



National Toxicology Program

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Draft Report on Carcinogens Concept Di- and Tri- Haloacetic Acids Found as Water Disinfection By-Products

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Gloria D. Jahnke, DVM, DABT

Project Leader

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

1 Objective and rationale

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 di- and tri-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).

The Office of the Report on Carcinogens (ORoC) plans to evaluate di- and tri-haloacetic acids identified in drinking water for possible listing in the RoC (see table below). These consist of seven chlorine and bromine containing di- or tri-acetic acids either regulated by EPA or being considered for regulation and three iodine containing acetic acids. 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. 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 regulation and on water disinfection processes.

Di- and Tri-Haloacetic Acids (HAA) Present in Drinking Water*

Halogen	Di-HAA	Tri-HAA
Chlorine	Dichloroacetic acid (DCA)	Trichloroacetic acid (TCA)
Bromine	Dibromoacetic acid	Tribromoacetic acid
Iodine	Diiodoacetic acid	
Chlorine and bromine	Bromochloroacetic acid	Bromodichloroacetic acid Dibromochloroacetic acid
Iodine and chlorine or bromine	Bromiodoacetic acid Chloriodoacetic acid*	

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

2 Background

Halogenated by-products are formed when naturally occurring organic matter, such as fulvic and humic acids derived from breakdown of vegetative material, or other organic materials, such as chemical pollutants, react with antimicrobial oxidizing agents or with halides in water (Loper 1980). Disinfectants used for drinking water include chlorine, chloramines, ozone, chlorine dioxide, and ultraviolet radiation all of which can kill or prevent replication of bacteria, viruses, and protozoa in the water supply (Richardson and Postigo 2015). The composition of by-products varies with the source and properties of the water, the disinfection method used, and the type of reactants present in the water.

Awareness of water disinfection by-products and potential health hazards began in the 1970s. Chloroform was identified as a by-product of chlorination of drinking water and shown to be a carcinogen in experimental animals (Rook 1974, NCI 1976). In addition, organic compounds in drinking water were shown to be mutagenic (Loper 1980). To protect public health and standardize U.S. public drinking water, the U.S. EPA established the Safe Drinking Water Act (1974) (EPA 2015b). This act applies to public water systems, not private wells, and regulates physical, chemical, biological or radiological substances or matter in water. U.S. EPA Disinfection Byproducts Rules (Stage 1, 1998; Stage 2, 2006) established maximum contaminant level (MCL) for each location within the water distribution system. Stage 2 requires for a sum of the concentrations of five haloacetic acids (HAA5 = dibromo-, dichloro-, monobromo-, monochloro-, trichloroacetic acids) as a running annual average of below 60 µg/L or 60 ppb and of total trihalomethanes as a running annual average of below 80 µg/L or 80 ppb (EPA 2010). Proposed haloacetic acid monitoring under EPA Fourth Unregulated Contaminant Monitoring Rule will add four additional HAAs (bromochloro-, bromodichloro-, dibromochloro-, tribromoacetic acids) to include the entire HAA9 class (EPA 2015c).

2.1 Human exposure

Based on estimates of U.S. population, water use, and use of chlorine-based disinfection (EPA 2005, 2006, Population Reference Bureau 2015, EPA 2015a, 2015b, American Chemistry Council 2016), over 250,000,000 people in the United States are exposed to

chlorinated drinking water. Haloacetic acids are generally formed at highest levels with chlorine, but they also can be formed by disinfection with chloramines, chlorine dioxide, and ozone (Richardson *et al.* 2007). Water chlorination is overwhelmingly the most commonly used 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, and chloramine (American Chemistry Council 2016). Water chlorination is also the most widely used disinfection process in the European Union (Connell 2016), and Canada (Canadian Chlorine Chemistry Council 2016).

The most common exposure route for disinfection by-products is ingestion of chlorinated drinking water; however, exposure via dermal and inhalation routes (e.g., showering and bathing) has been reported (Richardson *et al.* 2007). Other potential exposure sources include swimming pools and spas that use chlorine for disinfection (Cardador and Gallego 2011, Wang *et al.* 2014). The physical properties of disinfection by-products vary and so may affect absorption. For example, trihalomethanes are relatively more skin permeable than haloacetic acids as the acids are in an ionized form and have poor dermal penetration (Xu *et al.* 2002). In addition, research indicates potential focal sources of exposure such as point-of-use water treatment with iodine (e.g., iodine tinctures and tablets), cooking with iodized table salt and chlorinated tap water, and compounds used in medical imaging, such as iodinated X-ray contrast media (Richardson and Postigo 2015).

Data are limited on human exposure and on the number of haloacetic acids in treated water. Haloacetic acids were measured in the urine of swimmers after one hour of swimming; trichloroacetic acid, dichloroacetic acid and monochloroacetic acids were present at concentrations of ~ 4.400, ~ 2.300, and ~ 0.560 µg/L, respectively (Cardador and Gallego 2011). Dichloroacetic acid exposure to the general population has been reported as 10 to 40 µg/L from chlorine-based disinfection of drinking water and as 10 to 100 µg/L from swimming pool water (IARC 2014a). As part of the U.S. EPA Information Collection Request, occurrence data for HAA5 were collected from 500 treatment plants across the nation; the mean concentration was 23 µg/L for N = 11,251 values (McGuire *et al.* 2002). Concentration ranges of some di- and tri-haloacetic acids from tap water, finished drinking water, and other similar sources are reported in the table below.

Di- or Tri-Haloacetic Acid	Range, µg/L
Dichloroacetic acid	2.15–133
Dibromoacetic acid	0.9–19
Bromochloroacetic acid	BDL ^b –18
Trichloroacetic acid	3.28–13.25 ^a
Bromodichloroacetic acid	5.28–12.2
Dibromochloroacetic acid	BDL ^b –5.37
Tribromoacetic acid	0– approx. 10 ^c

Sources: McGuire *et al.* 2002, HSDB 2009a, 2009b, 2009c, 2009d, 2009e, 2009f, EPA 2011, IARC 2013, 2014a, 2014b.

^aOne study reported a value of 1,000 µg/L which appears to be an outlier.

^bBelow detection limit; detection limit not specified.

^cOne extreme value of ~ 20 µg/L was reported.

2.2 Problem formulation activities and identification of key issues

Problem formulation activities included initial scoping of the literature by an information specialist and discussion with scientists with substance specific expertise. The literature searches involved searching PubMed, and sources of other evaluations. Sorting the literature by chemical compound and evidence stream was done using Health Assessment Workplace Collaborative (HAWC) system of report management.

A preliminary review of the body of literature shows that although this group of chemicals has structural similarities, there are some apparent similarities and differences in metabolism and disposition. Thus, a key issue is whether some di- and tri-haloacetic acids found in drinking water should be evaluated individually, some as a chemical class, or all as a chemical class.

2.3 Concerns for potential carcinogenicity

Based on preliminary literature searches, over 19 reports of cancer studies in rats and mice for haloacetic acids were identified. Several reports had studies in both species (see table below). Unless noted, chemical exposure was through drinking water. Cancer studies were not located for tribromoacetic acid, chlorodibromoacetic acid, bromoiodoacetic acid, diiodoacetic acid, and chloroiodoacetic acid. Some di- and tri-haloacetic acids have been tested in animal cancer studies and most are positive which raises concern for their being a human health hazard.

Species	Dichloro-acetic acid	Trichloro-acetic acid	Dibromo-acetic acid	Bromochloro-acetic acid	Bromodichloro-acetic acid
Rat	3 studies	2 studies	1 study	1 study	1 study
Mouse	9 studies	4 studies	1 study	1 study; 1 study ^a	1 study

^adermal

Human exposure to haloacetic acids is primarily through disinfected drinking water in a mixture of what is estimated to be over 500 chemicals. A limited number of studies in people have assessed the amount of exposure to organic halogenated compounds formed during the disinfection process. Although there are numerous cancer studies in humans on chlorinated drinking water and a few studies on exposure to trihalomethanes, no studies of exposure specifically to di- or tri-haloacetic acids from drinking water were identified.

Although this group of chemicals has structural similarities, there are some apparent similarities and differences in metabolism, disposition, and tumor outcomes which would be addressed in the proposed cancer hazard assessment. For example, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) have been reviewed and potential mechanisms of

carcinogenicity proposed. DCA is genotoxic and is metabolized primarily through two pathways: cytochrome P450 reductive dehalogenation and a glutathione-dependent process through glutathione-S-transferase (GST) zeta1. Proposed carcinogenic mechanisms for DCA as well as some other di-haloacetic acids include the following (IARC 2014a, 2014b):

- DCA is genotoxic and glyoxylate, a metabolite of DCA, is mutagenic.
- Accumulation of toxic metabolic intermediates as dichloroacetic acid inhibits its own metabolism by inactivating GST-zeta1.
- Oxidative stress causing DNA damage.

Trichloroacetic acid (TCA) is primarily excreted via the urine and there is limited evidence from rodent studies of reductive dehalogenation to DCA. Further, TCA does not appear to be genotoxic and it has been proposed that TCA may act via multiple non-genotoxic mechanisms (IARC 2014a, 2014b) such as:

- Global DNA hypomethylation of *c-myc* promoter.
- Oxidative stress-lipid peroxidation and oxidative DNA damage.
- Selective enhancement of Jun-positive cells.
- Activation of PPAR alpha in rodents.

3 Objective and approach for conducting the cancer hazard evaluation

The objective of the NTP evaluation is to determine whether di- and tri-haloacetic acids found in drinking water should be listed in the RoC.

3.1 Cancer hazard evaluation

Based on problem formulation activities, the monograph will focus on (1) cancer studies in experimental animals to determine the level of carcinogenicity of HAAs, and (2) ADME, and mechanistic studies and other relevant data that will inform biological plausibility of any cancer findings in animals and whether the compounds have similar metabolic or mechanistic activities that would permit evaluation as a class. All potential cancer tumor sites will be evaluated.

The literature search for the monograph will include Scopus or Web of Science in addition to PubMed and all literature will be tagged and sorted for relevant articles using HAWC, similar to that described above. Studies will be excluded from consideration if they do not relate directly to an individual chemical or chemical class (for example, the chemical is a component of another chemical), or do not measure cancer endpoints or potential mechanisms of carcinogenicity.

In order to provide a framework for assessment of potential cancer mechanism of haloacetic acids, ORoC has developed literature searches based on the ten properties of carcinogens as reported by Smith *et al.* (2015). Properties include: (1) act as an electrophile either directly or after metabolic activation, (2) be genotoxic, (3) alter DNA

repair or cause genomic instability, (4) induce epigenetic alterations, (5) induce oxidative stress, (6) induce chronic inflammation, (7) induce immunomodulation, (8) modulate receptor-mediated effects, (9) cause immortalization, or (10) alter cell proliferation, cell death, or nutrient supply. Studies identified by these searches will be sorted for each of the characteristics using HAWC. Based on searches in PubMed, 227 mechanistic studies were identified. This mechanistic information plus available information on chemical disposition will also provide a framework for assessment of evaluating the di- and tri-haloacetic acids as a class. Key questions in evaluation of the mechanistic data are:

- What metabolic pathways related to carcinogenesis do these by-products have in common?
- Are there similar biological mechanisms of carcinogenesis among these by-products?
- Are there biological interactions among absorbed disinfection products that affect metabolism?

Metabolic, key events, and mechanistic data will be grouped according to properties of carcinogens and be evaluated as individual chemicals and also across chemical groupings, such as the number and type of halogen substitutions. How best to present these data for comparison will be determined by the available data and input from technical advisors and an information group (see Section 4.1).

The monograph will also review exposure using other evaluations and government sources to determine U.S. and worldwide population exposure to water disinfection by-products. Key questions related to exposure include:

- What are the effects of different water treatment methods (e.g., chlorine, chloramine, and ozone) on formation of haloacetic acids?
- What are data sources for levels of exposure to haloacetic acids, including iodinated and fluorinated forms?

3.2 Scientific and public input

The first step in the process is to establish an evaluation or monograph planning team and an information group to provide input on key questions and on protocol and monograph development; ORoC is soliciting technical support from experts on drinking water by-products. NTP toxicologists and U.S. EPA scientists from Office of Water, National Health and Environmental Effects Research Lab, and National Center for Environmental Assessment, along with ORoC staff, will provide technical expertise on current information on haloacetic acids and carcinogenicity. The information group will provide input on (1) key mechanistic questions listed above, (2) development of an approach and rationale for evaluation of some or all di- and tri-haloacetic acids as a potential class for carcinogenicity, which will be used to develop the protocol, and (3) evaluation of the data for the different haloacetic acids using that approach. ORoC also proposes to use the expertise of NTP laboratories to potentially help fill identified data gaps.

3.3 Protocol development

The protocol will outline literature search strategy, key issues, and focus of the monograph. Literature search strategy and identification of other evaluations will be developed with an information specialist and input from technical advisors. As outlined in the RoC Handbook (2015), exposure information will rely on reviews supplemented by key primary literature; cancer studies in experimental animals and mechanistic studies will rely on primary literature. The protocol will outline the study quality procedures for animal cancer studies and the approach for evaluating di- and tri-haloacetic acids as a class. Mechanistic and other relevant data will be grouped according to properties of carcinogens and be evaluated as individual chemicals and also across chemical groupings, such as the number and type of halogen substitutions. How best to present these data for comparison will be determined by the available data and input from technical advisors and the information group.

3.4 Next steps

Per the process for preparing the RoC, OROc will release the draft RoC monograph on di- and tri- haloacetic acids found as water disinfection by-products for peer review. Similar to the peer review of other candidate substances, the peer reviewers will be asked to comment on whether (1) there is evidence that a significant number of people residing in the United States are exposed and (2) whether the carcinogenicity and mechanistic information in the substance profile is clear, objectively presented, and supports NTP's preliminary listing recommendation.

4 Public health significance

Disinfection of the public water supply is an important public health initiative and has drastically reduced waterborne disease incidence. However, chemical disinfectants used in this process are necessarily powerful oxidizing agents and can react with organic material in drinking water and form disinfection by-products. The class and relative amount of by-products formed depend on a number of factors such as the water source, and method of disinfection. Halomethanes are in the greatest concentration (by weight of total halogenated by-products) and are regulated by the Federal government and inform local and state regulations. Haloacetic acids make up the second largest class by weight of total halogenated disinfection by-products in public water supplies and only five of at least nine are regulated. By evaluating haloacetic acids for cancer hazard and listing key mechanistic and cancer information on these chemicals, the *Report on Carcinogens* will identify chemicals that may be a factor in increasing cancer hazard and help to inform public health decisions on water distribution systems and disinfection processes.

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