2010). During controlled laboratory experiments reported by Raymer and Michael, several foods (frozen carrots and green beans, dried pinto beans, chicken, spaghetti, and lettuce) were cooked in haloacetic acid-spiked water, and uptake of the total HAAs available in the cooking water into the food was reported to be as high as 85% (for uptake of dibromoacetic acid by dried pinto beans) although uptake was generally in the range of 2% to 25% for other foods and HAAs. Raymer and Michael also estimated intakes of HAAs from food based on the uptake of HAAs from water containing the MCL of 60 μg/L and reported 3.75 μg per serving for cooked green beans and 6 μg per serving for cooked pasta.

Foods, both canned and fresh, are generally washed with or soaked in treated water and are cooked in treated water. The median amounts of HAAs expressed in μg per kg of food range from less than 1 μg/kg for milk to greater than 10 μg/kg for soft drinks, prepared salads, and minimally processed vegetables such as fruits or vegetables washed with chlorine-based chemicals in water (Cardador and Gallego 2016). Canned vegetables, fruit juices, and cheese fall between these levels. Canned vegetables generally contain several-fold higher levels of HAAs in the liquid phase than the solid phase because of their ionic and polar nature.

2.7.5 Potential exposure from other sources

Occupational exposure to swimming pool attendants

No data were found for other potential occupational exposures to HAAs, but limited information was found for exposure to swimming pool attendants. HAAs are neither volatile nor appreciably skin permeable (Xu *et al.* 2002, Regli *et al.* 2015), but studies of haloacetic acid exposure from indoor and outdoor swimming pools indicate that limited inhalation and dermal exposure also can occur (e.g., ingestion ~94% contribution, inhalation ~5%, and dermal ~1%) (Cardador and Gallego 2011). The authors also reported that HAAs (mostly dichloroacetic acid) can get into the atmosphere of indoor swimming pools through aerosols in ambient air and be inhaled.

The HAAs mono-, di-, and trichloroacetic acid have been measured in the urine of swimmers, and di- and trichloroacetic acid have been measured in the urine of swimming pool attendants (Kim and Weisel 1998, Cardador and Gallego 2011, IARC 2014). After 2 hours of exposure, indoor pool attendant urine samples contained 313 ng/L [0.313 μg/L] DCA and 120 ng/L [0.120

µg/L] TCA (Cardador and Gallego 2011). After 4 hours, indoor attendant urine samples contained 450 ng/L [0.45 µg/L] DCA and 139 ng/L [0.139 µg/L] TCA. Outdoor pool attendant urine samples contained 51 ng/L [0.051 µg/L] DCA after 2 hours of exposure and 58 ng/L [0.058 µg/L] DCA after 4 hours of exposure. No TCA was detected in the urine of outdoor pool attendants.

Releases to the environment

Although some haloacetic acids, such as trichloroacetic acid, dichloroacetic acid, and chloroacetic acid have some uses in industry or medicine, only chloroacetic acid (of the 13 haloacetic acids being reviewed) is on the Toxics Release Inventory (TRI) reporting list for 2015 (the most recent year for which TRI data are available). Total reported on- and off-site release of chloroacetic acid was approximately 5,470 pounds from 19 U.S. facilities in 2015 (TRI 2017). Calculations based on media-specific release data from TRI indicate that releases to air accounted for 94.8% of total releases, off-site disposal for 4.6%, and land for 0.6%.

2.7.6 Overall potential for exposure to HAAs

Individuals living in the United States are exposed to HAAs primarily from drinking treated tap water or other beverages prepared from treated water with additional exposure likely from foods that are prepared using treated water. Consumption of water from all foods and liquids per day has been estimated by CDC (Rosinger and Herrick 2016) to be 3.46 L for men over 20 and 2.75 L for women over 20. The contribution from plain water, i.e., tap water, is approximately 1/3 (33.3%) of the total. The Institute of Medicine estimates that 20% of total water consumption is derived from foods, and the remaining 46.7% would derive from beverages such as tea, coffee, soft drinks, and fruit drinks.

No data were identified that provided overall estimates for consumption of HAAs; however, based on the MCL for HAA5, and assuming that all water consumed contained HAAs at that level, total water consumption would result in exposure to approximately 210 µg per day for men and 165 µg per day for women in the United States. Data for U.S. water facilities serving communities of all sizes in 2011 indicate a median value for HAA5 of 20.1 ug/L with the 5^{th} percentile at 2.0 µg/L and the 95^{th} percentile at 59.0 µg/L. The median exposure would therefore be about 69 µg per day (5% to 95% = 6.9 to 204 µg per day) for men and 55 µg per day (5% to 95% = 5.5 to 162.2 µg per day) for women. At the median exposure and above, these levels will likely overestimate actual exposure for most people since the data reported above for beverages such as fruit drinks and soft drinks and for foods prepared with treated water suggest lower concentrations of HAAs compared with the typical water supply. In addition, any effort to determine total consumption of HAAs would need to take into account consumption of bottled water and point-of-use filtration methods in the home (Wright *et al.* 2006).

Relatively few data on potential exposure to HAAs from other sources such as swimming pools and spas; cooking and food; and point-of-use disinfection have been found, but the following information has been identified:

- HAAs form in swimming pools or spas disinfected with chlorine-based disinfectants and detectable levels of both dichloroacetic acid and trichloroacetic acid have been reported in swimming pool water and in urine samples from swimming pool attendants.

- Data was identified for HAAs in only a few foods, but each of the HAA9 components was detected in one or more common foods cooked in purified water that would not be expected to contain HAAs.
- Use of HAA-spiked water to cook food or to rinse food after cooking resulted in measurable uptake of HAAs. Estimates of possible intakes from such uptakes indicate several μg per serving could be consumed due to cooking food with treated water.
- Point of use disinfection methods based on iodine-containing disinfectants have been shown to result in formation of HAAs, but the use of these methods is limited.

2.8 Summary and synthesis

Disinfection of water has achieved tremendous public health benefits in the United States and worldwide through reduction in exposure of individuals to disease-causing microorganisms. However, a side effect of water treatment is that over 250,000,000 people in the United States are exposed to chlorinated drinking water, indicating that a significant number of people in the United States are exposed to mono-, di- and trihaloacetic acids found as water disinfection by-products. Ingestion of chlorinated drinking water is the most common exposure route for HAAs, but inhalation and dermal exposure also can occur. Other potential sources of exposure to HAAs include swimming pools and spas, cooking and food, and point-of-use disinfection. Disinfection by-products are formed from the reaction of chemical disinfectants (e.g., chlorine, chloramines, chlorine dioxide, or ozone) with organic precursors, and inorganic precursors (most often certain halide ions, i.e., bromide [Br^-] or iodide [I^-]). The primary factors affecting the formation of disinfection by-products are (1) source water quality and characteristics, (2) types and concentrations of precursors, and (3) type of disinfection method and dose.

Anthropogenic and natural sources of bromide and iodide can increase concentrations of these halide ions in source waters (e.g., due to incomplete removal or non-removal in wastewater treatment plants) and create brominated and iodinated HAAs and other disinfection by-products such as trihalomethanes and bromate.

Remediation of haloacetic acid disinfection by-products can be divided into three general approaches: (1) removal of precursors prior to disinfection, (2) optimization or modification of disinfection practices (e.g., altering disinfectant type, dose, or application point in the water treatment process), and (3) removal of disinfection by-products after formation.

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3 Disposition and Toxicokinetics

Disposition and toxicokinetics refer to how a chemical can enter and leave the body, what happens to it once it is in the body, and the rates of these processes. Disposition includes absorption, distribution, metabolism and excretion (ADME: Sections 3.1 to 3.3) while toxicokinetics (Section 3.4) refers to the mathematical description of the time course of disposition of a chemical in the body. A synthesis of the data is provided in Section 3.5.

Overall, the data indicate that the haloacetic acids are well absorbed following oral exposure, widely distributed, and excreted unchanged or as metabolites in the urine, or exhaled as carbon dioxide (CO_2). However, there are marked differences in disposition among these compounds that are related to both the number and types of halogen atom substitutions. Disposition studies in humans were available only for dichloro- and trichloroacetic acid while disposition data in experimental animals were available for most of the chlorinated and/or brominated acetic acids as well as some mixtures of these compounds. No disposition studies were identified for the iodinated acetic acids. The mechanistic implications of these data are discussed in Section 6.

3.1 Absorption

Haloacetic acids are rapidly and extensively absorbed from the gastrointestinal tract in humans and experimental animals (NTP 1992, EPA 2003, IARC 2004a, 2004b, NTP 2007a, 2009, EPA 2011a, IARC 2013a, 2013b, 2014a, 2014b, NTP 2015). Dermal absorption also occurs, but it is a minor route of exposure compared with ingestion. Absorption studies in humans and experimental animals are briefly reviewed below.

3.1.1 Human studies

Ingestion is the primary exposure pathway for di- and trihaloacetic acids because their chemical and physical properties (i.e., low volatility and high polarity) limit inhalation and dermal exposures (Kim and Weisel 1998, Cardador and Gallego 2011). Peak plasma concentrations were reached within 15 minutes to 1.5 hours following ingestion (Curry *et al.* 1991, Rogers 1995, Stacpoole *et al.* 1998, Kim *et al.* 1999, Froese *et al.* 2002, Cardador and Gallego 2011). However, oral bioavailability of dichloroacetic acid in human volunteers (8 men and 8 women) was highly variable (27% to 100% of a single 2 mg/kg dose) but less than 10% when administered at 20 μg/kg for 14 days (Schultz and Shangraw 2006).

Cardador and Gallego (2011) reported that ingestion accounted for about 94% of the total exposure while inhalation (from aerosol droplets) and dermal routes contributed about 5% and 1%, respectively in swimmers (adults and children) and workers exposed to haloacetic acids in outdoor and indoor swimming pools. However, di- and trihaloacetic acids are detectable in urine 5 to 30 minutes after wading or swimming in chlorinated swimming pools (Kim and Weisel 1998, Cardador and Gallego 2011).

Dermal permeability coefficients calculated for dichloro- and trichloroacetic acid in human subjects were very low (0.00002 to 0.008 cm/h) (Kim and Weisel 1998). The *in vitro* permeability coefficients of chloro-, dichloro-, trichloro-, bromo-, bromochloro-, and dibromoacetic acids in aqueous solution across human skin using diffusion chambers were also very low (0.0011 to 0.0026 cm/h) with lag times of 3.7 to 6.5 hours and the authors concluded

that the dermal dose from bathing in water containing these haloacetic acids would be insignificant compared to the estimated ingestion dose (Xu *et al.* 2002). Cases of accidental exposure to monochloroacetic acid show that it is readily absorbed through the skin and is corrosive to tissues (Kusch *et al.* 1990, Kulling *et al.* 1992).

3.1.2 Laboratory animal studies

All haloacetic acids administered orally to rats individually or in mixtures were detected in plasma within minutes after oral dosing (Schultz *et al.* 1999, Saghir and Schultz 2002, 2005). Oral bioavailability, mean absorption time, peak blood concentration, and time to peak blood concentration for di- and trihaloacetic acids in rats are shown in Figures 3-1 and 3-2. These data indicate that oral bioavailability was at or near 100% for trichloro-, bromodichloro-, and chlorodibromoacetic acid but was lower (30% to 81%) for dibromo-, bromochloro-, tribromo-, and dichloroacetic acid due to greater first pass metabolism (Schultz *et al.* 1999). The oral bioavailability of bromodichloroacetic acid in mice ranged from 28% to 73% and was lower than reported in rats (Merdink *et al.* 2001). The bioavailability of chloroacetic acids in rats was 100% (Saghir and Rozman 2003). Saghir and Schultz (2002) also showed that the oral bioavailability of dichloroacetic acid increased with dose and also increased in glutathione *S*-transferse-zeta (GST-ζ)-depleted rats due to decrease in GST-ζ mediated metabolism (see Figure 3-3 and Section 3.3). Maximum blood concentrations generally occurred around 1 hour for all di- and trihaloacetic acids, with the exception of dichloroacetic acid (8 hours) (Schultz *et al.* 1999). Maximum blood concentrations (molar basis) for the trihaloacetic acids were about 1.5 to six times greater than the corresponding dihaloacetic acids and reflect the relative bioavailability.

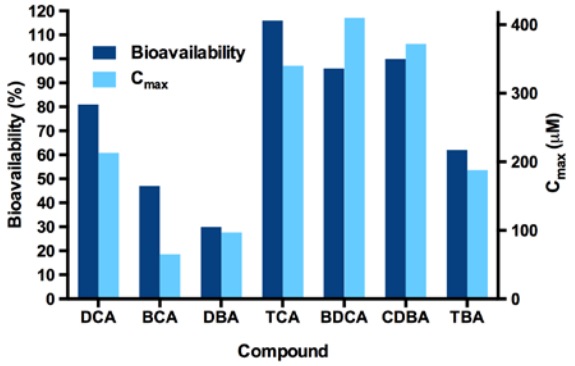

Figure 3-1. Oral bioavailability and peak blood concentration (C_{max}) of di- and trihaloacetic acids in rats

Source: Schultz *et al.* 1999

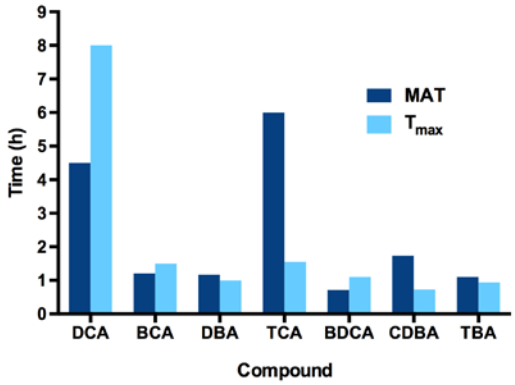

Figure 3-2. Mean absorption time (MAT) and time to peak blood concentration (T_{max}) of di- and trihaloacetic acids in rats

Source: Schultz *et al.* 1999

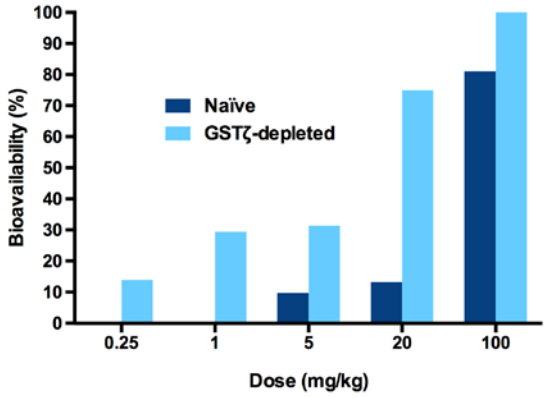

Figure 3-3. Oral bioavailability of dichloroacetic acid in naïve and GST-ζ-depleted rats

Source: Saghir and Schultz 2002.

Rats rapidly absorbed (within 15 minutes) over 95% of the applied dermal dose of monochloroacetic acid from the site of application (Saghir and Rozman 2003). However, much of the absorbed monochloroacetic acid was sequestered in deeper skin layers and served as a "depot" for continued absorption over the course of several hours. No other dermal studies were identified.

3.2 Distribution

The degree of reversible plasma protein binding for haloacetic acids is concentration-, species-, and haloacetic acid-dependent. Haloacetic acids show rapid and uniform distribution outside the vascular system to all body tissues with tissue:blood partition coefficients of the unbound fraction generally close to unity. Blood concentration-time profiles, blood:plasma ratios, plasma protein binding, volume of distribution, and tissue distribution data are discussed below.

3.2.1 Blood concentration-time profiles

Following i.v. dosing in rats, the blood concentrations of di- and trihaloacetic acids (brominated and chlorinated forms) show a short distribution phase followed by a rapdid log-linear decline with most concentrations reaching the detection limit within 12 hours (Schultz *et al.* 1999). Trichloroacetic acid was an exception with detectable blood concentrations after 24 hours. A similar pattern was seen after oral dosing; however, the dihaloacetic acids, particularly dichloroacetic acid, displayed a more complex plasma concentration-time profile characterized by multiple peaks appearing long after the initial absorption phase (Schultz *et al.* 1999, Saghir and Schultz 2002, 2005). This pattern was not due to enterohepatic recirculation (Schultz *et al.* 1999). Discontinuous absorption (i.e., region-dependent absorption) from the GI tract was proposed as a possible explanation (Saghir and Schultz 2002). The data suggest that dichloroacetic acid, and possibly other dihaloacetic acids, were absorbed in the upper portion of the GI tract to a higher degree than the trihaloacetic acids (Saghir and Schultz 2002, 2005).

3.2.2 Blood:plasma ratios and protein binding

Blood:plasma ratios in rats were close to unity for the dihaloacetic acids and indicate near equal distribution between erythrocytes and plasma (Schultz *et al.* 1999). Blood:plasma concentration

ratios for trihaloacetic acids were lower (0.66 to 0.82) and indicate preferential distribution to plasma. Monochloroacetic acid did not show significant binding to erythrocytes or hemoglobin but did bind to plasma proteins (Kaphalia *et al.* 1992).

Dihaloacetic acids exhibited much lower plasma protein binding in rats (6% to 11% bound) compared to trihaloacetic acids (50% to 80% bound) (Schultz *et al.* 1999). In addition, *in vitro* studies of trichloroacetic acid (doses of 0.01 to 1,000 μg/mL) found that humans have higher plasma binding capacity (75% to 87%) compared to rats (38% to 67%), dogs (54% to 65%), or mice (19% to 55%) (Templin *et al.* 1995, Lumpkin *et al.* 2003). The higher binding capacity in humans was attributed to more binding sites and slightly higher albumin concentrations (Lumpkin *et al.* 2003). Higher plasma protein binding increases residence time in the blood and reduces the proportion available for uptake by the tissues. Binding of trichloroacetic acid to plasma proteins is also nonlinear due to partial saturation of plasma binding at high doses in humans, rats, and mice (Yu *et al.* 2000, Lumpkin *et al.* 2003).

Both trichloro- and dichloroacetic acid formed adducts to hemoglobin and albumin in rats and mice, but much of the label associated with protein adduction could be accounted for by metabolic incorporation into the amino acid pool and subsequent *de novo* protein synthesis (Stevens *et al.* 1992). Mice incorporated a greater portion of the label into proteins than rats, which is consistent with a greater metabolic rate in the mouse. In contrast, Styles *et al.* (1991) did not find evidence of covalent binding of trichloroacetic acid to DNA or plasma proteins, and very little covalent binding was detected in the liver of mice.

3.2.3 Volume of distribution

Schultz *et al.* tested seven haloacetic acids in rats and did not find any statistically significant difference in steady-state apparent volume of distribution (ranging from 400 to 881 mL/kg) (Schultz *et al.* 1999). This range of values is comparable to the total body water volume of rats, suggesting that haloacetic acids evenly distribute outside of the vasculature and are not highly sequestered in peripheral tissues (Schultz *et al.* 1999). In addition, the similar volume of distribution across haloacetic acids, despite large differences in plasma protein binding, suggests that differences in protein binding are matched in peripheral tissues. Similarly, mice exposed i.v. to 5 to 100 mg/kg of bromodichloroacetate had a steady-state volume of distribution of about 380 mL/kg to 518 mL/kg (also consistent with distribution to total body water) and a blood/plasma ratio of 0.88 (Merdink *et al.* 2001). However, the volume of distribution for dichloroacetic acid in humans (190 mL/kg to 337 mL/kg following 10 mg/kg or 20 mg/kg i.v., respectively) was much lower than in rats (932 mL/kg, 100 mg/kg dose) and is consistent with greater plasma protein binding in humans (Lukas *et al.* 1980).

3.2.4 Tissue distribution

Abbas and Fisher (1997) reported tissue:blood partition coefficients ranging from 0.54 (lung) to 1.18 (liver) for trichloroacetic acid in mice. Following oral doses of various mixtures of mono-, di- and trihaloacetic acids, tissue concentrations were close to plasma concentrations and indicated a rapid equilibrium between plasma and tissues (Saghir and Schultz 2005). Monochloro- and monoiodoacetic acid distributed rapidly to peripheral tissues, particularly to organs that are rich in sulfhydryl groups, such as the liver and kidney (Hayes *et al.* 1973, Kaphalia *et al.* 1992). Distribution patterns were comparable at low (0.1 mmole/kg or ~9.5

mg/kg) and high doses (1 mmole/kg or ~95 mg/kg) of chloroacetic acid in rats (Kaphalia *et al.* 1992). Saghir *et al.* (2001) also reported rapid distribution of chloroacetic acid to tissues but distribution was slower at a toxic dose (75 mg/kg) compared to a non-toxic dose (10 mg/kg) in male rats. Overall, these data suggest that tissue:blood partition coefficients of haloacetic acids are close to unity and that they distribute uniformly without any significant sequestering in fat or peripheral tissues.

3.3 Metabolism and excretion

Metabolism of haloacetic acids is complex but is qualitatively similar in humans and experimental animals (Stacpoole *et al.* 1998, EPA 2003, IARC 2004a, 2004b, EPA 2011a, IARC 2014a, 2014b). A generalized metabolic scheme is shown in Figure 3-4. Although haloacetic acids share common metabolic pathways and metabolites, there are substantial differences in the extent of biotransformation and elimination between compounds and between species. These inter- and intraspecies differences in metabolism and metabolic capacity may explain differences in susceptibility to toxic effects of haloacetic acids. For example, mice have a higher capacity to metabolize dichloroacetic acid compared to rats (Larson and Bull 1992, Gonzalez-Leon *et al.* 1999).

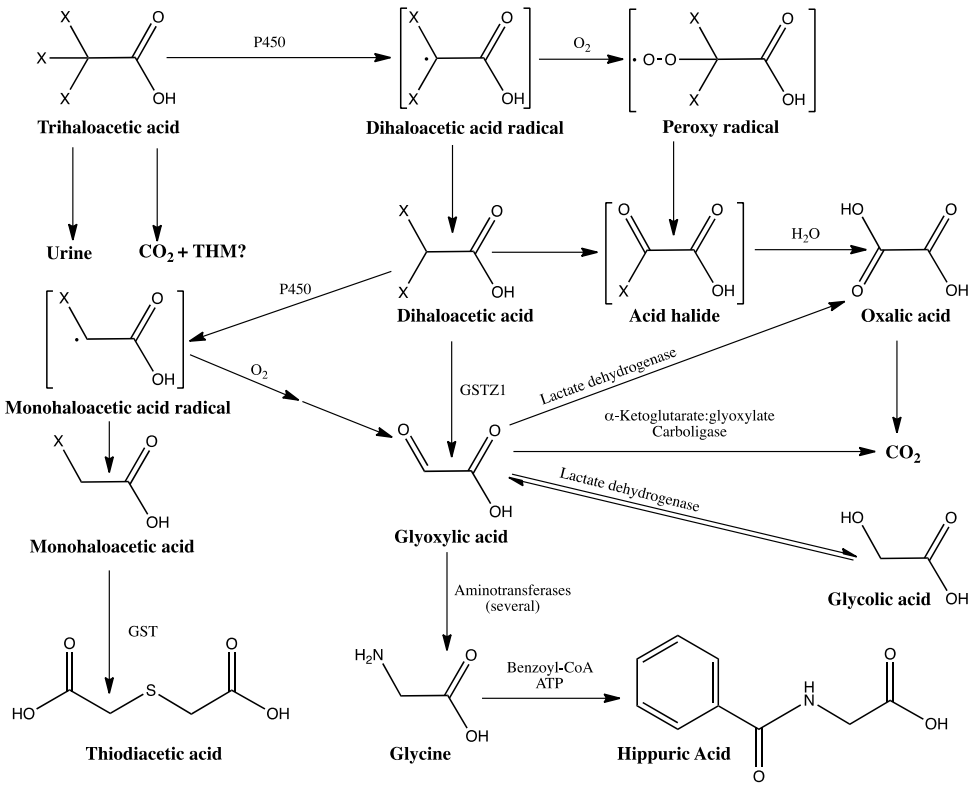

Figure 3-4. General metabolic pathways for tri- and dihaloacetic acids

GSTZ1 = glutathione S-tranferase zeta 1, THM = trihalomethane.
Adapted from: Xu *et al.* 1995, IARC 2013a, 2013b, 2014a, 2014b

The haloacetic acids fall into three broad groups based on their metabolism and renal clearance (Schultz *et al.* 1999, Saghir and Schultz 2005). These groups are broadly described as (1) low metabolism with moderate renal clearance (e.g., trichloroacetic acid), (2) moderate to high metabolism and high renal clearance (e.g., brominated trihaloacetic acids), and (3) high metabolism and low renal clearance (e.g., dihaloacetic acids). Because of the marked differences in the metabolism of various haloacetic acids, the metabolism and excretion of trihaloacetic acids and dihalo- and monohaloacetic acids are reviewed in separate subsections.

3.3.1 Trihaloacetic acid metabolism and excretion

Trihaloacetic acids are metabolized primarily by the microsomal fraction, but metabolism also occurs in the cytosolic subcellular fraction (Austin and Bull 1997, Saghir and Schultz 2005). Cytochrome P450 (CYP)-catalyzed reductive de-halogenation of trihaloacetic acids generates a dihaloacetic acid via a free radical intermediate and is then further metabolized by GSTs or can undergo further reductive dehalogenation to form a monohaloacetic acid (discussed in more detail below) (see Figure 3-4) (Stacpoole *et al.* 1998, Merdink *et al.* 2000, Saghir and Schultz 2005, Saghir *et al.* 2011). There is limited evidence for the direct decarboxylation of bromodichloroacetic acid to form CO_2 and a trihalomethane (Xu *et al.* 1995, Austin and Bull 1997). Pharmacokinetic simulations suggest that dichloroacetic acid forms slowly from trichloroacetic acid and is then rapidly metabolized and eliminated, resulting in generally nondetectable levels of dichloroacetic acid in the blood (Merdink *et al.* 1998).

Trichloroacetic acid is the least metabolized haloacetic acid in humans and experimental animals. Allen and Fisher (1993) estimated that in humans, 93% of trichloroacetic acid was excreted unchanged in urine while Paykoc and Powell (1945) reported that about 75% of an i.v. dose (1.5 to 3 g) administered to 6 patients was excreted unchanged in the urine after 10 days. Metabolism data in rats and mice show that about 45% to 84% of trichloroacetic acid is excreted unchanged in the urine after 24 to 48 hours, and the percent of unchanged trichloroacetic acid in the urine increases with dose (Green and Prout 1985, Larson and Bull 1992, Xu *et al.* 1995, Schultz *et al.* 1999, Yu *et al.* 2000). Metabolites detected in the urine of rodents exposed orally to trichloroacetic acid include dichloroacetic acid, monochloroacetic acid, glyoxylic acid, glycolic acid, oxalic acid, and some unidentified metabolites and accounted for only 7% to 13% of the administered dose (Larson and Bull 1992, Xu *et al.* 1995). About 4% to 15% was metabolized to CO_2 and about 1% to 8% was excreted in the feces (Green and Prout 1985, Larson and Bull 1992, Xu *et al.* 1995, Yu *et al.* 2000).

The metabolism of other trihaloacetic acids (i.e., bromodichloro-, chlorodibromo-, and tribromoacetic acid) was somewhat different from the pattern seen with trichloroacetic acid and indicates that bromine substitution enhances metabolism (Xu *et al.* 1995, Schultz *et al.* 1999). Mice administered 5, 20, or 100 mg/kg bromodichloroacetic acid eliminated 0% to 4% unchanged in the urine, 42% to 45% as urinary metabolites (primarily oxalate), 15% to 30% as CO_2, and 6% to 10% in the feces (Xu *et al.* 1995, Merdink *et al.* 2001). The large difference in urinary excretion of bromodichloroacetic acid between mice and rats was attributed to a more efficient renal tubular reabsorption mechanism and a greater rate of metabolism in mice compared to rats (Merdink *et al.* 2001).

In vitro data using rat or human microsomes showed that the rate of metabolism of brominated trihaloacetic acids (i.e., bromodichloro-, chlorodibromo-, and tribromoacetic acid) was directly

proportional to the number of bromines on the molecule and was further enhanced under reduced oxygen tensions that approximated tissue oxygen levels (Saghir *et al.* 2011). The brominated trihaloacetic acids were rapidly metabolized by liver microsomes to the dihaloacetic acid product corresponding to loss of a single bromine ion. Reduced oxygen tension (2% oxygen or nitrogen atmosphere) also enhanced the metabolic rate (2% oxygen was selected to be representative of tissue oxygen tension). Reductive dehalogenation of tribromoacetic acid produced dibromoacetic acid in a 1:1 molar ratio with the Br^- liberated and there was no evidence of additional metabolism. However, the amount of dibromoacetic acid formed only accounted for about 50% of the consumption of tribromoacetic acid, particularly at higher substrate concentrations, and suggests the possibility of covalent binding and/or nonmetabolic or degradative loss during chemical analysis.

Metabolism and elimination of haloacetic acids is also affected by exposure to mixtures of haloacetic acids or by pretreatment with dichloroacetic acid or trichloroacetic acid (Austin and Bull 1997, Barton *et al.* 1999, Gonzalez-Leon *et al.* 1999, Saghir and Schultz 2005). The primary difference in toxicokinetics of di- and trihaloacetic acids in rats was reduced clearance when administered in mixtures rather than as single compounds (discussed in more detail in Section 3.4) (Schultz *et al.* 1999, Schultz and Sylvester 2001, Saghir and Schultz 2002, 2005). Urinary recovery of trichloro-, bromodichloro-, chlorodibromo-, and tribromoacetic acid was lower in rats when administered in a mixture and is consistent with increased metabolism at relatively low doses (Saghir and Schultz 2005). However, the data also suggest that metabolism of trihaloacetic acids is possibly increased in mixtures when compared to studies where compounds were administered individually at similar doses.

Pretreatment with trichloroacetic acid inhibited both hepatic cytosolic (up to 70%) and microsomal (up to 30%) metabolism of bromodichloroacetic acid in mice but had little effect on dichloroacetic acid metabolism (Austin and Bull 1997). Pretreatment with dichloroacetic acid inhibited cytosolic metabolism of both dichloro- and bromodichloroacetic acid up to 70% but stimulated microsomal metabolism of bromodichloroacetic acid (1.3 fold); however, there was not a concomitant increase in dichloroacetic acid formation (possibly due to the direct decarboxylation of bromodichloroacetic acid to form CO_2 and bromodichloromethane).

3.3.2 Dihalo- and monohaloacetic acid metabolism and excretion

The dihaloacetic acids are extensively metabolized with low amounts of parent compound excreted in the urine (Larson and Bull 1992, Lin *et al.* 1993, Xu *et al.* 1995, Gonzalez-Leon *et al.* 1997, James *et al.* 1998, Schultz *et al.* 1999, NTP 2009). *In vivo* and *in vitro* studies show that mice have a significantly higher capacity to metabolize dichloroacetic acid than rats (Gonzalez-Leon *et al.* 1997, Gonzalez-Leon *et al.* 1999). When administered as part of a mixture of haloacetic acids, urinary elimination of parent dichloro-, bromochloro-, or dibromoacetic acids were all < 0.1% (Saghir and Schultz 2005).

Dihaloacetic acids are primarily metabolized in the cytosol to glyoxylate by a glutathione-dependent process that is catalyzed by GST-ζ (IARC 2013a, 2013b). The relative rates of glyoxylate formation among the three chlorinated/brominated dihaloacetates are: bromochloro- > dichloro- > dibromoacetic acid (Tong *et al.* 1998). Glyoxylate can be further metabolized to glycolate, oxylate, glycine, and CO_2 (Figure 3-4). Reductive dehalogenation of dihaloacetic acids to monohaloacetic acids is a minor pathway.

Dichloro-, dibromo-, and bromochloroacetic acid are mechanism-based, irreversible inhibitors of GST-ζ (Anderson *et al.* 1999, Schultz and Sylvester 2001, Saghir and Schultz 2002). Inhibition of GST-ζ by dihaloacetic acids reduces the extent of metabolism and increases the plasma half-life, and it has been reported in humans, dogs, and rodents (Tong *et al.* 1998, Anderson *et al.* 1999, Tzeng *et al.* 2000, Saghir and Schultz 2002, 2005, Maisenbacher *et al.* 2013). However, humans have a lower rate of dichloroacetic acid biotransformation by hepatic cytosol than rats or mice (Tong *et al.* 1998) and human GST-ζ is more resistant to inactivation than rodent or dog GST-ζ (Board and Anders 2011, Maisenbacher *et al.* 2013). Thus, the use of GST-ζ-depleted rats was shown to be a suitable model for evaluating the kinetics of dichloroacetic acid in humans based on similar *in vitro* intrinsic metabolic clearance values of low doses in human and rat GST-ζ-depleted liver cytosol (Saghir and Schultz 2002).

In vitro experiments using rat and human hepatic cytosol showed evidence of stereospecific metabolism of bromochloroacetic acid with more rapid elimination of the (−)-bromochloroacetic acid isomer compared to the (+)-bromochloroacetic acid isomer (Schultz and Sylvester 2001). *In vivo* studies in rats and mice administered a single i.v. dose also showed that the (−) isomer was eliminated about 1.5 to 2.5 times faster than the (+) isomer (NTP 2009). These data suggest that (+)-bromochloroacetic acid is a poor substrate for GST-ζ compared to (−)-bromochloroacetic acid and that another GST isoenzyme may be involved in the metabolism of bromine-substituted dihaloacetic acids.

Several polymorphic variants of GST-ζ have been identified in humans that differ in their susceptibility to inactivation (Fang *et al.* 2006, Board and Anders 2011, Li *et al.* 2012). Human liver samples homozygous or heterozygous for GST-ζ 1A exhibited a 3-fold higher capacity to dechlorinate dichloroacetic acid than samples carrying other alleles at a given level of expression (Li *et al.* 2012). GST-ζ haplotype also influenced dichloroacetic acid kinetics when administered to children with congenital mitochondrial diseases (Shroads *et al.* 2015). GST-ζ, also known as maleylacetoacetate isomerase (MAAI), is part of the tyrosine catabolism pathway and has been identified as a potential mode of action (see Section 5) (Schultz *et al.* 2002, Stacpoole *et al.* 2008, Board and Anders 2011, Stacpoole 2011).

3.4 Toxicokinetic data

The toxicokinetic properties of haloacetic acids in humans and experimental animals are reviewed below. The number and type of halogen substitutions, dose, exposure to mixtures, and age influence the biotransformation and elimination kinetics of haloacetic acids.

3.4.1 Human studies

Most of the toxico- or pharmacokinetic data in humans is for dichloroacetic acid and includes healthy subject volunteers and subjects with various diseases (e.g., lactic acidosis, malaria, liver disease) that were administered dichloroacetic acid as a therapeutic treatment. These studies indicate that the pharmacokinetics of dichloroacetic acid are dose dependent, there are no significant differences between men and women, but there are differences between diseased patients and healthy volunteers (see Appendix B, Table B-1). (Lukas *et al.* 1980, Curry *et al.* 1985, Curry *et al.* 1991, Krishna *et al.* 1994, Krishna *et al.* 1995, Krishna *et al.* 1996, Henderson *et al.* 1997, Schultz and Shangraw 2006). The distribution phase was generally slower and the plasma T½ was longer in diseased patients. The large interindividual differences in haloacetic

acid clearance in humans may be due to polymorphisms that alter GST-ζactivity and expression (Schultz and Shangraw 2006). The urinary excretion half-life of trichloroacetic acid in five healthy volunteers ranged from about 2.1 to 6.3 days and fit a single compartment exponential decay model (Bader *et al.* 2004). The few human studies that report on the pharmacokinetics of trichloroacetic acid showed a reduced volume of distribution and longer plasma half-life compared to dichloroacetic acid (see Appendix B, Table B-1). Pharmacokinetic modeling of trichloroacetic acid indicates that the volume of distribution is inversely related to body weight, where trihaloacetic acid distributes to about 7% to 14% of body weight in humans, compared to 25% to 51% for rats and 18% to 24% for mice (Allen and Fisher 1993). Systemic clearance of trichloroacetic acid in humans (0.028/hr/kg) also is slower than in rodents (0.045 to 0.1/hr/kg). The lower volume of distribution and clearance in humans compared to rodents is likely related to greater plasma protein binding in humans.

3.4.2 Experimental animal studies

Most of the toxicokinetic studies of the haloacetic acids were conducted in male F344 rats (see Appendix B, Table B-2). The data show that the steady-state apparent volume of distribution is similar for di- and tri-haloacetic acids while area under the concentration-time curve and clearance show considerable differences (Schultz *et al.* 1999). Dihaloacetic acids are primarily eliminated by biotransformation (i.e., nonrenal clearance) with very little parent compound excreted in the urine. As mentioned in Section 3.2, the blood concentration-time profiles for the dihaloacetic acids exhibited multiple peaks that resulted in some uncertainty in the start of the log-linear portion of the profiles and complicated calculations of toxicokinetic parameters. In contrast, trihaloacetic acids exhibited simpler blood concentration-time profiles with reduced metabolism and a higher contribution from renal clearance. Bromine substitution enhanced metabolism and increased both renal and nonrenal clearance, especially for the trihaloacetic acids (Figure 3-5). The combination of a similar distribution volume and increasing clearance with bromine substitution resulted in a progressive decrease in elimination half-lives. The data also show that total dose is an important facor for dihaloacetic acids because clearance is dose-dependent due to saturation and inhibition of the GST-ζ metabolic pathway (Figure 3-6).

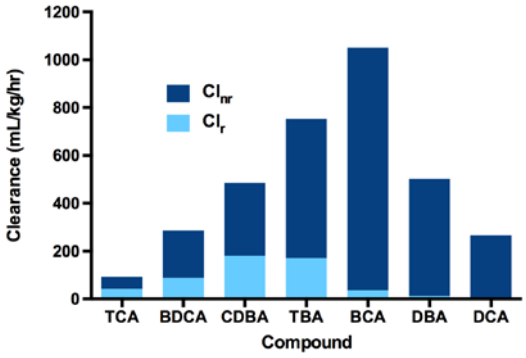

Figure 3-5. Comparison of renal (Clr) and nonrenal (Clnr) clearance of an equimolar i.v. dose (500 μmol/kg) of haloacetic acids in male rats.

Source: Schultz *et al.* 1999

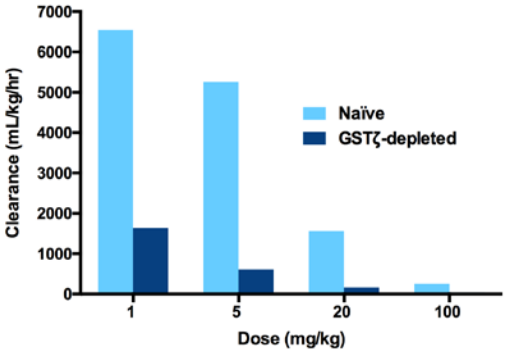

Figure 3-6. Clearance of dichloroacetic Acid in naïve and GST- ζ-depleted male rats.

Source: Gonzalez-Leon *et al.* 1997, Saghir and Schultz 2002

When haloacetic acids were administered to rats as mixtures rather than as single compounds, the primary difference was reduced clearance (with the exception of bromochloro- and dibromoacetic acids), suggesting competitive interactions between di- and trihaloacetic acids (Appendix B, Table B-2) (Saghir and Schultz 2005). The inability to show reduced clearance for bromochloro- or dibromoacetic acid when administered as part of a mixture rather than individually may be explained by dose. The available studies that administered these two haloacetic acids as single compounds used high doses (i.e., ≥ 500 µmol/kg) where metabolic clearance was likely reduced by metabolic saturation and/or GST-ζ depletion. The primary effect of GST-ζ-depletion for both mixtures and single compounds was reduced clearance of the dihaloacetic acids (Figure 3-7a). In contrast, GST-ζ depletion did not affect clearance of trihaloacetic acids (Figure 3-7b). In addition, data for bromochloroacetic acid, a chiral molecule, show stereospecific metabolism with faster elimination of the (−) stereoisomer compared to the (+) stereoisomer (Figure 3-8). James *et al.* (1998) showed that the peak plasma concentrations and area under the concentration-time curve were 5 to 6-fold higher in old rats while the elimination half-life was almost 2-fold slower compared to young rats (Appendix B, Table B-2).

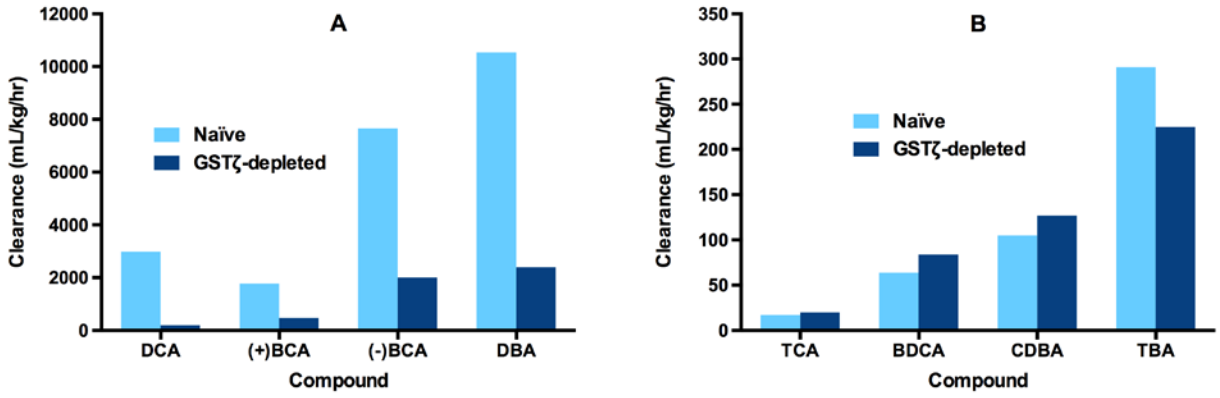

Figure 3-7. Clearance of dihaloacetic acids (A) and trihaloacetic acids (B) administered as mixtures of di- and trihaloacetic acids at equimolar i.v. doses (25 µmol/kg) to male rats

Source: Saghir and Schultz 2005.

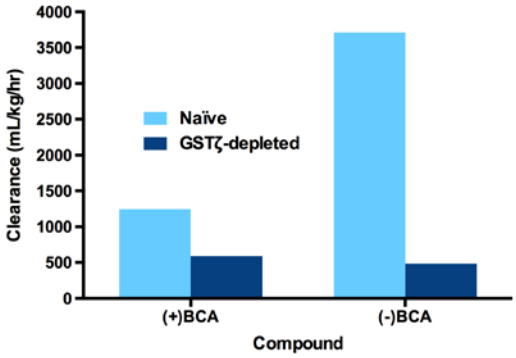

Figure 3-8. Stereospecific clearance of an i.v. dose (520 µmol/kg) of (−), (+)bromochloroacetic acids administered to naïve and GST-ζ-depleted male rats

Source: Schultz and Sylvester 2001.

The clearance values reported for the di- and trihaloacetic acids indicate that there are marked differences in the degree of tubular secretion and reabsorption (Schultz *et al.* 1999). The adjusted renal clearance values for the trihaloacetic acids approached or exceeded the glomerular filtration rate (GFR) for rats, suggesting tubular secretion is an important factor for the renal clearance of trichloroacetic acids. In contrast, adjusted renal clearance values for the dihaloacetic acids are much lower than the GFR (i.e., similar to the urine flow rate) and indicate that tubular reabsorption is important. Thus, the data support the proposition that urinary excretion of trihaloacetic acids is controlled by tubular secretion while urinary excretion of dihaloacetic acids is controlled by tubular reabsorption in rats.

Toxicokinetic data in mice were limited to a few studies with dichloro- and trichloroacetic acid and one study each with dibromo-, bromochloro-, and bromodichloroacetic acid (Appendix B, Table B-3). Although mice have a higher capacity to metabolize dichloroacetic acid than rats and appear to be less susceptible to GST-ζ inhibition, the available data in mice are generally consistent with the rat data (i.e., relative rate of clearance of dichloro- > bromodichloro- > trichloroacetic acid) (Larson and Bull 1992, Gonzalez-Leon *et al.* 1999, Merdink *et al.* 2001). Elimination kinetics for bromodichloroacetic acid in mice differed from that in rats and is best illustrated by comparing renal clearance (Merdink *et al.* 2001). Renal clearance adjusted for protein binding in mice suggested a very efficient tubular reabsorption process. In contrast, tubular secretion was the dominant renal process in rats (Schultz *et al.* 1999, Merdink *et al.* 2001).

Pretreatment with trichloroacetic acid had no appreciable effect on the toxicokinetics of a challenge dose of either trichloroacetic acid or dichloroacetic acid in mice (Gonzalez-Leon *et al.* 1999, Schultz *et al.* 2002). In contrast, pretreatment with dichloroacetic acid caused a significant increase in the blood concentration-time profile and reduced the clearance of dichloroacetic acid (Gonzalez-Leon *et al.* 1999). However, the impact of dichloroacetic acid pretreatment on clearance in mice was not as great as that observed in rats. As in rats, age was also shown to be an important factor (Schultz *et al.* 2002). Clearance of dichloroacetic acid in aged control mice was about 25% of that measured in young control mice. However, compared to age-matched controls, clearance was reduced in young but not aged mice, with a maximum effect observed at 16 hours or less recovery time (see Appendix B, Table B-3).

3.5 Synthesis

Some of the major differences and general trends in disposition and toxicokinetics of haloacetic acids are summarized below.

3.5.1 Absorption

Haloacetic acids are rapidly and extensively absorbed from the gastrointestinal tract. Dermal absorption also occurs, but it is a minor route of exposure compared to ingestion. Bioavailability increases with dose and is lower for the dihaloacetic acids than trihaloacetic acids due to greater first-pass metabolism.

3.5.2 Distribution

Haloacetic acids show rapid and uniform distribution outside the vascular system with tissue:blood partition coefficients generally close to unity. Binding to plasma proteins is much

higher for the trihaloacetic acids than dihaloacetic acids, decreases with dose, and is higher in humans than experimental animals. Oral administration of dihaloacetic acids, particularly dichloroacetic acid, results in a complex plasma concentration-time profile characterized by multiple peaks appearing long after the initial absorption phase.

3.5.3 Metabolism and excretion

Biotransformation is directly influenced by the number and types of halogen substituents on the molecule with bromine substitution increasing metabolism. Trihaloacetic acids are metabolized by both microsomal and cytosolic subcellular fractions with the primary pathway by cytochrome P450 (CYP) catalyzed reductive de-halogenation that generates a dihaloacetic acid via a free radical intermediate. Dihaloacetic acids are primarily metabolized in the cytosol to glyoxylate by a glutathione-dependent process that is catalyzed by GST-ζ. Glyoxylate may be further metabolized by a number of pathways to form the urinary metabolites glycine, oxalic acid, and glycolic acid, or decarboxylated to form CO_2. A minor metabolic pathway for dihaloacetic acids is reductive dehalogenation to a monohaloacetic acid. Dihaloacetic acids inhibit their own metabolism by inactivating GST-ζ, thus, slowing elimination; however, human GST-ζ is more resistant to inactivation than mouse or rat GST-ζ.

Elimination is also directly influenced by the number and types of halogen substituents on the molecule. Bromine substitution increases both renal and nonrenal clearance. Haloacetic acids can be broadly described by three patterns of elimination: (1) low metabolism with moderate renal clearance (trichloroacetic acid), (2) high metabolism and high renal clearance (bromodichloro-, chlorodibromo-, and tribromoacetic acid), and (3) high metabolism and low renal clearance (dihaloacetic acids).

3.5.4 Toxicokinetics

Human data show large interindividual differences in clearance of dichloroacetic acid that may be attributable to polymorphisms that alter GST-ζactivity and expression. Toxicokinetic studies in experimental animals show no significant differences in the apparent volume of distribution among the dihalo- and trihaloacetic acids while area under the concentration-time curve and clearance show considerable differences. The primary effect on haloacetic acid toxicokinetics when administered as mixtures was reduced clearance, which suggests competitive interactions between di- and trihaloacetic acids. GST-ζ depletion did not significantly affect toxicokinetics of trihaloacetic acids but significantly reduced clearance and increased area under the concentration-time curve of dihaloacetic acids.

4 Studies of Cancer in Experimental Animals

Introduction

This section reviews and assesses the evidence from carcinogenicity studies in experimental animals exposed to any of the thirteen haloacetic acids examined in this monograph. Experimental animal carcinogenicity studies were identified using methods described in the protocol (NTP 2017). A total of 24 publications were identified that reported on exposure of experimental animals to a haloacetic acid and met the following inclusion criteria: (1) reported on the presence or absence of neoplastic and related preneoplastic lesions, (2) had a concurrent or historical control group, and (3) either had an observational duration of 12 months or greater for rats and mice or were co-carcinogen exposure studies (initiation-promotion and other co-carcinogen studies). Five papers were excluded because they either duplicated other studies (Carter *et al.* 2003, Melnick *et al.* 2007, Kissling *et al.* 2009) or were not peer reviewed (Innes and Ulland 1968, Bull 1989).

Section 4.1 provides an overview of the studies, Section 4.2 assesses the quality of the studies, Section 4.3 reports the findings of the studies, organized by the type of neoplasms observed, and Section 4.4 provides a synthesis of the results from all studies.

4.1 Overview of the studies

The 19 remaining publications reported a total of 41 studies; 36 were carcinogenicity studies, 3 of which were in transgenic animals (NTP 2007b), and 5 were initiation-promotion studies (Gwynn and Salaman 1953, Herren-Freund *et al.* 1987, Pereira *et al.* 1997). These studies exposed rodents to seven of the thirteen haloacetic acids considered in this monograph; six of which were tested in long-term carcinogenicity studies: monochloroacetic acid (NTP 1992, DeAngelo *et al.* 1997), dichloroacetic acid (Bull *et al.* 1990, DeAngelo *et al.* 1991, Daniel *et al.* 1992, Richmond *et al.* 1995, DeAngelo *et al.* 1996, DeAngelo *et al.* 1999, Wood *et al.* 2015), dibromoacetic acid (NTP 2007a), bromochloroacetic acid (NTP 2009), trichloroacetic acid (Herren-Freund *et al.* 1987, Bull *et al.* 1990, Pereira and Phelps 1996, DeAngelo *et al.* 1997, Von Tungeln *et al.* 2002, DeAngelo *et al.* 2008), and bromodichloroacetic acid (NTP 2015). In addition, five initiation-promotion studies were identified for monoiodoacetic acid (Gwynn and Salaman 1953), dichloroacetic acid (Herren-Freund *et al.* 1987, Pereira *et al.* 1997), and trichloroacetic acid. All haloacetic acids were tested at multiple doses up to at least 1,000 mg/L for each of the six tested in long-term studies and up to 5,000 mg/L for dichloroacetic acid and trichloroacetic acid.

An overview of these studies is provided below (Table 4-1), and tables describing the study conditions and tumor findings are included in Appendix C, Tables C-8 through C-13. Exposure in almost all of these studies was by an oral route, mostly by drinking water, although two studies were by gavage (NTP 1992); one study used intraperitoneal exposure (Von Tungeln *et al.* 2002). Another study was by dermal application in a transgenic animal model: Tg.AC hemizygous mice (NTP 2007b).

Table 4-1. Overview of cancer studies in experimental animals

Species, strain, (sex)	Route	Exposure/study durations	Reference
Monochloroacetic acid			
Rat F344/N (M&F)	Gavage	104 wk/104 wk	NTP 1992
Mouse B6C3F$_1$ (M&F)	Gavage	104 wk/104 wk	NTP 1992
Rat, F344/M, (M)	Drinking water	104 wk/104 wk	DeAngelo *et al.* 1997
Monoiodoacetic acid			
Mouse albino "S" strain (NR)	Dermal	27 wk/30 wk	Gwynn and Salaman 1953[a]
Dichloroacetic acid			
Rat F344 (M)	Drinking water	100 wk/100 wk & 103 wk/103 wk	DeAngelo *et al.* 1996
Rat F344 (M)	Drinking water	60 wk/60 wk & 104 wk/104 wk	Richmond *et al.* 1995
Mouse B6C3F$_1$ (M)	Drinking water	60–75 wk/60–75 wk	DeAngelo *et al.* 1991
Mouse B6C3F$_1$ (M)	Drinking water	90–100 wk/90–100 wk	DeAngelo *et al.* 1999
Mouse B6C3F$_1$ (M)	Drinking water	61 wk/61 wk	Herren-Freund *et al.* 1987
Mouse B6C3F$_1$ (M)	Drinking water	61 wk/61 wk	Herren-Freund *et al.* 1987[a]
Mouse B6C3F$_1$ (M&F)	Drinking water	10 wk/94 wk	Wood *et al.* 2015
Mouse B6C3F$_1$ (F)	Drinking water	360 d/576 d & 360 d/360 d	Pereira 1996
Mouse B6C3F$_1$ (F)	Drinking water	44 wk/50 wk	Pereira *et al.* 1997[a]
Mouse B6C3F$_1$ (M)	Drinking water	52 wk/52 wk	Bull *et al.* 1990
Mouse B6C3F$_1$ (M)	Drinking water	104 wk/104 wk	Daniel *et al.* 1992
Mouse FVB Tg.AC (M&F)	Drinking water	26 wk/41 wk & 26 wk/26 wk	NTP 2007b[b]
Mouse p53 haploinsufficient (M&F)	Drinking water	26 wk/41 wk & 26 wk/26 wk	NTP 2007b[b]
Mouse FVB Tg.AC (M&F)	Dermal	39 wk/39 wk & 26 wk/26 wk	NTP 2007b[b]
Dibromoacetic acid			
Rat F344/N (M&F)	Drinking water	106 wk/106 wk	NTP 2007a
Mouse B6C3F$_1$ (M&F)	Drinking water	106 wk/106 wk	NTP 2007a
Bromochloroacetic acid			
Rat F344/N (M&F)	Drinking water	105 wk/105 wk	NTP 2009
Mouse B6CC3F$_1$ (M&F)	Drinking water	105 wk/105 wk	NTP 2009
Trichloroacetic acid			
Rat F344/N (M)	Drinking water	104 wk/104 wk	DeAngelo *et al.* 1997
Mouse B6C3F$_1$ (M)	Drinking water	60 wk/60 wk & 104 wk/104 wk	DeAngelo *et al.* 2008
Mouse B6C3F$_1$ (M)	Drinking water	61 wk/61 wk	Herren-Freund *et al.* 1987
Mouse B6C3F$_1$ (M)	Drinking water	61 wk/61 wk	Herren-Freund *et al.* 1987[a]
Mouse B6C3F$_1$ (F)	Drinking water	576 d/576 d & 360 d/360 d	Pereira 1996
Mouse B6C3F$_1$ (F)	Drinking water	44 wk/50 wk	Pereira *et al.* 1997[a]
Mouse B6C3F$_1$ (M)	Drinking water	52 wk/52 wk	Bull *et al.* 1990
Mouse B6C3F$_1$ (M&F)	IP inj.	15 d/20 mo & 15 d/12 mo	Von Tungeln *et al.* 2002
Bromodichloroacetic acid			
Rat F344/NTac (M&F)	Drinking water	104–105 wk/104–105 wk	NTP 2015
Mouse B6C3F$_1$ (M&F)	Drinking water	105 wk/105 wk	NTP 2015

[a] Initiation-promotion study.
[b] Transgenic animal model.

4.2 Study quality assessment

Each of these primary studies was systematically evaluated by two independent reviewers for its ability to inform the cancer hazard evaluation using a series of signaling questions related to the following study performance elements: study design, exposure conditions, outcome, confounding, reporting and analysis, and overall utility (see RoC Handbook). Details of each study assessment and quality criteria on a study-by-study basis are reported in Appendix C.

Most studies conducted by the NTP (1992, 2007a, 2007b, 2009, 2015) and some of the studies conducted by DeAngelo (1991, 1997, 2008) were considered the most informative because they used a sufficient number of experimental animals for a near lifetime exposure duration and tested three dose levels (except for NTP [1992] which used two dose levels) along with an untreated control. The rat NTP (1992) study had a moderate level of utility because the rats were very sensitive to monochloroacetic acid in the 13-week study and developed non-carcinogenic toxicity, which required the dose level to be reduced in the 2-year study. DeAngelo *et al.* (1997) and the NTP studies also included historical control data. Data on historical controls is useful for identifying rare tumors. If study controls exceed historical control levels for a tumor, this can indicate that the sensitivity of the study to detect neoplasms might be lower.

Many studies were considered somewhat less informative (moderate overall utility) primarily because of sensitivity issues (e.g., elements that limited their ability to detect a true effect, or evaluate dose-response effects) but would not be expected to cause false positives in the studies. The studies with overall rating of ++ either had fewer animals (Herren-Freund *et al.* 1987, DeAngelo *et al.* 1991, Daniel *et al.* 1992, Richmond *et al.* 1995, DeAngelo *et al.* 1996, DeAngelo *et al.* 1999, Von Tungeln *et al.* 2002, Wood *et al.* 2015), used only one sex of animal (Herren-Freund *et al.* 1987, DeAngelo *et al.* 1991, Daniel *et al.* 1992, Richmond *et al.* 1995, DeAngelo *et al.* 1996, Pereira 1996, DeAngelo *et al.* 1999), had a shorter exposure duration (Pereira 1996, Von Tungeln *et al.* 2002, Wood *et al.* 2015), had to decrease study duration due to hindleg paralysis (Richmond *et al.* 1995), tested only one dose level (Herren-Freund *et al.* 1987, DeAngelo *et al.* 1991, Daniel *et al.* 1992, DeAngelo *et al.* 1996, DeAngelo *et al.* 2008), or did not perform full necropsies (Herren-Freund *et al.* 1987, DeAngelo *et al.* 1991, Daniel *et al.* 1992, Richmond *et al.* 1995, DeAngelo *et al.* 1996, Pereira 1996, DeAngelo *et al.* 1999, Wood *et al.* 2015).

Only a few studies were considered to be of low quality (overall utility of +) because of not reporting chemical purity (Pereira *et al.* 1997), testing at only a single dose level (Gwynn and Salaman 1953), use of a small number of animals (Gwynn and Salaman 1953, Bull *et al.* 1990), the lack of full necropsies and instead focusing on specific target organs (Gwynn and Salaman 1953, Bull *et al.* 1990, Pereira *et al.* 1997) (in one study only a sample of gross liver lesions that were randomly picked were examined histologically [Bull *et al.* 1990] and in one study, lesions were classified based on “macroscopic” examination [Gwynn and Salaman 1953]).

Other studies rated as low utility were not conventional carcinogenicity studies and instead were initiation-promotion studies that did not include groups that exposed animals to the haloacetic acid promoter without the initiator (Gwynn and Salaman 1953, Pereira *et al.* 1997) or they used p53 haploinsufficient or Tg.AC hemizygous transgenic mice. The p53 haploinsufficient transgenic mice may not be able to detect non-genotoxic carcinogens (Tennant and Spalding 1996, Gulezian *et al.* 2000, Spalding *et al.* 2000). Concerns for the Tg.AC hemizygous mice

include production of false positive results from vehicle controls (Jacobs and Hatfield 2013), or minor skin abrasions (Fuhrman *et al.* 2005), and its inability to distinguish between promotion and *de novo* carcinogenicity (Jacobs and Hatfield 2013, Luijten *et al.* 2016). The use of the Tg.AC hemizygous mouse is no longer recommended by the FDA (Luijten *et al.* 2016). Another limitation of these studies was the small number of animals used for each treatment group, further limiting the ability to detect small changes in incidence.

4.3 Neoplastic findings from carcinogenesis studies

Results are summarized below for tumors induced by seven mono-, di-, and tri- haloacetic acids: monochloroacetic acid, monoiodoacetic acid, dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, trichloroacetic acid, and bromodichloroacetic acid. Section 4.3.1 discusses liver neoplasms, which are reported by themselves because they developed in mice in all positive studies. The other sections discuss other tumor sites (Section 4.3.2), studies with transgenic animals (Section 4.3.3), and initiation-promotion studies (Section 4.3.4). Table 4-3 in Section 4.4 summarizes results by individual haloacetic acid. The following text summarizes neoplastic findings across tumor sites and across haloacetic acids rather than providing a detailed study-by-study description. Information on the individual cancer studies can be found in tabular form in Appendix C.

4.3.1 Liver (see Table C-8 in Appendix C)

Overall, the data from studies that exposed rodents to haloacetic acids provide strong evidence that di- and trihaloacetic acids cause liver neoplasms in rodents. Liver neoplasms were significantly induced by all dihaloacetic acids (dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid) and trihaloacetic acids, (trichloroacetic acid, bromodichloroacetic acid) tested by exposure to male and female mice in the drinking water in well-conducted studies for at least 12 months. Significant increases in liver neoplasms for male rats were reported for dichloroacetic acid only.

The routes of exposure were primarily through drinking water with one study using intraperitoneal injection. Either route would be expected to result in initial exposure of the liver by uptake from the gastrointestinal tract circulation via the hepatic portal system. Exposure of the liver to the relatively high concentration of haloacetic acid prior to metabolism and distribution to other tissues could be a factor in the common occurrence of liver neoplasms.

All chronic cancer studies in this section are of moderate study quality except for Bull *et al.* (1990) which has lower study quality rating (see Appendix C). Some studies focused on the liver pathology only and did not do a full necropsy on the whole animal; limitations on study quality would not apply to histopathology results for this tissue.

Details on liver findings for the individual HAAs are discussed first, followed by a discussion of the findings across studies.

Table 4-2. Quality evaluations of cancer studies in experimental animals

Study	Study design		Exposure conditions				Outcome		Confound-ing	Reporting and analysis	Overall utility
	Animal model*	Statistical Power*	Purity	Dosing	Exposure duration*	Dose level*	Pathology methods	Equal treatment	Confounding	Statistical analysis	Overall utility
Monochloroacetic acid											
NTP 1992, Mouse M/F	+++	+++	+++	++	+++	++	+++	+++	+++	+++	+++
NTP 1992, Rat M/F	++	+++	+++	++	+++	++	+++	+++	+++	+++	++
DeAngelo *et al.* 1997, Rat M	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Monoiodoacetic acid											
Gwynn and Salaman 1953[a], Mouse NR	**0**	+	**NR**	+	++	+	+	**NR**	+	**0**	+
Dichloroacetic acid											
DeAngelo *et al.* 1996, Rat M (100 wk), 2 doses	++	++	+++	+++	+++	++	++	++	++	+++	++
DeAngelo *et al.* 1996, Rat M (103 wk), 2 doses	++	++	+++	+++	+++	+	++	++	++	+++	++
Richmond *et al.* 1995, Rat M (60 wk)	++	++	**NR**	+++	++	+++	++	++	+	++	++
Richmond *et al.* 1995, Rat M (100 wk)	++	++	**NR**	+++	+++	+++	++	++	+	++	++
DeAngelo *et al.* 1991, Mouse M (60/75 wk) 3 doses	++	++	+++	+++	+++	+++	++	++	++	+++	+++
DeAngelo *et al.* 1991, Mouse M (60 wk), 3 doses	++	+	+++	+++	+++	+	++	+++	++	+++	++
DeAngelo *et al.* 1999, Mouse M	++	++	+++	+++	+++	+++	++	++	++	+++	++
Herren-Freund *et al.* 1987, Mouse M	++	++	+++	+++	+++	+	++	+++	+++	+++	++
Herren-Freund *et al.* 1987[a], Mouse M	++	++	+++	+++	+++	+	++	+++	+++	+++	++
Wood *et al.* 2015, Mouse M	+++	++	++	+++	++	+++	+	+++	++	+++	++
Wood *et al.* 2015, Mouse F	+++	++	++	+++	++	++	+	+++	++	+++	++
Pereira 1996, Mouse F (360 d)	++	++	**NR**	+++	+++	+++	++	+++	++	+++	++

Study	Study design		Exposure conditions				Outcome		Confound-ing	Reporting and analysis	Overall utility
	Animal model*	Statistical Power*	Purity	Dosing	Exposure duration*	Dose level*	Pathology methods	Equal treatment	Confounding	Statistical analysis	Overall utility
Pereira 1996, Mouse F (576 d)	++	++	**NR**	+++	++	+++	++	+++	++	+++	++
Pereira *et al.* 1997[a], Mouse F	++	++	+	+++	+++	+++	++	+++	++	++	+
Bull *et al.* 1990, Mouse M	++	+	++	+++	+++	++	+	+	+	+	+
Daniel *et al.* 1992, Mouse M	++	++	+++	+++	+++	+	+	++	++	+++	++
NTP 2007b[b], Mouse M/F FVB Tg.AC dermal (39 wk), 3 doses	+	+	+++	++	+++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse M/F FVB Tg.AC dermal (26 wk), 3 doses	+	++	+++	++	++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse M FVB Tg.AC (41 wk), 3 doses	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse F FVB Tg.AC (41 wk), 3 doses	+	+	+++	++	+++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse M FVB Tg.AC (26 wk), 3 doses	+	++	+++	+	++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse F FVB Tg.AC (26 wk), 3 doses	+	++	++	+++	++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse M/F p53 haploinsufficient (41 wk), 3 doses	+	+	+++	++	+++	+++	+++	+++	+++	+++	+
NTP 2007b[b], Mouse M/F p53 haploinsufficient (26 wk), 3 doses	+	++	+++	++	++	+++	+++	+++	+++	+++	+
Dibromoacetic acid											
NTP 2007a Mouse M/F	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
NTP 2007a Rat M/F	+++	+++	+++	++	+++	+++	+++	+++	++	+++	+++

Study	Study design		Exposure conditions				Outcome		Confound-ing	Reporting and analysis	Overall utility
	Animal model*	Statistical Power*	Purity	Dosing	Exposure duration*	Dose level*	Pathology methods	Equal treatment	Confounding	Statistical analysis	Overall utility
Bromochloroacetic acid											
NTP 2009 Rat M/F	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
NTP 2009 Mouse M/F	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Trichloroacetic acid											
DeAngelo *et al.* 1997 Rat M	++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
DeAngelo *et al.* 2008 Mouse M (60 wk)	+++	+++	+++	+++	++	+++	+++	++	+++	+++	+++
DeAngelo *et al.* 2008 Mouse M (104 wk), 1 dose	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	++
DeAngelo *et al.* 2008 Mouse M (104 wk), 2 doses	+++	+++	+++	++	+++	++	+++	+++	+++	+++	++
Herren-Freund *et al.* 1987 Mouse M	++	++	+++	++	+++	+++	+	+++	+++	+++	++
Herren-Freund *et al.* 1987[a] Mouse M	++	++	+++	++	+++	+++	+	+++	+++	+++	++
Pereira 1996 Mouse F (576 d)	++	++	**NR**	+++	+++	+++	++	+++	++	+++	++
Pereira 1996 Mouse F (360 d)	++	++	**NR**	+++	++	+++	++	+++	++	+++	++
Pereira *et al.* 1997[a] Mouse F	++	++	**NR**	+++	+++	+++	++	+++	++	++	+
Bull *et al.* 1990 Mouse M	++	+	++	+++	+++	++	+	+	+	+	+
Von Tungeln *et al.* 2002 Mouse M/F (20 mo)	++	++	**NR**	+	+	++	++	++	++	+++	++
Von Tungeln *et al.* 2002 Mouse M/F (12 mo)	++	++	**NR**	+	+	++	++	++	++	+++	++
Bromodichloroacetic acid											
NTP 2015, Rat M/F	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
NTP 2015, Mouse M/F	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

*These factors contribute to the sensitivity of the study.
[a]Initiation/promotion study; [b]Transgenic animal model.

4.4 Neoplastic findings from carcinogenesis studies

Results are summarized below for tumors induced by seven mono-, di-, and tri- haloacetic acids: monochloroacetic acid, monoiodoacetic acid, dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, trichloroacetic acid, and bromodichloroacetic acid. Section 4.3.1 discusses liver neoplasms, which are reported by themselves because they developed in mice in all positive studies. The other sections discuss other tumor sites (Section 4.3.2), studies with transgenic animals (Section 4.3.3), and initiation-promotion studies (Section 4.3.4). Table 4-3 in Section 4.4 summarizes results by individual haloacetic acid. The following text summarizes neoplastic findings across tumor sites and across haloacetic acids rather than providing a detailed study-by-study description. Information on the individual cancer studies can be found in tabular form in Appendix C.

4.4.1 Liver (see Table C-8 in Appendix C)

Overall, the data from studies that exposed rodents to haloacetic acids provide strong evidence that di- and trihaloacetic acids cause liver neoplasms in rodents. Liver neoplasms were significantly induced by all dihaloacetic acids (dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid) and trihaloacetic acids, (trichloroacetic acid, bromodichloroacetic acid) tested by exposure to male and female mice in the drinking water in well-conducted studies for at least 12 months. Significant increases in liver neoplasms for male rats were reported for dichloroacetic acid only.

The routes of exposure were primarily through drinking water with one study using intraperitoneal injection. Either route would be expected to result in initial exposure of the liver by uptake from the gastrointestinal tract circulation via the hepatic portal system. Exposure of the liver to the relatively high concentration of haloacetic acid prior to metabolism and distribution to other tissues could be a factor in the common occurrence of liver neoplasms.

All chronic cancer studies in this section are of moderate study quality except for Bull *et al.* (1990) which has lower study quality rating (see Appendix C). Some studies focused on the liver pathology only and did not do a full necropsy on the whole animal; limitations on study quality would not apply to histopathology results for this tissue.

Details on liver findings for the individual HAAs are discussed first, followed by a discussion of the findings across studies.

Chlorine-only haloacetic acids

No liver tumors were induced by the only monohaloacetic acid tested, monochloroacetic acid, which was administered by gavage to rats and mice (NTP 1992) and in drinking water to rats (DeAngelo *et al.* 1997).

Dichloroacetic acid was tested for carcinogenicity in more studies than any other haloacetic acid, with 11 publications reporting 14 drinking water studies that varied by species (rat or mouse), sex, dose levels tested (from 50 mg/L up to 5,000 mg/L), and duration of exposure and observation (from 10 weeks to 104 weeks for chronic studies). These studies did not generally provide explanations for the choices of duration, or dose used. In general, most of the studies were in male mice only, a stop-exposure study was reported for male and female mice, two

studies were in female mice only, and two studies were in male rats only (see Table 4-1). Overall, increased incidences of liver tumors were reported for both sexes in mice and male rats over a range of doses and duration of exposure indicating a robust tumorigenic response for dichloroacetic acid.

In male mice, dichloroacetic acid induced a significant increase in hepatocellular adenoma and carcinoma at 5,000 mg/L for 60 weeks in a multiple-dose study for 60 to 75 weeks (50, 500, 5,000 mg/L) (DeAngelo *et al.* 1991) and at lower doses (starting at 1,000 mg/L) in a multi-dose, two-year study (0, 500, 1,000, 2,000, 3,500 mg/L, see Figure 4-1A) (DeAngelo *et al.* 1999). Three single high-dose studies of dichloroacetic acid in male mice yielded significant incidence rates for hepatocellular adenoma and carcinoma at 500 mg/L in a two-year study (Daniel *et al.* 1992) and 3,500 mg/L (DeAngelo *et al.* 1991) or 5,000 mg/L (Herren-Freund *et al.* 1987); in 60-week studies. Bull *et al.* (1990) reported that 50% (5 of 10) of male mice treated with 2,000 mg/L of dichloroacetic in the drinking water for one year developed hepatocellular carcinoma; however, only mice with gross liver lesions were histologically examined, including only two out of the 35 control mice in this study, which also had other study limitations (see Appendix C). In female mice, dichloroacetic acid caused significant increases in the incidence of hepatocellular adenoma at the mid (860 mg/L) and high dose (2,600 mg/L) and carcinoma at the high dose (2,600 mg/L) in an ~1.5 year study (Pereira 1996) (see Figure 4-1D); only hepatocellular adenoma was significantly increased at the high dose in a one-year study reported in the same publication.

In a stop-exposure study by Wood *et al.* (2015), 4-week-old mice were exposed to dichloroacetic acid in the drinking water for 10 weeks and then maintained on deionized water until 98 weeks of age for a total observation period of 94 weeks. In male mice, significant increases in the incidence of hepatocellular adenoma, carcinoma, and adenoma or carcinoma or hepatoblastoma (combined) were induced at the high dose (3,500 mg/L) with a significant positive trend for all three analyses. Female mice had significantly increased incidences of hepatocellular adenoma or carcinoma or hepatoblastoma combined at 1,000 mg/L and 2,000 mg/L with a significant positive trend. Wood *et al.* noted that the tumor incidence and number induced by the high dose level in their study were ≥ 85% of the same findings seen after continuous lifetime exposure.

Dichloroacetic acid significantly increased the incidence of hepatocellular neoplasms in male rats as reported in two studies described in the same publication (DeAngelo *et al.* 1996) that differed slightly in duration; in the study with a duration of 103 weeks, both the incidence of hepatocellular carcinoma and the combined incidence of hepatocellular adenoma and carcinoma were significantly increased at a dose of 2,500 mg/L, and in the second study with a duration of 100 weeks, the combined incidence of hepatocellular adenoma and carcinoma was significantly increased at a dose of 500 mg/L. A non-significant increase in hepatocellular adenoma was observed in male rats exposed to 500 mg/L (21% vs. 4% in controls), in a two-year study and at a high dose in a one-year study conducted by Richmond *et al.* (1995), which provides some support for the findings in the DeAngelo study.

Trichloroacetic acid was also tested in multiple studies (11 studies reported in 7 publications) in male and female mice but with only one study reporting results for male rats. In male mice, significantly increased incidences of hepatocellular adenoma and carcinoma were induced in two drinking water studies, one tested a single dose of 4,500 mg/L for 2 years and a multi-dose

study, which reported significantly increased incidences after 1 year at 5,000mg/L and after 2 years at 500 mg/L by DeAngelo and coworkers (DeAngelo *et al.* 2008) and in a single-dose study by Herren-Freund *et al.* (Herren-Freund *et al.* 1987) using a dose of 5,000 mg/L for 61 weeks. Significantly increased incidences of hepatocellular adenoma and carcinoma in male mice was observed at a much lower dose (500 mg/L) in the only study (multi-dose 50, 500 mg/L) that tested for carcinogenicity at doses less than 4,500 mg/L) (DeAngelo *et al.* 2008) (see Figure 4-1A). Bull *et al.* (1990) reported that a few hepatocellular carcinomas were observed in male mice in the mid-dose (1,000 mg/L, 2 of 5 animals) and high dose trichloroacetic acid groups (2,000 mg/L, 4 of 11 animals); however, there were only two control mice in this study, which had many study limitations (see Appendix C, Table C-3o). In female mice, trichloroacetic acid caused significant increases in the incidence of hepatocellular carcinoma at the mid (1,100 mg/L) and high-dose (3,300 mg/L) and adenoma at the high dose (3,300 mg/L) in an ~1.5 year study (Pereira 1996); the incidence of hepatocellular carcinoma was significantly increased at the high dose in a one-year study reported in the same publication. Trichloroacetic acid did not significantly increase the incidence of hepatocellular neoplasms in a 2-year, multi-dose (0, 50, 500, 5,000 mg/L) study in male rats (DeAngelo *et al.* 1997).

Intraperitoneal injection (2000 mg/L over two injections in neonatal male and female mice) of trichloroacetic acid did not induce liver tumors in male or female mice at either the 12-month or 20-month observation period (Von Tungeln *et al.* 2002).

Bromine-containing haloacetic acids: Dibromoacetic acid, bromochloroacetic acid, bromodichloroacetic acid

Exposure to dibromoacetic acid, bromochloroacetic acid, and bromodichloroacetic acid were tested by NTP in male and female mice and male and female rats exposed in the drinking water (NTP 2007a, 2009, 2015). Dibromoacetic acid was given at 50, 500, and 1,000 mg/L and the bromochloro- and bromodichloroacetic acids were given at 250, 500 and 1,000 mg/L Overall, the studies provide convincing evidence that these chemicals cause malignant tumors in mice: hepatocellular carcinoma in male and female mice and hepatobastoma in male mice, which were outside the historical control ranges, and positive dose-response trends. Additionally, female mice exposed to bromodichloroacetic acid had a significant increase in hemangiosarcoma in the liver at 1,000 mg/L. A summary of the findings for the different types of hepatocellular neoplasms and combinations is provided in Table 4-3 and the incidences of hepatocellular carcinoma and hepatoblastoma are provided in Figures 4-1 and 4-2. None of the bromine-containing dihaloacetic acids caused a significant increase in the incidence of hepatocellular neoplasms in rats.

Table 4-3. Hepatocellular neoplasms in mice exposed to bromine-containing haloacetic acids

HAA (Sex)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or carcinoma (combined)	Hepatoblastoma	All liver neoplasms combined
DBA (males)	Sig. increases at all doses; positive trend	Sig. increases at high dose; positive trend	Sig. increases at all doses; positive trend	Sig. increases at the mid and high dose; positive trend	Sig. increases at all doses; positive trend
DBA (females)	Sig. increases at mid and high doses; positive trend	Sig. increases at mid and high doses; positive trend	Sig. increases at mid and high doses; positive trend		
BCA (males)	Sig. increases at low and mid doses; positive trend	Sig. increases at mid and high doses; positive trend	Sig. increases at all doses; positive trend	Sig. increases at all doses; positive trend	Sig. increases at mid and high doses; positive trend
BCA (females)	Sig. increases at all doses; positive trend	Sig. increase at mid dose; exceeds historical controls at all doses	Sig. increases at all doses; positive trend		
BDCA (males)		Sig. increase at all doses; positive trend		Sig. increases at all doses; positive trend	Sig. increases at low and high dose; positive trend
BDCA (females)	Sig. increase at all doses; positive trend	Sig. increases at mid and high doses positive trend	Sig. increases at all doses; positive trend	Sig. increase at high dose; positive trend	

BCA = bromochloroacetic acid; DBA = dibromoacetic acid; BDCA = bromodichloroacetic acid; sig. = statistically significant.

Comparison of liver neoplasms findings across HAAs

Figures 4-1 and 4-2 compare the incidences of different types of hepatocellular neoplasms (carcinoma or hepatoblastoma) in male and female mice. (Data from studies with similar designs and/or similar laboratories were chosen for the graphs.) Incidences of combined liver neoplasms were not plotted because of the high background incidence of total liver neoplasms in controls. In general, no clear differences in the strength of the association with liver cancer by type or number of halogens was observed; however, the data are difficult to interpret because of differences in background carcinoma rates (especially between the chlorine-only haloacetic acids and bromine-containing haloacetic acids) and the high incidences of liver neoplasms in treated animals. There is some evidence to suggest that dichloroacetic acid but not the bromine-containing haloacetic acids cause liver neoplasms in rats. In contrast, the bromine-containing haloacetic acids may be linked to hepatoblastoma. Incidences of hepatoblastoma were significantly increased in male mice by the three bromine-containing haloacetic acids tested by NTP and in female mice by bromodichloroacetic acid. The only study to observe hepatoblastoma in mice exposed to a chlorine-only haloacetic acid was the stop-exposure study (Wood *et al.*

2015) which found a single hepatoblastoma at the lowest dose in males and none in females. However, the NTP studies also had the most complete necropsies and pathological evaluations of all of the studies reviewed, which might have contributed to identification of these tumors in mice exposed to bromine-containing haloacetic acids.

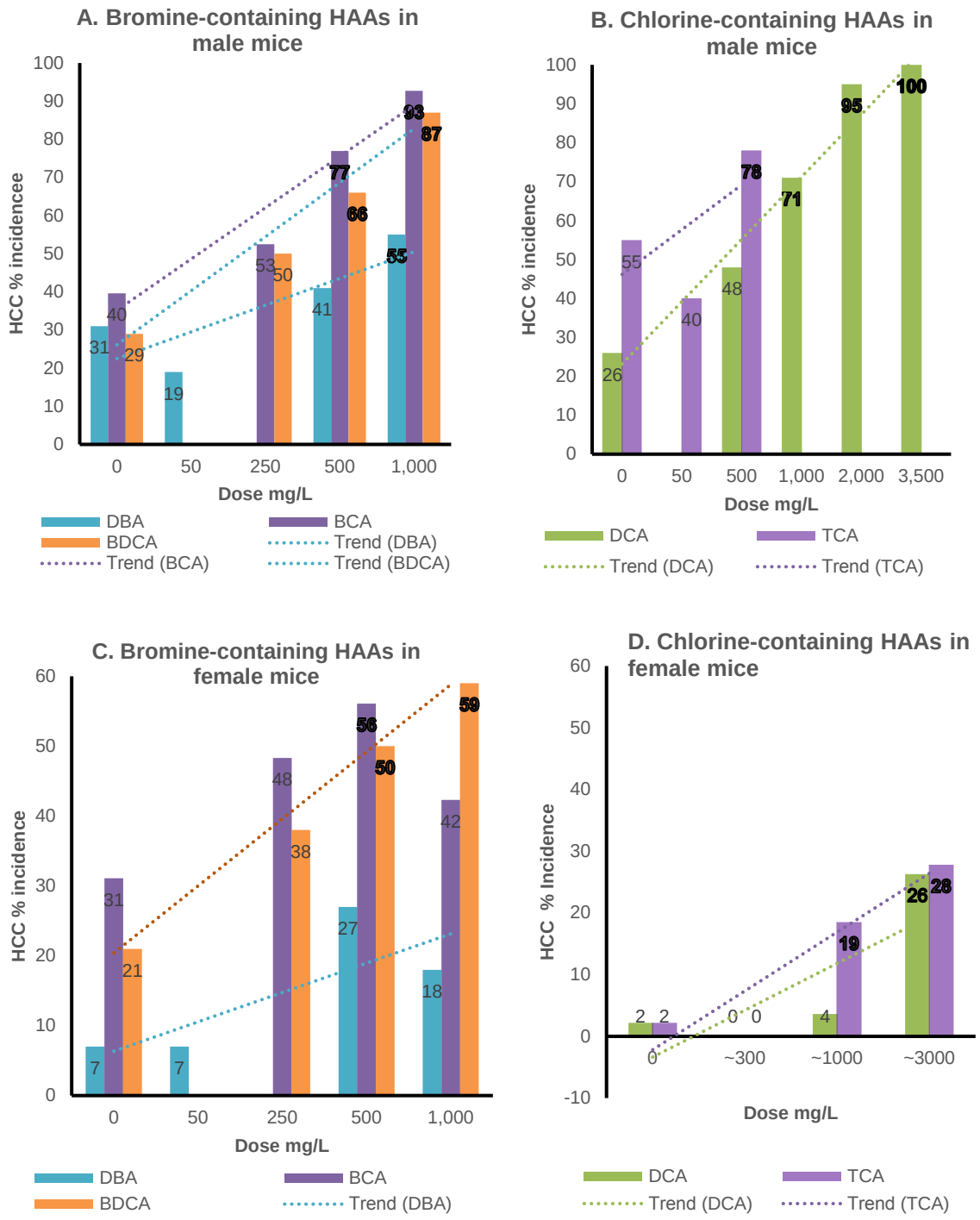

Figure 4-1. Hepatocellular carcinoma (HCC) in female and male mice exposed to di- and trihaloacetic acids (HAAs)

Source: NTP 2007a, NTP 2009, NTP for bromine-containing HAA in male (A) and female (C) mice; DeAngelo *et al.* 1999 for DCA in male mice (B) and DeAngelo *et al.* 2008 for TCA in male mice (B) and Pereira 1996 for DCA and TCA in female mice (D).
BCA = bromochloroacetic acid; BDCA = bromodichloroacetic acid; DBA = dibromoacetic acid; DCA = dichloroacetic acid; TCA = trichloroacetic acid.
The NTP and DeAngelo studies were 2 years in duration and the Pereira studies were 1.5 years in long. Bold numbers reflect statistically significant findings of each HAA compared to its concurrent control. Linear refers only to a positive trend and not the shape of the dose-response curve. Trends were not reported by the authors for DCA and TCA, but the NTP calculated trends based on the Cochran-Armitage test. In the NTP studies, BCA and BDCA were not tested at 50 mg/L and DBA was not tested at 250 mg/L. For the chlorine-only HAAs in males (C), the doses for DCA were 500, 1,000, 2,000, and 3,500 and for TCA they were 50 and 500 mg/L. In females (D), DCA was tested at 260, 860, and 2,600 mg/L and TCA was tested at 330, 1,100, and 3,300 mg/L (equimolar doses for DCA and TCA).

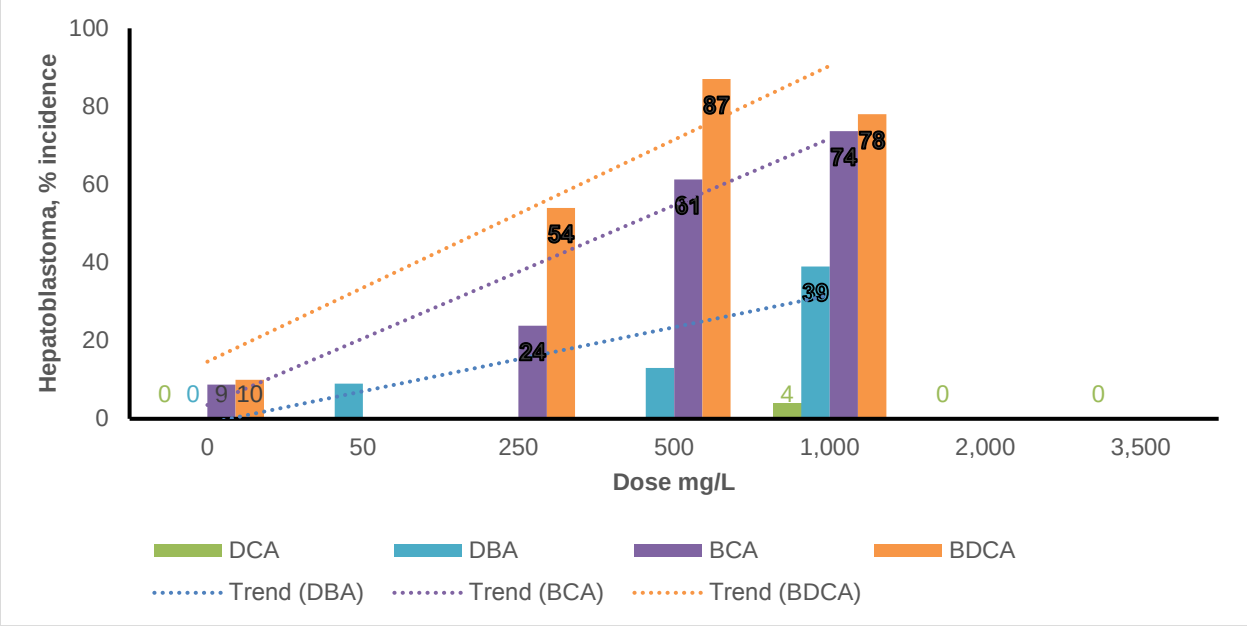

Figure 4-2. Hepatoblastoma in male mice exposed to di and trihaloacetic acids (HAAs)

Source: NTP 2007a, NTP 2009, NTP 2015for the bromine-containing HAA and Wood *et al.* 2015 for DCA (stop exposure study).
BCA = bromochloroacetic acid; BDCA = bromodichloroacetic acid; DBA = dibromoacetic acid; DCA = dichloroacetic acid. Bold numbers reflect statistically significant findings of each HAA compared to its control. Trend refers only to a positive trend and not the shape of the dose-response curve. BCA and BDCA were not tested at 50 mg/L and DBA was not tested at 250 mg/L. DCA was tested at 1,000, 2,000, and 3,500 mg/L, but no hepatoblastomas were reported for the 2,000 or 3,500 mg/L doses.

4.4.2 Other tumors (see Tables C-9 and C-10 in Appendix C)

The occurrence of tumors at multiple organ sites reinforces the evidence that some halogenated acetic acids have systemic carcinogenic activity in addition to the liver. In addition to liver tumors that developed with chronic exposure to the three brominated haloacetic acids tested by NTP in well-conducted studies, dibromoacetic acid (NTP 2007a), bromochloroacetic acid (NTP 2009), and bromodichloroacetic acid (NTP 2015) increased the incidences of neoplasms at organ sites outside the liver in both rats and mice. No increases in the incidence of neoplasms were observed after exposure to chlorine-only haloacetic acids; however, these tumors were reported only in studies conducted by NTP, which included full necropsies. Since most of the non-NTP

studies examined only the liver histologically, the presence of tumors at other systemic sites cannot be definitively ruled out (see Section 4.2, Table 4-2, and Appendix C).

Malignant mesothelioma was the only site to be induced by all three bromine-containing haloacetic acids and thus the findings are discussed across haloacetic acids. The findings for the other neoplasms are organized by the specific haloacetic acid. In the NTP studies, dibromoacetic acid was tested at 0, 50 (low), 500 (mid), and 1,000 (high) mg/L and bromochloroacetic acid and bromodichloroacetic were tested at 0, 250 (low), 500 (mid) and 1,000 (high) mg/L. The study on bromodichloroacetic acid differs from the other two haloacetic acids tested by NTP in that it was conducted in F344/NTac rats whereas the other two haloacetic acids were tested in F344/N rats. All three of these haloacetic acids were tested in B6C3F$_1$ mice. Details on the study designs and findings are provided in Appendix C, Tables C-4a to C-4d, C-5a to C-5d, C-7a to C-7d).

Malignant mesothelioma

Dibromoacetic acid (NTP 2007a), bromochloroacetic acid (NTP 2009), or bromodichloroacetic acid (NTP 2015) (Figure 4-3) increased the incidence of malignant mesothelioma in male rats with drinking water exposure. Both dibromoacetic acid and bromodichloroacetic acid caused significant positive dose-response trends. The historical control range for these tumors was exceeded for dibromoacetic acid and bromochloroacetic acid (NTP 2009) at all doses and for dibromoacetic acid and bromodichloracetic acid at the high dose. The strongest response was found for bromodichloroacetic acid, which significantly increased malignant mesothelioma at all doses, reaching 78% (poly-3 adjusted rate) for male rats exposed to the high dose.

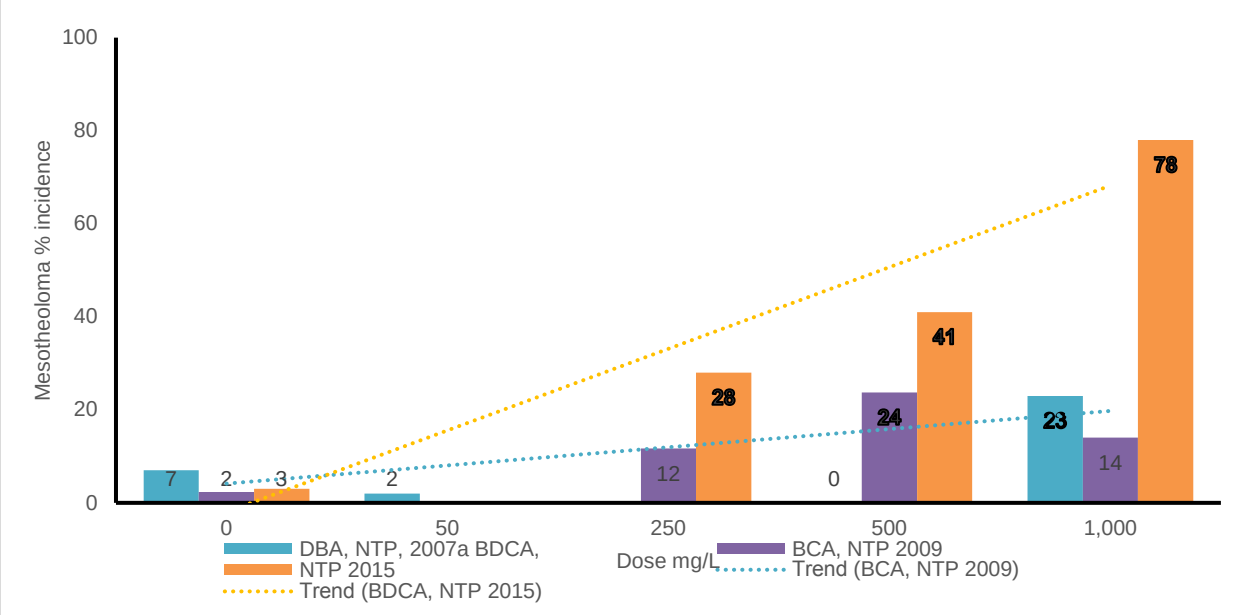

Figure 4-3. Malignant mesothelioma incidence in male rats exposed to bromochloroacetic acid (BCA), dibromoacetic acid (DBA), or bromodichloroacetic acid (BDCA) in drinking water

Note: DBA was not tested at 500 mg/L.
BCA = bromochloroacetic acid; BDCA = bromodichloroacetic acid; DBA = dibromoacetic acid.

Dibromoacetic acid

Mononuclear cell leukemia incidence was significantly increased by exposure to dibromoacetic acid (NTP 2007a) at the high dose in female rats with a positive dose-response trend. Historical control rates can help interpret these findings because the background rates of mononuclear cell leukemia in F344/N rats are high and variable. The poly-3 adjusted rates for the mid (35%) and high dose (47%) exceeded the historical control ranges in drinking water studies (20% to 30%) and thus increase the confidence that this is an exposure-related response. A significant increase in the incidence of mononuclear cell leukemia was observed in male rats at the low but not the mid and high dose and no dose-response was observed; the incidence of tumors for the high dose was somewhat lower than the incidence in controls which exceeded the historical control range. The poly-3 adjusted rates for the controls (37%), low (66%), and mid (56%) but not the high dose exceeded the historical control range of 26% to 34% for drinking water studies. Overall, the incidence of mononuclear cell leukemia in male rats may be related to exposure to dibromoacetic acid but the findings are inconclusive.

Lung adenoma and adenoma or carcinoma (combined) were also significantly increased by exposure to dibromoacetic acid (NTP 2007a) at the mid-dose in male mice and a significant dose response was observed for adenoma. The poly-3 adjusted incidence for lung adenoma or carcinoma in the mid (49%) and high dose (37%) and for adenoma at the mid (38%) and high dose (27%) exceeded the historical control range (12% to 26%) for drinking water studies. For female mice tumor incidence for lung adenoma or carcinoma at the high dose (15%) exceeded the historical control range of 2% to 12%. A significant dose response for adenoma was observed but none of the pairwise dose comparisons were statistically significant. Overall, the data provided indicate that the lung is a target organ site for dibromoacetic acid with the strongest evidence from male mice.

Bromochloroacetic acid

In addition to causing malignant mesothelioma in male rats, bromochloroacetic acid also increased the neoplasms of the mammary gland in female rats and large intestine in female and male rats.

Bromochloroacetic acid (NTP 2009) administered in the drinking water significantly increased the incidence of multiple mammary gland fibroadenomas in the mid- and high-dose groups in female rat. This haloacetic acid also caused dose-related increases in the incidence of large intestinal adenoma in both male and female rats. Significant increases in incidence (15.5%) were observed at the highest dose in female rats compared to controls. These are rare tumors with a historical control incidence in drinking water studies of 0% and can progress to malignant tumors of the large intestine.

A statistically significant increase in pancreatic islet adenoma in male rats exposed to bromochloroacetic acid was observed in the mid-dose groups compared to controls, which exceeded the historical control rates for both drinking water studies (6% to 10%) and studies by all routes (0% to 12%) for the poly-3 adjusted incidence for the 500 mg/L dose (9/50, 22%) (NTP 2009). However, the incidence was similar in the high-dose group (7%) to the concurrent controls (7%) and there were no significant increases in other types of pancreatic islet lesions (e.g., hyperplasia, carcinoma).

Bromodichloroacetic acid

Compared to controls, exposure to bromodichloroacetic acid (NTP 2015) significantly increased the incidence of mammary gland fibroadenoma (in all exposed groups), carcinoma (high dose), adenoma or carcinoma combined (mid and high dose), and all types of neoplasms combined (in all exposed groups) in female rats. Significant dose-response trends were observed for mammary gland carcinoma, fibroadenoma, and adenoma, carcinoma, or fibroadenoma combined.

Bromodichloroacetic acid treatment resulted in a non-statistically significant increase of large intestinal (cecum, colon, and rectum combined) adenoma in male rats with 2/50 each in the mid- and high-dose groups. No historical control data were available for the F344/NTac strain; however, large intestine tumors are very rare tumors in F344/N rats with a very low incidence of 0/699 for 2013 historical control incidence and 0% to 2% for the 2009 historical control range) (NTP 2015). Overall, the evidence was considered equivocal because of the small numbers of neoplasms in the exposed animals.

Skin tumors, keratoacanthoma, subcutaneous fibromas, and combined incidences of epithelial tumors (i.e., squamous-cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal-cell-adenoma, basal-cell carcinoma, or squamous-cell carcinoma [combined]) were significantly increased in male rats exposed to the highest dose of bromodichloroacetic acid (NTP 2015). Positive trends were observed for fibroma, keratoacanthoma, and basal-cell adenoma.

Harderian gland adenoma and adenoma or carcinoma (combined) incidences were significantly increased in the high- and mid-exposure groups in male mice exposed to bromodichloroacetic acid (NTP 2015). Significant positive dose-response trends were observed for adenoma and carcinoma as well as adenoma or carcinoma (combined). The poly-3-adjusted incidences of the combined neoplasms at all dose levels (low dose = 26%, mid dose = 38%, high dose = 51%) exceeded the historical control range of 12% to 14% for drinking water controls and 6% to 24% for all routes.

Increases in the incidence of brain and oral cavity neoplasms were observed after administration of bromodichloroacetic acid in the drinking water. For each tumor type, the increase in the incidence of the neoplasm was small and non-significant but exceeded the historical control range. However, the low number of Fischer 344/NTac historical controls limited further interpretation of these findings. Overall, the evidence is unclear whether these effects are related to treatment.

Synthesis across HAAs

Overall, bromodichloroacetic acid appears to have the strongest association with neoplasms in experimental animals because it induced the largest number of different types of neoplasms (liver, malignant mesothelioma, mammary gland, skin, and Harderian gland, large intestine, and possibly neoplasms of the brain and oral cavity). In addition, it appears to have the strongest association with mesothelioma, which was the only neoplasm induced by all three bromine-containing haloacetic acids. Bromochloroacetic acid induced similar types of neoplasms as bromodichloroacetic acid – mesothelioma and mammary gland tumors – whereas the bromine-only haloacetic acid caused different types of tumors – mesothelioma, lung, and mononuclear cell leukemia.

4.4.3 Transgenic studies

Two strains of male and female transgenic animals (Tg.AC hemizygous and p53 haploinsufficient mice) were used to test dichloroacetic acid in drinking water and Tg.AC hemizygous transgenic mice were used to test dichloroacetic acid by dermal exposure. The intent of these studies was to determine whether these animal models could serve as an adjunct to the 2-year rodent cancer assays for water disinfection by-products, given that dichloroacetic acid is positive in rodent cancer studies. The limitations of these transgenic models were discussed in Section 4.1. The expected neoplasms with exposure to carcinogens that affect the p53 gene are lymphoma or sarcoma in p53 haploinsufficient mice and squamous-cell papilloma or carcinoma of the skin or forestomach in Tg.AC hemizygous mice (Tennant *et al.* 2001, Eastmond *et al.* 2013).

One publication reported testing dichloroacetic acid administered in the drinking water in males and females of both strains of transgenic animals with two different study durations (NTP 2007b). After 41 weeks of exposure, dichloroacetic acid induced significant incidences of female Tg.AC hemizygous mice with multiple squamous-cell papilloma of the forestomach and lung alveolar/bronchiolar adenoma in males. No neoplasms or preneoplasms were significantly increased in p53 haploinsufficient mice.

Two dermal exposure studies of dichloroacetic acid were conducted in male and female Tg.AC hemizygous mice (NTP 2007b). Dermal exposure to dichloroacetic acid induced increased incidences of squamous-cell papilloma and epidermal hyperplasia of the skin at the site of application in both sexes. Although these increases were significant, this model is susceptible to false-positive findings of squamous-cell papilloma of the skin. It is not possible to distinguish dichloroacetic acid carcinogenic effects in this model system from a non-carcinogenic effect that leads to skin irritation and false-positive results.

Though these model systems are not ideal and can't be used directly to interpret the carcinogenic potential in experimental animals, the presence of neoplasms at the site of application offers evidence not only of increased incidences but also of a specific location where the exposure occurred and further supports the relationship of the results with treatment with dichloroacetic acid.

4.4.4 Initiation-promotion studies

Five initiation-promotion studies were identified that tested dichloroacetic acid, trichloroacetic acid, and monoiodoacetic acid. Dichloroacetic acid or trichloroacetic acid were administered in drinking water as a promoter in two sets of studies. In one set of studies, female mice were initiated by an intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) (Pereira *et al.* 1997). Dichloroacetic acid and trichloroacetic acid both significantly increased the multiplicity of hepatocellular adenomas and dichloroacetic acid significantly increased the multiplicity of foci of altered hepatocytes. In the second set of studies, male mice were injected intraperitoneally with ethylnitrosourea (ENU) (Herren-Freund *et al.* 1987). Dichloroacetic acid and trichloroacetic acid both significantly increased the incidence of hepatocellular adenoma and carcinoma at 2,000 and 5,000 mg/L above that caused by ENU alone. Further, 5,000 mg/L of either haloacetic acid alone, without ENU, significantly increased hepatocellular adenoma and carcinoma.

Monoiodoacetic acid was administered by dermal application as a promoter to mice (sex not reported) that were initiated by dermal application of DMBA (Gwynn and Salaman 1953). Monoiodoacetic acid significantly increased skin papillomas (exact histological classification not reported) in the high-dose group when compared to either acetone or acetic acid controls.

Only the Herren-Freund *et al.* (1987) initiation-promotion study included groups without the initiator being administered. The remaining initiation-promotion studies only examined the added effect of haloacetic acids to an already established carcinogenic process and so are not directly interpretable as to animal carcinogenicity (Pereira *et al.* 1997, Gwynn and Salaman 1953).

4.5 Synthesis

The evidence for the carcinogenic potential of haloacetic acids in experimental animals is strong as numerous studies have shown significant increases in the incidences of neoplasia from exposure to several haloacetic acids. All haloacetic acids, except for the monochlorohaloacetic acids that were tested, induced significantly increased incidences of liver neoplasms in mice or rats, which is consistent with a possible common carcinogenic mode of action (see Table 4-4).

Dichloroacetic acid and trichloroacetic acid induced hepatocellular adenoma and carcinoma in both sexes of $B6C3F_1$ mice, and in male Fischer 344 rats exposed to dichloroacetic acid.

Dibromoacetic induced hepatocellular adenoma and carcinoma in male and female mice, hepatoblastoma and lung tumors in male mice. In female rats, it induced mononuclear-cell leukemia and in male rats, malignant mesothelioma.

Bromochloroacetic acid caused tumors at several tissue sites in both rats and mice. It induced treatment-related malignant mesothelioma and adenoma of the large intestine in male rats, multiple fibroadenomas of the mammary gland in female rats, hepatoblastoma in male mice and hepatocellular adenoma and carcinoma in male and female mice.

Bromodichloroacetic acid had the most treatment-related cancer sites in both rats and mice. Male mice had treatment-related incidences of adenoma, adenoma or carcinoma (combined) of the Harderian gland, hepatoblastoma, and hepatocellular carcinoma. Female mice had increased incidences of hepatoblastoma, and hepatocellular adenoma and carcinoma. Male Fischer 344/NTac rats had increased incidences of malignant mesothelioma, epithelial tumors of the skin (combined) and subcutaneous fibroma, which typically does not progress to a malignancy. Female rats had treatment-related increases in incidences of fibroadenoma (includes multiple) and carcinoma of the mammary gland.

The mechanisms by which haloacetic acids might induce cancer are discussed in Section 5 and overall conclusions for the carcinogenicity of haloacetic acids are reported in Section 6.

Table 4-4. Results from cancer studies in experimental animals

Chemical (Route)	Neoplasms in Mice (Sex)	Neoplasms in Rats (Sex)	Reference
Monochloroacetic acid			
Carcinogenesis (Gavage)	None	Not tested	NTP 1992
Dichloroacetic acid			
Carcinogenesis (Drinking water)		Liver – hepatocellular carcinoma (M) Liver – hepatocellular adenoma or carcinoma (M)	DeAngelo *et al.* 1996
Carcinogenesis (Drinking water)	Liver – hepatocellular adenoma and carcinoma (M/F)		DeAngelo *et al.* 1991, 1999 Herren-Freund *et al.* 1987 Daniel *et al.* 1992 Wood *et al.* 2015 Pereira 1996
Dibromoacetic acid			
Carcinogenesis (Drinking water)	Liver – hepatocellular adenoma and carcinoma (M/F) Liver – hepatoblastoma (M) Lung – alveolar/bronchiolar adenoma (M) Lung – alveolar/bronchiolar adenoma or carcinoma (M)	Malignant mesothelioma (M) Mononuclear cell leukemia (F)	NTP 2007a
Bromochloroacetic acid			
Carcinogenesis (Drinking water)	Liver – hepatocellular adenoma and carcinoma (M/F) Liver – hepatoblastoma (M)	Malignant mesothelioma (M) Mammary gland – fibroadenoma (multiple only) (F) Large intestine – adenoma (rare) (M/F)	NTP 2009

Chemical (Route)	Neoplasms in Mice (Sex)	Neoplasms in Rats (Sex)	Reference
Trichloroacetic acid			
Carcinogenesis (Drinking water)	Liver – hepatocellular adenoma and carcinoma (M/F)		DeAngelo *et al.* 2008 Herren-Freund *et al.* 1987 Pereira 1996
Bromodichloroacetic acid			
Carcinogenesis (Drinking water)	Liver – hepatocellular adenoma (F) Liver – hepatocellular carcinoma (M/F) Liver – hepatoblastoma (M/F) Harderian gland – adenoma (M) Harderian gland – adenoma or carcinoma (combined) (M)	Malignant mesothelioma (M) Mammary gland – fibroadenoma (includes multiple) and carcinoma (F) Skin – fibroma (M) Skin – keratoacanthoma (M) Skin – squamous-cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal-cell adenoma, basal-cell carcinoma, or squamous-cell carcinoma (combined) (M)	NTP 2015

5 Human Cancer Studies

Water disinfection by-products include a complex mixture of chemicals created from reactions between water disinfection agents (such as chlorine) and organic matter in the water. These include a wide variety of compounds that lack reliable exposure measurement methods. Often, trihalomethanes as a class or a specific trihalomethane are used as a proxy measure for the complex mixture of chemicals in disinfected water (see Section 2.3.2 and Figure 2-5). To date, only one human epidemiological study has been identified that evaluates haloacetic acid exposures (i.e., any of the 13 individual chemicals or as a class or subclass) in humans and cancer risk. However, in addition to this cohort study, existing data in the primary literature of exposures to water disinfection mixtures or specific classes of water disinfection by-products (such as trihalomethanes) may serve as surrogates for chlorinated water. Findings from the cohort study are discussed below, along with a brief discussion of human cancer findings and any potential association with disinfection by-products. The discussion summarizes the review in the general remarks section of the International Agency for Research on Cancer (IARC) monograph on Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water (IARC 2013c) and updates the literature since that monograph.

5.1 Cohort Study

Jones *et al.* (2017) investigated the risk of kidney cancers from ingested nitrate and other disinfection by-products in the Iowa Women's Health Study (IWHS), a cohort of 41,836 post-menopausal women follow for up to 24 years, from 1986 to 2010. This study categorized women's drinking water exposure by source: public water sources, private well, and other. Exposure then to water disinfection products was split into time at that source (≤ 10 years or > 10 years), and the cancer analysis was done on women exposed to public water sources for greater than 10 years (n = 15,577). Exposure to haloacetic acids, including a mixture of five regulated haloacetic acids (monochloroacetic acid, monoacetic acid, dichloroacetic acid, dibromoacetic acid, trichloroacetic acid) and individual haloacetic acids (dichloroacetic acid, trichloroacetic acid and bromochloroacetic acid, which is unregulated) and trihalomethanes (total as well as individual) were estimated via and expert assessment that used available measure (data from plants, water source, quality, treatment, and disinfection type). A total of 266 incident kidney cancers were observed among all women in the study, with 125 occurring among women with greater than 10 years exposure to a public water source. No associations were seen between kidney cancer risk and either individual or combined haloacetic acid measures. The data for this study is presented in Table 5-1.

5.2 Other human cancer studies of disinfection by-products

The IARC working groups reviewed several epidemiological studies (both cohort and case-control studies) of chlorinated water or specific disinfection by-products (i.e., trihalomethanes as a class or chloroform) primarily on urinary bladder cancer and some other types of cancer but did not make a formal evaluation of potential cancer hazards from chlorinated water because the monographs were on individual disinfection by-products. In general, IARC (2013c) reported positive associations in all nine case-control studies of DBP or chlorinated water, or both, and urinary bladder cancer, including a dose-response relationship in some studies. Among these studies, 5 found an association between the highest exposure levels of chlorinated water and

bladder cancer (3 among all participants, 1 among men only and 1 for women only). Six studies found an association between trihalomethanes and bladder cancer, with 5 finding a dose-response relationship (3 of these for men only). One study (Cantor *et al.* 2010) found the association varied by genetic polymorphisms in GST and CYP genes. Results from three cohort studies were inconsistent, with only one study reporting a statistically significant risk to women for exposure to chloroform; however, no dose response was seen.

Increased risk for other types of cancer was seen in a limited number of studies, with two cohort studies reporting increased risks for lung, melanoma, breast, and esophageal cancers. Increased risks for kidney, brain, melanoma, and non-melanoma skin cancers were reported in four individual case-control studies. However, neither IARC (2004a, 2004b) nor IARC (2013a, 2013b) made a formal evaluation of specific disinfection by-products, as the studies were not specific for the individual disinfection by-products under review. This complexity of exposure and the correlation between the many surrogates of disinfection by-products and individual chemicals, such as haloacetic acids, make human epidemiological studies of individual chemicals difficult to conduct and interpret.

Since the IARC reviews, one case-control study in Spain was identified that found a non-statistically significant increased risk for colorectal cancer and brominated trihalomethanes, but did not find an association with total trihalomethanes (Villanueva *et al.* 2016). Another analysis within the Iowa Women's Health Study also found a non-significant increase in bladder cancer for those with the highest level of nitrate-nitrogen and trihalomethane exposure (Jones et al 2016). Based on the evidence to date, there appears to be some association with disinfection by-products and cancer, particularly with urinary bladder cancer, but the extent of the involvement of the haloacetic acids individually or as a class is unclear.

5.3 Preliminary level of evidence conclusion

Overall, the data from cancer studies in humans are inadequate to evaluate the relationship between human cancer and exposure specifically to the individual haloacetic acids, subclasses of haloacetic acid, or haloacetic acids as a class.

Table 5-1. Haloacetic acid exposure and kidney

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/ deaths	Hazard ratio (95% CI)	Co-variates controlled	Comments, strengths, and weaknesses
Jones *et al.* (2017) Cohort Study – The Iowa Women's Health Study USA 1986-2010	Women age 55-69 at baseline exposed to public water source for greater than 10 years. Total population N = 41,836 Women exposed to public water source > 10 years: n=15,577	**HAA5* (µg/L)**			Age, smoking status, pack-years of smoking, body mass index, ln-transformed NO_3-N	Exposure duration: Participants included in this analysis were exposed for at least 10 years at the same location. Confounding: While difficult to separate the effects of the individual disinfection by-products, this study attempts to control for some of these factors in the analysis, along with other related factors. Strengths: Long exposure duration for participants and low loss to follow-up. The study attempted to estimate exposure levels in a systematic way for participants. Weaknesses: Lack of regular measurement at all water facilities, and lack of individual measurement for exposure, and inability to full separate the effects of the individual disinfection by-products. Study enrollment restricted to premenopausal women.
		<1.89	38	Ref. 1.0		
		1.89-3.48	27	0.84 (0.51-1.4)		
		3.49-6.43	35	0.78 (0.49-1.2)		
		>6.43	25	0.65 (0.39-1.1)		
		P trend		*0.18*		
		Continuous	125	0.99 (0.98-1.0)		
		Years >30 µg/L HAA5+				
		0	109	Ref 1.0		
		<16	8	0.76 (0.37-1.6)		
		>=16	8	0.74 (0.36-1.5)		
		p trend		*0.32*		
		Continuous	125	0.99 (0.95-1.0)		
		Dichloroacetic Acid (µg/L)				
		<1.65	36	Ref 1.0		
		1.65-2.27	35	0.99 (0.62-1.58)		
		2.28-4.84	28	0.79 (0.48-1.30)		
		>4.84	26	0.75 (0.45-1.24)		
		p trend		*0.30*		
		Continuous		0.98 (0.95-1.01		
		Trichloroacetic Acid (µg/L)				
		<0.25	46	Ref 1.0		
		0.25-0.63	7	0.40 (0.18-0.89)		
		0.64-1.69	46	0.89 (0.58-1.36)		
		>1.69	26	0.68 (0.42-1.10		
		p trend		*0.26*		
		Continuous		0.99 (0.96-1.01)		

Reference, study design, location, and year	Population description & exposure assessment method	Exposure category or level	Exposed cases/ deaths	Hazard ratio (95% CI)	Co-variates controlled	Comments, strengths, and weaknesses
		Bromochloroacetic Acid (μg/L)				
		<0.1	52	Ref 1.0		
		0.1-0.94	18	1.20 (0.69-2.08)		
		0.95-1.89	28	0.76 (0.47-1.26)		
		>1.89	27	0.78 (0.49-1.25)		
		p trend		*0.25*		
		Continuous		0.95 (0.86-1.05)		

*HAA5 – regulated haloacetic acids (monochloracetic, trichloracetic, dichoroacetic, monobromoacetic, and dibromoacetic acids)

+ Number of years the annual average level was > ½ the MCL (maximum containment level) value

6 Mechanistic and Other Relevant Data

Of the 13 haloacetic acids found in drinking water, six were tested for carcinogenicity in a long-term assay in experimental animals (see Section 4). Liver tumors were the most common; however, the brominated haloacetic acids induced tumors at several other sites. As stated in the background and methods section, an objective of this monograph is to evaluate whether there are other relevant data that would allow the haloacetic acids to be evaluated as a class. The purpose of this section is to address the following key questions: (1) what are the biologically plausible modes of action through which these compounds may cause cancer in humans; (2) what are molecular initiating events and/or early and late key events associated with the potential modes of action; (3) and do the haloacetic acids, or subclasses, share a common mode(s) of carcinogenic action?

To facilitate the identification of potential mechanisms of cancer, the discussion of the body of literature was generally organized according to the 10 characteristics of carcinogens as defined by Smith *et al.*(2016) with a few modifications. The outline of this section provides a discussion of the data starting with early events and/or overall experimental support and is as follows: electrophilicity (Section 6.1); alteration of cellular metabolism (Section 6.2, somewhat related to altered nutrient supply, which is part of one of the characteristics of carcinogens); induction of oxidative stress (Section 6.3); genotoxicity and alteration of DNA repair (Section 6.4); induction of epigenetic alterations (Section 6.5); modulation of receptor-mediated effects (Section 6.6); inhibition of GST-ζ (Section 6.7, specific proposed mode of action related to electrophilicity), cell immortalization (Section 6.8); alteration of cell proliferation or cell death (Section 6.9); and induction of chronic inflammation or immunosuppression (Section 6.10). Studies that investigated global gene expression changes are relevant to multiple characteristics of carcinogens and are discussed in Section 6.11. Section 6.12 integrates the mechanistic data and provides a brief synthesis of the findings.

6.1 Electrophilicity

Haloacetic acids are recognized as electrophilic compounds due to electron withdrawal from the α-carbon by the halogen substituents, and SN2 reactivity (Plewa *et al.* 2010, Pals *et al.* 2011). The available data from screening studies for protein and DNA reactivity using complementary sets of *E. coli* strains (see Table 6-1) indicate that most haloacetic acids have a predominantly soft electrophilic nature and that the likely molecular initiating event is preferential reaction with protein sulfhydryl groups. However, three brominated species showed nonspecific reactivity (i.e., reaction with both proteins and DNA) in *E. coli* strains, and bromoiodoacetic acid was predicted to preferentially react with DNA in this screening assay. Reaction with thiol groups on proteins can cause indirect genotoxicity, or impair DNA repair through generation of reactive oxygen species (ROS) (Stalter *et al.* 2016). Other modes of action discussed below, including inhibition of pyruvate dehydrogenase kinase (PDK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and GST-ζ are consistent with the soft electrophilic nature of the haloacetic acids. Plewa *et al.* (2004b) showed that the rank order of cytotoxicity and genotoxicity of the monohaloacetic acids was correlated with their electrophilic reactivity (i.e., iodo- > bromo- ≫ chloroacetic acid). Furthermore, the brominated acetic acids consistently show a greater capacity to induce oxidative stress and a greater mutagenic and genotoxic potency compared to the chlorinated forms (see Sections 6.3 and 6.4). Whether or not this is related to a greater capacity of the brominated di- and trihaloacetic acids to react directly with DNA has not been confirmed.

Table 6-1. Electrophilc properties of haloacetic acids

Haloacetic acid	E_{LUMO} (eV)	E_{LUMO} (deprotonated)	TR_{GSH}[a]	TR_{GSH}[a]	TR_{DNA}	Classification[b]
Chloro-	4.54	9.43	0.72	0.69	0.92	–
Bromo-	4.47	8.68	2.30	2.05	0.84	GSH
Iodo-	2.88	7.18	2.68	1.61	0.81	GSH
Dichloro-	3.07	8.44	1.55	1.81	0.5	GSH
Dibromo-	2.76	7.51	1.70	1.25	1.9	GSH/DNA
Bromochloro-	3.11	7.78	2.24	1.61	3.18	GSH/DNA
Chloroiodo-	1.54	6.40	1.39	1.31	1.17	GSH
Bromoiodo-	1.60	6.46	0.59	0.64	1.47	DNA
Trichloro-	2.79	7.13	0.98	1.04	0.99	–
Tribromo-	2.42	6.12	2.39	2.13	2.61	GSH/DNA
Bromodichloro-	2.82	6.65	1.41	1.71	0.73	GSH
Chlorodibromo-	2.47	6.42	1.10	[c]	0.95	–

Source: Stalter *et al.* 2016.

E_{LUMO} = energy of the lowest unoccupied molecular orbital. A lower E_{LUMO} suggests a softer electrophile (Schultz *et al.* 2006).

TR_{GSH} = Toxic ratio of EC_{50} of *E. coli* GSH+/GSH–, TR > 1.2 indicates reaction with soft nucleophiles.

TR_{DNA}= Toxic ratio of EC_{50} of *E. coli* DNA repair +/DNA repair –, TR > 1.2 indicates reaction with hard nucleophiles.

[a]Values presented for two independent experiments.

[b]Indicates if compounds react preferentially with proteins (GSH), DNA, nonspecifically with both, or neither (–).

[c]Measured only in one experiment with two replicates.

6.2 Alteration of cellular metabolism

Modes of action that alter cellular energy metabolism include PDK and GAPDH inhibition (Pals *et al.* 2011, Dad *et al.* 2013, Wood *et al.* 2015, Pals *et al.* 2016). As mentioned above, inhibition of these enzymes is consistent with the soft electrophilic properties of haloacetic acids and results in disruption of cellular energy metabolism and oxidative stress. In the mitochondria, PDKs are a major gatekeeper of pyruvate entry into the tricarboxylic acid cycle while GAPDH is a cytosolic enzyme that catalyzes the sixth step of glycolysis (i.e., conversion of glucose to pyruvate) (Figure 6-1).

In one study, early life exposure to dichloroacetic acid for 10 weeks increased both the incidence and multiplicity of hepatocellular tumors in male and female mice at 98 weeks of age and was almost as carcinogenic as life-long exposures (see Section 4) (Wood *et al.* 2015). These authors noted that dichloroacetic acid has been characterized as a metabolic reprogramming agent because it is a structural analogue of pyruvate and inhibits PDK. PDK inhibition results in activation of the pyruvate dehydrogenase complex (PDH), thus, diverting pyruvate metabolism from the glycolytic pathway towards oxidative metabolism. Thus, a plausible mechanism of the latent carcinogenic effects of dichloroacetic acid could involve epigenetic effects leading to persistent changes in cell metabolism and PDH activation. Long-term induction of PDH and other oxidative pathways related to glucose metabolism can promote mitochondrial stress, cell aging, cell injury, DNA damage, and potentially lead to cancer. However, the current data do address how long PDH activation may persist after exposure to dichloroacetic acid (Wood *et al.* 2015).

The monohaloacetic acids inhibit GAPDH in a concentration-dependent manner and is highly correlated with compound reactivity following the rank order of iodo- > bromo- ≫ chloro-

(Hernández-Fonseca *et al.* 2008, Pals *et al.* 2011, Dad *et al.* 2013, Pals *et al.* 2016). GAPDH inhibition blocks glucose metabolism to pyruvate and causes decreased ATP production, mitochondrial stress, increased intracellular Ca^{2+}, generation of ROS, and genotoxicity. Dad *et al.* (2013) also showed that exogenous pyruvate supplementation enhanced ATP production in CHO cells and reduced genomic DNA damage. Treatment with calcium chelators also reduced DNA damage induced in CHO cells by bromoacetic acid (Pals *et al.* 2016). However, only chloroacetic acid (the weakest GAPDH inhibitor) has been tested for carcinogenicity in a long-term assay, and it was found to not be carcinogenic (NTP 1992).

In recent years, GAPDH has been implicated in other cell functions that are independent of its role in energy metabolism, including DNA repair, cell cycle progression, and cell death (Colell *et al.* 2009, Zhang *et al.* 2015).

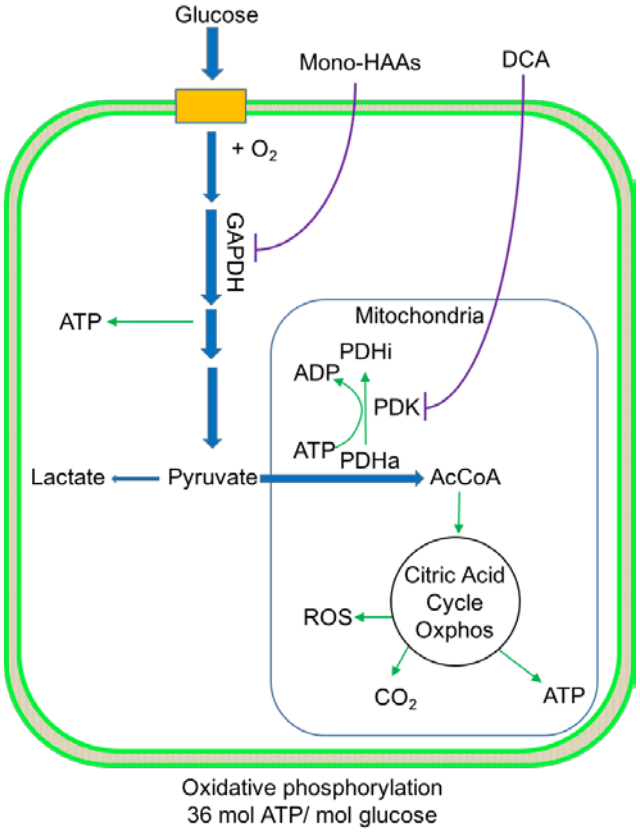

Figure 6-1. Inhibition of GAPDH and PDK by haloacetic acids and effects on glucose metabolism

In normal differentiated (quiescent) cells. glucose is converted to pyruvate via glycolysis. Under aerobic conditions, most pyruvate enters the mitochondria where it is converted to acetyl-CoA by the pyruvate dehydrogenase (PDH) complex and is used to produce ATP via oxidative phosphorylation. Dichloroacetic acid (DCA) inhibits pyruvate dehydrogenase kinase (PDK), thus, enhancing oxidative metabolism and generation of reactive oxygen species (ROS). Monohaloacetic acids inhibit GAPDH, thus, blocking formation of pyruvate and inducing mitochondrial stress, decreased ATP production, and generation of ROS. Adapted from Vander Heiden *et al.* 2009, Bruchelt *et al.* 2014, Lu *et al.* 2015.

6.3 Induction of oxidative stress

In vitro studies using human or rodent cells and *in vivo* studies in rodents also show strong evidence that oxidative stress is a common feature of haloacetic acids-induced toxicity and that treatment with antioxidants reduces the genotoxic and cytotoxic effects of haloacetic acids (Cemeli *et al.* 2006, Celik *et al.* 2009, Pals *et al.* 2011, Ondricek *et al.* 2012, Dad *et al.* 2013, El Arem *et al.* 2014a, El Arem *et al.* 2014b, El Arem *et al.* 2014c, Stalter *et al.* 2016). Numerous

studies confirm that all haloacetic acids that have been tested induce oxidative stress either through activating the oxidative stress-responsive nuclear factor E2 related factor/antioxidant response elements (Nrf2/ARE) pathway, lipid peroxidation, and/or inducing oxidative DNA damage (i.e., 8-OHdG adducts) in mammalian cells (Larson and Bull 1992, Austin *et al.* 1995, Austin *et al.* 1996, Hassoun and Ray 2003, Cemeli *et al.* 2006, Celik 2007, Hassoun and Dey 2008, Attene-Ramos *et al.* 2010, Plewa *et al.* 2010, Hassoun *et al.* 2010a, Hassoun *et al.* 2010b, Hassoun and Cearfoss 2011, Pals *et al.* 2011, Zhang *et al.* 2011, Pals *et al.* 2013, Hassoun and Cearfoss 2014, Hassoun *et al.* 2014, Wang *et al.* 2014, Hassoun and Mettling 2015, Procházka *et al.* 2015, Stalter *et al.* 2016).

EPA's toxicity forecaster (ToxCast) and the National Institutes of Health (NIH) Toxicology in the 21st century (Tox21) databases show that dibromoacetic acid (both ToxCast and Tox21), and tribromoacetic acid and bromochloroacetic acid (Tox21) were positive under the conditions of the assays to screen for an increase in activity of human Nrf2 transcriptional factor (oxidative stress) (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data). Bromoacetic acid, chloroacetic acid, bromodichloroacetic acid, dichloroacetic acid, and trichloroacetic acid were negative under conditions of the Tox21 assay. No other haloacetic acids were tested. The Nrf2-ARE signaling pathway is responsive to both electrophilic attack and oxidative stressors (Kensler *et al.* 2007).

In vitro studies (12 haloacetic acids) in human breast and liver cancer cell lines and *in vivo* (5 haloacetic acids) studies in rodents that compared biological markers of oxidative stress induced by three or more haloacetic acids reported the same general trends as follows: mono- > di- > trihaloacetic acids and iodinated > brominated ≫ chlorinated acetic acids (Figures 6-2 and 6-3) (Larson and Bull 1992, Austin *et al.* 1996, Pals *et al.* 2013, Stalter *et al.* 2016). Oxidative stress was measured *in vitro* by activation of the Nrf2/ARE pathway and *in vivo* by 8-OHdG and lipid peroxidation. Details on these studies, as well as data from a few other studies, are summarized in Appendix D (Table D-1). Thus, lipid peroxidation and oxidative damage to DNA potentially play a role in the carcinogenicity of haloacetic acids, and the significantly greater levels produced by brominated haloacetic acids suggest a greater potential to induce cancer than the chlorinated forms (Austin *et al.* 1996). Overall, dichloro- and trichloroacetic acid showed the weakest response.

Figure 6-2 Relative potency of haloacetic acids to induce oxidative stress in human cancer cell lines; (A) monohaloacetic acid), (B) di- and trihaloacetic acids

Sources: Stalter *et al.* 2016.

AREc32 assay: human breast cancer (MCF7 cell line), ARE-bla asssay: human hepatocellular carcinoma (HepG2) cell line. Relative potency estimates were derived by dividing all values by the lowest value reported in Table D-1 for the designated endpoint and represent a fold increase over the lowest estimate. CA = monochloro-, BA = monobromo-, IA = monoiodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, CIA = chloroiodo-, BIA = bromoiodo-, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloro-, CDBA = chlorodibromoacetic acid, AREc32 = activation of Nrf2-ARE oxidative stress response pathway in a human breast cancer cell line MCF7, ARE-bla = activation of oxidative stress response pathway in human hepatocellular carcinoma HepG2 cell line.

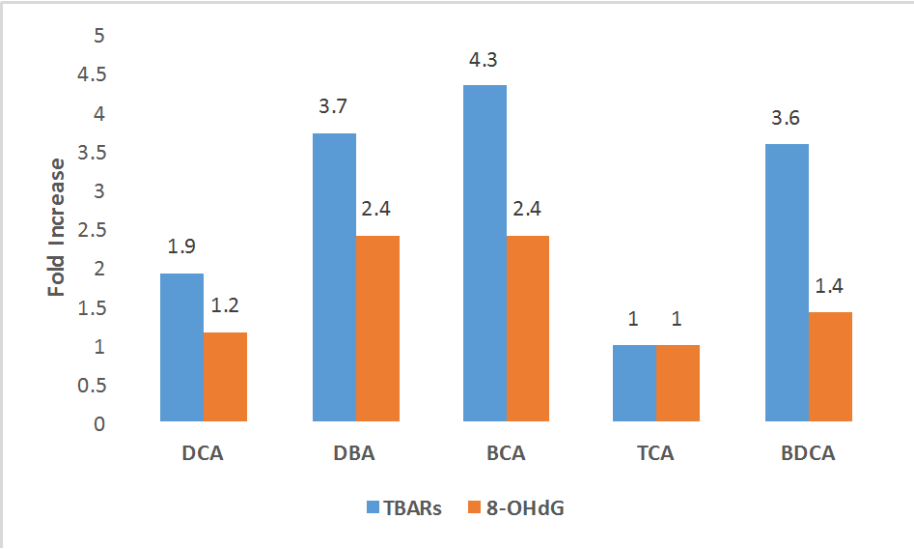

Figure 6-3. Relative potency of haloacetic acids to induce oxidative damage in mouse liver *in vivo*

Sources: Larson and Bull 1992, Austin *et al.* 1996.

Relative potency estimates were derived by dividing all values by the lowest value reported in Table D-1 for the designated endpoint and represent a fold increase over the lowest estimate. DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, TCA = trichloro-, BDCA = bromodichloroacetic acid, TBARS = thiobarbituric acid-reactive substances, 8-OHdG = 8-hydroxydeoxyguanosine.

6.4 Genotoxicity and/or alteration of DNA repair

Overall, haloacetic acids have been shown to have some mutagenic activity in bacterial and mammalian cells *in vitro* and mixed effects regarding DNA and chromosomal damage *in vitro* in

mammalian cells and *in vivo* in rodents (see Table 6-2). All 13 haloacetic acids have some published genotoxicity data and the mutagenic and genotoxic effects of most of these compounds have been reviewed by U.S. and international agencies (NTP 1992, EPA 2003, Richardson *et al.* 2007, NTP 2007a, 2009, EPA 2011a, IARC 2013a, 2013b, 2014a, 2014b, NTP 2015). In addition, the Chemical Effects in Biological Systems (CEBs) database also contains genotoxicity data for 9 haloacetic acids that have been investigated by the NTP (https://tools.niehs.nih.gov/cebs3/ui/). The genotoxicity data requested from CEBs is summarized in Appendix D, Table D-2). Dichloro- and trichloroacetic acid are the most extensively studied haloacetic acids, while genotoxicity data for the other compounds are more limited. Section 6.4.1 presents a brief summary of the findings. Studies that compared the mutagenicity/genotoxicity potency of three or more haloacetic acids are considered to be the most informative for evaluating patterns and are reviewed in more detail in Section 6.4.2. Many of these studies were included in the agency reviews.

6.4.1 Mutagenic and genotoxic effects

The mutagenic and genotoxic effects are summarized in Table 6-2 for 10 haloacetic acids (monochloro-, monobromo-, monoiodo-, dichloro-, dibromo-, bromochloro-, trichloro-, tribromo-, bromodichloro-, and chlorodibromoacetic acid). Chloroiodo-, bromoiodo-, and diiodoacetic acid are not included in this table because the only data available were limited to two studies that evaluated 12 haloacetic acids in the SOS umuC assay or the Comet assay in CHO cells. The results from these two studies are briefly mentioned in this section but are described in more detail in Section 6.4.2.

Bacteria

In general, mutagenicitiy and gentotoxicity (reverse mutations, SOS response, and prophage induction) results in various strains of *Salmonella typhimurium* and *Escherichia coli* were mostly negative for trichloroacetic acid; weakly positive or mixed for chloro-, dichloro-, and tribromoacetic acid; and generally positive for bromo-, iodo-, dibromo-, bromochloro-, bromodichloro-, and chlorodibromoacetic acid; metabolic activation was generally not required and did not usually enhance mutagenicity (Table 6-2). Positive results were more frequent when tested with tester strains designed to detect base-pair mutations (e.g., TA100) compared to tester strains detecting frameshift mutations (e.g., TA98). Cemeli *et al.* (2006) reported that the mutagenicity of iodoacetic acid (identified as the most potent mutagen among the haloacetic acids) was significantly reduced by treatment with antioxidants and supported the hypothesis that haloacetic acids induce genetic damage via an oxidative stress mechanism.

No studies were available for diiodoacetic acid. The only data for chloroiodo- and bromoiodoacetic acid was from a screening study for genetic damage in *Salmonella* using the SOS umuC assay and both were positive (Stalter *et al.* 2016). This study is described in more detail in Section 6.4.2.

Genetic effects in mammalian cells *in vitro*

In vitro genotoxicity studies were available for all 13 haloacetic acids. The most commonly investigated effects included DNA damage/strand breaks, gene mutations, micronuclei, and chromosomal aberrations while results for sister chromatid exchange, aneuploidy, and unscheduled DNA synthesis were only available for one haloacetic acid. Test systems included CHO cells, mouse lymphoma cells, mouse and rat hepatocytes, human lymphoblastoid cells, and a mouse fibroblast cell line (NIH3T3).

The strongest evidence for genotoxicity is that chloro-, bromo-, iodo-, dibromo-, bromochloro-, tribromo-, and chlorodibromoacetic acids induced DNA strand breaks, with weaker evidence for micronuclei formation (e.g., inconsistent findings across studies depending on the cell type) (Table 6-2). Data also suggest that some of the haloacetic acids may cause chromosomal aberrations and gene mutations albeit fewer chemicals were tested in these assays. Findings for trichloroacetic acid are less clear; however, this chemical is considered not to be genotoxic based on negative *in vitro* tests and inconsistent results *in vivo* (IARC 2014b). Although, two studies published after the IARC review reported that trichloroacetic acid induced micronuclei and chromosomal aberrations in human peripheral blood lymphocytes, there was no clear dose-response relationship, cytotoxicity issues, and deficiencies in reporting and methodology that made it difficult to interpret the results (Varshney *et al.* 2013, 2014). Ali *et al.* (2014) reported that treatment with antioxidants reduced DNA damage induced by the three monohaloacetic acids in human sperm and peripheral blood lymphocytes and micronuclei in human lymphocytes.

Several *in vitro* studies reported differences in DNA repair kinetics or altered expression of genes involved in DNA repair following exposure to the monohaloacetic acids or trichloroacetic acid (Komaki *et al.* 2009, Attene-Ramos *et al.* 2010, Muellner *et al.* 2010, Lan *et al.* 2016). Lan *et al.* (2016) reported that trichloroacetic acid induced strong responses in nucleotide excision repair and mismatch repair and a moderate response in double-strand break repair in a high-throughput toxicogenomic assay. No other haloacetic acid was tested. These results are consistent with oxidative damage to DNA. Komaki *et al.* (2009) reported that CHO cells treated with bromoacetic acid had a statistically significant slower rate of repair compared to cells treated with chloroacetic acid or iodoacetic acid. The different rates of genomic repair suggest that these compounds induce different DNA lesions and/or a different distribution of DNA lesions. These data are consistent with studies of monohaloacetic acids in nontransformed human cells that reported altered transcription profiles for genes involved in DNA repair, particularly the repair of double-strand DNA breaks (Attene-Ramos *et al.* 2010, Muellner *et al.* 2010). Dmitriev and Grodzinsky (1975) reported that blue-green algae (*Anacystis nidulans*) exposed to iodoacetic acid prior to irradiation had an increased number of single-strand breaks and a lower rate of subsequent DNA repair.

Genetic effects *in vivo*

Although limited, the available data indicate that the haloacetic acids are not strong genotoxicants *in vivo*. The evidence for genotoxicity across haloacetic acids or across different types of endpoints for the same haloacetic acids was largely inconsistent with some positive findings for DNA or chromosome damage.

In vivo genotoxicity studies were identified for eight haloacetic acids: chloro-, bromo-, dichloro-, dibromo-, bromochloro-, trichloro-, tribromo-, and bromodichloroacetic acid. Endpoints included DNA strand breaks, gene mutation, micronucleus formation, and chromosomal aberrations (Table 6-2). In general, only a few haloacetic acids were tested for each endpoint, and some haloacetic acids were tested for only a few endpoints, thus, limiting the ability to compare genotoxicity potential across haloacetic acids.

Dichloro- and trichloroacetic acid were the only haloacetic acids tested for DNA strand breaks in rodents. Results were mixed in liver but negative in other tissues (IARC 2014a, 2014b). Tribromoacetic acid did not induce DNA damage in zebrafish (Teixidó *et al.* 2015). Three haloacetic acids were tested for gene mutations with positive findings for dichloroacetic acid (lacI transgenic mouse liver assay), equivocal findings for chloroacetic acid (sex-linked recessive

lethal germ cell mutations in *Drosophila*), and negative findings for iodoacetic acid (sex-linked recessive lethal germ cell mutations in *Drosophila* (NTP 1992, IARC 2014a, Chemical Effects in Biological Systems (CEBS) 2017).

Eight haloacetic acids were tested for micronuclei in rodent or newt larvae, peripheral lymphocytes, or rodent bone marrow. Findings were mixed for dichloroacetic acid (NTP 2007b, IARC 2014a, Chemical Effects in Biological Systems (CEBS) 2017), trichloroacetic acid (IARC 2014b), and dibromoacetic acid (NTP 2007a, IARC 2013a) and were negative for bromo-chloroacetic acid (NTP 2015); bromodichloroacetic acid (NTP 2015); and chloro-, bromo-, and tribromoacetic acid (Giller *et al.* 1997). Chloroacetic acid did not cause chromosomal aberrations in CHO cells but there was some evidence that trichloroacetic can cause chromosome aberrations in bone marrow of mice and chickens when administered by i.p. injection.

Mutational spectra in liver tumors

Mouse liver tumors induced by three of the haloacetic acids (dichloro-, trichloro-, and bromodichloroacetic acid) showed different patterns in mutation frequency and/or spectra. A mutational analysis of liver tumors induced by dichloroacetic acid in male mice showed similar incidences of *H-ras* mutations (~50% to 62%) compared to spontaneous tumors in control animals (~58% to 69%) (Anna *et al.* 1994, Ferreira-Gonzalez *et al.* 1995). However, there was a shift in the spectrum of second exon *H-ras* mutations in dichloroacetic acid-treated animals, where dichloroacetic acid-induced tumors had a significantly lower incidence of CAA → AAA mutations and a significantly higher incidence of CAA → CTA mutations than spontaneous tumors. In contrast, only one *H-ras* codon 61 mutation was found out of 22 liver tumors (4.5%) examined from female mice exposed to dichloroacetic acid (Schroeder *et al.* 1997). In trichloroacetic acid-induced mouse liver tumors, both the incidence and mutational spectrum of *H-ras* mutations (45%) were not significantly different from spontaneous liver tumors, suggesting that trichloroacetic acid promotes the growth of spontaneously initiated hepatocytes (Ferreira-Gonzalez *et al.* 1995). Although these data suggest that liver tumor induction in male mice by both dichloro- and trichloroacetic acid involves activation of the *H-ras* protooncogene, the specific mechanisms are likely different between the two compounds (Ferreira-Gonzalez *et al.* 1995, IARC 2014a).

NTP (2015) also conducted a comparative mutation analysis of 30 hepatoblastomas and adjacent hepatocellular carcinomas in mice exposed to bromodichloroacetic acid. The incidence of *H-ras* mutations in bromodichloroacetic acid-induced hepatocellular carcinomas (13%) was about the same as observed in adjacent hepatoblastomas (7%) but was lower than in spontaneous hepatocellular carcinomas (55%). On the other hand, the incidence of *Ctnnb1 (ß-catenin)* mutations in treatment-related hepatocellular carcinomas (10%) was lower than in adjacent hepatoblastomas (23%) but higher than in spontaneous tumors (2%). There were no data for historical spontaneous hepatoblastomas in mice. *H-ras* codon 61 and *Ctnnb1* exons 2 and 3 mutation spectra were different for the two tumor types, and although the sample size is small, the data suggest that these tumors are distinct entities. Only two hepatoblastomas had *H-ras* mutations (both CAA → CTA) while three of four *H-ras* mutations in hepatocellular carcinomas contained CAA → CGA mutations and the other was a CAA → CTA mutation. There were no clear mutation spectrum patterns in the seven hepatoblastomas or the three hepatocellular carcinomas that contained *Ctnnb1* mutations.

Table 6-2. Summary of the mutagenic and genotoxic effects of haloacetic acids[a]

	Monohaloacetic acids			Dihaloacetic acids			Trihaloacetic acids			
Test system	**CA**	**BA**	**IA**	**DCA**	**DBA**	**BCA**	**TCA**	**TBA**	**BDCA**	**CDBA**
S. typhimurium										
TA100	(±)	+	+	±	+	+	–	±[b,c]	+	E[d]
TA98	(±)	nr	nr	±	±	–	–	–	+	–
Other strains	–	nr	nr	–	±	–	–	nr	+	nr
***E. coli* WP2**										
Reverse mutation	nr	nr	nr	–	nr	–	nr	nr	+	+
λ Prophage induction	nr	nr	nr	–[b]	nr	nr	–	nr	nr	nr
SOS chromotest	–	–	nr	(+)	+	nr	–	+	nr	nr
Mammalian cells										
DNA damage/strand breaks	+	+	+	–	+	+	–	+	–	(+)
Gene mutation	+	nr	nr	±	+	nr	–[d]	nr	nr	nr
Micronucleus formation	±	±	±	±	+	–	?	nr	–	nr
Chromosomal aberrations	–	nr	+	+	nr	nr	–	nr	nr	nr
Sister chromatid exchanges	+	nr	nr	nr	nr	nr	nr	nr	nr	nr
Aneuploidy	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
Unscheduled DNA synthesis	nr	nr	nr	nr	+	nr	nr	nr	nr	nr
In vivo										
DNA damage/strand breaks	nr	nr	nr	±	nr	nr	±	–[e]	nr	nr
Gene mutation	–[e]	nr	–[e]	+	nr	nr	nr	nr	nr	nr
Micronucleus formation	–[e]	–[e]	nr	±	±	–	±	–[e]	–	nr
Chromosomal aberrations	–	nr	nr	nr	nr	nr	+[f]	nr	nr	nr

Sources: NTP 1992, Kargalioglu *et al.* 2002, EPA 2003, Plewa *et al.* 2004a, Richardson *et al.* 2007, NTP 2007a, 2007b, 2009, Liviac *et al.* 2010, Plewa *et al.* 2010, Zhang *et al.* 2010, EPA 2011a, Varshney *et al.* 2013, IARC 2013a, 2013b, Ali *et al.* 2014, Varshney *et al.* 2014, IARC 2014a, 2014b, NTP 2015, Teixidó *et al.* 2015, Stalter *et al.* 2016, Chemical Effects in Biological Systems (CEBS) 2017.

CA = chloro-, BA = bromo-, IA = iodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloroacetic acid, CDBA = chlorodibromoacetic acid, nr = not reported/no data; – = negative, E = equivocal, ± = mixed results, (+) = weak positive, (±) = weak positive or negative, + = positive, ? = reported as positive but results are questionable due to deficiencies in reporting and methods.

[a] Results are reported without metabolic activation unless otherwise noted.

[b] Positive with metabolic activation.

[c] Positive in the fluctuation test (liquid media) with or without metabolic activation.

[d] Weak positive with metabolic activation in one study.

[e] Non-mammalian tests: newt larvae (micronucleus), zebrafish (DNA damage), Drosophila (sex-linked recessive lethal mutation)

[f] Only one oral study in mice, and two i.p. injection studies (mice and chickens).

6.4.2 Mutagenic and genotoxic potency

Mutagenic or genotoxic potency and cytotoxicity of three or more haloacetic acids were directly compared in several studies in bacteria (*S. typhimurium* strain TA100, SOS umuC assay) and mammalian cells (HGPRT mutations, Comet and p53-bla assays) (Kargalioglu *et al.* 2002, Plewa *et al.* 2004b, Richardson *et al.* 2008, Plewa *et al.* 2010, Zhang *et al.* 2010, Stalter *et al.* 2016, Zhang *et al.* 2016). These data are shown in Appendix D (Tables D-3 and D-4) and are illustrated as the fold increase relative to the least potent haloacetic acid in each of five *in vitro* genetox assays in Figure 6-4 (umuC) and Figure 6-5 (Ames TA100 strain, HGPRT mutations, p53-bla, and Comet). Data for the umuC assay are plotted separately because the general trends present in the other assays are not as apparent in the umuC assay (discussed below). The SOS umuC assay is a screening test and does not provide a direct measurement of DNA damage or mutagenesis but rather measures the activation of SOS umuC-dependent error prone DNA repair. Data for a few other studies shown in Appendix D, Tables D-3 and D-4 (Ono *et al.* 1991, Giller *et al.* 1997, Attene-Ramos *et al.* 2010, Escobar-Hoyos *et al.* 2013, Procházka *et al.* 2015) are not included in Figures 6-4 or 6-5 but show the same general patterns.

The data show that the mutagenic/genotoxic potency is highly influenced by both the number and type of halogen atoms with the general rank order of potency as: mono- > di- > trihaloacetic acids and iodinated > brominated ≫ chlorinated acetic acids and is consistent with that reported for oxidative stress in the previous section. In fact, all haloacetic acids that were active towards DNA in bacteria or mammalian cells also induced oxidative stress (Stalter *et al.* 2016).

Notable exceptions to the general trends reported above included the lack of mutagenic activity for the monohalogenated acetic acids and the high potency of brominated trihaloacetic acids, particularly tribromoacetic acid, in the SOS umuC assay (see Appendix D, Table D-3) (Stalter *et al.* 2016). Stalter *et al.* noted that induction ratios in the umuC assay were excluded from the data analysis when cytotoxicity exceeded 50%, so the lack of mutagenic activity by monohaloacetic acids in the umuC assay may be a result of cytotoxicity masking induction of the reporter gene. However, Zhang *et al.* (2016) showed that the monohaloacetic acids were active in the SOS umuC assay. Therefore, Figure 6-4 integrates data for the di- and trihaloacetic acids from Stalter *et al.* (2016) with data for the monohaloacetic acids from Zhang *et al.* (2016). Although no explanation was given for the high potency of the brominated trihaloacetic acids (especially tribromoacetic acid) in the umuC assay (Stalter *et al.* 2016), the trihaloacetic acids were negative or weakly positive in other bacterial systems and in mammalian cells. Tribromoacetic acid is not included in Figure 6-4 because it exhibited a 2,500-fold increase relative to chloro- and trichloroacetic acid and appears to be an outlier.

Exceptions to the general trends observed in the *in vitro* genetox studies summarized in Figure 6-5 include slightly more DNA damage in CHO cells exposed to dibromoacetic acid compared to bromoiodo- or diiodoacetic acid (Plewa *et al.* 2010), and dibromoacetic acid induced an 8-fold higher HGPRT mutant frequency in CHO cells than bromoacetic acid (Zhang *et al.* 2010).

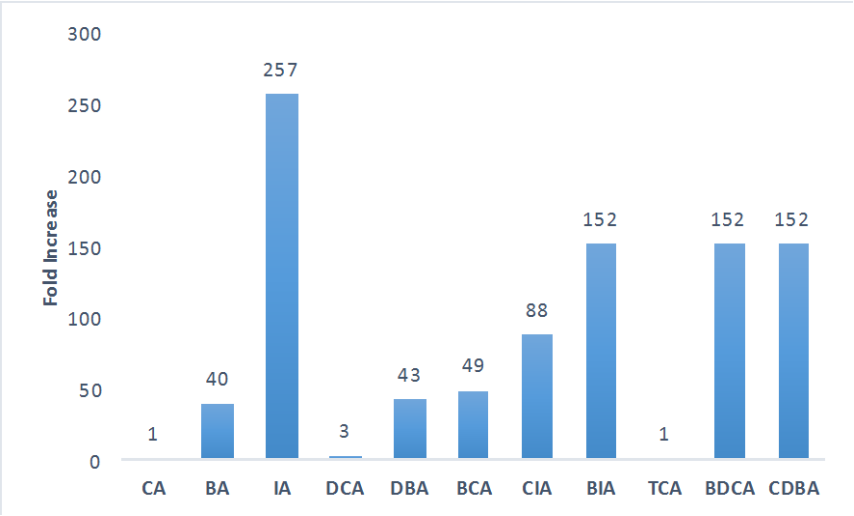

Figure 6-4. Relative genotoxicity potency estimates of haloacetic acids in the SOS-umuC assay

Sources: Stalter *et al.* 2016, Zhang *et al.* 2016.
CA= monochloro-; BA = monobromo-; IA = monoiodo-; DCA = dichloro-; DBA = dibromo-; BCA = bromochloro-; CIA = chloroiodo-; BIA = bromoiodo-; TCA = trichloro-; BDCA = bromodichloro-; CDBA = chlorodibromoacetic acid. Relative potency estimates were derived by dividing all values by the lowest value reported in Table D-3 for the designated endpoint and represent a fold increase over the lowest estimate. Data for the monohaloacetic acids, DCA, and TCA were derived from Zhang *et al.* (2016) and were estimated from a figure at an induction ratio of 1.5 using WebPlot Digitizer (http://arohatgi.info/WebPlotDigitizer/app/). Tribromoacetic acid value > 2,500 omitted due to scale.

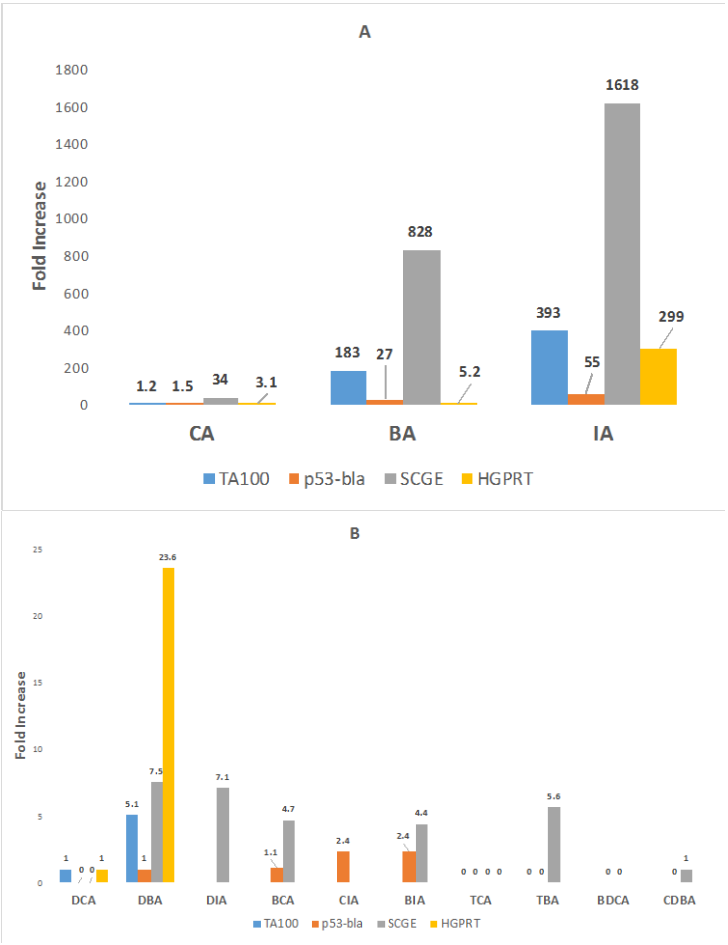

Figure 6-5. Relative genotoxicity potency estimates of haloacetic acids in bacteria and mammalian cells

Sources: Kargalioglu *et al.* 2002, Plewa *et al.* 2004b, Plewa *et al.* 2010, Zhang *et al.* 2010, Stalter *et al.* 2016.
CA= monochloro-, BA = monobromo-, IA = monoiodo-, DCA = dichloro-, DBA = dibromo-, DIA = diiodo-, BCA = bromochloro-, CIA = chloroiodo-, BIA = bromoiodo-, TCA = trichloro-, TBA = tribromoacetic acid, BDCA = bromodichloro-, CDBA = chlorodibromoacetic acid, Relative potency estimates were derived by dividing all values by the lowest value reported in Tables D-3 and D-4 for the designated endpoint and represent a fold increase over the lowest estimate. Value of zero indicates that the compound was tested but inactive. (A) Monohaloacetic acid), (B) Di- and trihaloacetic acids.

6.5 Induction of epigenetic alterations

Three haloacetic acids (dichloro-, dibromo-, and trichloroacetic acid) induced hypomethylation of DNA and the promoter regions of oncogenes (*c-myc* and insulin-like growth factor 2 [IGF-II]) genes in rodents. Hypomethylation of the promoter regions leads to increased expression of these genes and may represent early events in the hepatocarcinogenicity of these haloacetic acids (Tao *et al.* 2004a, Tao *et al.* 2005, IARC 2013a, 2014a, 2014b). In contrast, the global methylation pattern in liver tumor and nontumor DNA harvested from mice after 98 weeks following early-life exposure to dichloroacetic acid was not altered (Wood *et al.* 2015). DNA methylation status has been suggested as a possible screening tool for predicting the potential carcinogenicity of the haloacetic acids (Pereira *et al.* 2001, Tao *et al.* 2004b, Kuppusamy *et al.* 2015). However, no clear potency trends were observed. Findings for the three compounds are summarized across studies below and the data from the individual studies are reported in Appendix D, Table D-5.

Dose-dependent hypomethylation of DNA from normal liver tissue, liver tumor, and normal kidney tissue; hypomethylation in the promoter regions of IGF-II, *c-jun*, and *c-myc* genes; and increased mRNA expression of these genes were reported in mice exposed to dichloro-, dibromo-, or trichloroacetic acid (Tao *et al.* 1998, Tao *et al.* 2000a, 2000b, Pereira *et al.* 2001, Pereira *et al.* 2004a, Tao *et al.* 2004a, Tao *et al.* 2004b, Tao *et al.* 2005). Dibromoacetic acid also induced liver and kidney DNA hypomethylation in male rats (Tao *et al.* 2004a, Tao *et al.* 2005). Hypomethylation patterns varied depending on the exposure conditions and type of tissue (tumor vs. non tumor).

Several studies show that hypomethylation was correlated with the carcinogenic and tumor-promoting activity of both dichloro- and trichloroacetic acid in the liver and kidney of rodents but that the mechanisms of their carcinogenic activity may be different (Tao *et al.* 1998, Tao *et al.* 2000b, Pereira *et al.* 2001, Pereira *et al.* 2004a, Tao *et al.* 2004b, Tao *et al.* 2005). Dichloro- and trichloroacetic acid-induced hypomethylation of the promoter region of the *c-myc* gene in mouse liver coincided with enhanced cell proliferation and suggests that these compounds induce hypomethylation by inducing DNA replication and inhibiting methylation of the newly synthesized DNA (Ge *et al.* 2001). When co-administered with chloroform, both trichloroacetic acid and dichloroacetic acid induced hypomethylation and promoted kidney tumors in male (but not female) mice (Pereira *et al.* 2001). Hypomethylation induced by haloacetic acids was also prevented by prior treatment with methionine, suggesting that haloacetic acids deplete S-adenosyl methionine levels (Tao *et al.* 2000a, 2000b, Pereira *et al.* 2004a, Pereira *et al.* 2004b, Tao *et al.* 2005).

6.6 Modulation of receptor-mediated effects

Overall the data suggest that the carcinogenic activity of trichloroacetic acid in mouse liver is consistent with peroxisome proliferation (IARC 2004b, 2014b). The lack of a carcinogenic response in rats exposed to trichloroacetic acid may be explained by a much lower extent of peroxisome proliferation compared to mice (DeAngelo *et al.* 1989). In contrast, rats were more sensitive to the hepatocarcinogenicity of dichloroacetic acid than mice (DeAngelo *et al.* 1996).

Peroxisome proliferation, as measured by palmitoyl-CoA oxidation, appears to be a common effect of the seven haloacetic acids tested *in vitro* but with significant differences in potency and efficacy in cultured rat hepatocytes (Appendix D, Figure D-1) (Walgren *et al.* 2004). The lowest effective concentrations, based on palmitoyl-CoA oxidation, varied over about two orders of magnitude. Within the monohalo-substituted series, iodoacetic acid was more potent than bromo- and chloroacetic acid, respectively, however, no clear patterns were observed for the different halogen substitutions for the di- or trihaloacetic acid series. While monobromo- and monoiodoacetic acid induced significant increases in palmitoyl-CoA oxidation at the lowest concentrations (50 to 100 µM), higher concentrations were cytotoxic. Thus, the cytotoxicity of the monohaloacetic acids would likely limit their effectiveness as PPARα activators *in vivo*. Overall, dibromoacetic acid was the most effective inducer of palmitoyl-CoA reaching 6.2-fold at the maximum concentration tested (3 mM). Dichloro-, trichloro-, and tribromoacetic acid were modest inducers of palmitoyl CoA with much flatter concentration/response curves. However, these data are not consistent with the available *in vivo* data discussed below.

In vitro studies using human cells also reported variable results. Peroxisome proliferation (as measured by palmitoyl-CoA oxidation) was not detected in one study using human cell lines exposed to dichloro- or trichloroacetic acid; however, palmitoyl-CoA oxidation was not detected in control human hepatocytes in these studies (Walgren *et al.* 2000a, 2000b). Other *in vitro* studies have shown that human PPARα is activated by both dichloro- and trichloroacetic acid (IARC 2014b). Trichloroacetic acid at concentrations > 1 mM induced comparable dose-related transactivation in human and mouse PPARα *in vitro* (Maloney and Waxman 1999).

In vivo studies in rats and/or mice exposed to brominated or chlorinated haloacetic acids in drinking water showed a dose-related increase in acyl-CoA oxidase activity or hepatic peroxidome proliferation for dibromo- and trichloroacetic acid and the positive control (clofibric acid) but not for other haloacetic acids tested (chloro-, dichloro-, bromochloro-, or bromodichloroacetic acid) (DeAngelo *et al.* 1989, Xu *et al.* 1995, Parrish *et al.* 1996, Tao *et al.* 2004a, NTP 2015). Dichloroacetic acid produced a small, but significant increase only at the high dose while the response to dibromoacetic acid was dose related up to 1 g/L but then declined at 3 g/L (Parrish *et al.* 1996).

Trichloroacetic acid and dichloroacetic acid are relatively weak PPARα agonists requiring mM concentrations (DeAngelo *et al.* 1996, DeAngelo *et al.* 1999, Laughter *et al.* 2004, Corton 2008, DeAngelo *et al.* 2008). However, dose-response characteristics for dichloroacetic acid show that PPARα is not activated at concentrations that induce liver tumors in either rats or mice and indicates that the mode of action of dichloroacetic acid is PPARα-independent (DeAngelo *et al.* 1996, DeAngelo *et al.* 1999). In contrast, dose-response characteristics for trichloroacetic acid show that there is a relatively good correlation between trichloroacetic acid-induced liver tumors and induction of markers of PPARα activation in the mouse (DeAngelo *et al.* 2008). In addition, markers of PPARα activation are elevated at trichloroacetic acid doses that are below or coincident with doses that induce mouse liver tumors and trichloroacetic acid-induced mouse liver tumors have properties similar to those induced by classic peroxisome proliferators (Corton 2008, DeAngelo *et al.* 2008).

6.7 Inhibition of GST-ζ

GST-ζ inhibition has been suggested as a specific mode of action relevant to dihaloacetic acid-induced liver cancer in rodents; however, the available data are insufficient to fully define the key events or to assess their necessity or sufficiency for carcinogenicity. Several polymorphic

variants of GST-ζ have been identified that differ in their susceptibility to inactivation (Blackburn *et al.* 2000, Hayes and Strange 2000, Tzeng *et al.* 2000, Blackburn *et al.* 2001, Fang *et al.* 2006, Cantor *et al.* 2010, Shroads *et al.* 2010, Board and Anders 2011, Li *et al.* 2012). Inhibition of GST-ζ by successive or continuous doses of dihaloacetic acids reduces metabolism and prolongs the plasma half-life (Gonzalez-Leon *et al.* 1999, Schultz *et al.* 2002).

GST-ζ, also known as maleylacetoacetate isomerase (MAAI), catalyzes the penultimate step in the tyrosine catabolism pathway and metabolizes maleylacetoacetate and maleylacetone to fumarylacetoacetate and fumarylacetone, respectively (Cornett *et al.* 1999, Schultz *et al.* 2002, Anderson *et al.* 2004, Stacpoole *et al.* 2008, Theodoratos *et al.* 2009, Board and Anders 2011, Stacpoole 2011). These reactive metabolites may accumulate following inhibition of GST-ζ, react with macromolecules, and induce oxidative stress (Blackburn *et al.* 2006). Hereditary tyrosinemia type 1 is a metabolic disease caused by a deficiency of the enzyme involved in the last step of tyrosine catabolism. Individuals with this disease develop hepatocellular carcinoma at a young age (Tanguay *et al.* 1996, Stacpoole 2011).

Schultz *et al.* (2002) reported that dichloroacetic acid reduced MAAI activity over 80% in young mice but not in old mice suggesting that reduced MAAI activity is unlikely to be the sole carcinogenic mode of action for dichloroacetic acid and may be important only during the early stages of exposure. This conclusion is further supported by observations that GST-ζ-deficient mice do not spontaneously develop hepatocellular carcinoma (Fernández-Cañón *et al.* 2002, Schultz *et al.* 2002).

6.8 Cell immortalization

Iodo- and dibromoacetic acid (the only haloacetic acids tested) caused cell transformation in an immortalized aneuploid mouse cell line (NIH3T3 or Balb/c 3T3 cells) (Fang and Zhu 2001, Wei *et al.* 2013). This assay is responsive to the later stages in carcinogenic transformation (i.e., induction of morphologically transformed foci (Tanaka *et al.* 2012). Moreover, Wei *et al.* (2013) reported that iodoacetic acid transformed cells also exhibited anchorage-independent growth, agglutination with concanavalin A, and formed aggressive fibrosarcomas when injected into Balb/c nude mice. Cell transformation assays are capable of detecting both genotoxic and non-genotoxic carcinogens and validation studies have shown generally good concordance with rodent bioassay results and reproducibility (Corvi *et al.* 2012, Creton *et al.* 2012, Tanaka *et al.* 2012).

6.9 Alteration of cell proliferation and cell death

Some data suggest that dichloro- and trichloroacetic acid alter cell proliferation and apoptosis; however, the data are inconsistent and the effects appear to be transitory (reviewed in EPA 2003, 2011a, IARC 2014a, 2014b). Several studies reported hepatocyte proliferation, increased thymidine incorporation in hepatic DNA, and increased cell division rates in trichloroacetic acid-induced hepatic foci and tumors (Styles *et al.* 1991, Dees and Travis 1994, Pereira 1996, Stauber and Bull 1997, Channel *et al.* 1998, Ge *et al.* 2001, DeAngelo *et al.* 2008, IARC 2014b). Increased labeling of hepatic DNA was observed at sub-necrotic doses suggesting that cell proliferation was not due to regenerative hyperplasia (Dees and Travis 1994). Studies with dichloroacetic acid reported increased cell proliferation of *c-jun*-positive hepatocytes, reparative hyperplasia in the liver, increased numbers of hepatic foci and hyperplastic nodules, increased cell replication rates in altered foci, promotion of growth and survival of initiated cells, and a dose-related decrease in apoptosis (Sanchez and Bull 1990, Richmond *et al.* 1991, Snyder *et al.* 1995, Stauber and Bull 1997, Stauber *et al.* 1998, IARC 2014a). Other studies have suggested

that dichloro- and trichloroacetic acid are not direct-acting mitogens; however, dichloroacetic acid inhibited apoptosis and synergistically enhanced the mitogenic response to epidermal growth factor (EGF) in cultured rat hepatocytes (DeAngelo *et al.* 1991, Walgren *et al.* 2005).

Increased cell proliferation has been associated with increased expression of IGF-II as this gene has both mitogenic and anti-apoptotic activity in the liver (Tao *et al.* 2004b). As mentioned above (Section 6.5), both trichloro- and dichloroacetic acid increased *c-myc* and IGF-II expression and enhanced cell proliferation in rodent liver (Ge *et al.* 2001, Tao *et al.* 2004a, Tao *et al.* 2004b). Stauber *et al.* (1998) reported that both dichloro- and trichloroacetic acid promoted the formation of anchorage-independent mouse hepatocytes *in vivo* and *in vitro* in a dose-dependent manner. The phenotypes of the anchorage-independent colonies promoted by dichloroacetic acid were primarily *c-jun* positive while those promoted by trichloroacetic acid were mainly *c-jun* negative, consistent with the phenotypes from liver tumors induced by these compounds. These data suggest that the mode of action of dichloro- and trichloroacetic acid may be to selectively stimulate the clonal expansion of phenotypically different populations of initiated cells.

There are some *in vitro* data that suggest trichloroacetic acid and dichloroacetic acid, but not iodoacetic acid, inhibit gap-junctional intercellular communication (Si *et al.* 1987, Klaunig *et al.* 1989, Benane *et al.* 1996). Loss of gap junctional cellular communication may be an important step in carcinogenesis (Aasen *et al.* 2016). No data were available for other haloacetic acids regarding effects on gap-junctional intercellular communication. Benane *et al.* (1996) reported a dose- and treatment time-related inhibitory response for both haloacetic acids in a normal liver epithelial cell line from male Sprague-Dawley rats. The lowest concentration and shortest time to reduce gap-junctional intracellular communication, as measured by dye transfer, was 1 mM over 1 hour for trichloroacetic acid compared to 10 mM over 6 hours for dichloroacetic acid. Thus, trichloroacetic acid appeared to be more potent than dichloroacetic acid. Trichloroacetic acid significantly reduced dye transfer in mouse hepatocytes at 0.1 to 1 mM after 4 hours treatment but not after 8 or 24 hours (Klaunig *et al.* 1989). In contrast, dye transfer was not affected in rat hepatocytes exposed to trichloroacetic acid concentrations up to 1 mM for as long as 24 hours.

6.10 Induction of chronic inflammation or immunosuppression

The evidence that haloacetic acids induce chronic inflammation or immunosuppression is generally weak and inconsistent and neither process has been identified as a potential mode of action for dichloro-, trichloro-, bromochloro-, or dibromoacetic acid (IARC 2013a, 2013b, 2014a, 2014b). Nevertheless, some data regarding these effects were available for the chloro-, bromo-, iodo-, dichloro-, dibromo-, bromochloro-, trichloro-, and bromodichloroacetic acids and are briefly reviewed here.

In the two-year cancer bioassay studies, mild chronic inflammation in the liver was reported in male mice chronically exposed to trichloroacetic acid (DeAngelo *et al.* 2008) or dichloroacetic acid (Daniel *et al.* 1992) but not in rats or mice exposed to dibromo-, bromochloro-, or chlorodibromoacetic (NTP 2007a, 2009, 2015). None of the 12 haloacetic acids evaluated activated the NF-κB stress-response pathway for inflammation *in vitro* using the human THP-1 leukemia cell line (Stalter *et al.* 2016). However, Pals *et al.* (2013) reported that the three monohaloacetic acids, at non-cytotoxic concentrations, upregulated cyclooxygenase-2 (COX-2) in nontransformed human intestinal epithelial cells (line FHs 74 Int), suggesting a possible inflammatory response. Bromo- and iodoacetic acids also modulated the MAPK pathway in FHs 74 Int cells that suggests a response to cell stress and inflammation (Attene-Ramos *et al.* 2010).

Few studies have investigated the effects of haloacetic acids on the immune system. Neither dichloro- or trichloroacetic acid were immunotoxic in rats exposed to concentrations of up to 5 g/L in drinking water for 90 days (Mather *et al.* 1990). Ohashi *et al.* (2013) reported that dichloroacetic acid improved immune function and increased antitumor immunotherapeutic activity. Dibromoacetic acid did not significantly affect humoral immunity or innate immune function in mice at concentrations up to 1 g/L in drinking water for 28 days (Smith *et al.* 2010a). Other studies have suggested that monoiodo-, dichloro-, dibromo-, or trichloroacetic acid induce some immune responses, including increased serum IgG and IgM in autoimmune-prone $MRL^{+/+}$ mice, histological changes in the thymus and spleen, increased immune cell apoptosis, T cell activation and increased cytokine expression, and suppressed *in vitro* immune functions (Si *et al.* 1987, Cai *et al.* 2007, Gao *et al.* 2008, Pan *et al.* 2015, Gao *et al.* 2016).

6.11 Effects on gene expression

Toxicogenomics studies were available for chloro-, bromo-, iodo-, dichloro-, bromochloro-, trichloro-, and bromodichloroacetic acid and are discussed separately for the monohaloacetic acids and the di- and trihaloacetic acids. These data show that the haloacetic acids induce gene expression changes relevant to several of the characteristics of carcinogens and possible modes of action discussed in this monograph.

Comparative human cell toxicogenomic analysis reported that the monohaloacetic acids altered the transcription levels of genes involved in stress response to DNA damage and regulation of different stages in cell cycle progression or apoptosis and provide support for several of the proposed modes of action (Attene-Ramos *et al.* 2010, Muellner *et al.* 2010, Pals *et al.* 2013). The major cell pathways affected are shown in Table 6-3. Most of these pathways show a strong association with carcinogenesis (Khanna and Jackson 2001, Plewa and Wagner 2015).

Table 6-3. Transcriptome pathways in human cells induced by monohaloacetic acids

Pathway	Chloro-	Bromo-	Iodo-
ATM signaling	+	+	+
Cell cycle control	+	+	
Cyclins and cell cycle regulation	+		
MAPK signaling		+	+
p53 signaling		+	+
BRCA1, BRCA2, and ATR mediated cancer susceptibility and dsDNA repair	+	+	+
Nrf2/ARE-dependent ROS	+	+	+
PTGS2 (COX2)-mediated	+	+	+
MPO, LPO and NOX5 ROS	+	+	+
GSH/GSR	+	+	+
Peroxiredoxin oxidative stress	+	+	

Source: Plewa and Wagner 2015.
Blank cell = pathway not affected.

Several gene expression studies show that the di- and trihaloacetic acids induce expression changes in genes involved in oxidative stress-responsive pathways, DNA damage and repair, cell cycle progression, cell proliferation, metabolism, cancer progression, and apoptosis. However, the data are insufficient to determine any clear patterns in gene expression profiles that are

related to physiochemical or toxicological properties of the haloacetic acids. These studies compared gene expression changes in normal liver tissue, preneoplastic liver nodules, and liver tumors from mice exposed to dichloro-, trichloro-, or bromodichloroacetic acid and control mice; mouse sperm or rat mesotheliomas exposed to bromochloroacetic acid; normal mammary gland tissue and tumors in rats exposed to bromodichloroacetic acid; and yeast treated with trichloroacetic acid and the data are summarized in Appendix D, Table D-6) (Nelson *et al.* 1990, Choi and Park 1996, Thai *et al.* 2001, 2003, Tully *et al.* 2005, Kim *et al.* 2006, NTP 2015, Lan *et al.* 2016).

Mice exposed to dichloroacetic acid for 4 weeks showed similar gene expression profiles in liver tissue as observed in dichloroacetic acid-induced liver tumors (Thai *et al.* 2001, 2003). Trichloroacetic acid activated pathways involved in oxidative DNA damage and tumor progression (Nelson *et al.* 1990, Lan *et al.* 2016) while bromochloroacetic acid altered expression of genes involved in cell communication and adhesion, cell cycle and cell proliferation, metabolism, signal transduction, apoptosis, invasion, and metastasis (Tully *et al.* 2005, Kim *et al.* 2006). Distinct gene expression profiles were observed across four different tissues following bromodichloroacetic acid exposure: hepatocellular carcinomas, hepatoblastomas, and adjacent normal liver tissue from exposed rats as well as normal liver tissue from controls (NTP 2015). Gene expression changes in treatment-related nontumor liver tissue were consistent with neoplastic signaling and may suggest that microenvironment changes preceded neoplastic transformation due to chemical treatment. However, these gene expression changes may also have been influenced by the microenvironment of the adjacent hepatocellular carcinomas. The gene expression profiles of mouse hepatoblastomas were similar to early embryonic mouse livers, suggesting that these tumors arose from the transformation of a hepatic stem or multipotent progenitor cell, while hepatocellular carcinomas arose from transformed hepatocytes. Significant upregulation of eight genes was found in mammary gland tumors from rats exposed to bromodichloroacetic acid but were not found in spontaneous tumors; five of these genes were associated with Tgf-beta signaling and an aggressive tumor phenotype (NTP 2015, Harvey *et al.* 2016).

6.12 Mode of action integration and synthesis

Overall, the data suggest that the haloacetic acids may induce cancer through electrophilic reactions with macromolecules leading to altered gene expression, inhibited protein function, oxidative stress, and mutagenic and genotoxic effects. The data suggest that the mechanisms are complex and likely involve multiple interactions of toxicokinetic factors and modes of action, as well as unknown factors and modes of action that may differ somewhat among the various subclasses of haloacetic acids based on halogen substitution patterns.

The potential modes of action and key events associated with the characteristics of carcinogens are listed in Table 6-4. Most compounds have been associated with most of the 10 characteristics of carcinogens and all the potential modes of action are relevant to humans. Figure 6-6 identifies the specific haloacetic acids and subclasses that have been linked to a particular mode of action through *in vitro* and/or *in vivo* testing. The identification of the potential modes of action of the haloacetic acids is limited because not all 13 haloacetic acids have been tested for all of the key events. Biologically plausible modes of action with moderate to strong experimental support include oxidative damage, epigenetic alterations (i.e., DNA hypomethylation) leading to gene expression changes, GAPDH and PDK inhibition leading to metabolic reprogramming and oxidative stress, disruption of tyrosine catabolism by dihaloacetic acids via inhibition of GST-ζ, and PPARα activation. These effects are further supported by transcriptomic analyses showing

that haloacetic acids affect expression of genes involved in oxidative stress response, DNA damage and repair, cell growth and proliferation, tissue remodeling, apoptosis, angiogenesis, cancer progression, fatty acid metabolism, and xenobiotic metabolism. Direct genotoxicity does not appear to be a primary mode of action for the haloacetic acids and, overall, the data suggest that oxidative stress is responsible for the mutagenic and genotoxic effects of these compounds.

Table 6-4. Possible modes of carcinogenic action for haloacetic acids and the 10 characteristics of carcinogens

Characteristic(s) of carcinogens	Mode of action	Key events
Electrophilicity	Irreversible binding to macromolecules	1. Haloacetic acids have an electrophilic structure that can react with peptides, proteins, or DNA to form adducts. 2. Protein or DNA adducts result in altered activity or DNA damage that advances acquisition of multiple critical traits contributing to carcinogenesis..
Altered nutrient supply, electrophilicity, induction of oxidative stress	Reprogramming cellular energy metabolism (inhibition of pyruvate dehydrogenase kinase (PDK)	1. Haloacetic acids' inhibition of PDK increases pyruvate dehydrogenase complex activity and oxidative metabolism. 2. Increase in oxidative metabolism leads to an increase in reactive oxygen species (ROS) and oxidative stress. 3. Oxidative stress leads to acquisition of multiple, critical traits contributing to carcinogenesis.
Altered nutrient supply, electrophilicity, induction of oxidative stress	Inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1. Haloacetic acids' inhibition of GAPDH leads to inhibition of glycolysis. 2. Inhibition of glycolysis leads to reduced ATP levels and repressed pyruvate generation. 3. Reduced pyruvate leads to mitochondrial stress, ROS generation, cytotoxicity, and DNA damage.
Induction ofoxidative stress	Oxidative stress	1. Haloacetic acids induce oxidative stress through multiple pathways. 2. Oxidative stress can cause mutations and damage to proteins, lipids, and DNA. 3. Mutations and damage to macromolecules activate cell-signaling pathways, induce genomic instability, and cell transformation and lead to cancer.
Genotoxicity and/or alteration of DNA repair	Mutagenicity and genotoxicity	1. Haloacetic acids induce genetic damage in critical genes or form pro-mutagenic adducts. 2. Insufficient or mis-match repair of genetic damage leads to fixed genetic damage. 3. Unrepaired genetic damage leads to clonal expansion of initiated cells. 4. Clonal expansion leads to tumor formation.
Induction of epigenetic alterations	DNA hypomethylation	1. Haloacetic acids induce epigenetic changes (particularly DNA hypomethylation) that alter gene expression, DNA repair, and cell phenotype. 2. These changes advance acquisition of multiple critical traits contributing to carcinogenesis.

Characteristic(s) of carcinogens	Mode of action	Key events
Electrophilicity, induction of oxidative stress	Glutathione-S-transferase zeta (GST ζ) inhibition	3. Haloacetic acids bind to GST-ζ causing irreversible inhibition. 4. Deficiency in GST-ζ results in reduced metabolism and clearance of dihaloacetic acids, higher levels of tyrosine metabolites, oxidative stress, and activation of stress-response pathways. 5. Accumulation of tyrosine metabolites and oxidative stress lead to tumor formation.
Modulation of receptor mediated effects	Peroxisome proliferator-activated receptor α (PPARα) activation	1. Haloacetic acids activate PPARα in the liver. 2. PPARα activation leads to altered cell proliferation and apoptosis. 3. Alterations in cell proliferation and apoptosis cause clonal expansion of initiated cells. 4. Clonal expansion of initiated cells leads to tumor formation.
Cell immortalization, induction of epigenetic alterations, genotoxicity	Cell transformation	1. Haloacetic acids induce genetic and/or epigenetic alterations in the target cell. 2. These changes lead to altered gene expression and signal transduction and acquisition of a malignant phenotype including blocked cellular differentiation and morphological transformation, acquisition of an unlimited lifespan, genetic instability, anchorage-independent growth, foci formation, clonal expansion, and tumor formation.
Alteration of cell proliferation or cell death	Sustained cellular proliferation and suppression of apoptosis	1. Haloacetic acids induce sustained cell proliferation, cytotoxicity and reparative hyperplasia, and/or decreased programmed cell death (apoptosis) in target tissues. 2. Increased cell proliferation and reduced apoptosis. 3. Increases the probability that initiated cells will form and survive. 4. Survival of initiated cells leads to clonal expansion, foci formation, and tumor formation.

Sources: Pals *et al.* 2011, EPA 2011b, Dad *et al.* 2013, IARC 2013a, 2013b, 2014a, 2014b, Wood *et al.* 2015, Smith *et al.* 2016

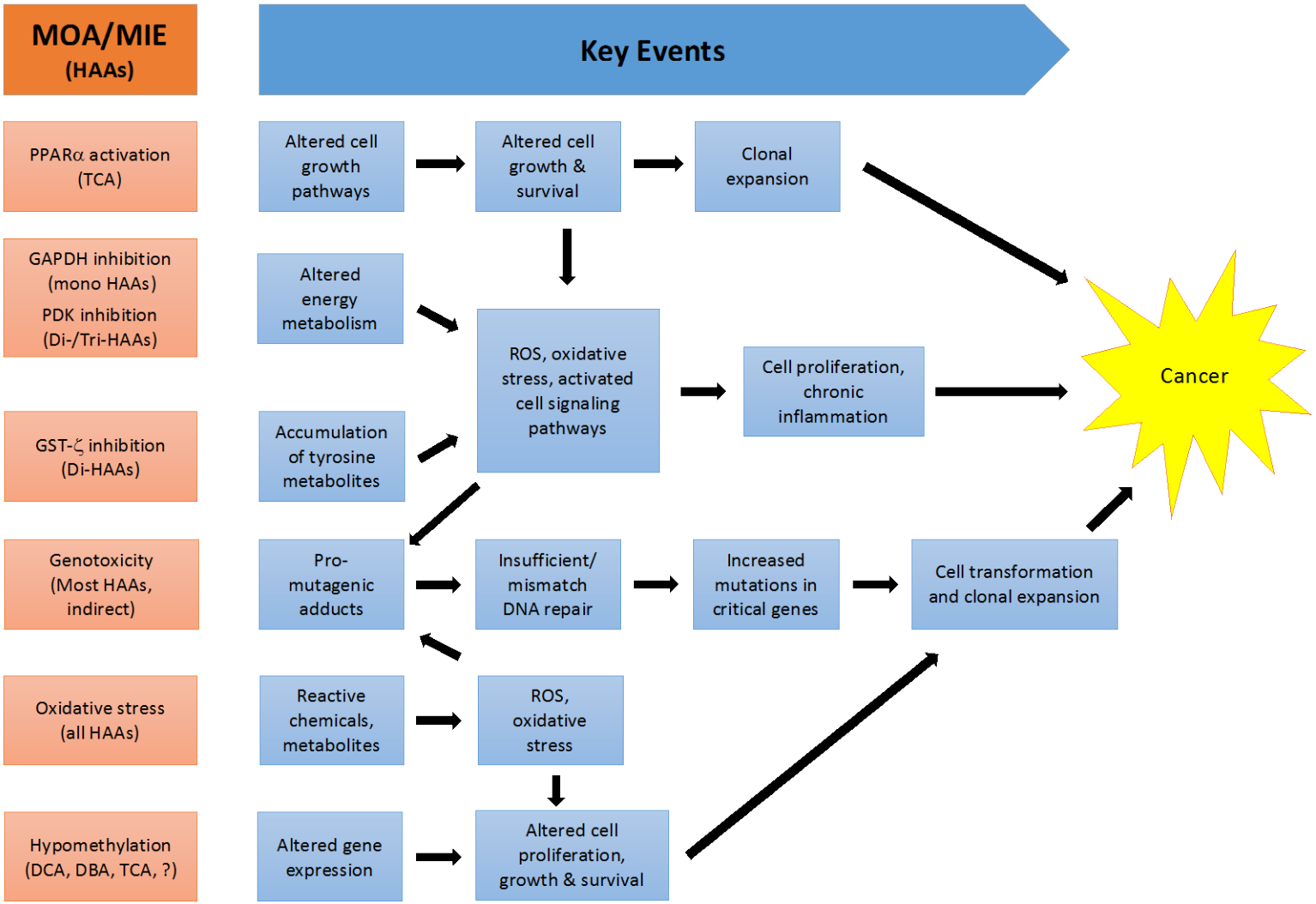

Figure 6-6. Interactions of potential modes of action and key events associated with haloacetic acid-induced carcinogenicity

MOA = mode of action, MIE = molecular initiating event, HAA = haloacetic acid, PPARα = peroxisome proliferator-activated receptor alpha, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, PDK = pyruvate dehydrogenase kinase, GST-ζ = glutathione *S*-transferase-zeta, ? = uncertain.

7 Evaluation of Haloacetic Acids as a Class or Subclass(es)

Since only 6 of the 13 haloacetic acids considered in this monograph have been tested for carcinogenicity in experimental animals, the primary purpose of this section is to evaluate whether or not the carcinogenic potential of any or all of the haloacetic acids that have not been tested in long-term cancer bioassays could be reasonably predicted based on application of read-across like principles and methods. The key questions addressed in this section are as follows:

1. Can haloacetic acids be evaluated as a class or subclasses for potential carcinogenicity based on a common mode(s) of carcinogenic action or key intermediate events that are relevant to carcinogenicity?
2. Can a read-across (e.g., QSAR) model and/or other models help inform the evaluation of the carcinogenicity of haloacetic acids that do not have animal carcinogenicity studies?

7.1 Approach and methods

A read-across like approach was used to determine whether haloacetic acids could be evaluated as a class or subclasses or if this approach could be applied to individual haloacetic acids that do not have cancer data. Read-across is a data gap filling technique used with an analogue or category (group of chemicals whose physico-chemical, metabolic, and toxicological properties are likely to be similar or follow a regular pattern based on structural similarity) approach (Patlewicz *et al.* 2015, ECHA 2008). In the read-across approach, endpoint information for a tested chemical (source) is used to predict the same endpoint for another chemical (target) which is considered similar (Patlewicz *et al.* 2015, Schultz *et al.* 2015). Haloacetic acids as a class were judged to fit the general definition of a chemical category based on chemical structure, properties, and generally consistent patterns in toxicokinetics and toxicology. OECD (2017) defines a chemical category as, "a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity." In this context, the use of a read-across like approach is intended to predict carcinogenicity for haloacetic acids that have not been tested in a cancer bioassay.

Two general approaches are described here: (1) a category approach in which a group of haloacetic acids, consisting of either all 13 molecules or a chemically defined subclass with fewer haloacetic acids can be evaluated together, and (2) evaluaton of individual haloacetic acids using metabolism data and/or an analogue approach. The first approach would use basic read-across like methods to determine if data gaps can be filled and would include an evaluation of all the relevant data presented in the previous sections of this monograph to determine if it is feasible to consider all haloacetic acids or a specific subclass of haloacetic acids as a category based on carcinogenicity (Section 7.2). The second approach will determine if individual haloacetic acids (without cancer data) could be evaluated as metabolites and/or with an analogue approach to read-across based on similar metabolic pathways and toxicokinetic data when compared to related chemicals (i.e., analogues) with cancer data (Section 7.3). The methods for the two variations of the category approach are described below in Sections 7.1.1 for all 13 haloacetic acids and 7.1.2 for potential subclasses, and the methods for the analogue approach are described in Section 7.1.3. The application of the category approach for all 13 haloacetic acids is discussed in Section 7.2, that for a subclass of haloacetic acids in Section 7.3, and the analogue approach in Section 7.4.

7.1.1 Approach for evaluating haloacetic acids as a class

The primary methods for the read across like approach for evaluating haloacetic acids as a class include (1) a comparison of the mechanistic data based on the characteristics of carcinogens across the different haloacetic acids, (2) an assessment of QSAR evaluations of haloacetic acids reported in the literature for biological effects, and (3) a comparison of cancer potency using QSAR modeling and reported cancer data for haloacetic acids.

The general trends in potency estimates for various mechanistic endpoints and chemical properties of the 13 haloacetic acids are shown in a heat map in Table 7-1. This table includes toxicokinetic data, physicochemical properties (pKa, E_{LUMO}, and binding affinity for proteins and DNA), *in vitro* and *in vivo* data for several key events potentially associated with carcinogenicity (see Figure 6-6), and animal carcinogenicity. Each row in the table is independently scored and ranked on a color scale of red (high potency), pink (moderate high potency), white or light blue (moderate potency) and blue (low potency or inactive). Most of the data in Table 7-2 were derived from the potency estimates discussed in Section 6 and tabulated in Appendix D.

Carcinogenic potency was assessed using two quantitative measures (mg/kg body weight per day), a QSAR model for predicting TD_{50}s (chronic dose rate that would induce tumors in half the animals tested) and benchmark doses (BMDs) as 95% lower confidence corresponding to a 10% response level (BMDLs). The TD_{50} values were predicted for all 13 haloacetic acids using a readily available open source QSAR model - the ADMET Predictor™ version 7.2 chronic carcinogenicity model (http://www.simulations-plus.com/software/admet-property-prediction-qsar/) for both rats and mice based on the parent compound structure and default settings. BMDL values for trichloro- and dichloroacetic acids were available from EPA's Integrated Risk Information System (IRIS) database (available at https://www.epa.gov/iris) and BMDL values for combined liver tumors in male mice (dibromo-, bromochloro-, and bromodichloroacetic acid were available from NTP studies (CEBS database available at https://cebs.niehs.nih.gov/multistage/). Predicted BMDLs and estimated TD_{50}s (reported as reciprocals) are included in Table 7-1.

In addition, a subjective measure of carcinogenic potency was derived from the results of the cancer bioassays and results are included in Table 7-2. Values of 0 to 3 were assigned as follows: 0 = negative response, 1 = liver tumors in mice only, 2 = liver tumors in mice and rats, and 3 = liver and other tumor sites in rats and mice.

7.1.2 Approach for evaluating subclasses of halocetic acids

The approach for evaluation of subclasses of haloacetic acids is essentially the same as for the set of 13 haloacetic acids, but by making subclasses based on similar chemical structures, e.g., number of halogens or type of halogen substitutions, it might be possible to apply the read-across like methods described above to a set of molecules with more consistent chemical characteristics.

7.1.3 Approach for evaluating halocetic acids for a potential analogue approach

The approach for identifying potential analogues among haloacetic acids uses one molecule with tumorigenicity data as a source chemical to inform the potential tumorigenicity of a second haloacetic acid that has not been tested in a cancer bioassay. Reasons for selecting a source chemical or chemicals for a target haloacetic acid include metabolism, which could provide direct evidence that the tumorigenic source chemical is metabolized to the target chemical and similarities of physicochemical properties that are related to potential key events in carcinogenicity.

7.2 Evaluation of haloacetic acids as a class

Overall, studies of metabolism, clearance, cytotoxicity, genotoxicity, and other non-cancer adverse effects among the haloacetic acids provide some evidence that haloacetic acids could be considered as a class or subclass. As mentioned, in Section 6, the haloacetic acids are generally considered soft electrophiles and the likely molecular initiating event is reaction with protein sulfhydryl groups. Reaction with proteins can cause indirect genotoxicity through generation of reactive oxygen species (ROS) (Stalter *et al.* 2016). The available studies found a strong relationship for greater toxic potency with larger halogen size (i.e., iodo- > bromo- ≫ chloro-) and decreasing toxicity with an increasing degree of halogenation (i.e., mono- > di- > tri-) (see Table 7-2). There are some exceptions to the general trends (e.g., trichloroacetic acid is the strongest PPARα agonist while dichloroacetic acid shows low activity for many of the key events). In cases where data were available for only a few of the haloacetic acids (e.g., GST-ζ inhibition, *in vivo* genetic effects or oxidative stress), the trends are not as evident because of incomplete data.

QSAR techniques have also successfully predicted several biological properties of the haloacetic acids as a category and have established similarity patterns among these chemicals. Two independent *in vitro* studies investigated different biological effects (oxidative stress and genotoxicity, and neural tube defects in mouse embryo cultures) of the haloacetic acids and reported that although the relative potency of the mono-, di-, and trihaloacetic acids was not highly correlated with any single property, there was a strong correlation when two chemical properties were considered together as independent variables: energy of the lowest unoccupied molecular orbital (E_{LUMO}) and the acid dissociation constant (pKa) (Richard and Hunter 1996, Stalter *et al.* 2016). A series of QSAR models further indicated that these two molecular parameters account for two opposing trends in the data: E_{LUMO} was related to the intrinsic reactivity and correlated with increasing potency with halogen size, especially among the monohaloacetic acids. On the other hand, pKa was related to transport and bioavailability and correlated with decreasing potency with an increasing degree of halogenation within a series (i.e, bromo- > dibromo- > tribromoacetic acid) and increasing potency with halogen size among the di- and trihaloacetic acids (Richard and Hunter 1996). Related parameters that have shown moderate to strong correlations with cytotoxicity and/or genotoxicity of haloacetic acids include the log octanol/water partition coefficient (log P), carbon-halogen bond length, and relative S_N2 reactivity (Plewa *et al.* 2004b, Plewa *et al.* 2010, Pals *et al.* 2011).

Although the data show consistent trends in potency for many of the potential key events involved in the toxicity of these compounds, their relationship to carcinogenic potency is unknown. In addition, the available carcinogenicity data do not appear to support evaluating haloacetic acids as a class. Using a subjective measure of carcinogenicity (described above), the trend observed with the carcinogenicity data is somewhat consistent with the trends observed with other endpoints (i.e., brominated > chlorinated forms and dichloroacetic acid > trichloroacetic acid) but does not distinguish between di- or trihaloacetic acids containing at least one bromine atom. Moreover, chloroacetic acid also presents a challenge because the data do not explain the absence of rodent carcinogenicity when this compound shows a greater or similar genotoxic and oxidative stress potency than the di- or trihaloacetic acids. However, the maximum dose tested by oral exposure in mice was much lower (100 mg/L) than that used for the other tested haloacetic acids (at least 1000 mg/L) because of high toxicity of chloracetic acid. The estimated TD_{50}s and published BMDLs for carcinogencity do not follow these general trends. The QSAR predicted TD_{50}s for carcinogenicity of all 13 haloacetic acids based on structural attributes and default settings were within one order of magnitude of each other, did

not predict that chloroacetic acid would be inactive as a rodent carcinogen, and predicted that trichloroacetic acid was the most potent. The published BMDL values (liver tumors in male mice) for dichloro-, dibromo-, bromochloro-, trichloro-, and bromodichloroacetic acid ranged from 1.5 to about 25 mg/kg/day, thus, they do not provide sufficient separation to determine a potency trend with any degree of confidence and also indicated that trichloroacetic acid was the most potent.

In addition, the mechanisms by which haloacetic acids induce carcinogenic effects in experimental animals have not been conclusively determined and remain as a large source of uncertainty for read-across, especially for an apical endpoint as complex as carcinogenicity. There is also evidence that, at least for dichloroacetic acid and trichloroacetic acid, the modes of action are likely different based on species affected and dose response of tumor formation, liver tumor mutation spectra, toxicokinetics, PPARα activation, liver pathology, and tumor phenotypes (Bull *et al.* 1990, Anna *et al.* 1994, Ferreira-Gonzalez *et al.* 1995, Pereira and Phelps 1996, Stauber *et al.* 1998, Bull 2000, Bull *et al.* 2002, Corton 2008).

Based on the lack of a well-defined mechanism or mode(s) of action, combined with evidence that at least some of the haloacetic acids do not share a common mode(s) of action, the lack of a clear trend in carcinogenic potency, the absence of carcinogenic activity of chloroacetic acid in rodents, and the lack of a suitable QSAR model, the current data do not support considering all 13 haloacetic acids as a chemical class.

7.3 Potential haloacetic acid subclasses

Although the current data were judged insufficient to support evaluating the set of 13 haloacetic acids as a category, the data were examined to determine if subclasses of haloacetic acids (see Table 7-2) could potentially be evaluated using either a category approach. Potential analogues within each subclass were also considered to determine if haloacetic acids with cancer data could be matched with a target chemical (without cancer data) to predict the carcinogenicity of that target chemical.

Overall, available data did not identify any subclass (i.e., category based on number or type of haloagen substitution) of haloacetic acids that could be evaluated for carcinogenicity because of the lack of a common mode of action, the lack of adequate cancer data, and/or inconsistencies in the cancer data among the members of that subclass. Of the subclasses of haloacetic acids, the confidence for a potential category for using read-across approaches was highest for subclasses that include brominated haloacetic acids, which are more similar with respect to tumor profiles, than those subclasses that included the chlorianted haloacetic acids (i.e. chloroacetic acid, dichloroacetic acid and trichloroacetic acid). The subclass with the highest potential was the di- and trihaloacetic acids which includes three members with cancer data (dichloracetic acid, bromochloroacetic acid, dibromoacetic acid) and three members without cancer data (chlorodibromoacetic acid, tribromoacetic acid, bromoiodoacetic acid). A strength of this approach is that chlorodibromoacetic acid and tribromoacetic acid are metabolized to the two dihaloacetic acids with cancer data. However, a read-across approach for the other members of a subclass without cancer data (bromoidooacetic acid) is more uncertain because of the lack of direct experimental evidence for effects of substitution of bromine or chlorine atoms with iodine on tumorigenicity. Additional toxicological data on key events may contribute to a better understanding of the mode(s) of action and could reduce uncertainty in the read-across of haloacetic acids in the future.

Table 7-1. Comparison of relative potency estimates for mechanistic endpoints and chemical properties of haloacetic acids

Endpoint	Monohaloacetic acids			Dihaloacetic acids						Trihaloacetic acids			
	CA	BA	IA	DCA	DBA	DIA	BCA	CIA	BIA	TCA	TBA	BDCA	CDBA
Toxicokinetics[a]													
Oral bioavailability (%)	100			81	30		47			100	62	96	100
Unbound fraction (%)	27			94	89		93			53	18	49	55
Total clearance (mL/kg/h)	262			267	491		1037			92.5	754	286	486
Renal (% of total)	59			1.1	2.6		3.6			45.5	22.7	31.1	37.4
Non-renal (% of total)	41			98.9	97.4		96.4			54.5	77.2	68.9	62.6
Chemical properties[b]													
pKa	2.97	2.96	2.95	1.41	1.39		1.4	1.47	1.67	0.66	0.03	0.05	0.04
E_{lumo} (deprotonated)	9.43	8.68	7.18	8.44	7.51		7.78	6.40	6.46	7.13	6.12	6.65	6.42
TR_{GSH}	0.71	2.18	2.15	1.68	1.48		1.93	1.35	0.62	1.01	2.26	1.56	1.1
TR_{DNA}	0.92	0.84	0.81	0.5	1.9		3.18	1.17	1.47	0.99	2.61	0.73	0.95
Oxidative stress in vitro[c]													
AREc32	3.7	192	278	0.17	8.3		7.1	45.5	38.5	N	2.3	0.5	0.2
ARE-bla	4	90.9	196	0.06	4		2.2	10	18.9	N	1.5	0.25	0.46
Oxidative stress *in vivo*[d]													
8-OHdG				1.4	2.9		2.9			1.2		1.7	
TBARS				129	250		290			67		240	
Genetox *in vitro*													
SOS-umuC [c,e]	60	2400	15400	180	2564		2941	5263	9091	60	142860	9091	9091
Ames TA100 (-S9) [f]	27	5465	14129	35	148					N	N		
Ames TA100 (-S9) [g]				5.2	61.9		60.6			N	1.2	31.6	1.7
Comet CHO cells [h]	2439	58820	114900	N	556	500	333		313	N	400	N	71
HGPRT CHO cells [i]	8.7	14.6	836	2.8	66.2					N			
P53-bla [c]	5882	105260	212770	N	3846		4348	9091	9091	N	N	N	N
Genetox *in vivo* [j]				0.5	1		N			N		N	
PPARα *in vitro*[k]	2	20	100	1	1					2	1		
PPARα *in vivo* [l]	N			2	3.5		N			4.3		N	
GST-ζ inhibition [m]				45	83		81			N			
Animal carcinogenicity													
Species/tumor site[n]	N			2	3		3			1		3	
TD_{50}s rat (predicted)[o]	0.0041	0.0025	0.0015	0.0071	0.0029	0.0012	0.0044	0.0028	0.0019	0.0076	0.0027	0.0052	0.0037
TD_{50}s mouse (predicted)[o]	0.0021	0.0012	0.0007	0.0024	0.0011	0.0004	0.0016	0.0010	0.0007	0.0027	0.0011	0.0019	0.0014
BMDL[p]				0.49	0.04		0.08			0.67		0.06	

Relative potency scale: red = high, pink = high moderate, white or light blue = moderate, blue = low, purple = negative (N), gray = no data (rows are scored independently).
[a] Schultz *et al.* 1999 (di- and trihaloacetic acids); Kaphalia *et al.* 1992, Saghir *et al.* 2001, Saghir and Rozman 2003 (chloroacetic acid)
[b] TR = toxicity ratio; TR_{GSH} > 1.2 suggest the compound is a soft electrophile and preferentially reacts with protein. TR_{DNA} > 1.2 indicates hard electrophile and preferentially reacts with DNA, both values > 1.2 indicates unspecific reactivity; lower values of E_{LUMO} indicate compound is a softer electrophile, lower pKa indicates stronger acid and greater dissociation at physiological pH (Stalter *et al.* 2016).
[c] Reciprocal of the effective concentration that elicits an induction ratio of 1.5 ($EC_{IR1.5}$ representing a 1.5-fold or 50% effect increase compared to the control) (Stalter *et al.* 2016).
[d] 8-OHdG/10^5 dG liver, TBARS: nmol malondialdehyde/g liver (Larson and Bull 1992, Austin *et al.* 1996).
[e] Reciprocal of $EC_{IR1.5}$ (Stalter *et al.* 2016, Zhang *et al.* 2016).
[f] Mean revertants/µmol (Kargalioglu *et al.* 2002, Plewa *et al.* 2004a).
[g] Mean revertants/µmol (NTP 1992, 2007a, 2009, 2015, CEBs database).
[h] Reciprocal of genotoxic potency (Plewa *et al.* 2010).
[l] Mutant frequency/mM (Zhang *et al.* 2010).
[j] 1 = positive, 0.5 = weak positive, 0 = negative (NTP 2007a, 2009, IARC 2013a, 2013b, 2014a, 2014b, NTP 2015).
[k] Reciprocal of the LEC (lowest effective concentration) (Walgren *et al.* 2004).
[l] Fold increase compared to control (Acyl-CoA- oxidase activity [Xu *et al.* 1995, Parrish *et al.* 1996, NTP 2015]).
[m] % reduction enzyme activity compared to controls (Anderson *et al.* 1999, Gonzalez-Leon *et al.* 1999).
[n] 0 = no evidence of carcinogenicity, 1 = liver tumors in mice only, 2 = liver tumors in rats and mice, 3 = multiple tumor sites in rats and mice.
[o] Reciprocal of predicted TD_{50} from ADMET Predictor™ software.
[p] Reciprocal of the Benchmark dose low (BMDL), mg/kg/day based on combined liver tumors in male mice (EPA at https://www.epa.gov/iris and CEBS at https://cebs.niehs.nih.gov/multistage/).

Even though no category could be identified for the 13 haloacetic acids as a class or for subclasses within the set, application of an analogue approach (see Section 7.1.3) is possible for chlorodibromoacetic acid and tribromoacetic acid, which is described in Section 7.4. Potential analogues within each subclass are identified in the Rationale column.

Table 7-2. Evaluation of subclasses of haloacetic acids

Subclass	Members[a]	Confidence for potential category	Rationale
Monohaloacetic acids	CA, BA, IA	No	CA tested negative in a long-term cancer bioassay. IA was positive in the cell transformation assay. Insufficient data to read-across to BA or IA.
Dihaloacetic acids	DCA, DBA, DIA, BCA, CIA, BIA	Low	DCA, DBA, and BCA are rodent carcinogens and are potential analogues for read-across to the iodinated compounds; however, more uncertainty regarding impact of iodine substitution on carcinogenicity.
Trihaloacetic acids	TCA, TBA, BDCA, CDBA	Low	TCA and BDCA tested positive in cancer bioassays. TCA may cause liver cancer via different mechanisms than other haloacetic acids. *Potential analogues:* BDCA could be used as an analogue for potential read-across to CDBA. There is more uncertainty with read across to TBA.
Chlorinated acetic acids	CA, DCA, BCA, CIA, TCA	No	All but CIA have been tested in long-term cancer bioassays; however, the negative findings of CA in cancer studies present a challenge for read-across, and result in increased uncertainty. *Potential analogues:* BCA could be a potential analogue for read-across to CIA
Brominated acetic acids	BA, DBA, BCA, BIA, TBA, BDCA, CDBA	Maybe Low/moderate	DBA, BCA, and BDCA are rodent carcinogens. TBA is metabolized to DBA and CDBA is metabolized to BCA. The uncertainty for this subclass is based on lack of carcinogenicity with CA and a possible unique mode of action for the monohaloacetic acids. *Potential analogues:* BCA is a potential analogue for read-across to BIA, but more uncertainty as noted above. BDCA is a potential analogue for read-across to CDBA

Iodinated acetic acids	IA, DIA, CIA, BIA	No	None of the members have been tested in a long-term cancer bioassay. The modes of action may not be the same for the mono- and dihaloacetic acids containing iodine.
Brominated di- and trihaloacetic acids	DBA, BCA, BIA, TBA, BDCA, CDBA	Moderate	DBA, BCA, and BDCA are rodent carcinogens. Based on similarity of tumor profiles and trends in metabolism, toxicokinetics, physicochemical properties, and potential key events, the mode of action for this subclass is possibly similar. However, the similarities are greater within the dihalo- and trihaloacetic acids than across these molecules. *Potential analogues:* TBA is metabolized directly to DBA and CDBA is metabolized to BCA. In addition, BDCA is a potential analogue for read-across to both CDBA and TBA. Read-across to BIA is more uncertain as noted above.

CA = chloro-, BA = bromo-, IA = iodo-, DCA = dichloro-, DBA = dibromo-, BCA = bromochloro-, DIA = diiodo-, CIA = chloroiodo-, BIA = bromoiodo, TCA = trichloro-, TBA = tribromo-, BDCA = bromodichloroacetic acid, CDBA = chlorodibromoacetic acid.

7.4 Individual di- and tribromohaloacetic acids.

Based on their metabolism to known rodent carcinogens, both bromochloroacetic acid and dibromoacetic acid are predicted to cause cancer in rodents. Chlorodibromo- and tribromoacetic acid are directly metabolized to bromochloro- and dibromoacetic acid, respectively (see Section 3 and 7.4.1 below) and both dihaloacetic acids, as well as bromodichloroacetic acid (which could also be a potential analogue), have been shown to be rodent carcinogens (see Section 4). Thus, dibromoacetic acid was selected an analogue for read-across to tribromoacetic acid and bromochloroacetic acid is an analogue for read-across to chlorodibromoacetic acid based on their metabolism and similar physiochemical properties, toxicokinetics properties, and biological effects *in vivo* and *in vitro* (e.g., similar potencies for genetic and oxidative stress) (see Table 7-1). Supporting evidence for these conclusions is discussed below.

7.4.1 Metabolism and toxicokinetics

Trihaloacetic acids are primarily metabolized by reductive dehalogenation that generates a dihaloacetic acid via a free radical intermediate (Xu *et al.* 1995, Austin and Bull 1997, Anderson *et al.* 1999, Saghir *et al.* 2011). Saghir *et al.* (2011) demonstrated that reductive dehalogenation of tribromoacetic acid *in vitro* by a rat liver enzyme preparation (microsomes) produced dibromoacetic acid in a 1:1 molar ratio with the Br^- liberated and there was no evidence of additional oxidative metabolism. This study also demonstrated that bromodichloro-, chlorodibromo-, and tribromoacetic acid were rapidly metabolized by rat liver microsomes to the di-haloacetic acid product corresponding to the loss of a single Br^-. Further, substitution of bromines enhanced metabolism, thus, tribromo- and chlorodibromo- are metabolized to their corresponding di-haloacetic acid to a much greater degree than trichloroacetic acid (Schultz *et al.*

1999, Saghir *et al.* 2011). (See Section 3 for a more complete discussion of metabolism and toxicokinetics.)

7.4.2 Carcinogenicity data

Near lifetime exposure to bromodichloroacetic acid and dibromoacetic acid in the drinking water caused liver tumors in mice as well as tumors at other tissue sites (listed in Table 7-3) in rats and mice. Thus, chlorodibromoacetic acid and tribromoacetic acid are predicted to be animal carcinogens based on metabolism to bromochloroacetic acid and dibromoacetic acid, respectively. Moreover, all of the di- and tri- haloacetic acids tested caused liver tumors in mice, which increases the confidence that chlorodibromoacetic acid and tribromoacetic acid would specifically cause liver tumors in mice. The three tested brominated di-and trihaloacetic acids, (dibromo-, bromochloro- and bromodichloroacetic acid), which are the postulated source chemicals, also caused malignant mesothelioma in male rats. Based on the greater metabolism of the brominated trihaloacetic acids compared to trichloroacetic acid, and the generally greater biological and carcinogenic activity with bromine substitution for chlorine, it is expected that the tumor profiles of chlorodibromoacetic and tribromoacetic acids may be more similar to their direct metabolites (bromochloroacetic acid and dibromoacetic acid, respectively) than the carcinogenicity of trichloroacetic acid and its metabolite dichloroacetic acid. The dihalogenated metabolites may not account for all of the carcinogenic potential of the trihaloacetic acids because bromodichloroacetic acid induced more tumor types in rodents than its primary metabolite, dichloroacetic acid.

Table 7-3. Tumor profiles in source chemicals and predicted tumor profiles in target chemicals

Endpoint	Source chemicals			Target chemicals	
	BCA (metabolite)	DBA (metabolite)	BDCA	CDBA	TBA
Rats (sex)	**Yes**	**Yes**	**Yes**	**Predicted**	**Predicted**
Liver (M/F)	No	No	No		
Mononuclear cell leukemia (F)	No	Yes	No		
Malignant mesothelioma (M)	Yes	Yes	Yes	Likely site	Likely site
Mammary (F)	Yes	No	Yes		
Skin	No	No	Yes		
Mice (sex)	**Yes**	**Yes**	**Yes**	**Predicted**	**Predicted**
Liver (M/F)	Yes	Yes	Yes	Very likely site	Very likely site
Lung (M)	No	Yes	No		
Harderian Gland (M)	No	No	Yes		

BCA = bromochloro-, DBA = dibromo-. BDCA = bromodichloro-, CDBA = chlorodibromo-, TBA = tribomoacetic acid, Likely or very likely tumor sites are based on metabolism to BCA and DBA, tumor profiles of metabolites or source chemicals and support from mechanistic and other relevant data

7.4.3 Supporting mechanistic data

In general, chlorodibromoacetic acid and tribromoacetic acid have similar toxicokinetic and biological effects compared with other brominated haloacetic acids that cause cancer in rodents (Tables 7-21, and 7-4). Physicochemical properties also show that the electrophilicity increases with bromine content and that the trihaloacetic acids are stronger acids than the dihaloacetic acids. The available data for these two chemicals provide evidence that they also cause oxidative stress and genetic effects, e.g., mutations in bacteria and DNA strand breaks in cultured cells. These events are characteristic of other human carcinogens and support biological plausibility for the carcinogenicity of chlorodibromoacetic and tribromoacetic acid in humans.

7.4.4 Conclusions

The set of 13 haloacetic acids considered in this monograph did not form a category to evaluate all of them as a class because of (1) the lack of a well-defined mechanism or mode(s) of action, (2) data that suggest that at least some of the haloacetic acids do not share a common mode(s) of action, (3) the absence of carcinogenicity of chloroacetic acid, and (4) the lack of a clear trend in carcinogenic potency. QSAR model predictions of cancer potency were not consistent with the trends in the key events data. Therefore, the current data are inadequate to support considering all 13 haloacetic acids as a chemical class.

Subclasses of haloacetic acids based on number and types of halogen substitutions were also considered for making read-across predictions of carcinogenicity. The major limitations in the confidence for the read-across analyses are the lack of cancer data for any iodohalacetic acids or monoacetic acids, the negative findings for chloroacetic acid (even though tested at a much lower dose in mice than other haloacetic acids), and data suggesting that trichloroacetic acid may cause cancer by different modes of action than dichloroacetic acid. None of the subclasses of haloacetic acids considered for read across were considered to have sufficient evidence at this time to support their use as a category.

Consideration of one subclass with six di- and tribrominated haloacetic acids (dibromo-, bromochloro-, bromoiodo-, tribromo-, bromodichloro-, and chlorodibromoacetic acid) led to identification of two individual trihaloacetic acids as target chemicals for read across based on metabolism and supporting mechanistic data. Cancer predictions were made for two members of this class that did not have cancer data: (1) tribromoacetic acid which is metabolized to dibromoacetic acid (which is the source analogue) and (2) chlorodibromoacetic acid, which is metabolized to bromochloroacetic acid (which is the source analogue). Bromodichloroacetic acid is also a rodent carcinogen with similar physicochemical, toxicokinetic, and toxicological properties as the target chemicals, which provides additional support for the conclusion that both tribromoacetic acid and chlorodibromoacetic acid are reasonably anticipated to be rodent carcinogens.

8 Overall Cancer Evaluation and Preliminary Listing Recommendation

The purpose of this monograph is to assess the carcinogenicity data of 13 haloacetic acids found in water disinfection by-products and to determine whether the scientific evidence meets the RoC criteria for listing as a class, subclasses, or as individual haloacetic acids. The overall evaluation integrates the assessments of the animal cancer studies (Section 4, Section 8.1), mechanistic and other relevant data (Section 6, Section 8.2), as well as the read across analysis for evaluating haloacetic acids as a class or subclasses, to reach preliminary listing conclusions (see Figure 1 in Background and Methods).

Overall, the data from cancer studies in humans are inadequate to evaluate the relationship between human cancer and exposure specifically to the individual haloacetic acids, subclasses of haloacetic acid, or haloacetic acids as a class, as only one study was identified that provided risk estimates specific for a class of, or for individual haloacetic acids. However, studies on water disinfection by products suggest the potential for cancer risk from chlorinated water and increase the confidence of the relevance of the animal cancer studies (conducted at higher doses) to humans.

8.1 Evidence of carcinogenicity from studies in experimental animals

There is sufficient evidence of carcinogenicity for dichloroacetic acid (DCA), dibromoacetic acid (DBA), bromochloroacetic acid (BCA), and bromodichloroacetic acid (BDCA) from studies in experimental animals.

The conclusion for each of these haloacetic acids is based on significantly increased incidences of malignant tumors or a combination of benign and malignant tumors at several organ sites in rodents or in several species by exposure in drinking water (see Section 4, Tables 4-1 and 4-3, and Table 8-1 below). Exposure to dichloroacetic acid induced liver tumors (hepatocellular adenoma and carcinoma) in male Fischer 344 rats and in male and female $B6C3F_1$ mice. Exposure to dibromoacetic acid, bromochloroacetic acid, and bromodichloroacetic acid induced liver tumors (hepatocellular adenoma, carcinoma) in both sexes and, hepatoblastoma in male $B6C3F_1$ mice and tumors at other sites in male and female Fischer 344 or Fischer 344 N/Tac rats (for bromodichloroacetic acid). These three haloacetic acids all induced malignant mesothelioma in male rats and bromochloroacetic acid and bromodichloroacetic acid induced mammary gland tumors (fibroadenoma, carcinoma) in female rats. Bromodichloroacetic acid exposure also resulted in malignant skin tumors in male rats. Bromochloroacetic acid induced tumors in the large intestine in both sexes of rats. Although the large intestinal tumors were adenoma, they are considered to be supportive of a carcinogenic response because of their rarity and progression to malignant tumors. Dibromoacetic acid also induced lung tumors (alveolar/bronchiolar carcinoma) in male $B6C3F_1$ mice and bromodichloroacetic acid induced Harderian gland (benign or malignant) tumors in male $B6C3F_1$ mice.

The evidence of carcinogenicity from studies in experimental animals is not sufficient to meet the RoC criteria for listing monochloroacetic acid, monoiodoacetic acid, or trichloroacetic acid (TCA). Exposure to monochloroacetic acid by gavage did not induce tumors in male and female

$B6C3F_1$ mice or Fischer 344 rats. Monoiodoacetic acid applied dermally in a co-carcinogen study with 7,12 dimethylbenz(a)anthracene (DMBA) in mice (strain and sex not given) resulted in papillomas. In addition, monoiodoacetic acid transformed NIH3T3 cells in a cell-transformation assay that resulted in aggressive fibrosarcomas after injection into Balb/c mice. Exposure to trichloroacetic acid in drinking water induced liver tumors in male and female $B6C3F_1$ mice but not in male F344 rats (female rats not tested) and did not induce tumors in male mice with intraperitoneal injection. No cancer studies in experimental animals were available for monobromoacetic acid, diiodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, tribromoacetic acid, and chlorodibromoacetic acid.

Table 8-1. Evidence of cancer in experimental animals

	DCA				DBA				BCA				TCA				BDCA			
	Rats		Mice		Rats		Mice		Rats		Mice		Rats		Mice		Rats		Mice	
Neoplasm or tissue	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Liver	X		X	X			X	X			X	X			X	X			X	X
Mononuclear-cell leukemia						X														
Malignant mesothelioma					X				X								X			
Mammary gland										X								X		
Lung							X													
Skin																	X			
Harderian gland																			X	
Large intestine									X	X										

DCA = dichloroacetic acid, DBA = dibromoacetic acid, BCA = bromochloroacetic acid, TCA = trichloroacetic acid, BDCA = bromodichloroacetic acid.

8.2 Summary of mechanistic data and read across approach

Key mechanistic cancer-initiating events are not known for the HAAs but most likely involve multiple biochemical pathways as several potential molecular initiating events have been identified. The selected haloacetic acids may induce cancer through binding to macromolecules, for example, causing oxidative stress through perturbation of energy metabolism within the cell or affecting regulation of genes involved in carcinogenicity.

Haloacetic acids did not form a category to evaluate as a class or a subclass because of lack of a well-defined mechanism or mode(s) of action and lack a clear trend in carcinogenic potency. QSAR model predictions of cancer potency were not consistent with cancer data. Therefore, the current data are inadequate to support considering haloacetic acids as a chemical class or subclass.

8.3 Preliminary listing recommendation

These preliminary listing recommendations are based on applying the RoC listing criteria to the body of scientific evidence provided in this monograph (https://ntp.niehs.nih.gov/go/rocprocess).

Dichloroacetic acid, dibromoacetic acid, chlorobromoacetic acid, and bromodichloroacetic acid are *reasonably anticipated to be human carcinogens* based on *sufficient evidence* from studies in experimental animals and supporting mechanistic data that demonstrate biological plausibility of its carcinogenicity in humans.

- Dichloroacetic acid – liver tumors (male and female mice, male rats)
- Dibromoacetic acid – liver tumors (male and female mice), malignant mesothelioma (male rats), mononuclear-cell leukemia (female rats), lung tumors (male mice)
- Bromochloroacetic acid– liver tumors (male and female mice), malignant mesothelioma (male rats), mammary gland tumors (female rats), large intestinal tumors (male and female rats)
- Bromodichloroacetic acid– liver tumors (male and female mice), malignant mesothelioma and skin tumors (male rats), mammary gland and Harderian gland tumors (female rats)

Chlorodibromoacetic acid is *reasonably anticipated to be human carcinogen* based on (1) metabolism studies that provide convincing evidence that chlorodibromoacetic acid is metabolized to bromochloroacetic acid, (2) sufficient evidence for the carcinogenicity of bromochloroacetic acid from studies in experimental animals, and (3) supporting mechanistic data that demonstrate biological plausibility of its carcinogenicity in humans. These mechanisms are biologically plausible in humans.

Tribromoacetic acid is *reasonably anticipated to be a human carcinogen* based on (1) metabolism studies that provide convincing evidence that tribromoacetic acid is metabolized to dibromoacetic acid, (2) sufficient evidence for the carcinogenicity of dibromoacetic acid from studies in experimental animals, and (3) supporting mechanistic data that demonstrate biological plausibility of its carcinogenicity in humans. These mechanisms are biologically plausible in humans.

Distribution and mechanistic information relating to properties of carcinogens on almost all of the 13 HAAs is available (see Section 6); however, the data are inadequate to group all 13 haloacetic acids as a class or into subclasses based on the type and number of halogens.

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Abbreviations

^{1}H NMR:	proton nuclear magnetic resonance
8-OHdG:	8-hydroxydeoxyguanosine
ACGIH:	American Conference of Governmental Industrial Hygienists
ADD:	average daily dose
ADME:	absorption, distribution, metabolism, and excretion
AEGL:	Acute Exposure Guideline Level
AhR:	aryl hydrocarbon receptor
ALL:	acute lymphocytic leukemia
ALT:	serum alanine aminotransferase, alanine aminotransferase
ANOVA:	analysis of variance
AOP:	adverse outcome pathway
ARE:	antioxidant response element
ARNT:	aryl hydrocarbon nuclear translocator
AST:	serum aspartate aminotransferase, aspartate aminotransferase
atm:	atmosphere
ATSDR:	Agency for Toxic Substances and Disease Registry
AWWA:	American Water Works Association
BCA:	bromochloroacetic acid
BDCA:	bromodichloroacetic acid
BDL:	below detection limit
BIA:	bromoiodoacetic acid
BMD:	benchmark dose
BMDL:	benchmark dose low
CA:	chromosomal aberration
CASRN:	Chemical Abstracts Service registry number
CDBA:	chlorodibromoacetic acid
CDC:	Centers for Disease Control and Prevention
CDR:	Chemical Data Reporting Rule
CEBS:	Chemical Effects in Biological Systems database
CERHR:	Center for the Evaluation of Risks to Human Reproduction
CHO:	Chinese hamster ovary

CIA:	chloroiodoacetic acid
CIN:	chromosomal instability
cm^2:	centimeters squared
CO_2:	carbon dioxide
CT:	chlorine concentration (C) times contact time (T)
Cx:	connexin
Cx32:	gap junction beta 1-protein; connexin32
DBA:	dibromoacetic acid
DBP:	disinfection by-product
DBPR:	Disinfectants and Disinfection Byproducts Rule
DCA:	dichloroacetic acid
DIA:	diiodoacetic acid
DLMI:	dominant lethal mutation index
DLMR:	dominant lethal mutation rate
DNA:	deoxyribonucleic acid
dw:	drinking water
EASE:	Estimation and Assessment of Substance Exposure
EC_{50}:	concentration of a drug that gives a half-maximal response
E_{HOMO}:	energy of the highest occupied molecular orbital
E_{LUMO}:	energy of the lowest unoccupied molecular orbital
EPA, USEPA:	Environmental Protection Agency, United States Environmental Protection Agency
EQ:	exposure quartiles model
Erk MAPK:	extracellular signal-regulated kinase mitogen activated pathway
EUSES:	European Union System for the Evaluation of Substances
EWG:	Environmental Working Group
Exp.:	exposed
F:	female
FDA:	Food and Drug Administration
FLARE:	fragment length analysis with repair enzyme
FR:	*Federal Register*
ft:	feet
FTE:	full-time equivalent

FU:	follow-up
G:	guanine
GAC:	Genetic Alterations in Cancer
GC/MS:	gas chromatography/mass spectroscopy
GFR:	glomerular filtration rate
GI:	gastrointestinal
GIS:	Geographic Information System
GM:	geometric mean
GSH:	glutathione
GSSH:	oxidized glutathione
GST:	glutathione-*S*-transferase
GST- ζ:	glutathione-*S*-transferase zeta
HAA:	haloacetic acid
HAA5:	sum of five haloacetic acids (bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, and trichloroacetic acid)
HAA9:	sum of nine haloacetic acids (bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, and trichloroacetic acid, bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid)
Hb:	hemoglobin
HBV:	Hepatitis B virus
HCB:	hexachlorobenzene
HCL:	hairy-cell leukemia
HCV:	Hepatitis C virus
HETA:	Health Hazard Evaluation and Technical Assistance
HGPRT:	hypoxanthine-guanine phosphoribosyltransferase
HHE:	Health Hazard Evaluation
HHS:	Department of Health and Human Services
HIC:	highest ineffective concentration
HID:	highest ineffective dose
HIV:	Human immunodeficiency virus
HOBr:	hypobromous acid
HOCl:	hypochlorous acid

HOI:	hypoiodous acid
HPLC:	high-performance liquid chromatography
hr:	hour
HWE:	healthy worker (hire or survival) effect
I:	inconclusive
i.p.:	intraperitoneal
i.v.:	intravenous
IARC:	International Agency for Research on Cancer
ICD-9:	International Classification of Diseases, Ninth Revision
ICD-O-2:	International Classification of Diseases for Oncology (revision 2)
IDLH:	immediately dangerous to life and health
in:	inch
IOM:	Institute of Medicine
IRIS:	Integrated Risk Information System
IUR:	Inventory Update Rule
JEM:	job-exposure matrix
kg:	kilogram
L:	liter
LEC:	lowest effective concentration
LED:	lowest effective dose
LHC:	lymphohematopoietic cancer
LOD:	limit of detection
Log D:	logarithm of the n-octanol/buffer solution (pH 7.4 or 4.0) distribution coefficient
Log K_{ow}, logP:	logarithm of octanol/water partition coefficient
LOH:	loss of heterozygosity
M:	male
m^3:	cubic meter
MAAI:	maleylacetoacetate isomerase
MAPK:	mitogen activated protein kinases
MBA:	monobromoacetic acid
MCA:	monochloroacetic acid
MCL:	maximum contaminant level

MG:	methylguanine
mg:	milligram
MIA:	monoiodoacetic acid
MIE:	molecular initiating event
mL:	milliliter
MM:	multiple myeloma
MN:	micronuclei
MOA:	mode of action
mol:	mole
MS:	mass spectrometry
N:	number
NA	not available; not applicable
NCE:	normochromatic erythrocyte
NCTR:	National Center for Toxicological Research
ND:	not detected; not determined; not done
ng:	nanogram
NHANES:	National Health and Nutrition Examination Survey
NHL:	non-Hodgkin lymphoma
NIEHS:	National Institute of Environmental Health Sciences
NIH3T3:	mouse fibroblast cell line
NIH:	National Institutes of Health
NIOSH:	National Institute for Occupational Safety and Health
NLM:	National Library of Medicine
NOES:	National Occupational Exposure Survey
NOM:	natural organic matter
NOS:	not otherwise specified
NPL:	National Priorities List
NR:	not reported, none reported
Nrf2:	nuclear factor (erythroid derived-2)-like 2, nuclear factor E2-related factor 2
ns:	not specified
NS:	not significant
nt:	nucleotides

NT:	not tested
NTP:	National Toxicology Program
OHAT:	Office of Health Assessment and Translation
OR:	odds ratio
OSHA:	Occupational Safety and Health Administration
OTM:	olive tail moment
pKa:	acid dissociation constant
p.o.:	per os (oral administration)
PBZ:	personal breathing zone
PCE:	polychromatic erythrocyte
PCNA:	proliferating cell nuclear antigen
PDH:	pyruvate dehydrogenase
PDK:	pyruvate dehydrogenase (PDH) kinase
PEL:	permissible exposure limit
PGE_2:	prostaglandin E_2
PPARα:	peroxisome proliferator-activated receptor alpha
ppm:	parts per million
ppt:	parts per trillion
QSAR:	quantitative structure-activity relationship
R:	estimated daily production of adducts
r:	correlation coefficient
RAHC:	Reasonably anticipated to be a human carcinogen
RBC:	red blood cell
REL:	recommended exposure limit
RLV:	Rauscher-leukemia virus
RoC:	Report on Carcinogens
ROS:	reactive oxygen species
RQ:	reportable quantity
RR:	relative risk
RTG:	relative total growth
s.c.:	subcutaneous
SAFE:	significance analysis of function and expression

SCE:	sister-chromatid exchange
SCGE:	single cell gel electrophoresis (Comet assay)
SD:	standard deviation
SDWA:	Safe Drinking Water Act
SIC:	Standard Industrial Classification
sig:	statistically significant
SIR:	standardized incidence ratio
SMR:	standardized mortality ratio
S_N2:	substitution, nucleophilic, with 2 molecules in the rate-determining step
SOCMI:	synthetic organic chemical manufacturing industry
SRR:	standardized rate ratio, standardized relative risk
SSB:	single strand break
STS:	soft tissue sarcoma
TBA:	tribromoacetic acid
TBARS:	thiobarbituric acid-reactive substances
TCA:	trichloroacetic acid
TD_{50}s:	chronic dose rate that would induce tumors in half the animals tested
TDS:	Total Diet Study
THAAs:	total haloacetic acids
TL:	tail length
TLC:	thin-layer chromatography
TLV-TWA:	threshold limit value time-weighted average
TM:	tail moment
t_{max}:	time to maximum concentration in plasma
TMD:	tail moment dispersion coefficient
TRI:	Toxics Release Inventory
TSCA:	Toxic Substances Control Act
TSFE:	time since first employment
TTHMs:	total trihalomethanes
UCMR4:	Fourth Unregulated Contaminant Monitoring Rule
UDS:	unscheduled DNA synthesis
UK:	United Kingdom

V_D:	apparent volume of distribution
VOC:	volatile organic compound
WBC:	white blood cell
WHO:	World Health Organization
wt%:	weight percent
yr:	year or years
µg:	microgram

Glossary

Alkylating potential: The likelihood that a hydrogen will be replace by an alkyl group, especially in a biologically important molecule.

Ames assay: The Ames *Salmonella*/microsome mutagenicity assay is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations.

Amniotic fluid: The protective fluid surrounding the developing fetus within the amniotic sac of a pregnant female.

Aneuploidy: An abnormality involving a chromosome number that is not an exact multiple of the haploid number (one chromosome set is incomplete).

Apoptosis: Cell deletion by fragmentation into membrane-bound particles, which are phagocytosed by other cells.

Apurinic site: A location in DNA (also in RNA but much less likely) that has neither a purine nor a pyrimidine base, either spontaneously or due to DNA damage.

Arabinose resistance: The L-arabinose resistance test with *Salmonella typhimurium* (Ara test) is a forward mutation assay that selects a single phenotypic change (from L-arabinose sensitivity to L-arabinose resistance) in a unique tester strain (an araD mutant).

ARE-bla: Activation of oxidative stress response pathway in human hepatocellular carcinoma HepG2 cell line.

AREc32: Activation of Nrf2-ARE oxidative stress response pathway in a human breast cancer cell line MCF7.

Aroclor 1254-induced liver: Liver tissue treated with the polychlorinated biphenyl mixture Aroclor 1254 used as a source of S9 fraction for mutagenic and genotoxic effects testing.

Atomic size: The size of an atom measured as the atomic radius or the mean distance from the center of the nucleus to the outer boundary of the electron cloud.

Attrition bias: Systematic differences between comparison groups in withdrawals or exclusions of participants from the results of a study.

Basal-cell adenoma: A benign tumor of major or minor salivary glands or other organs composed of small cells showing peripheral palisading.

Basal-cell carcinoma: The most common type of skin cancer. It begins in the lowest layer of the epidermis (the outer layer of the skin), called the basal cell layer. It usually develops on sun-exposed areas, especially the head and neck. Basal cell cancer grows slowly and is not likely to spread to distant parts of the body.

Biexponential process: A process of drug (or xenobiotic) clearance with two phases with different rates. The first phase often involves rapid distribution of a drug to peripheral tissues,

while the second phase represents clearance mechanisms that eliminate the drug from the body. (See "Two-compartment pharmacokinetic model.")

Bioavailability: The degree to which a drug or other substance becomes available to the target tissue after administration.

Biodegradation: Biotransformation; the conversion within an organism of molecules from one form to another. A change often associated with change in pharmacologic activity.

Biotransformation: The chemical conversion of substances by living organisms or enzyme preparations.

Boiling point: The boiling point of the anhydrous substance at atmospheric pressure (101.3 kPa) unless a different pressure is stated. If the substance decomposes below or at the boiling point, this is noted (dec). The temperature is rounded off to the nearest °C.

Bond dissociation energy: The amount of energy needed to cause homolytic cleavage of a covalent bond. It is one of numerous measures of bond strength.

Carbon nanotubes: A tube-shaped material, made of carbon, having a diameter measuring on the nanometer scale. A nanometer is one-billionth of a meter.

Carcinoma: Cancer that begins in the skin or in tissues that line or cover internal organs.

Cell cycle arrest: A regulatory process that halts progression through the cell cycle during one of the normal phases (G1, S, G2, M).

Chemical Data Reporting Rule: Chemical Data Reporting (CDR) is the new name for Inventory Update Reporting (IUR). The purpose of Chemical Data Reporting is to collect quality screening-level, exposure-related information on chemical substances and to make that information available for use by the U.S. Environmental Protection Agency (EPA) and, to the extent possible, to the public. The IUR/CDR data are used to support risk screening, assessment, priority setting and management activities and constitute the most comprehensive source of basic screening-level, exposure-related information on chemicals available to EPA. The required frequency of reporting currently is once every four years.

Coagulation and flocculation: Addition of chemicals to source water to allow particles to bind together and form larger particles called floc.

Cochran-Armitage trend test: A statistical test used in categorical data analysis when the aim is to assess for the presence of an association between a variable with two categories and a variable with *k* categories. It modifies the chi-square test to incorporate a suspected ordering in the effects of the *k* categories of the second variable.

Comet assay: Single cell gel electrophoresis for assessment of DNA damage in presumptive target tissues.

Congenital lactic acidosis: A rare disease caused by mutations in mitochondrial DNA (mtDNA) that affect the ability of cells to use energy and cause too much lactic acid to build up in the

body, a condition called lactic acidosis. The word "congenital" means that the underlying condition that increases risk of developing lactic acidosis is present at birth.

Connexin proteins: A group of transmembrane proteins that form the intermembrane channels of gap junctions. They are used by inorganic ions and most small organic molecules to pass through cell interiors.

Conversion factor: A numerical factor used to multiply or divide a quantity when converting from one system of units to another.

CpG island: A short region of DNA in which the frequency of the CG sequence is higher than in other regions. "p" indicates that "C" and "G" are connected by a phosphodiester bond.

Critical temperature: The temperature at and above which a gas cannot be liquefied, no matter how much pressure is applied.

Delocalization of electron cloud: The spatial distribution of electrons shared among the atoms in a molecule.

Differential selection: Selective pressure for self renewal. Gene mutations that confer a growth or survival advantage on the cells that express them will be selectively enriched in the genome of tumors.

Dihaloacetic acids: Carboxylic acids in which two halogen atom takes the place of two hydrogen atoms in acetic acid.

Disinfection: Application of oxidants to water *(*chlorine, chloramine, chlorine dioxide, or ozone) or ultraviolet (UV) light to kill disease-causing microorganisms or to render them inactive.

Disposition: The description of absorption, distribution, metabolism, and excretion of a chemical in the body.

Dominant lethal mutation assay: The dominant lethal assay identifies germ cell mutagens by measuring the ability of a chemical to penetrate gonadal tissue and produce embryonic death due to chromosomal breakage in parent germ cells.

Double acid conjugate: A compound formed by the joining of two acids.

Ecological study: A study in which the units of analysis are populations or groups of people rather than individuals.

Electronegativity: A measure of the tendency of an atom to attract a bonding pair of electrons.

Electrophilic reactivity: The tendency of a charge or neutral molecule to be attracted to an electron rich center.

Electrophilic substitution reaction: A substitution reaction in which the new group introduced into the molecule was an electrophile.

Epigenetic mechanisms: Changes in gene function that do not involve a change in DNA sequence but are nevertheless mitotically and/or meiotically heritable. Examples include DNA

methylation, alternative splicing of gene transcripts, and assembly of immunoglobulin genes in cells of the immune system.

FDA Good Laboratory Practice Regulations: A quality system codified by the U.S. Food and Drug Administration that prescribes operating procedures for conducting nonclinical laboratory studies that support or are intended to support applications for research or marketing permits for products regulated by the Food and Drug Administration.

Filtration: Passage of water through porous media to remove particles remaining from sedimentation.

Fisher's exact test: The test for association in a two-by-two table that is based on the exact hypergeometric distribution of the frequencies within the table.

Follow-up: Observation over a period of time of a person, group, or initially defined population whose appropriate characteristics have been assessed to observe changes in health status or health-related variables.

Fulvic acid: A family of organic acids, natural compounds, components of the humus, which is a fraction of soil organic matter.

Gap junctional cell communication: Intercellular communcations through specialized connection between adjacent cells that directly connect the cytoplasm of the two cells, allowing molecules, ions, and electrical impulses to pass through.

Genomic instability: An increased propensity for genomic alterations that often occurs in cancer cells. During the process of cell division (mitosis) the inaccurate duplication of the genome in parent cells or the improper distribution of genomic material between daughter cells can result from genomic instability.

Glioma: A cancer of the brain that begins in glial cells (cells that surround and support nerve cells).

Haloacetic acids: Carboxylic acids in which one or more hydrogen atoms on the alpha carbon of acetic acid is replaced by halogen atoms. Haloacetic acids are commonly formed as disinfection by-products during water purification with chlorine-based disinfectants.

Halogen: One of a class of reactive nonmetallic chemical elements that form strongly acidic compounds with hydrogen and simple salts with cationic elements. Members of the class of halogens in order of increasing atomic weight are fluorine, chlorine, bromine, iodine, and astatine.

Harderian gland: An orbital gland of the majority of land vertebrates.

Hard nucleophile: A molecule that is highly polarized and tend to react with oxygen atoms in DNA or RNA.

Healthy worker hire effect: Initial selection of healthy individuals at time of hire so that their disease risks differ from the disease risks in the source (general) population.

Healthy worker survival effect: A continuing selection process such that those who remain employed tend to be healthier than those who leave employment.

Hemangiosarcoma: A type of cancer that begins in the cells that line blood vessels.

Henry's Law constant: The ratio of the aqueous-phase concentration of a chemical to its equilibrium partial pressure in the gas phase. The larger the Henry's law constant the less soluble it is (i.e., greater tendency for vapor phase). The relationship is defined for a constant temperature, e.g., 25°C.

Hepatoblastoma: An uncommon malignant liver cancer composed of tissue resembling fetal liver cells, mature liver cells, or bile duct cells.

Hepatocellular adenoma: A benign tumor that starts from hepatocytes, i.e., liver cells.

Hepatocellular carcinoma: A malignant tumor that starts from hepatocytes, i.e., liver cells.

Hepatoma: A liver tumor.

Hereditary tyrosinemia type 1: A metabolic disease caused by a deficiency of the enzyme involved in the last step of tyrosine catabolism.

Historical control range: Tumor rates found in control animals, usually those of the same species and strain as the test animals and exposed by the same route of administration.

Host-mediated assay: This assay evaluates the genotoxicity of a substance to microbial cells introduced (e.g., by intravenous injection) into a host animal. The host animal receives the test compound orally, and therefore acts as a source of chemical metabolism, distribution and excretion of the test compound.

Humic acid: A brown, melanin-tinted mixture of polymers, found in soils and water and resulting from breakdown of organic matter.

Immersion cleaning: A process in which a tank containing cleaning solvent at a temperature below its boiling point is used for metal parts cleaning. To use the vapor degreaser, the operator places the parts to be cleaned in a metal wire basket, removes the cover, and lowers the basket of parts by hand into the cleaning solvent. After a brief period of time, the operator raises the basket and allows the parts to drip-dry inside the degreaser.

Ionizability: The ability of an atom or molecule to lose or gain an electron, just becoming either positively or negatively charged.

Keratoacanthoma: A low-grade, or slow-growing, benign skin tumor that looks like a tiny dome or crater.

Keratosis: A localized horny overgrowth of the skin, such as a wart or callus.

Leaving group: A fragment that leaves a molecule as either an anion or neutral molecule.

Loss of heterozygosity: If there is one normal and one abnormal allele at a particular locus, as might be seen in an inherited autosomal dominant cancer susceptibility disorder, loss of the normal allele produces a locus with no normal function. When the loss of heterozygosity

involves the normal allele, it creates a cell that is more likely to show malignant growth if the altered gene is a tumor suppressor gene.

Lung adenoma: A benign tumor of the lung.

Lymphoma: Cancer of the lymph nodes.

Lymphokine-activated killer cell: Killer cell lymphocytes activated in the presence of interleukin-2 (IL-2). Lymphokine-activated killer cells (LAKs) are cytotoxic effector cells with an exceptionally wide target cell spectrum including normal and malignant cells of different origins. LAKs exhibit a profound heterogeneity with regard to phenotype surface marker expression; it remains to be determined if they represent a unique cell lineage.

Malignant mesothelioma: A rare, aggressive form of cancer that develops in the lining of the lungs, abdomen, or heart.

Melting point: The melting point of the substance at atmospheric pressure (101.3 kPa). When there is a significant difference between the melting point and the freezing point, a range is given. In case of hydrated substances (i.e., those with crystal water), the apparent melting point is given. If the substance decomposes at or below its melting point, this is noted (dec). The temperature is rounded off to the nearest °C.

Metabolic activation: The chemical alteration of an exogenous substance by or in a biological system. The alteration may inactivate the compound or it may result in the production of an active metabolite of an inactive parent compound.

Metaplasia: A change of cells to a form that does not normally occur in the tissue in which it is found.

Methemoglobin: A form of hemoglobin found in the blood in small amounts. Unlike normal hemoglobin, methemoglobin cannot carry oxygen. Injury or certain drugs, chemicals, or foods may cause a higher-than-normal amount of methemoglobin to be made. This causes a condition called methemoglobinemia.

Micronuclei: Small nuclei separate from, and additional to, the main nucleus of a cell, produced during the telophase of mitosis or meiosis by lagging chromosomes or chromosome fragments derived from spontaneous or experimentally induced chromosomal structural changes.

Miscible: A physical characteristic of a liquid that forms one liquid phase with another liquid (e.g., water) when they are mixed in any proportion.

Molecular chaperone: Any of a diverse group of proteins that oversee the correct intracellular folding and assembly of polypeptides without being components of the final structure.

Molecular weight: The molecular weight of a substance is the weight in atomic mass units of all the atoms in a given formula. The value is rounded to the nearest tenth.

Monohaloacetic acids: Haloacetic acids containing only one halogen on the alpha carbon of acetic acid; one of fluoracetic acid, chloroacetic acid, bromoacetic acid, or iodoacetic acid.

Mononuclear cell leukemia: The most common type of leukemia in rats, also known as large cell granular lymphocyte leukemia (LGL) .

Morphologically transformed foci: Groups of cells transformed so they lose contact inhibition for their group and multiply to forms foci.

Multiple myeloma: A type of cancer that begins in plasma cells (white blood cells that produce antibodies). Also called Kahler disease, myelomatosis, and plasma cell myeloma.

Mutations: A change in the structure of a gene, resulting from the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes. The genetic variant can be transmitted to subsequent generations.

National Health and Nutrition Examination Survey: A program of studies designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations.

Natural killer cells: A type of white blood cell that contains granules with enzymes that can kill tumor cells or microbial cells. Also called large granular lymphocytes.

NF-$_{\kappa}$B activation: Activation of a protein complex (nuclear factor kappa-light-chain-enhancer of *activated* B cells) that controls transcription of DNA, cytokine production and cell survival.

Non-differential misclassification: The probability of erroneous classification of an individual, a value, or an attribute into a category other than that to which it should be assigned is the same in all study groups.

Non-Hodgkin lymphoma: A heterogeneous group of malignant lymphomas; the only common feature being an absence of the giant Reed-Sternberg cells characteristic of Hodgkin disease.

Normochromatic erythrocyte: A mature erythrocyte that lacks ribosomes and can be distinguished from immature, polychromatic erythrocytes by stains selective for RNA.

Nrf2: A protein that controls how certain genes are expressed. These genes help protect the cell from damage caused by free radicals (unstable molecules made during normal cell metabolism). Also called NFE2L2 and nuclear factor (erythroid-derived 2)-like 2.

Octanol/water partition coefficient (log *K*ow): A measure of the equilibrium concentration of a compound between octanol and water.

One-compartment model: A pharmacokinetic modeling approach that models the entire body as a single compartment into which a drug is added by a rapid single dose, or bolus. It is assumed that the drug concentration is uniform in the body compartment at all times and is eliminated by a first order process that is described by a first order rate constant.

Ozone-depleting substance: A family of man-made compounds that includes, but are not, foot and eye protection, protective hearing devices (earplugs, muffs) hard hats, respirators and full body suits.

Phase I metabolism: Metabolism of drugs or other xenobiotic molecules, usually by oxidation or hydrolysis and involving a cytochrome P450 monooxygenase.

Phase II metabolism: A conjugation reaction that forms a covalent linkage between a functional group on a xenobiotic molecule and glucuronic acid, sulfate, glutathione, amino acid, or acetate.

p53 haploinsufficient mice: Mice in which one copy of the p53 gene has been lost.

Papilloma: A small solid benign tumor with a clear-cut border that projects above the surrounding tissue.

Personal breathing zone: A sampling area as close as practical to an employee's nose and mouth, (i.e., in a hemisphere forward of the shoulders within a radius of approximately nine inches) so that it does not interfere with work performance or safety of the employee.

Peroxisome proliferation: The process by which multifunctional cellular organelles increase in number within the cell.

Personal protective equipment: Specialized clothing or equipment, worn by an employee to minimize exposure to a variety of hazards. Examples of PPE include such items as gloves

Physiological pH: The normal pH of blood; it is generally considered to be 7.4.

Placental barrier: The semipermeable layer of tissue in the placenta that serves as a selective membrane to substances passing from maternal to fetal blood.

Plaque assay: An assay for antibody production by single lymphocytes using cells isolated from the spleen or lymph nodes of animals injected with sheep red blood cells as an antigen. Incubation of the antibody-forming cells together with sheep red cells in an agar layer with exposure to guinea pig serum as complement results in formation of microscopic plaques (i.e., circular areas of hemolytic clearance around a lymphoid cell) due to release of hemolysin.

Plate incorporation: A commonly used procedure for performing a bacterial reverse mutation test. Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. In the plate-incorporation method, these suspensions are mixed with an overlay agar and plated immediately onto minimal medium. After two or three days of incubation, revertant colonies are counted and compared with the number of spontaneous revertant colonies on solvent control plates.

Point emission: A release that can be identified with a single discharge source or attributed to a specific physical location.

Poly-3 trend test: A survival-adjusted statistical test that takes survival differences into account by modifying the denominator in the numerical (quantal) estimate of lesion incidence to reflect more closely the total number of animal years at risk.

Poly-3 trend test: A survival-adjusted statistical test that takes survival differences into account by modifying the denominator in the numerical (quantal) estimate of lesion incidence to reflect more closely the total number of animal years at risk.

Polychromatic erythrocyte: A newly formed erythrocyte (reticulocyte) containing RNA.

Polyethylene terephthalate: A synthetic resin made by copolymerizing ethylene glycol and terephthalic acid, widely used to make polyester fibers.

Prophage lambda (λ): A virus in *Escherichia coli* (*E. coli*) bacteria that has integrated itself into the host *E. coli* DNA.

Proto-oncogene: A gene involved in normal cell growth. Mutations (changes) in a proto-oncogene may cause it to become an oncogene, which can cause the growth of cancer cells.

***P*$_{trend}$:** Level of statistical significance of a change over time in a group selected to represent a larger population.

Renal clearance: A pharmacokinetic measurement of the volume of plasma from which a substance is completely removed per unit time.

Reverse osmosis: A process of water purification in which water passes through a porous membrane by application of hydrostatic pressure greater than the osmotic pressure removing ions, molecules, and larger particles from drinking water.

Sarcoma: A malignant tumor of connective or other nonepithelial tissue.

Saturable asymmetric transport: A process by which a molecule is carried across a cell membrane or cell barrier in one direction by a process that can be saturated at high concentrations.

Sebaceous gland adenoma: A benign epithelial neoplasm composed of *sebaceous gland*–like structures or *tumors* with well-recognized *sebaceous* differentiation by microscopic examination.

Sedimentation: Transfer of floc particles to basins where they either settle to the bottom or are removed by skimming.

Selection bias: An error in choosing the individuals or groups to take part in a study. Ideally, the subjects in a study should be very similar to one another and to the larger population from which they are drawn (for example, all individuals with the same disease or condition). If there are important differences, the results of the study may not be valid.

Sister-chromatid exchange: The exchange during mitosis of homologous genetic material between sister chromatids; increased as a result of inordinate chromosomal fragility due to genetic or environmental factors.

SKF-525A: An inhibitor of drug metabolism and cytochrome P-450 activity.

Soft nucleophile: A molecule with lower polarization that tends to bind with thiol or amino groups on proteins.

Soft tissue sarcoma: A cancer that begins in the muscle, fat, fibrous tissue, blood vessels, or other supporting tissue of the body.

Solubility: The ability of a substance to dissolve in another substance and form a solution. The Report on Carcinogens uses the following definitions (and concentration ranges) for degrees of solubility: (1) *miscible* (see definition), (2) *freely soluble*- capable of being dissolved in a specified solvent to a high degree (> 1,000 g/L), (3) *soluble*- capable of being dissolved in a specified solvent (10–1,000 g/L), (4) *slightly soluble*- capable of being dissolved in a specified

solvent to a limited degree (1-10 g/L), and (5) practically insoluble- incapable of dissolving to any significant extent in a specified solvent (< 1 g/L).

SOS umuC assay: An assay using *Salmonella typhimurium* TA1535/pSK1002 that is used to evaluate the ability of testing substance or sample to induce DNA damage. The system is based on alterations in the induction of SOS response as a consequence of DNA damage.

Specific gravity: The ratio of the density of a material to the density of a standard material, such as water at a specific temperature; when two temperatures are specified, the first is the temperature of the material and the second is the temperature of water.

Spot test: Qualitative assay in which a small amount of test chemical is added directly to a selective agar medium plate seeded with the test organism, e.g., *Salmonella*. As the chemical diffuses into the agar, a concentration gradient is formed. A mutagenic chemical will give rise to a ring of revertant colonies surrounding the area where the chemical was applied; if the chemical is toxic, a zone of growth inhibition will also be observed.

Squamous-cell carcinoma: A type of malignant skin cancer that begins in the squamous cells. Squamous cells are the thin, flat cells that make up the epidermis, or the outermost layer of the skin.

Squamous-cell papilloma: A generally benign papilloma that arises from the stratified squamous epithelium of the skin, lip, oral cavity, tongue, pharynx, larynx, esophagus, cervix, vagina or anal canal. Squamous cell papillomas are a result of infection with human papillomavirus (HPV).

Steric bulk: An indicator of the stability of the spatial arrangement of atoms in a molecule.

T-helper cell: A type of immune cell that stimulates killer T cells, macrophages, and B cells to make immune responses. A helper T cell is a type of white blood cell and a type of lymphocyte. Also called CD4-positive T lymphocyte.

Tg.AC: A transgenic mouse model with the ability to mount a tumorigenic response within 6 months in skin paint assays when dosed topically with nonmutagenic carcinogens.

Time-weighted average: The average exposure concentration of a chemical measured over a period of time (not an instantaneous concentration).

Tissue:blood partition coefficient: The ratio of tissue chemical concentration to that of the venous outflow of the tissue when at equilibrium; it is an important parameter required for physiological based pharmacokinetic models.

Toxicokinetics: The mathematical description (toxicokinetic models) of the time course of disposition of a chemical in the body.

Transitions: DNA nucleotide substitution mutation in which a purine base is substituted for another purine base (adenine → guanine or guanine → adenine) or a pyrimidine base for another pyrimidine base (cytosine → thymine or thymine → cytosine).

Transversions: DNA nucleotide substitution mutation in which a purine base (adenine or guanine) is substituted for a pyrimidine base (cytosine or thymine) or vice versa.

TR_{DNA}: Toxic ratio of EC_{50} of *E. coli* DNA repair +/DNA repair –, TR > 1.2 indicates reaction with hard nucleophiles.

TR_{GSH}: Toxic ratio of EC_{50} of *E. coli* GSH+/GSH–, TR > 1.2 indicates reaction with soft nucleophiles.

Trihaloacetic acids: Molecules in which all three hydrogens on the alpha carbon of acetic acid have been replaced by halogen atoms of either the same halogen or mixed halogens.

Trihalomethanes: Compounds in which three halogen atoms replace hydrogen atoms in a molecule of methane.

Tubular reabsorption: The process by which the nephron removes water and solutes from the tubular fluid (pre-urine) and returns them to the circulating blood.

Tubular secretion: The transfer of materials from peritubular capillaries to the renal tubular lumen; it is the opposite process of reabsorption. This secretion is caused mainly by active transport and passive diffusion.

Two-compartment pharmacokinetic model: A two-compartment pharmacokinetic model resolves the body into a central compartment and a peripheral compartment. The central compartment generally comprises tissues that are highly perfused such as heart, lungs, kidneys, liver and brain. The peripheral compartment comprises less well-perfused tissues such as muscle, fat and skin. A two-compartment model assumes that, following drug administration into the central compartment, the drug distributes between that compartment and the peripheral compartment. However, the drug does not achieve instantaneous distribution (i.e., equilibrium), between the two compartments. After a time interval (t), distribution equilibrium is achieved between the central and peripheral compartments, and elimination of the drug is assumed to occur from the central compartment.

Type-I error: The error of rejecting a true null hypothesis, i.e., declaring that a difference exists when it does not.

Type-II error: The error of failing to reject a false null hypothesis, i.e., declaring that a difference does not exist when in fact it does.

Vapor density, relative: A value that indicates how many times a gas (or vapor) is heavier than air at the same temperature. If the substance is a liquid or solid, the value applies only to the vapor formed from the boiling liquid.

Vapor pressure: The pressure of the vapor over a liquid (and some solids) at equilibrium, usually expressed as mm Hg at a specific temperature (°C).

Volume of distribution: The theoretical volume that would be necessary to contain the total amount of an administered drug at the same concentration that it is observed in the blood plasma.

Xenobiotic metabolism: A set of metabolic pathways that modify the chemical structure of compounds foreign to an organism's normal biochemistry, such any drug or poison.

National Toxicology Program
U.S. Department of Health and Human Services

Interagency Review Draft
Report on Carcinogens
Draft Profiles

Selected Haloacetic Acids Found as Water Disinfection By-products:

Dichloroacetic acid

Dibromoacetic acid

Bromochloroacetic acid

Bromodichloroacetic acid

Tribromoacetic acid

Chlorodibromoacetic acid

June 6, 2017

Office of the Report on Carcinogens
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
U.S. Department of Health and Human Services

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Haloacetic acids found as water disinfection by-products (Selected)

Also known as HAAs

Introduction

Disinfection of the public water supply is an important aspect of public health in the prevention of disease transmission in the United States and worldwide (Calderon 2000). Haloacetic acids are formed as by-products during the disinfection of water due to reaction between organic molecules, such as humic acid, in the source water and chlorine-based disinfection agents (chlorine, chloramine, and chlorine dioxide).

Human exposure to haloacetic acids results from their formation as water disinfection by-products, including exposure to mixtures of various disinfection by-products. Only one epidemiological study was identified that evaluated the relationship between human cancer risk and exposure specific to several individual haloacetic acids and a mixture of five regulated haloacetic acids (monochloroacetic, trichloroacetic, dichloroacetic, monobromoacetic, and dibromoacetic acids) (Jones *et al.* 2017). This study did not find an association with exposure to haloacetic acids in drinking water and kidney cancer. Several human epidemiological studies on exposure to chlorinated water or proxies for mixtures of disinfection water by-products (such as trihalomethanes) found an association between chlorinated water and an increased risk of urinary bladder cancer (reviewed by IARC 2013, Villanueva *et al.* 2016). Although these studies are not specific for haloacetic acids, they provide some information concerning the potential for cancer risk from water disinfection by-products in humans and increase the confidence in the relevance of the cancer bioassays in experimental animals (conducted at higher doses) to humans.

Currently, public health to potential health effects from chlorinated water is protected by regulating specific or classes of water disinfection by-products In contrast to the existing epidemiological studies, which cannot disentangle effects of different types of water disinfection by-products, there is an adequate database of toxicological studies that are specific for particular water disinfection by-products, such as haloacetic acids, and can help inform public health decisions. Haloacetic acids make up about 36% by weight of halogenated by-products in disinfected water and are second to trihalomethanes in abundance of halogenated by-products by weight. Two trihalomethanes – chloroform and bromodichloromethane – are listed as *reasonably anticipated to be human carcinogens* in the Report on Carcinogens. EPA regulates a mixture of five haloacetic acids (see Regulations and Guidelines).

NTP emaluated13 haloacetic acids identified in chlorinated treated drinking water, six of these are individually listed as *reasonably anticipated to be a human carcinogen* in the Report on Carcinogens:

- Dichloroacetic acid
- Dibromoacetic acid
- Bromochloroacetic acid
- Tribromoacetic acid
- Bromodichloroacetic acid
- Chlorodibromoacetic acid

The available data are inadequate to evaluate haloacetic acids either as a class or as subclasses, such as those based on the number of halogen substitutions (e.g. mono-, di- or tri-) or type of halogen substitution (e.g. chlorine, bromine, and iodine), or to list the other seven identified haloacetic acids (see Report on Carcinogens Monograph on Haloacetic Acids):

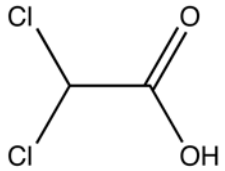
- Monochloroacetic acid
- Monobromoacetic acid
- Monoiodoacetic acid
- Diiodoacetic acid
- Bromoiodoacetic acid
- Chloroiodoacetic acid
- Trichloroacetic acid

The profiles for the six selected haloacetic acid follow this introduction. The listings for dichloroacetic acid, bromochloroacetic acid, dibromoacetic acid, and bromodichloroacetic acid are based on carcinogenicity studies in experimental animals, whereas, the listings for chlorodibromoacetic acid and tribromoacetic acid are based on other relevant and mechanistic data. Most of the supporting mechanistic and other relevant information and data on properties, use, production, exposure, and U.S. regulations to limit exposure is common to all six listed haloacetic acids and therefore is combined in one section following the discussions of the key carcinogenicity data, which are the basis for the listings.

Dichloroacetic acid

CAS No. 79-43-6

Reasonably anticipated to be a human carcinogen[1]



Carcinogenicity

Dichloroacetic acid is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic studies that demonstrate biological plausibility of its carcinogenicity in humans. (See Selected Haloacetic Acids, Carcinogenicity for other relevant data on haloacetic acids.)

[1] NTP's preliminary listing recommendation proposed for RoC.

Cancer Studies in Experimental Animals

Dichloroacetic acid caused benign (hepatocellular adenoma) and malignant (hepatocellular carcinoma) liver tumors in both sexes of mice and in male rats after drinking water exposure. Male and female mice exposed for near lifetime had significant increases in benign and malignant liver tumors (Herren-Freund *et al.* 1987, DeAngelo *et al.* 1991, Daniel *et al.* 1992, Pereira 1996, DeAngelo *et al.* 1999). In a stop-exposure study, male and female mice were exposed at weaning (4 weeks of age) to dichloroacetic acid in drinking water for a ten-week period, followed by no further exposure to this chemical for 80 weeks (Wood *et al.* 2015). Significant increases in benign and malignant liver tumors were reported for both sexes and tumor incidence approached levels found with near lifetime exposures. Near lifetime exposure of male rats to dichloroacetic acid also increased the incidence of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) (DeAngelo *et al.* 1996).

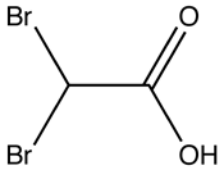
Cancer Studies in Humans

The available data from epidemiological studies are inadequate to evaluate the relationship between human cancer risk and exposure specifically to dichloroacetic acid. A cohort study of post-menopausal women did not find an association between exposure to dichloroacetic acid in the drinking water and kidney cancer risk (Jones *et al.* 2017).

Dibromoacetic acid

CAS No. 631-64-1

Reasonably anticipated to be a human carcinogen[2]



Carcinogenicity

Dibromoacetic acid is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic studies that demonstrate biological plausibility of its carcinogenicity in humans. (See Selected Haloacetic Acids, Carcinogenicity for other relevant data on haloacetic acids.)

Cancer Studies in Experimental Animals

Drinking water exposure to dibromoacetic acid caused benign and malignant tumors at several tissue sites in mice and rats. Significant increases in the incidences of benign (hepatocellular adenoma) and malignant (hepatocellular carcinoma) liver tumors in male and female mice, and hepatoblastoma, a malignant liver tumor, in male mice were reported. In addition, in male mice, benign (alveolar/bronchiolar adenoma) and benign or malignant (alveolar/bronchiolar carcinoma) (combined) lung tumors were above the background range (historical control values)

[2] NTP's preliminary listing recommendation proposed for RoC.

and were significant in incidence. Male rats had a significant increase in malignant mesothelioma of the abdominal/pelvic (peritoneum) lining and female rats had a significant increase in mononuclear-cell leukemia with dibromoacetic acid exposure (NTP 2007).

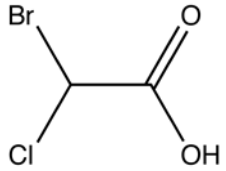
Cancer Studies in Humans

No epidemiological studies were identified that evaluated the relationship between human cancer risk and exposure specifically to dibromoacetic acid. A cohort study of post-menopausal women did not find an association between exposure to haloacetic mixture that included dibromoacetic acid and kidney cancer risk (Jones *et al.* 2017).

Bromochloroacetic acid

CAS No. 5589-96-8

Reasonably anticipated to be a human carcinogen[3]



Carcinogenicity

Bromochloroacetic acid is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic studies that demonstrate biological plausibility of its carcinogenicity in humans. (See Selected Haloacetic Acids, Carcinogenicity for other relevant data on haloacetic acids.)

Cancer Studies in Experimental Animals

Drinking water exposure to bromochloroacetic acid caused liver tumors in both sexes of mice and other tumors at several tissue sites in both sexes of rats. Bromochloroacetic acid caused benign (hepatocellular adenoma) and malignant (hepatocellular carcinoma) liver tumors in male and female mice and hepatoblastoma, a malignant liver tumor, in male mice. Male rats had an increase in malignant mesothelioma of the abdominal/pelvic (peritoneum) lining and female rats had an increase in multiple fibroadenomas of the mammary gland. In addition, very rare adenomas of the large intestine were reported in both sexes of rats with a significant tumor incidence in male rats. Both sexes had positive trends in tumor incidences. Fibroadenoma of the mammary gland and adenoma of the large intestine can progress to malignancy (NTP 2009).

Cancer Studies in Humans

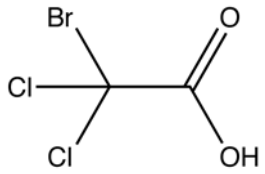
The available data from epidemiological studies are inadequate to evaluate the relationship between human cancer risk and exposure specifically to bromochloroacetic acid. A cohort study of post-menopausal women did not find an association between exposure to bromochloroacetic acid in the drinking water and kidney cancer risk (Jones *et al.* 2017).

[3] NTP's preliminary listing recommendation proposed for RoC.

Bromodichloroacetic acid

CAS No. 71133-14-7

Reasonably anticipated to be a human carcinogen[4]



Carcinogenicity

Bromodichloroacetic acid is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic studies that demonstrate biological plausibility of its carcinogenicity in humans. (See Selected Haloacetic Acids, Carcinogenicity for other relevant data on haloacetic acids.)

Cancer Studies in Experimental Animals

Drinking water exposure to bromodichloroacetic acid caused tumors at several tissue sites in mice and rats. Bromodichloroacetic acid exposure induced dose-related significant increases in hepatocellular carcinoma and hepatoblastoma, a malignant liver tumor, in male and female mice and hepatocellular adenoma in female mice. In addition, increased incidences of benign and benign or malignant (combined) Harderian gland (an accessory lacrimal gland of the eye) tumors were reported for exposed male mice. Bromodichloroacetic acid caused malignant mesothelioma of the abdominal/pelvic (peritoneum) lining and multiple types of skin tumors in male rats. Skin tumors included fibroma (a benign skin tumor), keratoacanthoma and several types of skin tumors that can progress to malignancy (squamous-cell papilloma, keratoacanthoma, sebaceous gland adenoma, basal-cell adenoma, basal-cell carcinoma, or squamous-cell carcinoma [combined]) were reported for male rats. Exposed female rats developed mammary gland fibroadenoma (including multiple tumors) and carcinoma (NTP 2015).

Cancer Studies in Humans

No epidemiological studies were identified that evaluated the relationship between human cancer risk and exposure specifically to bromodichloroacetic acid.

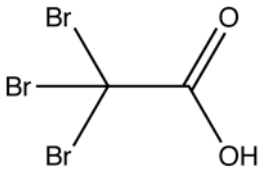
Tribromoacetic acid

CAS No. 75-96-7

Reasonably anticipated to be a human carcinogen[5]

[4] NTP's preliminary listing recommendation proposed for RoC.

[5] NTP's preliminary listing recommendation proposed for RoC.



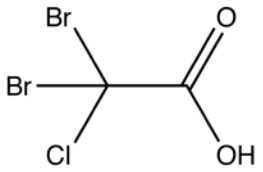
Carcinogenicity

Tribromoacetic acid is *reasonably anticipated to be a human carcinogen* based on (1) metabolism studies that provide convincing evidence that tribromoacetic acid is metabolized to dibromoacetic acid, (2) sufficient evidence for the carcinogenicity of dibromoacetic acid from studies in experimental animals, and (3) supporting mechanistic data that demonstrate biological plausibility of its carcinogenicity in humans. No cancer studies in humans or experimental animals with exposure to tribromoacetic acid were identified.

Oral exposure studies in rats report non-renal clearance of tribromoacetic acetic is 77.2%, indicating potential metabolism of this compound *in vivo* (Schultz *et al.* 1999). *In vitro* studies using a rat liver enzyme preparation (microsomes) demonstrated that tribromoacetic acid is metabolized to dibromoacetic acid, which accounts for ~50% of the consumption of tribromoacetic acid with no evidence of additional metabolism and minimal direct decarboxylation; human microsomes were only used in metabolism studies with chlorodibromoacetic acid and showed almost complete metabolism to chlorobromoacetic acid (Saghir *et al.* 2011). In addition, metabolism studies have provided convincing evidence that tribromoacetic acid rapidly loses a bromide ion under oxygen tension conditions that mimic those measured in liver tissue. Near lifetime exposure to dibromoacetic acid in drinking water caused liver and lung tumors in mice and malignant mesothelioma and mammary tumors in rats (NTP 2007, see dibromoacetic acid profile). Mechanistic studies show that tribromoacetic acid (similar to dibromoacetic acid) is mutagenic in bacteria and causes oxidative stress and damages DNA in cultured cells. These events are characteristic of other human carcinogens and support biological plausibility for the carcinogenicity of tribromoacetic acid in humans. (See Selected Haloacetic Acids, Carcinogenicity for relevant data on haloacetic acids.) Therefore, it is reasonably anticipated that chronic oral exposure to tribromoacetic acid would result in neoplasia and these findings are relevant to humans.

Chlorodibromoacetic acid

CAS No. 5278-95-5



Reasonably anticipated to be a human carcinogen[6]

[6] NTP's preliminary listing recommendation proposed for RoC.

Carcinogenicity

Chlorodibromoacetic acid is *reasonably anticipated to be human carcinogen* based on (1) metabolism studies that provide convincing evidence that chlorodibromoacetic acid is metabolized to bromochloroacetic acid, (2) sufficient evidence for the carcinogenicity of bromochloroacetic acid from studies in experimental animals, and (3) supporting mechanistic data that demonstrate biological plausibility of its carcinogenicity in humans. No cancer studies in humans or experimental animals with exposure to chlorodibromoacetic acid were identified.

Oral exposure studies in rats report that non-renal clearance of chlorodibromoacetic acid is 62.6% of total clearance, suggesting potential metabolism of this compounds *in vivo*. (Schultz *et al.* 1999). Chlorodibromoacetic acid is metabolized to bromochloroacetic acid *in vitro* using a rat liver enzyme preparation (microsomes) and the metabolism of chlorodibomoacetic acid resulted in equimolar formation of bromochloroacetic acid in studies using microsomal preparations either from rat liver or from human liver (Saghir *et al.* 2011). Metabolism studies have provided convincing evidence that chlorodibromoacetic acid rapidly loses a bromide ion under oxygen tension conditions that mimic those measured in liver tissue. Near lifetime exposure to bromochloroacetic acid in drinking water caused liver and Harderian gland tumors in mice and malignant mesothelioma, mammary gland tumors, and skin tumors in rats (NTP 2009, see bromochoroacetic acid profile). Mechanistic studies show that chlorodibromoacetic acid (similar to bromochloroacetic acid) is mutagenic in bacteria, and causes oxidative stress in cultured cells; these events are characteristic of other human carcinogens and support biological plausibility for the carcinogenicity of chlorodibromoacetic acid in humans. (See Selected Haloacetic Acids, Carcinogenicity for other relevant data on haloacetic acids). Therefore, it is reasonably anticipated that chronic oral exposure to chlorodibromoacetic acid would result in neoplasia and these findings are relevant to humans.

Selected Haloacetic Acids

Carcinogenicity

The mechanisms by which these haloacetic acids cause cancer in experimental animals are not known and most likely involve multiple modes of action. Although many of the haloacetic acids cause similar effects *in vitro* and *in vivo*, the available mechanistic and other relevant data are insufficient to enable haloacetic acids to be evaluated as a class or subclass. (See Section 6, Report on Carcinogens Monograph on Haloacetic Acids Found as Water Disinfection By-Products).

Absorption and Metabolism

Haloacetic acids are rapidly absorbed from the gastrointestinal tract when consumed by drinking or eating and are found in the blood and body tissues at approximately equal concentrations. Metabolism of di- and trihaloacetic acids is complex, but the limited data available suggest that metabolism of trichloroacetic acid to dichloroacetic acid is similar in rodents and humans. However, excretion of trichloroacetic acid in the urine, which removes it from the body, appears to be slower in humans than in rodents (Stacpoole *et al.* 1998, EPA 2003, IARC 2004a, 2004b, EPA 2011, IARC 2014a, 2014b).

The three trihaloacetic acids (bromodichloroacetic acid, chlorodibromoacetic acid, and tribromoacetic acid) discussed here are all metabolized by liver enzymes (cytochromes P450) to remove one of the halogens, usually a bromine atom, to form a dihaloacetic acid (dichloroacetic acide, bromochloroacetic acid, and dibromoacetic acid, respectively). An important effect of this metabolism for the potential mechanism(s) of carcinogenesis is that a highly reactive molecule (a free radical) is formed as part of the process. The chemical nature of bromine makes it the most likely halogen to be removed in this conversion from tri- to dihaloacetic acid (Saghir *et al.* 2011). Trichloroacetic acid is the least metabolized of the haloacetic acids and remains in blood longer than other trihaloacetic acids and most dihaloacetic acids because it is more likely to bind to proteins in blood which reduces its availability for metabolism; it is mainly excreted unchanged in the urine. Dihaloacetic acids are metabolized to a greater extent than trichloroacetic acid and the other trihaloacetic acids and formation of the metabolites glyoxylate, glycolate, oxylate, glycine, and carbon dioxide reflect that metabolism.

Studies on Mechanisms of Carcinogenesis

Haloacetic acids have a weak positive charge (i.e., are weak electrophiles) and are attracted to macromolecules (proteins, lipids, DNA, RNA) with a weak negative charge (weak nucleophiles), such as thiol or amino groups on proteins; stronger electrophiles can also bind with oxygen in DNA and RNA. The body of data suggests that the selected haloacetic acids may induce cancer through reactions with macromolecules leading to oxidative stress, mutagenic and genotoxic effects, inhibition of enzymes leading to oxidative stress, and regulation of genes involved in carcinogenicity.

Most of the available mechanistic data on the haloacetic acids are from *in vitro* studies measuring oxidative stress, genotoxicity, and toxicity. In general, from *in vitro* studies, dihaloacetic acids are more genotoxic, cytotoxic, and mutagenic than trihaloacetic acids and bromine substitution has a more potent effect on these properties than chlorine substitutions (Kargalioglu *et al.* 2002, Plewa *et al.* 2004a, Stalter *et al.* 2016). All of the listed haloacetic acids caused oxidative stress (assessed in different types of *in vitro* assays), which leads to the generation of reactive oxygen species that can damage DNA and cause mutations. In addition, exposure to some di-and tri-haloacetic acids (dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, and bromodichloroacetic acid, which were the only ones tested) also caused oxidative stress (as evidenced by 8-hydroxy-deoxyguanosine DNA adducts and lipid peroxidation) in experimental animal studies; the strongest response was for the brominated haloacetic acids (Larson and Bull 1992, Austin *et al.* 1996). The types of pathways that generate oxidative stress may vary across the haloacetic acids and multiple pathways may be involved (Cemeli *et al.* 2006, Celik *et al.* 2009, Pals *et al.* 2011, Ondricek *et al.* 2012, Dad *et al.* 2013, El Arem *et al.* 2014a, El Arem *et al.* 2014b, El Arem *et al.* 2014c, Stalter *et al.* 2016).

Overall, the data suggest that haloacetic acids do not bind to DNA and most likely cause genotoxicity through oxidative stress. Both the dihaloacetic acids (dichloroacetic acid, dibromoacetic acid, and bromochloroacetic acid) and trihaloacetic acids (tribromoacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid) in this profile caused mutations in bacteria (see Section 5 in the Report on Carcinogens Monograph on Haloacetic Acids found as Water Disinfection By-Products). Evidence for other types of genotoxicity is limited because only a few haloacetic acids (mostly the dihaloacetic acids) were tested for each type of damage and the findings for each endpoint were not always consistent across different haloacetic acids. The strongest evidence is that bromine-containing haloacetic acids (dibromoacetic acid,

tribromoacetic acid, and bromochloroacetic acid) damage DNA (e.g., cause DNA strand breaks) and dibromoacetic acid and dichloroacetic acid damage chromosomes (i.e., micronuclei formation) and induce gene mutations *in vitro* (see Section 5 in the Report on Carcinogens Monograph on Haloacetic Acids found as Water Disinfection By-Products).

Some studies provide some insight into one of the mechanisms leading to oxidative stress. Dichloroacetic acid and potentially other haloacetic acids have been shown to affect energy metabolism within the cell by inhibiting the mitochondrial enzyme complex (pyruvate dehydrogenase complex), which enhances oxidative metabolism, potentially increasing reactive oxygen species and DNA damage and mutations, if not correctly repaired (Pals *et al.* 2011). Another mechanism by which some haloacetic acids (dichloroacetic acid, dibromoacetic acid, and bromochloroacetic acid) can cause oxidative stress is via the inhibition of an enzyme involved in dihaloacetic acid metabolism, glutathione-S-transferase (GST) zeta, which results in reduced metabolism and clearance of dihaloacetic acids, oxidative stress, and activation of stress-response pathways (Anderson *et al.* 1999, Gonzalez-Leon *et al.* 1999).

Other studies suggest a potential mechanism by which some haloacetic acids (dichloroacetic acid and dibromoacetic acid) cause cancer by regulation of genes related to carcinogenicity (e.g., *c-myc*, *c-jun*, or *IGF-II* genes that can promote cell growth, cell division, or cell death). Dichloroacetic acid treatment induced hypomethylation (loss of a methyl group in one of the DNA nucleotides) in the promoter region of the *c-myc* gene in liver, kidney and urinary bladder tissues in mice, enhanced cellular proliferation in mouse liver (Ge *et al.* 2001), promoted liver tumors in mice (Tao *et al.* 2000), and promoted kidney tumors in male mice (Pereira *et al.* 2001).

Properties

Haloacetic acids are water soluble non-volatile chemicals that vary in number and type of halogen substitutions at the alpha carbon of acetic acid. The selected haloacetic acids have either two or three halogen substitutions with either chlorine or bromine (Table 1). The physical-chemical characteristics of each haloacetic acid depend on the type and number of halogen atoms in the molecule.

At physiological pH, these haloacetic acids are in their ionized form, with the acid strength increasing as the pKa decreases. Therefore, trihaloacetic acids are stronger acids than dihaloacetic acids. There are three physical-chemical properties likely to be related to the toxicity of the haloacetic acids because they describe the ability of the molecules to enter cells and their potential reactivity with other molecules within a cell: (1) the octanol-water partition coefficient (log P), (2) the negative log of the acid dissociation constant (pK_a), and (3) the energy of the lowest unoccupied molecular orbital (E_{LUMO}) (Table 1). The toxic potency correlates with electrophilic reactivity (alkylating potential) of a haloacetic acid and increases with chlorine to bromine substitution (Plewa *et al.* 2004, Pals *et al.* 2011). Electrophilic reactivity is inversely related to the bond dissociation energy and E_{LUMO}.

U.S. EPA regulates five of the most common haloacetic acids (HAA5) in the public water supply. These haloacetic acids are: monochloroacetic acid, dichloroacetic acid, monobromoacetic acid, dibromoacetic acid, and trichloroacetic acid (see Regulations, below).

In order to support national drinking water standards, Information Collection Rule data were collected for nine common haloacetic acids (HAA9): monochloroacetic acid, dichloroacetic acid, monobromoacetic acid, dibromoacetic acid, trichloroacetic acid, bromochloroacetic acid, chlorodibromoacetic acid, bromodichloroacetic acid, and tribromoacetic acid.

Table 1. Physical and chemical properties of haloacetic acids (selected)

Haloacetic acid	Molecular weight	Solubility in water (g/100 mL)[c]	Vapor pressure (mm Hg)[a,c]	Octanol-water partition coefficient (log P)[b]	Dissociation constant (pK_a)[b]	Energy of lowest unoccupied molecular orbital, E_{lumo} (eV)[b,d]
Dichloroacetic acid	128.9	100 (@ 20°C)	0.179	0.92	1.41	8.44
Dibromoacetic acid	217.8	211	0.023	0.7	1.39	7.51
Bromochloroacetic acid	173.4	25	0.14	0.61	1.4	7.78
Tribromoacetic acid	296.7	20	0.00028	1.71	0.03	6.12
Bromodichloroacetic acid	207.8	0.49	0.036	1.53	0.05	6.65
Chlorodibromoacetic acid	252.3	0.24	0.0052	1.62	0.04	6.42

Sources: [a] PubChem 2017, except chloroacetic acid, bromoacetic acid, and dichloroacetic acid from ChemIDplus 2017, [b]Stalter *et al.* 2016.
[c]Reported at 25°C (298.15°K) unless noted otherwise.
[d]Deprotonated (acetate form)

Use

Although the focus of this profile is exposure to haloacetic acids found in drinking water, dichloroacetic acid is also used for commercial purposes. It is used as a chemical manufacturing intermediate (*e.g.*, for glyoxylic acid), as a laboratory reagent in polyethylene terephthalate production, as a skin cauterizing agent, as a medicinal disinfectant (*e.g.*, a substitute for formalin), as a treatment for congenital lactic acidosis, and it has been proposed as a targeted cancer therapeutic agent (IARC 2014a). Chloroacetic acid is used in the manufacture of organic chemicals including cellulose ethers (used mainly for drilling muds, detergents, food, and pharmaceuticals), glycine, thioglycolic acid, dyes, synthetic caffeine, and as a post-emergence contact herbicide and defoliant (PubChem 2016b). Tribromoacetic acid has been used in organic synthesis (HSDB 2009). Diiodoacetic acid has been used as a chemical intermediate (HSDB 2009). Bromoacetic acid has been used for organic synthesis and abscission of citrus fruit (HSDB 2009). Iodoacetic acid has been used as a food additive and as an intermediate in pharmaceuticals, herbicides, antipyretic, anti-inflammatory, and analgesics (HSDB 2003). Dibromoacetic acid and bromochloroacetic acid have been reported to be used only in research (IARC 2013).

Formation and removal of disinfection by-products

The purpose of water treatment is to remove contaminants and disease-causing agents *from drinking water (*CDC 2015*). The most common steps in conventional water treatment are (1) coagulation and flocculation, (2) sedimentation, (3) filtration, (4) disinfection, and (5) storage (*CDC 2015, EPA 2016a*).* Water disinfection is regulated by the U.S. EPA through Surface Water Treatment Rules (SWTRs) that established maximum contaminant level goals (MCLGs) for viruses, bacteria, such as *Legionella*, and other organisms such as the protozoa species *Giardia lamblia* and *Cryptosporidium*. The presence of haloacetic acids in disinfected drinking water in the United States is well established, but knowledge of the chemical and physical processes that lead to their formation is important to help control their levels as required by law and to protect public health. The factors (see Figure 1) that determine the type and amount of disinfection by-products formed during water treatment include (1) the presence of organic matter and inorganic matter in the source water, (2) the disinfecting chemicals used, and (3) the length of time the organic matter is exposed to the disinfecting chemicals, (4) the temperature at which the disinfection process takes place, and (5) the pH of the water during the disinfection process. The organic molecules in source water are often extremely large, complex molecules and intermediate molecules will form as a result of exposure to disinfecting chemicals; further reaction between these intermediate molecules and disinfecting chemicals during the disinfection process and storage will result in formation of halogenated by-products, including haloacetic acids. (For more information, see Section 2 of the Report on Carcinogens Monograph on Haloacetic Acids found as Water Disinfection By-Products.)

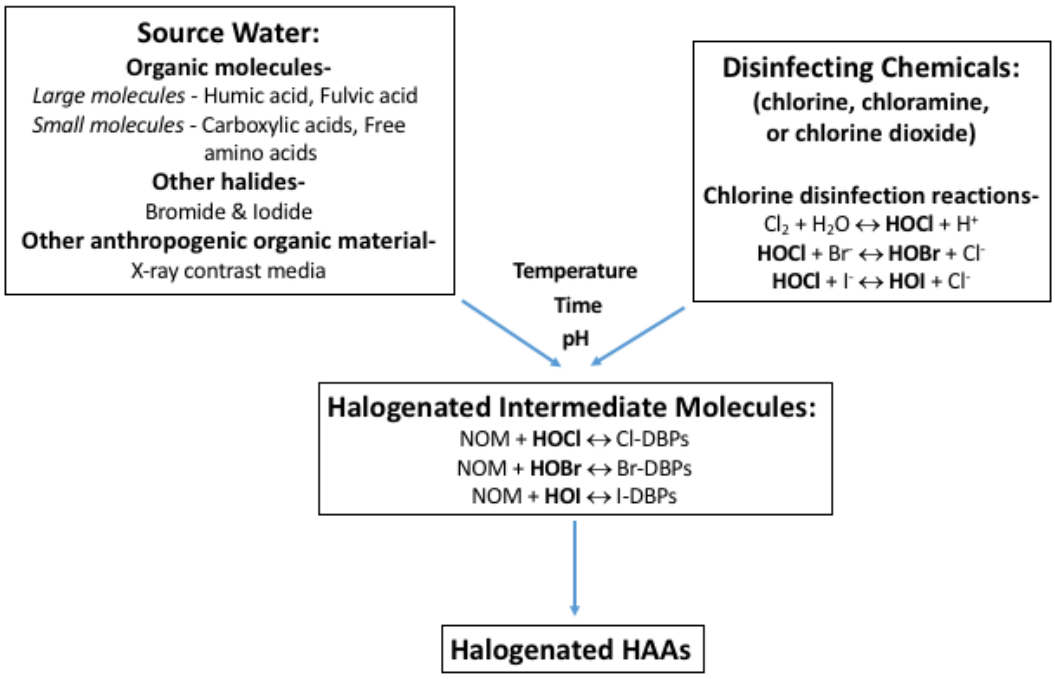


Figure 1. Major factors affecting the formation of halogenated disinfection by-products

Organic molecules in source water plus naturally occurring or anthropogenic bromide and iodide react with various chlorine-containing disinfecting chemicals to form halogenated intermediate molecules and ultimately the halogenated HAAs. HOBr = hypobromous acid; HOCl = hypochlorous acid; HOI = hypoiodous acid; NOM = natural organic matter.

Remediation of haloacetic acid disinfection by-products can be divided into three general approaches: (1) removal of precursors prior to disinfection, (2) optimization or modification of disinfection practices (*e.g.*, altering disinfectant type, dose, or application point in the water treatment process), and (3) removal of disinfection by-products after formation. Up to 80% removal of precursors can be achieved prior to water disinfection with alum coagulation in combination with use of ion exchange resins; membrane filtration can remove up to 99% of precursors by nanofiltration; and activated charcoal can remove up to 91% of precursors. Disinfection practices can be modified using ozone and ultraviolet irradiation which do not leave a disinfectant residual in the water or changing the pre-oxidation chemical to a non-chlorinated one. Removal of haloacetic acids after their formation can be accomplished with biologically active granular activated charcoal filtration.

Exposure

Disinfection of water has achieved tremendous public health benefits in the United States and worldwide through reduction in exposure of individuals to disease-causing microorganisms. Over 250,000,000 people in the United States are exposed to chlorinated drinking water, indicating that a significant number of people in the United States are exposed to haloacetic acids found as water disinfection by-products. Humans are exposed to haloacetic acids from drinking plain tap water, consumption of beverages and food that came in contact with treated water, and ingestion, dermal, and inhalation exposure from swimming pools and spas (both occupational and recreational) where water is disinfected. In addition, people are potentially exposed to

dichloroacetic acid at the workplace to dichloroacetic acid from its use as a chemical intermediate or from medicinal disinfection.

Occurrence of haloacetic acids in treated water

The highest levels of selected HAAs that have been detected are for dichloroacetic acid, chlorobromoacetic acid, and bromodichloroacetic acid (Table 2). National occurrence data from the American Water Works Association (AWWA) for HAA5 for U.S. water disinfection systems serving populations greater than 100,000 people from 1997 to 2014 indicate that 95th percentile HAA5 concentrations have been generally decreasing since 2000 and have been at or below the USEPA maximum contaminant level (MCL) of 60 μg/L for HAA5 since 2004 (Seidel *et al.* 2017). There is some evidence that smaller facilities might have had more difficulty meeting the regulatory limits (i.e. USEPA maximum contaminant level [MCL] of 60 μg/L for HAA5). During the time period from 1997 to 2004, U.S. EPA data indicate that 5% (95th percentile) of smaller systems (i.e., serving fewer than 10,000 people) exceeded the HAA5 MCL. Data for U.S. water facilities serving communities of all sizes in 2011 indicate a median value for HAA5 of 20.1 μg/L with the 5th percentile at 2.0 μg/L and the 95th percentile at 59.0 μg/L.

Table 2. Concentration ranges for di- and trihaloacetic acids in tap water, finished drinking water, and other similar sources

Di-, or trihaloacetic acid	Range (μg/L)	Reference
Dichloroacetic acid	10.4 (1.3–32)	EPA 2016b
Dibromoacetic acid	2.1 (0.63–12)	EPA 2016b
Chlorobromoacetic acid	BDL–18	HSDB 2009a, IARC 2013
Tribromoacetic acid	0–approx. 10	McGuire *et al.* 2002
Bromodichloroacetic acid	5.28–12.2	HSDB 2009b
Chlorodibromoacetic acid	BDL–5.37	HSDB 2009c

Overall potential exposure to haloacetic acids

Daily exposure to mixtures of haloacetic acids from consumption of chlorine-treated tap water in the United States is estimated to be about 69 μg per day (5% to 95% = 6.9 to 204 μg per day) for men and 55 μg per day (5% to 95% = 5.5 to 162.2 μg per day) for women based on median levels of mixtures of haloacetic acids in U.S. water facilities (2011 levels for all facilities reported above). Consumption of water from all foods and liquids per day has been estimated by CDC (Rosinger and Herrick 2016) to be 3.46 L for men over 20 and 2.75 L for women over 20, and the contribution from plain water, i.e., tap water, is approximately 1/3 (33.3%) of the total.

Sources of exposure other than drinking water

In addition to ingesting haloacetic acids by drinking plain tap water, humans can also be exposed to them from other beverages prepared with treated water such as tea, coffee, fruit drinks, and soft drinks or by ingesting food that came in contact with treated water, *e.g.*, rinsing or washing foods before or after cooking or cooking in treated water. Low levels of haloacetic acids may also be present in natural foods. The median amounts of HAAs expressed in μg per kg of food range from less than 1 μg/kg for milk to greater than 10 μg/kg for soft drinks, prepared salads,

and minimally processed vegetables such as fruits or vegetables washed with chlorine-based chemicals in water (Cardador and Gallego 2016). Canned vegetables, fruit juices, and cheese fall between these levels. The Institute of Medicine estimates that 20% of total water consumption is derived from foods, and the remaining 46.7% would derive from beverages such as tea, coffee, soft drinks, and fruit drinks (in addition to the 33.3% provided by tap water).

The disinfection of water for swimming pools and spas often results in higher levels of haloacetic acids than in disinfected tap water because of the use of a higher chlorine residual and higher temperatures than in typical water distribution systems (Parinet *et al.* 2012, Chowdhury *et al.* 2014). Dichloroacetic acid and trichloroacetic acid are the most abundant haloacetic acids detected in swimming pools (Teo *et al.* 2015). For U.S. swimming pools disinfected with chlorine, concentrations of dichloroacetic acid, the most abundant of the listed haloacetic acids, have been reported to range from 52 μg/L to 6,800 μg/L, (Kanan 2010, Teo *et al.* 2015). In contrast, brominated haloacetic acids, *i.e.*, monobromoacetic acid, bromochloroacetic acid, dibromoacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid, occur at the highest concentrations in seawater swimming pools treated with chlorine bleach as disinfectant; levels of mixtures of haloacetic acids (HAA9) ranged from 417 μg/L to 2,233 μg/L for different seawater pools tested (Parinet *et al.* 2012). Dermal (~ 1% of total exposure from swimming pools and spas) and inhalation (~ 5% exposure of haloacetic acids from swimming pools and spas) are not considered to be a major source of exposure to haloacetic acids as they are neither volatile nor appreciably skin permeable (Xu *et al.* 2002, Regli *et al.* 2015). However, exposure can also occur from ingestion of treated pool water (~94%) (Cardador and Gallego 2011) and haloacetic acids have been detected in the urine of swimming pool attendants and swimmers. Urinary levels of dichloroacetic acid of indoor swimming pool attendants were higher with longer exposure (4 hours, 450 ng/L) than shorter exposure (2 hours, 313 ng/L) and higher than outdoor pool attendants (51 ng dichloroacetic acid for 2 hour exposure) (Cardador and Gallego 2011).

Regulations

Coast Guard, Department of Homeland Security

Minimum requirements have been established for safe transport of chloroacetic acid solution on ships and barges.

Department of Transportation (DOT)

Chloroacetic acid (molten, solid, and solution); bromoacetic acid (solid and solution); dichloroacetic acid; trichloroacetic acid; and trichloroacetic acid solution are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Environmental Protection Agency (EPA)

Clean Air Act

National Emission Standards for Hazardous Air Pollutants: Chloroacetic acid is listed as a hazardous air pollutant.

New Source Performance Standards: Manufacture of chloroacetic acid is subject to certain provisions for the control of volatile organic compound emissions.

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 100 lb for chloroacetic acid.

Emergency Planning and Community Right-To-Know Act

EPCRA Section 302: Threshold planning quantity (TPQ) = 100 lb for chloroacetic acid (solids in powder form with particle size < 100 µm or solution or molten form); = 10,000 lb for all other forms of chloroacetic acid.

EPCRA Section 304: Reportable quantity (RQ) = 100 lb for chloroacetic acid.

Toxics Release Inventory: Chloroacetic acid is a listed substance subject to reporting requirements.

Federal Insecticide, Fungicide, and Rodenticide Act

The end-use concentration of chloroacetic acid in acetic acid, chloro-, sodium salt, reaction products with 4,5-dihydro-2-undecyl-1H-imidazole-1-ethanol and sodium hydroxide when ready for use as an ingredient in an antimicrobial pesticide formulation applied to dairy processing equipment and food-processing equipment and utensils is not to exceed 42 parts per million (ppm).

Safe Drinking Water Act

Maximum contaminant level for HAA5 = 60 µg/L.

Food and Drug Administration (FDA)

Maximum permissible level of HAA5 in bottled water = 60 µg/L.

Chloroacetic acid is permitted in food package adhesives with an accepted migration level up to 10 parts per billion (ppb).

Food containing any added or detectable level of chloroacetic acid is deemed to be adulterated.

Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value – time-weighted average (TLV-TWA) = 0.5 ppm for dichloroacetic acid and trichloroacetic acid.

Dichloroacetic acid and trichloroacetic acid are listed as confirmed animal carcinogens with unknown relevance to humans.

Potential for dermal absorption for dichloroacetic acid.

Environmental Protection Agency (EPA)

Integrated Risk Information System (IRIS) oral reference dose (RfD) = 4×10^{-3} mg/kg b.w. per day for dichloroacetic acid; = 2×10^{-2} mg/kg b.w. per day for trichloroacetic acid.

IRIS oral cancer slope factor = 5×10^{-2} per mg/kg b.w. per day for dichloroacetic acid; = 7×10^{-2} per mg/kg b.w. per day for trichloroacetic acid.

IRIS drinking water unit risk = 1.4×10^{-6} per µg/L for dichloroacetic acid; = 2×10^{-6} per µg/L for trichloroacetic acid.

National Institute for Occupational Safety and Health (NIOSH)

Recommended exposure limit (REL) = 1 ppm (10-h TWA) for trichloroacetic acid.

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