

Report on Carcinogens Protocol: Haloacetic Acids Found as Water Disinfection By- Products

Running title- Haloacetic Acids: RoC Protocol

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Protocol: Methods for Preparing the Draft Report on Carcinogens Monograph on “Haloacetic Acids Found as Water Disinfection By-Products”

Background information

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 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). 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 from chemicals and organic material in the water of a large number of unintended compounds that 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%). Two of four U.S. EPA regulated trihalomethanes, chloroform and bromodichloromethane, are listed in the Report on Carcinogens (RoC) as *reasonably anticipated to be a human carcinogen*; the other two chemicals, chlorodibromomethane and bromoform, do not have available cancer data. Haloacetic acids (HAAs) the second largest group by weight (36%) of total halogenated disinfection by-products found in public water supplies (Liang and Singer 2003).

The potential class of di- and tri-haloacetic acids found as water disinfection by-products was nominated by the National Toxicology Program (NTP) for possible review based on structural similarities and NTP cancer bioassays on several haloacetic acids. A concept for reviewing di- and tri- HAAs as a potential class was presented to the NTP Board of Scientific Counselors in April 11, 2016. Based on a request from the U.S. EPA Office of Water and advice from technical experts the review was expanded to include monoacetic acids as well as di- and tri- halogenated forms (Table 1-1).

The Office of the Report on Carcinogens (ORoC) has identified thirteen HAAs formed from water disinfection processes: mono-, di-, or tri- halogen substituted acetic acids with chlorine, bromine, or iodine substitutions. All bromine and chlorine substituted HAAs are either regulated by EPA or being considered for regulation of amounts in disinfected drinking water; however, some haloacetic acids found drinking water are not monitored or regulated and may have health consequences.

ORoC plans to evaluate mono-, di-, and trihaloacetic acids identified in drinking water for possible listing in the RoC (see table below). As proposed in the concept, an Information Group was convened on September 9, 2016 to discuss approaches to evaluate of some or all of these chemicals as a class. ORoC is in the process of exploring suggested approaches and has met with NTP scientists to discuss data needs and how to obtain this information in short-term experiments. As part of the evaluation, ORoC will assess whether some or all of these chemicals can be considered members of a class of carcinogens or if they should be considered separately. Moreover, information from this ORoC evaluation can help to inform public health decisions on water regulation and on water disinfection processes.

Table 1. Selected haloacetic acids (HAA) present in drinking water

Mono-HAA	CAS RN	Di-HAA	CAS RN	Tri-HAA	CAS RN
Chloroacetic acid	79-11-8	Dichloroacetic Acid	79-43-6	Trichloroacetic acid	76-03-9
Bromoacetic acid	79-08-3	Dibromoacetic Acid	631-64-1	Tribromoacetic acid	75-96-7
Iodoacetic acid	64-69-7	Diiodoacetic Acid	598-89-0	Bromodichloroacetic acid	71133-14-7
		Bromochloroacetic acid	5589-96-8	Chlorodibromoacetic acid	5278-95-5
		Bromiodoacetic acid	71815-43-5		
		Chloroiodoacetic acid*	53715-09-6		

* Chloroiodoacetic acid is formed by iodized salt being added to chlorinated drinking water.

Cancer hazard evaluation

The purpose of the cancer evaluation component is to assess the available scientific evidence, apply the RoC listing criteria¹ to this evidence, and reach a preliminary level of evidence conclusion from studies in humans and recommendation for a listing status in the RoC. Briefly, the RoC listing criteria for the two listing categories are as follows:

Known to be a human carcinogen

- Sufficient evidence of carcinogenicity from studies in humans

Reasonably anticipated to be a human carcinogen

- Limited evidence of carcinogenicity from studies in humans
- Sufficient evidence of carcinogenicity from studies in experimental animals, OR
- Substance belongs to a structurally related class of substances that are listed in the RoC, OR
- Convincing relevant information that the agent acts through a mechanism indicating it would likely cause cancer in humans.

The goal of the cancer hazard evaluation component of the draft RoC monograph is to conduct an assessment of the scientific literature, utilizing information from the primary literature as well as from authoritative and other reviews. The monograph will evaluate the level of evidence from studies of cancer in experimental animals, as well as from studies reporting on mechanistic and related data such as metabolism and genotoxicity. The evaluation will be informed by the utilization of tables that will provide pertinent data on HAAs.

Conclusions regarding the carcinogenicity in experimental animals, as well as mechanistic and related data, are based on scientific judgment with consideration of all relevant data.

¹For the formal Report on Carcinogens listing criteria, see <http://ntp.niehs.gov/go/15209>.

Protocol components

This protocol discusses the methods that will be used to prepare the cancer evaluation component of the draft monograph on thirteen HAAs found as water disinfection by-products (hereinafter referred to as HAAs).

- Part 1: Preliminary Outline of the Draft RoC Monograph
- Part 2: Methods for Evaluating Properties and Human Exposure Information
- Part 3: Methods for Evaluating Human Cancer Studies
- Part 4: Methods for Evaluating Cancer Studies in Experimental Animals
- Part 5: Methods for Evaluating Mechanistic and Other Relevant Data
- Part 5: Methods for Data Integration

Appendix A provides the literature search strings that are specific for haloacetic acids.

1 Preliminary Outline of Draft RoC Monograph

The draft RoC monograph on Haloacetic Acids Found as Water Disinfection By-Products (HAAs) focuses on the relationship between exposure to HAAs and cancer. The cancer evaluation component of the monograph is organized by topic and includes several sections, as described below. Since no human cancer studies were identified on exposure to any individual HAA, a brief summary of available information for human exposures to disinfected water and cancer will be included in the monograph introduction or final section. Appendices to the monograph contain additional information, such as descriptions of the quality evaluation of the carcinogenesis studies in experimental animals and data tables.

The major sections in the cancer hazard evaluation component are as follows:

1.1 Properties and Human Exposure

This section includes details on the chemical properties of the HAAs under review and the current available information on human exposure in the United States to these water disinfection by-products.

1.2 Disposition and Toxicokinetics

This section discusses the available information on absorption, distribution, metabolism, and excretion of HAAs in humans and experimental animals.

1.3 Studies in Experimental Animals

This section (1) reviews and assesses the quality and utility of the available studies of cancer in experimental animals exposed to HAAs to inform the cancer hazard evaluation, (2) interprets the study findings and identifies treatment-related cancer sites in each study, and (3) integrates the findings across studies for each HAA.

1.4 Mechanisms and Other Relevant Data

This section assesses the strength of mechanistic and related evidence for effects resulting from exposure to HAAs. It includes relevant studies on cytotoxicity and genotoxicity conducted *in vivo* or *in vitro*.

1.5 Overall Cancer Evaluation

The final section of the draft RoC monograph integrates the evidence from the cancer studies in experimental animals with mechanistic and relevant data and applies the RoC listing criteria to reach the NTP preliminary listing recommendation. This section will also discuss the evidence for possibly grouping some or all of these chemicals as members of a class of carcinogens, based on their carcinogenic and/or biochemical properties.

1.6 Appendices

The appendices to the monograph contain additional information, including descriptions of the quality evaluation of carcinogenesis studies in experimental animals, literature search strategy, or important supplemental information.

2 Methods for Evaluating Properties and Human Exposure Information

2.1 Objectives

Human exposure to HAAs for people, focusing on people residing in the United States, will be assessed in this section of the monograph by reviewing available data on formation, production factors (source water, disinfectant, process, and remediation methods) levels of exposure in the general population as well as occupational exposure. The general approach for identifying the relevant literature, drafting subsections which will incorporate information on key questions (see below), and examples of tabular and graphical presentation of data are described in Part C of the [Handbook for Preparing Report on Carcinogens Monographs](#) (herein after referred to as RoC Handbook) (NTP 2015).

2.1.1 Key questions

- What HAAs are found as water disinfection by-products and what is their amount in relation to other chemical by-products formed with water disinfection?
- Are a significant number of people residing in the United States exposed to these chemicals?
- How is exposure measured and what federal regulations and guidelines limit exposures?
- How is exposure controlled; what remediation methods are used or proposed?
- What factors in the disinfection process influence formation and type of halogen substitutions of the HAAs?
- What ways can the general population be exposed to HAAs; what occupations or activities pose a greater risk of exposure?

2.1.2 Subsections

- Substance identification and properties
- Water treatment and formation of disinfection byproducts
- Remediation of HAAs
- Exposure to HAAs
- Summary and synthesis

2.1.3 Approach

The information presented in this section will rely primarily on secondary literature sources as described in Section 1 of Part C of the RoC Handbook. Information used for exposure assessment will also come from reports from the U.S. EPA Office of Water and other sources and include published meeting proceedings, manuscripts, and Federal Register Notices.

2.2 Literature search strategy

Data related to methods, properties, occurrence, and human exposure to HAAs are collected from secondary sources and authoritative reviews and other online resources outlined in the RoC Handbook (see Sections B and C). Potentially relevant literature was identified by a search for “occurrence” in the PubMed citation database. The review of results from citation databases searched for other topics of the monograph, such as the Mechanistic section, provides additional

potentially relevant literature. Such results are identified during screening and reviewed for relevance.

Table 2-1. Literature search strategy for haloacetic acids

Substance-specific search concepts	Exposure
Haloacetic acids (class)	Occurr*
Dihaloacetic acids (class)	
Trihaloacetic acids (class)	
13 individual HAAs names, synonyms and CAS numbers	
Excluded: TCA acid peels, TCA solub*, TCA precipitable, HPV, anogenital wart treatments	
Disinfection by-products	
Treated water	
Water treatment	

3 Methods for Evaluating Human Cancer Studies

3.1 Epidemiological studies

No epidemiological studies were identified evaluating exposure specific for HAAs using the literature search strategy outlined below. Human exposures to HAAs formed as water disinfection by-products are to the mixture of by-products and our evaluation of carcinogenicity is based on individual exposures and cancer outcomes in experimental animals. There are existing data in the primary literature of exposures to water disinfection mixtures or specific classes of water disinfection by-products (such as trihalomethanes) that may serve as surrogates of chlorinated water. A brief discussion of the human cancer findings and any potential association with disinfection by-products will be discussed in either the introduction or final section the monograph. The discussion will summarize 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 2013) and update the literature since that monograph.

3.2 Identification and selection of relevant literature

Search concepts used to search the three scientific databases (PubMed, Scopus, and Web of Science) for potential HAA exposures and cancer studies in humans are provided in the table below. The search strategy used terms related to HAAs or relevant exposure scenarios combined (using “and”) with search terms for epidemiologic studies and with search terms for the outcome, i.e., cancer. Retrieved citations are screened for inclusion/exclusion using [Health Assessment Workplace Collaborative \(HAWC\)](#) software. HAWC software is an open-source, modular, content-management system designed to facilitate synthesis of multiple data sources, integrating and documenting workflow from literature search to data extraction, synthesis, and interpretation. Citation analysis of articles, reports, and reviews identified from this search strategy are used to identify any additional primary studies or other relevant literature.

Table 3-1. Literature search strategy for human cancer studies

Substance-specific search concepts ^a	Epidemiologic search concepts ^b	Cancer endpoint concepts
Dihaloacetic acids (class)	RoC Epidemiological (Human)	RoC Cancer Search Strings
Trihaloacetic acids (class)	Studies Search Strings	
13 individual HAAs names, synonyms, and CAS numbers		
Disinfection by-products, water disinfection, water treatment (General concepts)		

^a For full search string see Appendix.

^b See [RoC Handbook Appendix: Standard search strings for databases searches](#) for search terms for epidemiological studies and cancer

4 Methods for Evaluating Cancer Studies in Experimental Animals

4.1 Objectives

This section describes the procedures used to prepare the cancer studies in the experimental animal section of the draft RoC monograph, and to reach a level of evidence conclusion on the carcinogenicity of HAAs with animal cancer data. The general approach for identification and selection of the relevant literature, systematic extraction of data from the experimental animal studies, assessment of the utility of the individual studies in experimental animals, cancer hazard evaluation, and examples of table templates and figures are described in Part E of the RoC Handbook (<http://ntp.niehs.nih.gov/go/rochandbook>).

The objective of the evaluation of the studies in experimental animals is to reach a preliminary level of evidence conclusion [sufficient, not sufficient] for the carcinogenicity of HAAs by applying the RoC listing criteria to the body of knowledge (Section 4.1.1). Because HAAs may potentially be defined as a class rather than as individual HAA compounds, the application of the RoC listing criteria and preliminary level of evidence conclusions from studies in experimental animals may not be discussed until the overall cancer hazard conclusions, which is after the evaluation of the mechanistic data.

4.1.1 *RoC listing criteria for sufficient evidence of carcinogenicity from studies in experimental animals*

“Increased incidence of malignant and/or a combination of malignant and benign tumors

- in multiple species or at multiple tissue sites OR
- by multiple routes of exposure, OR
- to an unusual degree with regard to incidence, site, or type of tumor, or age at onset.”

4.1.2 *Key questions*

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

4.1.3 *Approach*

The steps for conducting the cancer evaluation include:

- Selection of the literature included in the cancer hazard evaluation of the experimental animal studies (RoC Handbook, Part E, Section 1; Section 4.2 below)
- Systematic extraction of the data from experimental animal studies (RoC Handbook, Part E, Section 3; Section 4.3 below)
- Assessment of the quality of the individual studies (RoC Handbook, Part E, Section 4; Section 4.4 below)

- Cancer hazard evaluation of the evidence from studies in experimental animals (RoC Handbook, Part E, Section 5; Section 4.5 below)

Details on the approach to be used to identify and report on relevant studies, including the factors used for study quality assessment, is provided following the brief description of the literature search.

4.2 Literature search strategy

The identification of the relevant literature includes developing strategies for searching for citations and inclusion/exclusion questions for selecting the relevant citations from the searches.

The following approaches for identifying literature are employed:

- Literature searches of three scientific databases – PubMed, Scopus, and Web of Science – are conducted for this section using the specific search terms for the substance (see Appendix) combined (using “and”) with search terms for cancer and experimental animals.
- For HAAs, sources searched for studies that might not be included in the scientific databases listed above were the Carcinogenic Potency Data Base (Gold 2011), PHS-149 (*Survey of Compounds Which Have Been Tested for Carcinogenic Activity*) (Lee *et al.* 1988), the Chemical Carcinogenesis Research Information System (CCRIS) (NLM 2011), and IARC monographs.

Table 4-1. Literature search strategy for cancer studies in experimental animals

Substance-specific search concepts ^a	Animal species concepts ^b	Cancer search concepts ^b
Haloacetic acids (class)	RoC Experimental Animal	RoC Cancer Search Strings
Dihaloacetic acids (class)	Studies Search Strings	
Trihaloacetic acids (class)		
13 individual HAAs names, synonyms and CAS numbers		
Disinfection by-products, water disinfection, water treatment (General concepts)		

^aFor full search string Appendix of this document.

^bSee [RoC Handbook Appendix: Standard search strings for databases searches](#) for search terms for experimental animals and cancer

Citations are screened for primary studies on cancer in experimental animals using the procedures outlined in the Appendix of the protocol and HAWC (<http://hawcproject.org>)

Studies are initially included in the study if they meet the following inclusion criteria:

- Measure neoplastic (benign, malignant) endpoints.
- Have non-cancer data that are informative for a cancer assessment, such as reporting preneoplastic lesions.
- Describe non-neoplastic lesions that are considered part of a morphologic continuum to neoplasia.
- Provide information on chronic study dose selection (such as a subchronic or short-term toxicity study used for chronic study dose selection).

- Co-carcinogen studies

Studies that have no concurrent control group or poor reporting of study design or of results may be excluded from further consideration.

4.3 Systematic extraction of data from the cancer studies in experimental animals

Detailed information regarding study data and methods abstraction from individual studies is described in the [RoC Handbook](#), Part D, Section 3. Briefly, data is selected and entered into NTP Table Builder, a database specifically created for entering information from scientific publications in a systematic manner using standardized instructions and questions. The database contains “fields” that are specific for the different types of extracted information (e.g., such as species, strain, sex, route, dosing regimen, duration, and results). Questions and guidelines are available to describe the specific type of information that should be summarized or entered into each field; and selected fields are used to populate tables in the monograph.

4.4 Assessment of the utility of the individual studies in experimental animals

Over 50 cancer studies in experimental animals were identified and reviewed for study quality by two independent reviewers. Exposure to the HAA in most studies was by drinking water and in a few cases by gavage.

Chronic carcinogenicity studies were located for six of the thirteen haloacetic acids: monochloroacetic acid, dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, trichloroacetic acid, and bromodichloroacetic acid. In addition, iodoacetic acid was tested as a promoter in an initiation-promotion study.

Each primary study is 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: population, exposure conditions, outcome assessment, potential confounding, and statistics and reporting. Each element contains questions related to potential for bias as well as questions related to study sensitivity, which is the ability of the study to detect a “true” risk (see below for question). Studies are also evaluated for elements relevant to external validity (interpreting the findings for relevance to humans). The response for answering the signaling question (see below) of whether there is a potential bias or limitation is based on a comparison of the study element with that of the “ideal” study for a specific endpoint and exposure to the candidate substance. The signaling questions listed in the table below briefly review the major questions in the RoC Handbook and discuss issues specific for HAA animal cancer studies. Some issues specific for the evaluation of the quality of HAA studies are: (1) Studies may focus on liver pathology only and not provide information on whether a full necropsy was performed. Thus, these studies may not be as informative for evaluating the complete carcinogenicity of the HAA tested. (2) Neutralization of pH and sodium ion concentration in the drinking water across all dose groups (including control group) is important as these differences can affect the animal’s physiology and water palatability. HAAs are acidic and acidity varies with concentration in water. In addition, neutralizing dose groups of differing acidities with sodium hydroxide would vary the sodium ion content of the dose groups. Therefore, all dose groups would need to be normalized as to sodium ion concentration as well as to a neutral pH range.

Table 4-2. Signaling questions^a

Question	Type of question
Is there concern that the study design did not include randomization of animals to dosed groups?	Study domain
Is there concern that the concurrent control group was not adequate for evaluating effects across treatment groups?	Study domain
Are historical control data reported? (No rating given).	Study domain
Are there concerns about the age of the animals for evaluating potential effects?	Study domain Sensitivity
Is there concern that the animal model is not sensitive for detecting an effect?	Study domain Sensitivity
Is there concern that there is inadequate statistical power (number of animals per dose and control group) to detect a neoplastic effect, if present?	Study domain Sensitivity
Is there concern that the chemical characterization and dose formulations and delivery of the chemical were not adequate to support attribution of any neoplastic effects to the substance?	Exposure conditions
Is there concern that the dosing regimen was not adequate for detection of a neoplastic effect (if present) or attribution of any neoplastic effects to the substance?	Exposure conditions
Is there concern that the exposure duration period was not adequate for detection of a neoplastic effect, if present?	Exposure conditions Sensitivity
Is the study design adequate to evaluate dose-response relationships (e.g., more than one dose)	Exposure conditions Sensitivity
Is there concern that the methods used to assess tumor outcome or the pathology procedures were not adequate for attribution of the effects to the exposure?	Outcome assessment
Is there concern that not all treatment and control groups were assessed in the same way and in balanced blocks, to avoid bias?	Outcome assessment
Is the study duration (observation period) adequate to detect a neoplastic effect, if present?	Outcome assessment Sensitivity
Is there concern for potential confounding?	Confounding
Is there concern that reporting of the data and statistical analysis are inadequate for evaluating the results?	Reporting and analysis
Is there concern that different types of tumors were not accurately combined in the analysis?	Reporting and analysis

^aQuestions have been shortened somewhat; see RoC handbook for further details.

Responses to signaling questions

- **Minimal concerns:** Information from study designs and methodologies indicate that they are close to the ideal study characteristics and that the potential for bias is unlikely or minimal (+++).
- **Some concerns:** Study designs or methodologies are less than ideal, indicating possible bias (++)

- Major concerns: Study designs or methodologies suggest that the potential for a specific type of bias is likely (+).
- Inadequate: Study designs or methodologies suggest that the bias is critical and would make the study not informative for cancer hazard evaluation.
- No information: Inadequate information in the study to evaluate the level of concern.

Study level judgment signaling questions

The overall utility (ability of the study to inform the cancer hazard evaluation) of a study is based on consideration of both the potential for bias (limitations) and study sensitivity.

- **High** (low concerns about most potential biases and high sensitivity)
- **Moderate** (some concerns about many potential biases)
- **Low** (major concerns about several biases)
- **Inadequate** (critical concerns about some potential biases)

4.5 Cancer hazard evaluation

The findings of each study are interpreted with respect to their limitations and strengths and the evidence from the studies is integrated across studies. The most informative studies (highest quality and sensitivity) are given the most weight, and positive findings from these studies are considered to provide evidence of a treatment-related tumor effects. The evaluation also considers questions related to external validity including concerns whether the route of exposure was not adequate for evaluating the potential for human carcinogenicity and whether tumor formation occurs by a mechanism that would not operate in humans. Findings in experimental animals are considered to be relevant to humans unless there is *compelling* evidence to suggest otherwise. Although the relevance of the route of the exposure to humans is considered, the findings of a similar tumor site by multiple routes of exposure strengthens the evidence for carcinogenicity.

The preliminary level of evidence for carcinogenicity is reached by applying the RoC listing criteria to the body of knowledge. Because HAAs may potentially be defined as a class rather than as individual HAA compounds, the application of the RoC listing criteria and preliminary level of evidence conclusions from studies in experimental animals may not be discussed until the overall cancer hazard conclusions, which is after the evaluation of the mechanistic data.

5 Methods for Evaluating Disposition and Toxicokinetics, Mechanistic Studies, and Other Relevant Data

The purpose of this section is to provide background information important for understanding potential mechanisms of carcinogenicity and includes relevant discussions on the roles of absorption, distribution, metabolism, and excretion (ADME), toxicokinetics, genotoxicity, cancer mechanisms, and other relevant data potentially involved in the carcinogenicity of some or all of the HAAs.

5.1 Objectives for evaluation of disposition and toxicokinetic studies

This section provides an overview of available information on the HAAs ADME and toxicokinetics in experimental animals and humans. This information as it applies to potential mechanism(s) of chemical carcinogenicity will be discussed in the mechanistic section.

5.1.1 Key questions

- How are HAAs absorbed (inhalation, ingestion, dermal exposure)?
- What is known about metabolism, distribution, and excretion for HAAs?
- 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 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?

5.1.2 Literature search strategy

Information for ADME is collected from general sources and reviews together with sources identified by a review of the search results for other topics (e.g., mechanistic searches). If additional searches are required for special topics for ADME they will be conducted using substance-specific search concepts (Haloacetic acids (class); Dihaloacetic acids (class); Trihaloacetic acids (class); 13 individual HAAs names, synonyms and CAS numbers; disinfection by-products, water disinfection, water treatment (General concepts)) combined with terms for the specific topics identified for ADME. Results of those searches in PubMed, Scopus, and Web of Science databases will be screened to identify potentially useful literature for ADME.

See [RoC Handbook Appendix: Standard search strings for databases searches](#)

5.1.3 Approach

Information from authoritative and other reviews and reports, e.g., IARC, EPA, NTP publications, will help to identify established data and primary issues to provide targeted searches of primary literature and also help to identify more recent publications from primary sources. EPA, NTP, and IARC have reviewed six of the haloacetic acids considered in this document (chloroacetic acid, dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, trichloroacetic acid, and bromodichloroacetic acid) and data presented in these agency reviews will be checked against the primary literature and supplemented with additional studies of these and other mono-, di- and trihaloacetic acids from the primary literature. Primary literature is cited to adequately address an

issue, such as to obtain detailed information on key questions or to interpret conflicting information. (See Part F of the RoC Handbook [NTP 2015].)

5.2 Objectives for evaluation of mechanistic studies and other relevant data

The purpose of this section is to discuss and assess the potential mechanisms of carcinogenicity for HAAs. It will include relevant discussions on the characteristics of carcinogens and other mechanisms that are potentially involved in the carcinogenicity of some or all of the HAAs.

5.2.1 Key questions

- Is there evidence that HAAs produce effects identified in the 10 characteristics of carcinogens and if so, which HAAs produce these effects?
 - Do the *in vivo* studies (if any) correlate with *in vitro* studies?
 - Are there patterns (based on relative potencies) in the results based on number or type of halogen substitution?
- What are the major modes of action for the carcinogenicity of HAAs?
- Can potential cancer pathways and key events be identified?
- How do potential modes of action relate to the ten characteristics of carcinogens?
- How do potential modes of action relate to bio-physical properties of HAAs?

5.2.2 Literature search strategy

Literature searches of three scientific databases – PubMed, Scopus, and Web of Science – are conducted for this section using the specific search terms for the substance (see Appendix) combined (using “and”) with search terms developed to target the ten characteristics of carcinogens. These terms target a broad range of mechanistic and genotoxic concepts.

Table 5-1. Literature search strategy for mechanistic studies and other relevant data

Substance ^a	Key mechanistic concepts ^b
Haloacetic acids (class)	RoC Characteristics of Carcinogens Search String
Dihaloacetic acids (class)	
Trihaloacetic acids (class)	
13 individual HAAs names, synonyms and CAS number	

^a For full search string, see Appendix of this document.

^b See [RoC Handbook Appendix: Standard search strings for databases searches](#) for search terms for each of the listed key mechanistic concepts.

Mechanistic findings in reviews from various U.S. and international agencies and researchers will be summarized (*e.g.*, IARC, EPA, NTP). In addition, Chemical Effects in Biological Systems (CEBs database) also will be searched for genotoxicity data for several haloacetic acids (available at <https://tools.niehs.nih.gov/cebs3/ui/>). In addition, *in silico* databases such as Tox21 and ToxCast will be searched for mechanistic findings on the HAAs.

5.2.3 Approach: Steps in the evaluation of mechanistic studies and other relevant data

Evaluation of mechanistic data will be organized according to the characteristics of carcinogens (Smith *et al.* 2016) as well as emerging characteristics (see below). For each characteristic the text will (1) discuss the evidence available for the individual 13 HAAs from relevant *in vitro* and *in vivo* data and (2) will also discuss the evidence across the 13 HAAs. The latter will be based on physical and chemical characteristics and will evaluate whether the response for each characteristic varies by the number and type of halogens. The section will focus on studies that compare activity of several HAAs within the same testing platform. Trends in potencies for individual HAAs across various testing platforms may help identify modes of action and the associated early and late key events that could lead to cancer. The tentative organization is as follows:

Subsections

- Is electrophilic
- Alters nutrient supply
- Induces oxidative stress
- Is genotoxic and/or alters DNA repair
- Induces epigenetic alterations
- Modulates receptor-mediated effects
- GST- ζ inhibition
- Causes cell immortalization
- Alters cell proliferation and cell death
- Induces chronic inflammation or immunosuppression
- Affects gene expression
- Mode of action integration and synthesis

6 Overall Cancer Evaluation and NTP Listing Recommendation

This section evaluates all the data on the HAAs and makes a determination as to (1) whether HAAs can be grouped into a class or subclass, and (2) whether the scientific data for HAA as a class, subclass or for individual HAAs meet the RoC listing criteria for listing in the RoC and if so, for which listing category.

The purpose of this section is to integrate the body of evidence from cancer studies in experimental animals, and humans (if available) with disposition and mechanistic studies or other relevant data and apply the RoC listing criteria to reach the NTP preliminary listing recommendation. An assessment will be made as to whether some or all of these HAA chemicals can be considered members of a class of carcinogens, based on chemical properties and modes of action, or if they should be considered separately based on animal cancer data.

6.1 Key questions

Animal cancer data is available for some of the HAAs and a key question is how do these cancer outcomes relate to mechanistic information and/or physico-chemical properties of the HAAs?

Do all or some of the HAAs share common modes of action and key events?

Does the mechanistic and other relevant data provide adequate support for considering some or all of the HAAs as a well-defined, structurally related class?

Does the evidence for HAA as a class or subclass, or for individual HAAs meet the RoC listing criteria?

6.2 Approach

Collectively, a large body of toxicologic and mechanistic data are available for the HAAs included in this review; though dichloroacetic acid and trichloroacetic acid are the most extensively studied. Some of these studies evaluated the relationship of physico-chemical properties of the HAAs to their potencies. In order to understand how these factors relate to cancer outcomes *in vivo*, a table listing the HAAs organized by number and type of halogen substitutions, key events, and physico-chemical properties will be employed with consideration of cancer outcomes from studies in experimental animals (Patlewicz *et al.* 2013). Qualitative comparisons of potencies across the various HAAs for various mechanistic and cancer endpoints may help to identify potential modes of action, their associated key events relating to carcinogenicity, and data gaps. This integrated information will help with the assessment of the HAAs and determination of whether mechanistic information and physico-chemical properties allow grouping of some or all of them into chemical and mechanistic class(es) of carcinogens, or whether they should be considered for potential listing based primarily on animal cancer data.

Considerations for listing based on available (1) physico-chemical and disposition data, (2) mechanistic and other relevant data including mode(s) of action and key events, and (3) overall synthesis will be discussed and integrated with cancer findings from studies in experimental animals and epidemiology studies. Finally, preliminary RoC listing recommendations for the HAAs based on RoC listing criteria will be reported based on these findings.

7 References

1. EPA. 2005. *Economic Analysis for the Final Stage 2 Disinfectants and Disinfection Byproducts Rule*. EPA 815-R-05-010. U.S. Environmental Protection Agency. 432 pp.
2. EPA. 2015a. *About Private Water Wells*. U.S. Environmental Protection Agency. Updated on 11/17/15. <http://www.epa.gov/privatewells/about-private-water-wells>.
3. EPA. 2015b. *Basic Information about Your Drinking Water*. U.S. Environmental Protection Agency. Updated on 11/30/15. <http://www.epa.gov/your-drinking-water/basic-information-about-your-drinking-water>.
4. IARC. 2013. *Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-water*, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. vol. 101, Lyon, France: International Agency for Research on Cancer. 610 pp.
5. IPCS. 2000. *Disinfectants and Disinfectant By-Products*. Environmental Health Criteria 216. Geneva, Switzerland: World Health Organization. 529 pp.
6. Liang L, Singer PC. 2003. Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environ Sci Technol* 37(13): 2920-8. (Supported by the American Water Works Association Research Foundation. Authors affiliated with University of North Carolina, NC.)
7. Gold LS. 2011. *The Carcinogenic Potency Database*. Last updated 9/1/11. <https://toxnet.nlm.nih.gov/cpdb/>.
8. Lee A, Campbell B, Varley T. 1988. Survey of Compounds Which Have Been Tested for Carcinogenic Activity. USPHS Publication No. 149 (PHS-149).
9. NLM (National Library of Medicine, US). 2011. Last updated 2011. *Chemical Carcinogenesis Research Information System (CCRIS: A Toxnet Database)*. <https://toxnet.nlm.nih.gov/newtoxnet/ccris.htm>.
10. NTP. 2015. *Handbook for Preparing Report on Carcinogens Monographs*. Research Triangle Park, NC: National Toxicology Program. 89 pp. <http://ntp.niehs.nih.gov/go/rochandbook>.
11. Patlewicz G, Ball N, Booth ED, Hulzebos E, Zvinavashe E, Hennes C. 2013. Use of category approaches, read-across and (Q)SAR: general considerations. *Regul Toxicol Pharmacol* 67(1): 1-12.
12. Richardson SD, Plewa MJ, Wagner ED, Schoeny R, Demarini DM. 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res* 636(1-3): 178-242.
13. Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Coglianò VJ, Straif K. 2016. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect* 124(6): 713-721.

Appendix A: Literature Search Strategy

This section provides the search strings for the thirteen haloacetic acids that are specific for the draft RoC monograph (Table A-1). The literature search terms for the HAAs include terms for the class(es) of chemical, names, and synonyms for the individual chemicals, database index terms (MeSH terms), and CAS numbers.

Trichloroacetic acid is associated with large sets of results that were not relevant to this evaluation because of its use to precipitate proteins, and treat anogenital warts. Targeted exclusion of such results was accomplished using a select set of terms combined with the boolean term “Not.”

Table A-1. Literature search strings for haloacetic acids

Database	Formatted Search Strings ^a
PubMed	<p>((Haloacetic-acid*[tiab]) OR Dihaloacetic-acid*[tiab]) OR Trihaloacetic-acid*[tiab]) OR (“dichloroacetic acid”[nm] OR 79-43-6[rn] OR dichloroacetate[tiab] OR “dichloroacetic acid”[tiab] OR “Bichloracetic acid”[tiab] OR “Dichloracetic acid”[tiab] OR “Dichlorethanoic acid”[tiab] OR “Dichloroethanoic acid”[tiab]) OR (“Trichloroacetic Acid”[mh] OR 76-03-9[rn] OR “Trichloroacetic Acid”[tiab] OR Trichloroacetate[tiab] OR “Trichloracetic acid”[tiab]) OR (“Dibromoacetic acid”[nm] OR 631-64-1[rn] OR dibromoacetate[tiab] OR “Dibromoacetic acid”[tiab]) OR (“tribromoacetic acid”[nm] OR 75-96-7[rn] OR tribromoacetate[tiab] OR “tribromoacetic acid”[tiab]) OR (“Dichlorobromoacetic acid”[tiab] OR Bromodichloroacetate[tiab] OR “bromodichloroacetic acid”[nm] OR bromodichloroacetic-acid[tiab] OR 71133-14-7[rn]) OR (“Dibromochloroacetic acid”[tiab] OR 5278-95-5[rn] OR bromochloroacetate[tiab]) OR (“bromochloroacetic acid”[nm] OR 5589-96-8[rn] OR “bromochloroacetic acid”[tiab] OR bromochloroacetate[tiab] OR “Chlorobromoacetic acid”[tiab]) OR (Diiodoacetic-acid[tiab] OR “598-89-0”[tiab] OR Diiodoacetate[tiab]) OR (71815-43-5[rn] OR Bromoiodoacetic-acid[tiab] OR Bromoiodoacetate[tiab]) OR (Chloroiodoacetic-acid[tiab] OR “Chloro(iodo)acetic acid”[tiab] OR 53715-09-6[rn] OR “2-Chloro-2-iodoacetic acid”[tiab] OR “Acetic acid, 2-chloro-2-iodo-”[tiab] OR “chloro-iodoacetic acid”[tiab] OR Chloroiodoacetate[tiab]) OR (“Monochloroacetic acid”[tiab] OR “Monochloracetic acid”[tiab] OR “79-11-8”[rn] OR Chloroacetic-acid[tiab] OR “Chloroacetic acid”[nm] OR “Chloracetic acid”[tiab]) OR (Bromoacetic-acid[tiab] OR Bromoacetate[tiab] OR Monobromoacetic-acid[tiab] OR 79-08-3[rn]) OR (“Iodoacetic acid”[mh] OR 64-69-7[rn] OR Monoiodoacetic-acid[tiab] OR Monoiodoacetate[tiab] OR Monoiodine-acetate[tiab] OR Iodoacetate[tiab] OR Iodoacetic-acid[tiab]))</p> <p>NOT</p> <p>(trichloro-acetic-acid-peel*[tiab] OR trichloroacetic-acid-peel*[tiab] OR trichloracetic-acid-peel*[tiab] OR Trichloroacetic-Acid-solub* OR Trichloroacetic-Acid-insolub* OR Trichloroacetic-Acid-precipit* OR TCA-solub* OR TCA-insolub* OR TCA-precipit* OR anogenital-wart*[tiab] OR genital-wart*[tiab] OR “Condylomata Acuminata”[Mh] OR “Human papillomavirus”[tiab] OR “Human papillomavirus 31”[mh] OR “Sexually transmitted diseases”[tiab])</p> <p>General concepts:</p> <p>(Disinfection-ByProduct*[tiab] OR Disinfection-By-Product*[tiab] OR water-disinfect*[tiab] OR disinfected-water[tiab]) OR (“treated water”[tiab] OR “water treatment”[tiab]) AND (“2010”[Date - Entrez] : “3000”[Date - Entrez])</p>

Database	Formatted Search Strings ^a
Scopus	<p>((TITLE-ABS-KEY ("Haloacetic acid*" OR "Dihaloacetic acid*" OR "Trihaloacetic acid*")) OR (TITLE-ABS-KEY ("dichloroacetic acid" OR "79-43-6" OR dichloroacetate OR "dichloroacetic acid" OR "Bichloroacetic acid" OR "Dichloroacetic acid" OR "Dichlorethanoic acid" OR "Dichloroethanoic acid" OR "76-03-9" OR "Trichloroacetic Acid" OR trichloroacetate OR "Trichloroacetic acid" OR "Dibromoacetic acid" OR "631-64-1" OR dibromoacetate OR "Dibromoacetic acid" OR "tribromoacetic acid" OR "75-96-7" OR tribromoacetate OR "tribromoacetic acid" OR "Dichlorobromoacetic acid" OR bromodichloroacetate OR "bromodichloroacetic acid" OR bromodichloroacetic-acid OR "71133-14-7" OR "Dibromochloroacetic acid" OR "5278-95-5" OR bromochloroacetate OR "bromochloroacetic acid" OR "5589-96-8" OR "bromochloroacetic acid" OR bromochloroacetate OR "Chlorobromoacetic acid")) OR (TITLE-ABS-KEY ("Diiodoacetic acid" OR "598-89-0" OR diiodoacetate OR "594-68-3" OR "71815-43-5" OR "Bromiodoacetic acid" OR bromiodoacetate OR "Chloroiodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR chloroiodoacetate)) OR (TITLE-ABS-KEY ("Monochloroacetic acid" OR "Monochloroacetic acid" OR "79-11-8" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Iodoacetic acid" OR "64-69-7" OR "Monoiodoacetic acid" OR monoiodoacetate OR "Monoiodine acetate" OR iodoacetate OR "Bromoacetic acid" OR bromoacetate OR "Monobromoacetic acid" OR "79-08-3")))</p> <p>AND NOT</p> <p>(TITLE-ABS-KEY("trichloro-acetic acid peel*" OR "trichloroacetic acid peel*" OR "trichloroacetic acid peel*" OR "Trichloroacetic-Acid solub*" OR "Trichloroacetic Acid insolub*" OR "Trichloroacetic Acid precipit*" OR "TCA solub*" OR "TCA insolub*" OR "TCA precipit*" OR "anogenital wart*" OR "genital wart*" OR "Condylomata Acuminata" OR "Human papillomavirus*" OR "Sexually transmitted diseases*"))</p> <p>General concepts:</p> <p>(((TITLE-ABS-KEY ("Disinfection ByProduct*" OR "Disinfection By-Product*")) OR (TITLE-ABS-KEY (water W/2 disinfect*))) OR ((TITLE-ABS-KEY (water W/2 treatment* OR "treated water")) AND (PUBYEAR > 2009)))</p>
Web of Science	<p>(TS=("Haloacetic acid*" OR "Dihaloacetic acid*" OR "Trihaloacetic acid*")) OR (TS=("dichloroacetic acid" OR "79-43-6" OR dichloroacetate OR "dichloroacetic acid" OR "Bichloroacetic acid" OR "Dichloroacetic acid" OR "Dichlorethanoic acid" OR "Dichloroethanoic acid" OR "76-03-9" OR "Trichloroacetic Acid" OR Trichloroacetate OR "Trichloroacetic acid" OR "Dibromoacetic acid" OR "631-64-1" OR dibromoacetate OR "Dibromoacetic acid" OR "tribromoacetic acid" OR "75-96-7" OR tribromoacetate OR "tribromoacetic acid" OR "Dichlorobromoacetic acid" OR Bromodichloroacetate OR "bromodichloroacetic acid" OR "bromodichloroacetic-acid" OR "71133-14-7" OR "Dibromochloroacetic acid" OR "5278-95-5" OR bromochloroacetate OR "bromochloroacetic acid" OR "5589-96-8" OR "bromochloroacetic acid" OR bromochloroacetate OR "Chlorobromoacetic acid")) OR (TS=("Diiodoacetic acid" OR "598-89-0" OR Diiodoacetate OR "71815-43-5" OR "Bromiodoacetic acid" OR Bromiodoacetate OR "Chloroiodoacetic acid" OR "Chloro(iodo)acetic acid" OR "53715-09-6" OR "2-Chloro-2-iodoacetic acid" OR "chloro-iodoacetic acid" OR Chloroiodoacetate)) OR (TS=("Monochloroacetic acid"</p>

Database	Formatted Search Strings ^a
	<p>OR "Monochloroacetic acid" OR "79-11-8" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Chloroacetic acid" OR "Iodoacetic acid" OR "64-69-7" OR "Monoiodoacetic acid" OR Monoiodoacetate OR "Moniodine acetate" OR Iodoacetate OR "Bromoacetic acid" OR Bromoacetate OR "Monobromoacetic acid" OR "79-08-3"))</p> <p>NOT</p> <p>(TS=("trichloro-acetic acid peel*" OR "trichloroacetic acid peel*" OR "trichloroacetic acid peel*" OR "Trichloroacetic-Acid solub*" OR "Trichloroacetic Acid insolub*" OR "Trichloroacetic Acid precipit*" OR "TCA solub*" OR "TCA insolub*" OR "TCA precipit*" OR "anogenital wart*" OR "genital wart*" OR "Condylomata Acuminata" OR "Human papillomavirus*" OR "Sexually transmitted diseases*"))</p> <p>General concepts:</p> <p>((TS=("Disinfection ByProduct*" OR "Disinfection By-Product*") OR (TS=(water n/2 disinfect*))) OR (TS=("treated water" OR water NEAR/2 treatment*) AND (PY=(2010-2016))))</p>

^aSearch terms were developed in consultation with an information specialist.