National Toxicology Program

Peer Review of Draft Report on Carcinogens (RoC) Monograph on Haloacetic Acids Found as Water Disinfection By-products

July 24, 2017

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

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I. Attendees*

Peer-Review Panel

Weihsueh Chiu (Chair), Texas A&M University
Mathias Attene Ramos, George Washington University
Julia H. Carter, Wood Hudson Cancer Research Laboratory
Lawrence H. Lash, Wayne State University
Shahid Parvez, Indiana University
Consolato Sergi, University of Alberta
Susan C. Tilton, Oregon State University
Stephen M. Roberts, University of Florida

National Toxicology Program Board of Scientific Counselors Liaison

Daniel Kass, Vital Strategies (by webcast)

National Institute of Environmental Health Sciences Staff

Windy Boyd
John Bucher
Michael DeVito
Virginia Guidry
Michelle Hooth
Gloria Jahnke
Ruth Lunn
Suril Mehta
Cynthia Rider
Andrew Rooney
Amy Wang
Mary Wolfe

Report on Carcinogens Contract Support Staff

Whitney Arroyave, Integrated Laboratory Systems (ILS)
Stanley Atwood, ILS
Canden Byrd, ICF
Susan Dakin, Independent Consultant
Andrew Ewens, ILS
Sanford Garner, ILS
Alton Peters, ILS

Public Attendees

Joel Cheny, Israel Chemicals Ltd.

II. Welcome and Introductions

The National Toxicology Program (NTP) Peer Review Panel for the Draft Report on Carcinogens (RoC) Monograph on Haloacetic Acids Found as Water Disinfection By-products convened on July 24 in Rodbell Auditorium, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Weihsueh Chiu served as chair. Dr. Daniel Kass attended by webcast as the NTP Board of Scientific Counselors (BSC) liaison. Representing the NTP were Dr. John Bucher, Associate Director, NTP; Dr. Mary Wolfe, Director, NTP Office of Liaison, Policy, and Review; and Dr. Ruth Lunn, Director, Office of the RoC. Dr. Wolfe served as the Designated Federal Official.

*The meeting was webcast. Individuals who viewed the webcast are not listed except as noted.

Dr. Chiu called the meeting to order at 9:55 a.m., welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. Bucher welcomed the Panel and thanked them for their service. Dr. Wolfe read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Chiu briefed the Panel and the audience on the format for the peer review.

III. Process for Preparing the Draft RoC Monograph

Dr. Ruth Lunn, Division of the NTP (DNTP), presented background information about the RoC and the process and methods used to prepare the Draft RoC Monograph on Haloacetic Acids Found as Water Disinfection By-products. She noted that the RoC is congressionally mandated and identifies substances that pose a cancer hazard for U.S. residents. It is prepared for the Secretary of Health and Human Services (HHS) by the NTP and is cumulative, including substance profiles for newly listed substances and for all substances listed in previous reports.

Dr. Lunn outlined the four-part formal process for preparing the RoC: (1) selection of substances for evaluation, (2) preparation of draft RoC monographs, (3) peer review and finalization of the RoC monographs, and (4) approval of the substance profiles by the HHS Secretary and release of the RoC. The process incorporates public comment, scientific input, and peer review of the scientific information.

Dr. Lunn outlined the steps of the process that had been completed for selection and evaluation of haloacetic acids (HAAs) found as water disinfection by-products. A literature search was conducted, and a public request for information in October 2015 received no responses. A draft concept document explaining the rationale and proposed approach for the review was released for public comment in March 2016 and presented to the BSC in April 2016. The decision to evaluate HAAs found as water disinfection by-products for listing in the RoC was based on widespread exposure of the U.S. population via drinking water, concerns about toxicity in humans, and the availability of a database with a large number of cancer studies in animals and mechanistic data. Dr. Lunn noted that two trihalomethane (THM) water disinfection by-products (chloroform and bromodichloromethane) were already listed in the RoC, but that none of the HAAs had been reviewed.

A protocol was developed for preparing the Draft RoC Monograph, specifying the methods for evaluating study quality, addressing issues, and integrating data. An informational group of external and government scientists was convened to discuss approaches for determining whether HAAs could be evaluated as a chemical class or as subclasses. The objective of the monograph was to evaluate the relevant scientific information, assess its quality, apply RoC listing criteria to the information, and reach a listing recommendation. Thirteen individual HAAs were evaluated, as well as the possibility of evaluating HAAs as a class or as subclasses.

Dr. Lunn provided a brief overview of the monograph contents. She emphasized that the evaluation of whether a significant number of persons residing in the U.S. were exposed to these haloacetic acids is a scientific judgment as to whether the information in the draft monograph supports that conclusion and not a formal exposure assessment. She noted that the information presented in monograph on chemical properties, disposition and toxicokinetics, and mechanistic and other relevant data was used to inform the class and subclass assessments, which used read-across-like approaches; collectively all this information was integrated in the overall cancer hazard evaluation. Dr. Lunn reviewed the RoC criteria for listing a substance as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen,* and, in particular, the

criteria for the level of evidence (sufficient or not sufficient) from studies in experimental animals. She emphasized that the listing recommendations are based on scientific judgment considering all relevant information.

Dr. Lunn said the draft monograph would be revised based on the NTP's review of the peer-review comments. The revised monograph, the peer-review report, and the NTP's response to the peer-review review report would be provided to the BSC, after which the monograph would be finalized.

The charge to the Peer-Review Panel was as follows:

- Comment on whether the Draft RoC Monograph on Haloacetic Acids Found as Water Disinfection By-products is technically correct, clearly stated, and objectively presented.
- Provide an opinion on whether there is currently or was in the past significant human exposure to haloacetic acids found as disinfection by-products.

The Panel would be asked to vote on the following questions:

- Whether the scientific evidence supports the NTP's conclusions on the level of evidence for carcinogenicity from cancer studies in animals for six individual haloacetic acids found as water disinfection by-products.
- Whether the scientific evidence supports the NTP's preliminary policy decision on the listing status of six individual haloacetic acids found as water disinfection by-products.

IV. Public Comments

IV.A. Written Public Comments

The NTP received written public comments on the draft monograph from the Environmental Working Group and the American Chemistry Council. The comments from the American Chemistry Council incorporated by reference that organization's previous comments on the draft concept document. The written comments were posted to the meeting webpage and distributed to the Panel prior to the meeting.

IV.B. Oral Public Comments

No oral public comments were made.

V. Peer Review of the Draft RoC Monograph on Haloacetic Acids Found as Water Disinfection By-products

V.A. Cancer Evaluation Component

V.A.1 Properties and Human Exposure

V.A.1.1 Presentation on Properties and Human Exposure

Dr. Sanford Garner, ILS, presented an overview of the key information in the draft monograph on properties and human exposure. Thirteen HAAs have been identified in disinfected water, which differ by number and size(s) of substituted halogen atoms. Five of these (the HAA5) are

regulated in drinking water by the U.S. Environmental Protection Agency (EPA): monochloroacetic acid (MCA), monobromoacetic acid (MBA), dichloroacetic acid (DCA), dibromoacetic acid (DBA), and trichloroacetic acid (TCA). The other HAAs found in drinking water are bromochloroacetic acid (BCA), tribromoacetic acid (TBA), bromodichloroacetic acid (BDCA), chlorodibromoacetic acid (CDBA), monoiodoacetic acid (MIA), diiodoacetic acid (DIA), chloroiodoacetic acid (CIA), and bromoiodoacetic acid (BIA).

The biological reactivity of HAAs varies with the number and size(s) of substituted halogen atoms. The bioavailability of HAAs is expected to decrease with decreasing negative log of the acid dissociation constant (pK_a), which decreases with decreasing number of halogen atoms. The reactivity of HAAs may decrease with decreasing energy of the lowest unoccupied molecular orbital (E_{LUMO}), which decreases with decreasing number of halogen atoms and with the addition of less electrophilic halogens within the mono-, di-, and tri-HAAs.

A significant number of U.S. residents are exposed to HAAs found as water disinfection by-products, based on exposure of over 80% of the population to disinfected water from community drinking-water systems. Less common exposure routes are through dermal contact with or inhaling vapors from water in swimming pools and spas, ingestion of foods cooked or prepared with disinfected water, and ingestion of water treated with iodine tables for point-of-use disinfection. Although large community water treatment facilities are now compliant with EPA's HAA5 maximum contaminant level, smaller facilities have higher median HAA5 levels, with 95th percentile levels sometimes exceeding the maximum contaminant level. Factors affecting the types and amounts of HAAs formed in disinfected water include temperature, pH, choice of disinfecting chemical, and duration of treatment. Remediation methods include removal of precursors from the source water, selection of disinfection methods that produce lower levels of by-products, or removal of by-products from the treated water.

V.A.1.2 Peer-Review Comments on Properties and Human Exposure

Dr. Shahid Parvez, first reviewer, stated that the draft monograph described the key physical and chemical properties of HAAs in great detail, and that the details were backed by peer-reviewed publications. However, he suggested citing peer-reviewed studies to support the discussion of physical-chemical properties likely to be related to the toxicity of HAAs. He offered to provide some key references, which were not essential for reaching conclusions, but would strengthen the monograph.

Dr. Parvez found the temporal analysis of HAA exposure to be comprehensive and representative of U.S. drinking-water levels of HAAs. However, he questioned the percentages cited for THMs and HAAs as shares by weight of total halogenated disinfection by-products found in public water supplies (page ii, 1st paragraph). He said the numbers were too high for U.S. water systems and did not reflect variation due to varying concentrations of organic molecules in source water or type and concentration of disinfecting chemicals. He suggested instead citing a range based on EPA's Nationwide DBP Occurrence Study.

Dr. Parvez suggested mentioning the potential effect of the use of filters on HAA exposure, though he acknowledged that little literature is available on the subject. He mentioned his own unpublished pilot study showing that the use of commercially available filters significantly reduced the levels of regulated THMs and HAAs. Discussion of this issue would not change the conclusions about exposure, but would strengthen the monograph. Dr. Parvez was pleased to see a detailed discussion of non-drinking-water sources of exposure to HAAs. He suggested that the

monograph specify the categories of medications that contain halogens (such as anti-depressants) and acknowledge the importance of considering the dermal and inhalation routes (including exposure through the use of cleaning products and disinfectants) in estimating exposure. He noted that the discussion of HAA remediation methods was accurate and backed by scientific studies.

Dr. Matias Attene Ramos, second reviewer, found the description of the chemical properties of HAAs to be thorough and correct. However, he noted that E_{LUMO} does not always correlate with the toxicity properties of HAAs (as stated in the last paragraph of page 3). It correlates well for brominated and iodinated HAAs, but with chlorinated HAAs, the difference in toxicity is much greater than the difference in E_{LUMO}. The same point applies to most of the molecular descriptors of polarizability or soft electrophilicity. He noted that the discussion of the correlation between THM and HAA levels (Section 2.7.2) supported the notion that THM levels are not a good surrogate for HAA exposure in epidemiological studies. In reference to Table 2-3, Dr. Attene Ramos noted that removal of HAAs after formation by filtration using biologically active granular activated charcoal works very well. He asked whether data were available on how commonly this remediation method is used, especially in places with high levels of formation of brominated and iodinated HAAs.

V.A.1.3 Panel Discussion on Properties and Human Exposure

Dr. Consolato Sergi agreed that the discussion of human exposure was well done and comprehensive. He suggested that the monograph mention daily variation in HAA exposure levels. Levels of disinfection by-products are probably higher in the morning than in the afternoon, which could be important for epidemiological studies. Dr. Julia Carter commented that disinfection by-products will also show seasonal variation, due to seasonal variation in the occurrence of organic molecules in the source water. Dr. Parvez agreed that HAA levels show huge temporal variability, but he considered that for the end point of cancer, long-term exposure was more important.

Dr. Susan Tilton asked whether spatial and geographic differences in the presence of organic material and halogens in source water contributed as much as the choice of disinfection method to variation in exposure to disinfection by-products. Dr. Parvez said that information on spatial variability could be important for evaluation of cancer risk, especially for exposure in coastal areas, because of seepage of bromine- and iodine-rich seawater.

With respect to the use of remediation by filtration of HAAs, Dr. Garner said that no data had been found, but that this would be explored further.

Summarizing the peer-review comments, Dr. Chiu said the monograph sections on Properties and Human Exposure were clear and technically accurate overall. A few studies on properties and exposure could be added for completeness, but would not affect the conclusions. It was noted that E_{LUMO} was useful for explaining toxicity properties within classes of HAAs, but not across chlorinated, brominated, and iodinated HAAs, and was less useful for chlorinated HAAs than for the other classes. The data suggest that THMs are not good surrogates for HAAs in epidemiologic studies. Overall, the panel agreed that there was widespread exposure to HAAs across the United States and concurred with the statement that a significant number of persons living in the United States are exposed to HAAs found as water disinfection by-products.

V.A.2 Studies of Cancer in Experimental Animals

V.A.2.1 Presentation on Studies of Cancer in Experimental Animals

Dr. Gloria Jahnke, DNTP, presented an overview of the key information in the draft monograph on studies in experimental animals. Data from chronic carcinogenicity studies, studies with transgenic animals, or co-carcinogen studies were available for six HAAs (MCA, DCA, DBA, BCA, TCA, and BDCA).

Nineteen publications met the inclusion criteria for relevance to the carcinogenicity hazard evaluation. Five types of signaling questions were used to assess the utility of the studies for informing the hazard evaluation: study design/population, exposure conditions, outcome measurement and assessment, confounding, and analysis and reporting. Responses to the questions were reported in terms of degree of concern and reason for concern, and overall study utility was rated as high, moderate, low, or inadequate. The most informative studies were those of chronic carcinogenicity. Studies were conducted on mono-, di-, and tri-HAAs, in both mice and rats, and in both sexes. The bulk of the studies were on DCA and TCA. At least one study of high utility was found for each of the HAAs evaluated. Initiation-promotion studies on DCA and TCA and studies on DCA in transgenic animals provided some supporting information.

The primary tumors observed were liver tumors. MCA caused no increased tumor incidence (liver or otherwise) in rats or mice of either sex. DCA and TCA caused hepatocellular carcinoma (HCC) in mice of both sexes. In male rats, DCA caused HCC, but no increased incidence of tumors were observed with TCA. DBA, BCA, and BDCA caused HCC in mice of both sexes and hepatoblastoma primarily in male mice, but no tumors in male rats. DBA, BCA, and BDCA were not carcinogenic in female rats (TCA was not tested in female rats). HCC incidence in male mice showed significant dose reponses with both chlorinated and brominated HAAs. Drinking-water exposure to DBA, BCA, and BDCA also caused tumors at sites in addition to the liver; all three caused malignant mesothelioma in male rats, and BCA and BDCA both caused mammary-gland fibroadenoma in female rats. DBA caused mononuclear-cell leukemia in female rats and bronchioalveolar adenocarcinoma in male mice, BCA caused adenoma of the large intestine (rare, progressive tumors) in male and female rats, and BDCA caused several types of skin tumors in male rats and tumors of the Harderian gland in male mice.

Dr. Stephen Roberts asked for clarification on the exclusion of non-peer-reviewed studies. Dr. Jahnke explained that non-peer-reviewed studies were not automatically excluded, but to be included in the evaluation, they had to be newly peer-reviewed. In this case, the data from one of the two excluded non-peer-reviewed publications were also reported in a later, peer-reviewed publication, so the data were considered. The other non-peer-reviewed publication was not considered useful enough to justify peer-reviewing it for inclusion. Dr. Lunn noted that the complete literature search strings could be found in the protocol for preparing the draft monograph.

With respect to the final table in Dr. Jahnke's presentation, which summarized the tumor sites observed in experimental animals exposed to each HAA, Dr. Sergi noted that it would be clearer to identify all tumor sites by the name of the organ or tissue, rather than some by tumor type. Dr. Tilton asked whether the absence of results for cancer other than liver tumors for a given HAA meant that none of the studies had evaluated extrahepatic tumors or that no extrahepatic tumors had been found. Dr. Jahnke clarified that for each HAA, each of the tumor sites listed had been evaluated in at least one study. Dr. Parvez suggested that statistical significance be

indicated in a footnote. Dr. Jahnke noted that all results shown in the summary table were statistically significant except those for the large intestine, which were included because of this tumor's rarity.

V.A.2.2 Peer Review Comments on Studies of Cancer in Experimental Animals

Dr. Roberts, first reviewer, said that the scientific information from cancer studies in experimental animals was clearly and objectively presented and scientifically correct. He stated that the monograph presented the appropriate amount of information and that Appendix C was useful for those wanting more detail. He concurred with Dr. Tilton that tables summarizing results of cancer studies need to differentiate between negative results and the absence of results. He agreed that the approach to assessing the utility of animal carcinogenicity studies was systematic, transparent, and objective.

Dr. Sergi, second reviewer, said that the scientific information provided was clear, technically accurate, and factually presented. Study quality and sensitivity were expertly summarized and presented systematically and objectively, and he agreed with the overall evaluations of study quality. Dr. Sergi suggested adding information on which studies used step-sectioning and of which organs; this may be especially important for the kidney in full necropsy studies. The monograph should note whether gross examinations were made of the surfaces of the urinary bladder, ureters, and renal pelvis, with a magnifying lens or by naked eye, bilaterally or unilaterally. He was pleased to see the statement of concerns about the use of Tg.AC hemizygous mice.

Dr. Sergi commented that hepatoblastoma is a highly malignant tumor originating in embryonal precursor cells, and that its occurrence in male and female mice exposed to brominated HAAs may be relevant to HAA exposure of infants via formula made with drinking water, as well as for future studies on the effects of embryonic exposure during pregnancy. He also stressed the importance of identifying the type of mesothelium in which tumors were seen; their occurrence only in peritoneal (not pleural) mesothelium is related to the route of administration. Finally, he noted that fibroadenoma of the mammary gland and adenoma of the large intestine should not be given equal weight in the synthesis, as fibroadenoma progresses to malignancy much more slowly than does adenoma of the large intestine.

Dr. Julia Carter, third reviewer, found the review of cancer studies in animals to be very well done, and she particularly appreciated the evaluation of each study's utility. She noted that the age of the animals at exposure in the drinking-water studies was not specified, and that age is important; for example, animals are more susceptible to carcinogenesis during puberty than as adults. Dr. Jahnke clarified that in most studies the rats and mice were 6 to 8 weeks old — young, but of reproductive age. Dr. Lunn mentioned that in a stop-exposure study of DCA in younger animals, tumor incidence was similar to that seen in the chronic-exposure studies.

Dr. Carter suggested that it would be helpful to have a checklist of organs that are important in human cancer (such as the ovary and uterus) indicating whether carcinogenesis of these organs has been studied in animals. She found it striking that BDCA had the strongest association with neoplasms, in that it caused tumors in the largest number of different sites.

V.A.2.3 Panel Discussion of Studies of Cancer in Experimental Animals

Dr. Sergi agreed with Dr. Carter on the importance of looking at potential effects of HAAs on the ovary and uterus, as well as the influence of age at exposure. Given concerns about kidney or urinary-bladder cancer in humans exposed to HAAs, it is important to note the primordial connection between the urinary and reproductive systems. He suggested that future studies look at the effects of HAAs in newborns and on embryogenesis. On the issue of timing, Dr. Carter noted that fibroadenomas occur earlier in women than in mice and rats, and that the timing of some lesions can differ between women and men and between male and female mice and rats.

Summarizing the peer-review comments, Dr. Chiu said the monograph section on Studies of Cancer in Experimental Animals was clear, technically correct, and objectively presented, in both assessment of the studies' utility and presentation of the findings. Overall, the section was well done, presenting the right levels of detail in the main text and the appendices. However, in tables, it would be useful to clarify which organs were not examined in a given study. The histological evaluation methods should be described in more detail. Discussion could be added on the potential relevance of hepatoblastomas, particularly for developmental exposure. The specific site of the mesothelioma should be identified, and more detail added about the progression rates of tumors of the mammary gland and large intestine. The ages of animals at exposure should be specified, and the potential importance of other organs, such as the ovary and uterus, for future research could be discussed.

The meeting was recessed at 11:40 a.m. and reconvened at 12:30 p.m.

V.A.3 Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

V.A.3.1 Presentation on Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

Mr. Stanley Atwood, ILS, presented an overview of the key information in the draft monograph on disposition and toxicokinetics and on mechanistic and other relevant data. HAAs are rapidly and extensively absorbed through the gastrointestinal tract, and inhalation and dermal exposure are limited. HAAs are distributed rapidly and uniformly to the tissues, with little or no bioaccumulation. Known metabolic pathways and metabolites of HAAs are similar in humans and rodents, but not all pathways have been completely described. The extent of metabolism is greater in di- than tri-HAAs and in brominated than chlorinated HAAs. Bioavailability is lower for di- than tri-HAAs, and first-pass metabolism is higher for brominated than chlorinated HAAs.

Tri-HAAs are metabolized primarily by cytochrome P450 reductive dehalogenation, but 20% to 80% is excreted unmetabolized in the urine. Di-HAAs are metabolized primarily by glutathione S-transferase zeta (GST-ζ) to glyoxylic acid (whose products are primarily excreted in the urine) or to carbon dioxide. A minor pathway for di-HAAs is P450 reductive dehalogenation to mono-HAAs, which are primarily excreted in the urine.

Evaluation of potential mechanisms of carcinogenicity was based on the ten key characteristics of carcinogens defined by Smith *et al.* (2016), focusing especially on electrophilic properties, induction of oxidative stress, and genotoxicity, which were also evaluated for trends according to number and size of halogens.

All HAAs are relatively soft electrophiles, able to bind to proteins. The mono-HAAs have been shown to inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH), thus reducing adenosine triphosphate (ATP) production. Di-HAAs, particularly DCA, block pyruvate dehydrogenase kinase (PDK), thus enhancing oxidative phosphorylation. E_{LUMO} plus pK_a has been shown to correlate with cytotoxicity, oxidative stress, and genotoxicity of HAAs, and GAPDH inhibition rate correlates with E_{LUMO}, cytotoxicity, and genotoxicity among the mono-HAAs.

All HAAs induce oxidative stress, which may be generated by metabolism via reductive dehalogenation, disruption of energy metabolism, GST-ζ inhibition, activation of the transcription factor Nrf2/antioxidant responsive element (Nrf2/ARE) pathway, oxidative damage to DNA, lipid peroxidation, and peroxisome-proliferator-activated receptor alpha (PPARα) activation. In studies of oxidative damage, the potency of HAAs decreased with increasing number of halogens and increased with increasing halogen atom size. Most HAAs have been shown to be genotoxic or mutagenic. The evidence is growing that the DNA damage induced by HAAs is associated with oxidative stress.

Other key characteristics of carcinogens that have been associated with at least one of the HAAs include hypomethylation (DCA, DBA, and TCA), which was correlated with carcinogenicity and tumor promoting activity by DCA and TCA; alteration of energy metabolism, though inhibition of GAPDH by mono-HAAs and PDK inhibition by DCA; PPARα activation by TCA; and cell transformation by DBA and MIA.

V.A.3.2 Peer-Review Comments on Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

Dr. Lawrence Lash, first reviewer, found the sections on absorption and distribution to be clear, but the metabolism section a bit difficult to follow. He suggested highlighting key points in a summary table or chart similar what was provided in the presentation. Overall, he found the section to be technically correct and objectively presented.

Dr. Lash found the section on mechanistic and other relevant data to be clear, technically correct, concise, and objectively and systematically presented. However, one statement (second sentence of the last paragraph on page 73) should be corrected or clarified: it is not true that "all potential modes of action are relevant to humans," as, for example, peroxisome proliferation is not generally considered relevant in humans. He suggested that the diversity of the mechanisms and some of the species differences should be made clearer.

Dr. Attene Ramos, second reviewer, agreed that the discussion of absorption, distribution, and metabolism was complete, but that a summary of key points about metabolism would be helpful. He found the mechanistic data to be complete, but raised some minor issues. With respect to Table 6-1 and the related text, he commented that the experimental design of the studies by Stalter *et al.* (2016) did not provide a basis for statements about differential binding affinity for DNA or protein. Although oxidative stress is clearly involved in HAA toxicity, based on observations of oxidative damage and results in ARE cell lines, this does not prove the formation of reactive oxygen species, which Dr. Ramos thought had been directly shown in only one study. He found the integration of the data to be logical and clear, showing that more than one molecular initiating event might be involved in HAA carcinogenesis.

V.A.3.3 Panel Discussion on Disposition and Toxicokinetics and Mechanistic and Other Relevant Data

Dr. Lash characterized the point about electrophilicity vs. oxidative stress as a "chicken-and-egg" argument, as the action of binding to reductants could cause oxidative stress; the result would be the same as that of a direct oxidation process.

Given the involvement of HAAs in disruption of energy metabolism, Dr. Sergi suggested adding a discussion of mitochondrial DNA mutations as a potential mechanism of liver carcinogenicity. Although data on mitochondrial DNA mutations are not available for HAAs, the plausibility of the mechanism could be discussed in the light of other studies on liver carcinogenesis.

Summarizing the peer-review comments, Dr. Chiu said the monograph section on Disposition and Toxicokinetics was reasonably concise, clear, technically correct, and objectively presented, except that the metabolism section could provide a clearer synthesis to highlight the main points. The section on Mechanistic and Other Relevant Data was concise, systematically presented, and technically correct. However, details could be added to the discussions of (1) PPARα and its relevance to humans, (2) the implications of study by Stalter *et al.* for conclusions about binding of HAAs, and (3) the molecular initiating event(s) for oxidative stress. A general discussion could be added on the potential role of mitochondrial dysfunction as a mechanism of liver cancer.

V.A.4 Evaluation of Haloacetic Acids as a Class or Subclass(es)

V.A.4.1 Presentation on Evaluation of Haloacetic Acids as a Class or Subclass(es)

Mr. Atwood presented an overview of the key information in the draft monograph on evaluation of HAAs as a class or subclass(es). To determine whether all 13 HAAs could be evaluated as a class, a read-across-like analysis was conducted. Potency to cause events involved in toxicity increased with decreasing number and increasing size of halogens, and these trends were related to the compounds' chemical properties. However, they were not consistently related to estimates of carcinogenic potency. The analysis was limited by the lack of a well-defined mechanism for HAA carcinogenicity and the lack of a potency metric for cancer. Published QSAR models (based on pK_a and E_{LUMO}) also successfully predicted the potency of 12 HAAs in causing oxidative stress and genetic damage in cultured mammalian cells and the potency of 10 HAAs in causing neural-tube defects in mouse embryo cultures, but failed to predict the carcinogenicity of HAAs in animals. In particular, in dose-response modeling of carcinogenic potency, DCA and TCA were predicted to be at least as potent carcinogens as the brominated HAAs, and MCA was predicted to be carcinogenic.

The same approach was then used to consider seven potential subclasses based on halogen number and size. The data were insufficient to support a read-across analysis for any subclasses.

The potential carcinogenicity of CDBA and TBA, for which no data on cancer in animals were available, was evaluated through a read-across approach within the tri-HAAs. CDBA and TBA are metabolized to known animal carcinogens (BCA and DBA); no other microsomal metabolites have been detected; and the physicochemical, toxicokinetic, and toxicological data support their similarity to other brominated HAAs known to cause cancer in animals. CDBA and TBA are predicted to cause tumors in both rats and mice, and their biological effects are directly relevant to humans.

V.A.4.2 Peer Review Comments on Evaluation of Haloacetic Acids as a Class or Subclass(es)

Dr. Tilton, first reviewer, said she believed that the use of the read-across-like approach to predicting carcinogenicity was justified based on correlation of the physical-chemical properties of HAAs with oxidative stress and genotoxicity end points linking key events potentially leading to carcinogenesis. These effects increased with halogen size and decreased with degree of halogenation. However, data to evaluate this trend across the HAAs were lacking for a number of other mechanistic end points, and other end points tended to organize based on characteristics other than halogen size and number. Dr. Tilton appreciated the way in which the end points for comparison were comprehensively summarized in Table 7-1, but since not all of the mechanistic data from Section 6 were used in the analysis, it would be helpful to provide a rationale for inclusion or exclusion of end points. It was not clear whether correlations between animal carcinogenicity and mechanistic end points were made quantitatively; inclusion of correlation values would be helpful.

The mechanistic data suggest that different or multiple mechanisms contribute to carcinogenicity among the potential HAA subclasses, whereas the read-across approach was based on the assumption of a common mechanism across all subclasses. When more mechanistic data are available across the HAAs, it might be possible to use approaches that can evaluate multiple mechanisms, or at least different mechanisms across subclasses, in predicting cancer outcomes.

Dr. Tilton agreed with the NTP's conclusion that the available data are inadequate to evaluate the carcinogenicity of HAAs as a class. She noted in particular that MCA did not cause cancer in rodents at the concentrations tested, but induced genotoxicity or oxidative stress to at least the same degree as the carcinogenic HAAs. Improved knowledge is needed of some of the toxicokinetic parameters and cancer outcomes, specifically for the iodinated HAAs and for MCA, as well as an expanded suite of mechanistic end points across all of the HAAs. The main limitations of the approach are the overall lack of data for mono-HAAs and the lack of correlation between *in vitro* toxicity studies and liver cancer data, particularly for MCA. Dr. Tilton agreed with the NTP's conclusion concerning evaluation of HAAs as subclasses for similar reasons. She also supported the NTP's approach to evaluating the carcinogenicity of CDBA and TBA and agreed with the conclusions.

In response to Dr. Tilton's comments on Table 7-1, Mr. Atwood noted that it included studies that compared at least three HAAs. A number of these studies looked at the mono-HAAs, for which the molecular initiating event appears to be GAPDH inhibition; however, no prototypical GAPDH inhibitor has been shown to be related to carcinogenesis. Understanding of the potential role of GAPDH inhibition in carcinogenesis is a data gap. Dr. Bucher expressed interest in hearing any suggestions Dr. Tilton might have about a quantitative method for correlating mechanistic end points with animal carcinogenicity.

Dr. Roberts, second reviewer, said that it was not clear to him how the NTP's approaches could be used to arrive at a listing recommendation of *reasonably anticipated to be a human carcinogen* for CDBA and TBA based on the language of the criteria for listing when there is less than sufficient evidence of carcinogenicity. He noted that no HAAs were listed in the RoC, and that the mechanisms by which HAAs cause cancer are not known. He interpreted the final paragraph, below the listing criteria, as guidance on applying the stated criteria for *reasonably anticipated to be a human carcinogen*, not as an additional way to satisfy the criteria. Aside from

any scientific issues, he therefore did not see how the listing criteria were satisfied for CDBA and TBA, and said that more explanation was needed in the monograph as to how the listing recommendation was reached. He also suggested that the wording of the criteria be clarified to address similar situations in the future.

Dr. Lunn noted that there is a precedent for substances being listed on the basis of being metabolites of listed substances. Dr. Bucher stated that since the basis for listing was the metabolism of CDBA and TBA to metabolites of other HAAs for which there is sufficient evidence of carcinogenicity from animal studies, their listing recommendations would depend on the order of voting. If the panel first voted to list the HAAs with animal cancer data as *reasonably anticipated to be human carcinogens*, that would provide the basis for listing their metabolites. However, this would be up to the Panel; if the Panel did not agree, then listing of CDBA and TBA would have to be brought up at a future meeting.

Dr. Roberts agreed that the proposed approach was more expeditious, though it conflicted with the wording in the criteria, which refers to listing of class members in a "previous" RoC. Dr. Lash responded that the proposed listing was based not on membership in a class, but solely on metabolism to substances for which there was sufficient evidence for listing. Dr. Roberts agreed with the argument for listing CDBA and TBA, but suggested that it might not be clear to all readers how the criteria were met, and that the wording of the criteria could be adjusted to make this an explicit criterion. Dr. Chiu said he had understood the final clause of the third criterion to apply in this case, given that "mechanisms" could include metabolism to substances for which there is evidence of carcinogenicity. Dr. Roberts suggested that since the evidence that can be brought to bear on evaluation of carcinogenicity is evolving, it might be time for the criteria to evolve, to recognize new approaches.

Dr. Roberts commented that finding the evidence for carcinogenicity of TCA insufficient despite the carcinogenicity of its metabolite DCA would appear to be inconsistent with the argument made for CDBA and TBA that a substance with a carcinogenic metabolite would itself be carcinogenic. He suggested providing further explanation of the rationale for the conclusions about TCA. Specifically, the toxicokinetic data showed that TCA was metabolized to DCA to a much smaller extent than TBA and CDBA were metabolized to their di-HAA metabolites. The case for the carcinogenicity of TBA and CDBA also would be strengthened by an explanation of how the situation with TCA differed. Dr. Carter noted that TCA was carcinogenic in mice, but by a mechanism not relevant to humans.

Dr. Roberts agreed with the approaches used to evaluate HAAs as a class or subclass(es) and concurred with the NTP's conclusions that the data were inadequate for those purposes, for the reasons clearly articulated in the monograph.

Dr. Attene Ramos, third reviewer, agreed that the NTP's approach to evaluation was logical, but that the data were insufficient to evaluate HAAs as a class or subclass(es).

V.A.4.3 Panel Discussion of Evaluation of Haloacetic Acids as a Class or Subclass(es)

Dr. Lash returned to the issue of seeming inconsistency in the evaluations of TCA vs. TBA and CDBA. The key difference is probably in the extent of metabolism and accumulation of metabolites, but this was not clearly enunciated in the monograph, especially in the case of TCA. He noted also that the differences between the mutagenesis profiles of TCA and DCA indicated that the effects observed with exposure to TCA were not due to DCA, which again was not

brought out in the monograph. He said that the NTP's approach to evaluation of HAAs as a class may be useful if certain conditions are met, such as adequate data on metabolism.

Dr. Chiu said that problems with artefactual formation of DCA may have affected the results of studies from the late 1990s and early 2000s; he was not sure the issue had been fully resolved. He suggested citing a study by Bradford, Kim, *et al.* from the late 2000s comparing trichloroethylene metabolism in several strains of mice; the amounts of DCA formed were very small, placing an upper limit on the amount of DCA that could be formed from TCA. Dr. Lash emphasized that the monograph should more fully explain why an extrapolation was not made from the carcinogenicity of DCA to that of TCA. Dr. Carter raised the issues of whether dose-response or threshold effects in metabolism might be involved and whether compounds were acting as promoters, rather than initiators. Dr. Chiu commented that it was unclear to what extent mouse liver tumors caused by TCA could be attributed to PPARα activation, given that there has been no knockout study of TCA carcinogenesis.

In summary, Dr. Chiu noted that the main issue discussed was how the RoC listing criteria were applied to the two HAAs without cancer data. Additional explanation is needed of how the approach taken to evaluating CDBA and TBA in the monograph is consistent with the language of the listing criteria. The monograph should also provide a clearer explanation as to why the approach taken with CDBA and TBA would not apply to TCA. Thought should be given to updating the listing criteria to reflect advances in the science with respect to using analogues and read-across approaches. The Panel agreed with the NTP's conclusions that HAAs could not be evaluated as a class or subclass(es), although subclass evaluations might be possible in the future with more data on mechanisms.

The meeting was recessed at 2:07 p.m. and reconvened at 2:19 p.m.

V.A.5 Overall Cancer Evaluation and NTP's Preliminary Listing Recommendations

V.A.5.1 Overall Cancer Evaluation

Presentation, Peer-Review Comments, Panel Discussion, and Level of Evidence of Human Cancer Studies

Dr. Jahnke presented an overview of the key information in the draft monograph on cancer studies in humans. She noted that the available data from epidemiological studies are inadequate to evaluate the relationship between human cancer risk and exposure specifically to individual HAAs or HAA as a class, or subclasses of HAAs. One recent cohort study that evaluated the association between HAA exposure and kidney cancer risk, using estimated HAA5, TCA, BCA, and DCA concentrations, found no increased risks. Several case-control studies found that exposure to chlorinated water or THMs (which may be surrogates for chlorinated water) was associated with increased the risk of urinary-bladder cancer; one study showed that this association varied by polymorphisms in *CYP* and *GST* genes; however, these studies cannot evaluate effects for specific disinfection water by-products.

Dr. Sergi, first reviewer, noted the potential biases in the cohort study, specifically, the lack of control for exposure to other contaminants (e.g., through occupational exposure, which is important for kidney cancer) and the lack of individual exposure measurements, which caused him to question its relevance. The study's weaknesses could be stressed more, but the information was technically accurate and factually presented.

Dr. Lash, second reviewer, was not clear on the rationale for mentioning the one Spanish case-control study of THMs. He also objected to the statement that this study found a "non-statistically significant increased risk," as there is no such thing.

Dr. Carter mentioned a study by Richmond (in the *Southern Medical Journal*) investigating colorectal cancer in Kentucky which found a higher incidence of cancer in urban areas, which used chlorinated drinking water from the Ohio River, than in rural areas, which used water mainly from cisterns and wells.

Dr. Sergi asked why a "submitted" (non-peer-reviewed) journal article had been cited (Seidel *et al.* 2017) and how it had been obtained prior to publication. Dr. Bucher clarified that non-peer-reviewed sources were acceptable for data on exposure (though not for data on which conclusions about carcinogenesis were based). Dr. Garner noted that the unpublished data were shared by Dr. Seidel during preparation of the draft monograph.

Action

Dr. Roberts moved, Dr. Sergi seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the available data from epidemiological studies were inadequate to evaluate the relationship between human cancer risk and exposure specifically to haloacetic acids (either as a class or as individual haloacetic acids).

Level of Evidence of Animal Cancer Studies

Dr. Jahnke briefly summarized the results of the animal cancer studies. Dr. Chiu noted that the Environmental Working Group in its written comment said the level of evidence of carcinogenicity for TCA from studies should classified as "sufficient," consistent with the evaluation by the California Office of Environmental Health Hazard Assessment.

Actions

Dr. Roberts moved, Dr. Sergi seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of bromochloroacetic acid from studies in experimental animals was sufficient, based on the occurrence of tumors in two species and at multiple sites in one species.

Dr. Roberts moved, Dr. Tilton seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of bromodichloroacetic acid from studies in experimental animals was sufficient, based on the occurrence of tumors in two species at multiple sites.

Dr. Lash moved, Dr. Attene Ramos seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of dibromoacetic acid from studies in experimental animals was sufficient, based on the occurrence of tumors in two species at multiple sites.

Dr. Attene Ramos moved, Dr. Tilton seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of chloroacetic acid from studies in experimental animals was not sufficient, because no tumors were reported.

Dr. Carter moved, Dr. Tilton seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of dichloroacetic acid from studies in

experimental animals was sufficient, based on the occurrence of tumors in two species at one site (liver).

Dr. Roberts moved, Dr. Sergi seconded, and the Panel agreed (6 yes, 1 no, 0 abstentions) that the level of evidence for carcinogenicity of trichloroacetic acid from studies in experimental animals was not sufficient, based on the occurrence of tumors in only one species at only one site. Dr. Lash voted no, not because he believed the evidence was sufficient, but because the way in which the insufficiency of the evidence for the carcinogenicity of TCA was presented was inconsistent with the arguments presented for the carcinogenicity of CBDA and TBA based on their metabolism. Dr. Chiu clarified that the vote was based on the panelists' scientific judgment on the sufficiency of the evidence, independent of how the evidence was presented. Dr. Lash said he wished to change his vote. In a revote, Dr. Roberts moved, Dr. Sergi seconded, and the Panel agreed (7 yes, 0 no, 0 abstentions) that the level of evidence for carcinogenicity of trichloroacetic acid from studies in experimental animals was not sufficient, based on the occurrence of tumors in only one species at only one site.

NTP's Conclusions and Preliminary Listing Recommendations

Dr. Jahnke summarized the NTP's preliminary conclusions about exposure to haloacetic acids found as water disinfection by-products, based on data available from studies in humans, data from cancer studies in experimental animals, and information on metabolism and properties of HAAs. The preliminary listing recommendations were that DCA, DBA, BCA, and BDCA are *reasonably anticipated to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data, and that CDBA and TBA are reasonably anticipated to be human carcinogens based on the metabolism of each to a rodent carcinogen and supporting mechanistic data.

Actions

Dr. Lash moved, Dr. Tilton seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list dichloroacetic acid as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data.

Dr. Tilton moved, Dr. Carter seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list dibromoacetic acid as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data.

Dr. Roberts moved, Dr. Sergi seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list bromochloroacetic acid as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data.

Dr. Carter moved, Dr. Sergi seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list bromodichloroacetic acid as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data.

Dr. Roberts moved, Dr. Tilton seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list chlorodibromoacetic acid as *reasonably*

anticipated to be a human carcinogen based on its metabolism to a rodent carcinogen and supporting mechanistic data.

Dr. Sergi moved, Dr. Carter seconded, and the Panel agreed unanimously (7 yes, 0 no, 0 abstentions) with the NTP's recommendation to list tribromoacetic acid as *reasonably anticipated to be a human carcinogen* based on its metabolism to a rodent carcinogen and supporting mechanistic data.

V.B. Draft RoC Substance Profiles

Dr. Jahnke summarized the purpose and contents of the draft substance profiles.

Dr. Parvez, first reviewer, said that the draft substance profile sections on properties and human exposure were scientifically and technically correct and cited sufficient literature, and he agreed with the exposure estimate for the U.S. population. He agreed that the profiles highlighted the key information from the cancer studies in experimental animals that supported the listing recommendation. The information on physical-chemical properties supported and explained the mechanisms of toxicity and carcinogenesis. Overall, the literature provided was sufficient and technically sound.

Dr. Carter, second reviewer, said that the data on cancer in experimental animals were succinctly summarized; the information was complete, technically correct, clearly presented, and well organized; and the listing recommendations were supported by the summary. Dr. Sergi, third reviewer, completely agreed with Dr. Carter's comments. Dr. Tilton, fourth reviewer, said that the mechanistic data were presented in a clear and accurate manner for a general audience. She suggested it would be helpful to highlight some of the species differences in the mechanisms, specifically relating to the degree of GST-ζ inhibition and to PPARα agonists and peroxisome proliferation, since that was seen to correlate with carcinogenicity of TCA.

VI. Closing Remarks on Draft RoC Monograph

In light of the public comment from the American Chemistry Council that control of HAA5 adequately controls for all other HAAs in drinking water, Dr. Chiu suggested adding to the exposure section a discussion of the extent to which control of HAA5 is known to control levels of other HAAs.

Dr. Roberts commented that, overall, the monograph was very well prepared. Although specific points could be clarified, he found the monograph to be very readable, presenting sufficient information in a concise format. He thought the synthesis sections were very effective in putting together the key information and explaining the thinking behind the interpretation, and that there was enough information in the appendices for those who wanted further supporting information. Dr. Chiu agreed with Dr. Roberts's comments on behalf of the Panel.

Dr. Bucher and Dr. Wolfe thanked the Panel for their hard work, helpful comments, and responsiveness.

The meeting was adjourned at 3:10 p.m.

VII. Literature Cited

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, *et al.* 2016. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect* 124(6): 713-721.

Stalter D, O'Malley E, von Gunten U, Escher BI. 2016. Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products. *Water Res* 91: 19-30.

VIII. Approval of the Peer Review Report by the Chair of the Peer Review Panel

This peer review report has been read and approved by the chair of the July 24, 2017 National Toxicology Program Report on Carcinogens Monograph Peer Review Panel.

Weihsueh Chiu, Ph.D.

Peer Review Panel Chair

Date: 10/23/2017