

Public Health Goals – Trihalomethanes in Drinking Water: Chloroform, Bromoform,
Bromodichloromethane, and Dibromochloromethane

Peer Review

Matias S. Attene Ramos

Department of Environmental and Occupational Health
Milken Institute School of Public Health
The George Washington University

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Introduction.

Below I provide a review of the scientific portions of the Draft Staff Report that pertain to my expertise in mechanism and mode(s) of action for chemical carcinogenesis, test methods in mammalian cancer bioassays, and evaluation of genotoxicity studies. This expertise is needed to evaluate the assessment of the cancer risk related to exposure to the trihalomethanes. In preparing this review I have read the Draft document “Public Health Goals – Trihalomethanes in Drinking Water: Chloroform, Bromoform, Bromodichloromethane, and Dibromochloromethane”. I have also read the “Plain English summary of the trihalomethane Public health Goals” and the Description of scientific assumptions, findings, and conclusions for the peer review”. As requested, I will focus my review on the evaluation of the literature related to cancer risk for each of the four trihalomethanes but I will include other comments when necessary.

Chloroform.

The toxicological profile presented in the draft is comprehensive and covers most of available literature. Most of the toxicological information comes from animal and in vitro studies. Human data is mostly available through occupational studies. The genotoxicity and mutagenicity data is presented and discussed properly. Specifically, the experimental reasons that could lead to the variation observed in the genotoxicity tests are explained clearly and concisely. Nevertheless the toxicological profile for Chloroform (and this is true for each of the four chemicals discussed in the draft) would benefit of including some of the biological test results information present in curated online databases like PubChem¹. These results are currently more difficult to interpret than traditional toxicological studies but nevertheless contribute to the description of the biological activities of the chemical of interest and to the possible elucidation of a mechanism of action, and they will become more common and informational in the near future. The discussion of the available carcinogenicity data is sound and complete.

¹ Pubchem <https://pubchem.ncbi.nlm.nih.gov/> Accessed June 2019

Bromoform.

The toxicological profile is clear and comprehensive covering the published literature regarding the effect of Bromoform. Similarly to Chloroform, it would be informational to add at least a table including some of the active bioassays for this chemical. The genotoxicity and mutagenicity information is well described and organized including the human study that links Bromoform to genotoxicity in swimmers (Kogevinas 2010). I would advise to divide table 6.5, page 106 to separate the mutagenicity assay (e.g. Ames) from the genotoxicity/DNA damage response or at least to change the table's title. It will be useful to include more information in the table to separate the DNA damage response assays (e.g. SOS Chromotest) from the direct DNA damage measurement (e.g. SCGE assay). The carcinogenicity literature is complete and well discussed.

Bromodichloromethane

The toxicological profile is complete and well described. I would again include at least a table discussing the publically available bioassay data similarly to the previous chemicals. The genotoxicity and mutagenicity data seems to be mostly discussed correctly. The Robbianno et al. (2004)² manuscript is cited under human effects even though it uses an in vitro assay using primary human and rat kidney cells as well as whole animals (rats). I recommend to remove it from the "effect in humans" section and to add it to the main in vitro section. Also similarly to Bromoform, I would split table 7.6 (page 144) between mutation and genotoxic assays. The terms genotoxicity and mutagenicity are used interchangeably which is confusing. . The discussion of the available carcinogenicity data is sound and complete.

Dibromochloromethane

The toxicological profile is complete and reflects the somehow limited information about this chemical compound. The inclusion of a table containing the bioactivity results for Dibromochloromethane available in the above mentioned databases will enhance the toxicological profile including relevant mechanistic data. The genotoxicity and mutagenicity data is covered entirely but I would still suggest to divide the mutagenicity from the DNA damage assays in table 8.4 page 184. The discussion of the carcinogenicity classification of the compound is accurate and reflects the limited information available for this chemical.

Mechanisms of action of carcinogenicity:

The division of the literature review between Brominated THMs and Chloroform is logical based on data availability, chemical composition, and possible mechanisms of action. For the brominated THMs the main proposed mechanisms for kidney, colon and liver cancer are well discussed. It is likely that in some of these organs more than one mechanism are responsible for the observed tumors in animals. For example the liver tumors could be a result not only of the cytotoxicity/cell regeneration effect of the chemical but also of the possible genotoxic/mutagenic properties of the chemicals including GST mediated adduct formation. The possible mechanisms of kidney and liver carcinogenicity for chloroform are well described and discussed and include a wealth of data. The role of cytotoxicity in tumor formation is clearly discussed

² Toxicology. 2004 Nov 15;204:187-95. DOI: 10.1016/j.tox.2004.06.057

and the data (or lack of) suggests that it is likely that more than one molecular mechanism is involved in the development of tumors. This is not trivial since it affects the public health goals limits estimation.

Final conclusions:

The reports gives a comprehensive description of the literature covering the toxicological studies for the four Trihalomethanes of interest. It clearly discusses the know cancer and non-cancer effects for this four chemicals. The possible mechanisms of carcinogenicity are discussed extensively and their conclusions are sound. The methodologies utilized to calculate the acceptable daily doses and the cancer potency values for each of the chemicals are clearly explained and seem appropriated. The assumptions made are reasonable and more importantly consistent. This is also true for the estimation of the public health goal levels for cancer and health protective concentrations for non-cancer effects presented in the report

Cristina Villanueva, PhD
Associate Research Professor
ISGlobal-Barcelona Institute of Global Health
Barcelona Biomedical Research Park (PRBB)
Doctor Aiguader, 88
08003 Barcelona, Spain
Tel. +34 93 214 73 44
cristina.villanueva@isglobal.org
www.isglobal.org

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**Comments to Public Health Goals, Trihalomethanes in Drinking water:
Chloroform, Bromoform, Bromodichloromethane, Dibromochloromethane.
First public review draft, October 2018.**

This is a comprehensive review of the state of the art of chloroform, bromoform, bromodichloromethane, dibromochloromethane exposure, pharmacokinetics, toxicology and epidemiological evidence. It includes a clear and detailed description of the methodology followed to estimate public health goals (PHG) in drinking water. The PHG values, estimated based on evidence from animal models, yield threshold values that reasonably match with human epidemiological evidence for bladder cancer, the most consistently related outcome with THM exposure. Other outcomes associated with THM exposure, including developmental or pregnancy outcomes, show less consistent associations in human observational studies, and this is consistent with a higher value for PHG for non-cancer outcomes. The document is of great quality and value. A few minor issues have been identified, that could be considered for further improvement.

Comments to specific questions:

(a) For each proposed PHG, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in deriving the PHG based on cancer effects.

(b) For each proposed health protective concentration, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in deriving the health protective concentration based on non-cancer health effects.

The PHG estimates are based on animal studies and I do not have the expert knowledge to judge about the quality of these studies. The procedure to estimate cancer and non-cancer protective drinking water concentrations is clear, and cover the relevant scientific aspects. The explanations in the text are clear and justify the exclusion/inclusion of studies seem to be sensible.

From my perspective, a point that remains obscure is the estimation of multi-route exposure from tap water use, and specifically the contribution of the 3 exposure routes to the total exposure. The authors mention, that the CalTOX has been used, but the input data or the studies used for calculations are not indicated. In addition, the estimates from CalTOX do not match with some of the findings from specific studies, and some quotations from different studies give contradictory information. This information do not seem to be used for the PHG

estimation, and this inconsistency is not critical for the purposes of this report. However, this information is highly valuable from the human exposure assessment perspective and epidemiology. These type of estimates have not been published and could get the attention from the scientific community. For this reason, it is important to clarify this point and provide reliable and informed estimates that could be used for others.

A general aspect that is disregarded in this report is the fact that the 4 THMs occur in combination (and also together with other DBPs). The procedure to reach PHG are conducted independently for each of the 4 THMs. This disregards the fact that there may be interactions, and the sum of independent effects may not be the same than the effect to the combined exposure, which is the real exposure in the population. The issue of mixtures is scientifically complex and probably there is not enough evidence to address this properly. This does not invalidate the methods used, but it would be good to raise this idea and some thoughts about it somewhere in the text, as part of the scientific uncertainties, and acknowledge that this evaluation assumes independence of effects between the 4 THMs.

(c) For each chemical reviewed, please comment on whether a relevant study useful for assessing dose-response relationship or otherwise informing the PHG development was missed.

I have included some references throughout my review (see below) that could be considered to complement the report. They refer mainly to human studies for non-cancer outcomes and mechanistic studies, and none of these are used to estimate the PHG. In this sense, they are not worrisome omissions.

(d) PHGs must be protective of known sensitive subpopulations. Please comment on whether each PHG is health protective.

From the epidemiological perspective, the most consistent evidence is for bladder cancer. The largest pooled and meta-analysis of bladder cancer (Costet et al 2011) shows increased odds ratios at total THM levels of 5 µg/L compared to ≤5 µg/L. The sum of the PHG for the four THMs gives 1.06 µg/L, leading to a reasonable threshold that is coherent with the epidemiological evidence in human populations.

Specific comments:

SUMMARY

- 1) Page 2, "Necessity of Disinfection", second paragraph. The sentence "Of the more than 250 DBPs that have been identified", is not completely accurate or update. According to a detailed review by Richardson et al. (2007), more than 600 DBPs have been reported in the literature. I would suggest to update the figure of 250 to 600 DBPs and cite this reference (Richardson SD et al. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research 2007; 636: 178-242). The same comment can be applied to the Introduction, page 7, second paragraph, where the same sentence is quoted.

1. INTRODUCTION

PURPOSE

- 2) Page 7, second paragraph. "... disinfection by chlorination or chloramination leaves residual toxic byproducts in the drinking water such as THMs..." Chlorine dioxide also produces THM, and could be added here.
- 3) Page 7, second paragraph.
See comment 1)

2. PRODUCTION, USE AND ENVIRONMENTAL OCCURRENCE

- 4) Page 9, second sentence. "... occurrence and exposure are provided Table 2.1" typographic error, "in" is missing between "provided" and "Table 2.1."

ENVIRONMENTAL OCCURRENCE

- 5) Drinking Water. Page 12. Table 2.2. If possible, it would be informative to include the number of measurements, and some measure of dispersion (e.g. standard deviation).
- 6) Page 13. Swimming Pools section. There are many more studies on swimming pools that could be mentioned here, and I could suggest a few. However, since the main focus of this report is drinking water, I think it is reasonable to keep this section brief. If necessary, I could provide references if requested.
- 7) Page 15. Food and beverages section. I could think of a similar comment, there are more publications reporting THMs e.g. in bottled water. Perhaps it could be mentioned somewhere earlier in the text, that the main focus of this report is public drinking water, and some data is provided on other environmental sources as examples, without being necessarily exhaustive.

3. EXPOSURE TO THMS VIA TAP WATER

- 8) Page 17. Ingestion of THMs in Tap Water. "... age-specific intake rates are normalized to body weight and expressed as liters of water ingested per kilogram of body weight per day..." it is not clear in the text where the values of body weight by age group are taken from, and it would be informative. In addition, the authors could consider to include this information in Table 3.1.
- 9) Page 17. Table 3.1. Table foot note indicates that ingestion rate for pregnant women is slightly higher than that denoted for "adult". Where is it taken from, and what value is assigned?

MULTI-ROUTE EXPOSURE ESTIMATES FROM TAP WATER USE

- 10) Page 21. "OEHH uses the CalTOX to determine the dermal and inhalation exposures to THMs resulting from their presence in tap water". The values in table A4 (page 305) do not match with some of the references mentioned before, e.g. Jo et al.

1990a,b (page 18), where “The dermal and inhalation routes were estimated to contribute an equivalent amount of chloroform to body burden during showering”, and Jo et al. 2005 (page 20), where “THM exposure estimates from ingestion were similar to those from showering”. According to this, the crude estimates for the contribution of the different exposure routes to the total exposure seem to be equivalent (approx. 33% each). However, the estimates in thable A3 are disproportionally high for ingestion and low for dermal.

The reader would like to know what references or data is used as inputs for the CalTOX, and explanations to understand how these values are produced. This is important from the perspective of exposure assessment in human populations and epidemiological studies. This type of estimates showing the contribution of the different exposure routes have not been published, and they are very valuable. For this reason, it should be clear how they have been estimated, which may explain the difference with the expectations based on some specific references.

4. PHARMACOKINETICS

ABSORPTION

11) Page 24. “Using USEPA methodology Xu et al. (2002) estimated that the daily dose from bathing (dermal absorption) was 40-70% of the daily ingestion dose”. These figures do not match with the numbers in the Appendix 1, Table A.3, Page 304 (page 304), where dermal absorption contributes around 3% tot total chloroform exposure. The reader would like to understand what is the reason for those differences. Information requested in comment #10, could help to clarify this.

12) The following relevant references could be considered:

- Ashley, D. L. *et al.* Changes in blood trihalomethane concentrations resulting from differences in water quality and water use activities. *Arch Environ Occup Heal* **60**, 7–15 (2005).
- Backer, L. C. *et al.* Exogenous and endogenous determinants of blood trihalomethane levels after showering. *Environ Health Perspect* **116**, 57–63 (2008).
- Gordon, S. M. *et al.* Changes in breath trihalomethane levels resulting from household water-use activities. *Environ Health Perspect* **114**, 514–521 (2006).
- Kim, E., *et al.* Estimating Exposure to Chemical Contaminants in Drinking Water. *Environ Sci Technol* **38**, 1799–1806 (2004).
- Nuckols, J. R. *et al.* Influence of tap water quality and household water use activities on indoor air and internal dose levels of trihalomethanes. *Environ Health Perspect* **113**, 863–870 (2005).
- Xu, X. & Weisel, C. P. Human respiratory uptake of chloroform and haloketones during showering. *J Expo Anal Environ Epidemiol* **15**, 6–16 (2004).
- Xu, X. & Weisel, C. P. Dermal uptake of chloroform and haloketones during bathing. *J Expo Anal Environ Epidemiol* **15**, 289–296 (2005).

DISTRIBUTION

13) The following relevant reference could be considered:

Leavens, T. L. *et al.* Disposition of bromodichloromethane in humans following oral and dermal exposure. *Toxicol Sci* **99**, 432–445 (2007).

5. TOXICOLOGICAL PROFILE: CHLOROFORM

IMMUNOTOXICITY

14) Page 73. Effects in Humans. Vlaanderen et al. 2017 evaluated short-term changes in immune markers after THM exposure during swimming. The authors could consider to mention this study. [Vlaanderen, J. *et al.* Acute changes in serum immune markers due to

swimming in a chlorinated pool. *Environ Int* **105**, 1–11 (2017).] The same comment applies for “Immunotoxicity” section for bromoform, bromodichloromethane and dibromochloromethane.

NEUROTOXICITY

15) Page 75. Effects in humans. The authors could consider to add the following references, either here or in the appendix C3. Epidemiologic studies not used in the review of disinfection by-products:

- Grandjean, P. & Landrigan, P. J. Developmental neurotoxicity of industrial chemicals. *Lancet* **368**, 2167–2178 (2006). - Here, chloroform used as a solvent is classified as neurotoxic in humans.
- Villanueva, C. M. *et al.* Drinking water disinfection by-products during pregnancy and child neuropsychological development in the INMA Spanish cohort study. *Environ Int* **110**, 113–122 (2018). The authors evaluated the association between estimates of DBP exposure during pregnancy, including THMs, and child neuropsychological outcomes at 1 and 4–5 years of age.

16) Page 76. Effects in animals. The authors could consider the following reference:

- Guariglia, S. R., Jenkins Jr., E. C., Chadman, K. K. & Wen, G. Y. Chlorination byproducts induce gender specific autistic-like behaviors in CD-1 mice. *Neurotoxicology* **32**, 545–553 (2011).

The authors observe autistic like behaviors in male mice after gestational and postnatal exposure to chloroform and bromoform in drinking water. However, this is in co-exposure with perchloroethylene.

CARCINOGENICITY

17) Page 84. Effects in humans. The reference Villanueva et al 2006 is based on the same population as Villanueva et al. 2004. The main analysis on the association between THMs and bladder cancer is reported in Villanueva et al. 2004. It is not clear the added value of quoting also Villanueva et al. 2006. The same comment applies in other parts of the text where the meta and pooled analyses are cited.

6. TOXICOLOGICAL PROFILE: BROMOFORM

IMMUNOTOXICITY

18) Page 115. See comment #14.

NEUROTOXICITY

19) Page 115. See comment #16, and also consider the citation Villanueva et al. 2018 in comment #15.

7. TOXICOLOGICAL PROFILE: BROMODICHLOROMETHANE

IMMUNOTOXICITY

20) Page 156. See comment #14.

NEUROTOXICITY

21) Page 158. Consider the citation Villanueva et al. 2018 in comment #15. In addition, for animal evidence you could also consider this reference: Moser, V. C., Phillips, P. M., McDaniel, K. L. & Sills, R. C. Neurotoxicological evaluation of two disinfection by-products, bromodichloromethane and dibromoacetonitrile, in rats. *Toxicology* **230**, 137–144 (2007).

8. TOXICOLOGICAL PROFILE: DIBROMOCHLOROMETHANE

IMMUNOTOXICITY

22) Page 192. See comment #14.

NEUROTOXICITY

23) Page 192. Consider the citation Villanueva et al. 2018 in comment #15.

9. MECHANISMS OF ACTION OF CARCINOGENICITY

24) Page 200. Epigenetic effects, including DNA methylation and gene expression are proposed mechanisms of action that are not covered in this section. If there is a reason for not including them, this should be clarified in the text. Otherwise, this should be mentioned in the text. Some –not exhaustive list of- suggested references about this topic are:

- Coffin, J. C. *et al.* Effect of trihalomethanes on cell proliferation and DNA methylation in female B6C3F1 mouse liver. *Toxicol Sci* **58**, 243–252 (2000).
- Espín-Pérez, A. *et al.* Blood transcriptional and microRNA responses to short-term exposure to disinfection by-products in a swimming pool. *Environ Int* **110**, 42–50 (2018).
- Pereira, M. A. *et al.* Effect of chloroform on dichloroacetic acid and trichloroacetic acid-induced hypomethylation and expression of the c-myc gene and on their promotion of liver and kidney tumors in mice. *Carcinogenesis* **22**, 1511–1519 (2001).
- Tao, L. *et al.* DNA hypomethylation induced by drinking water disinfection by-products in mouse and rat kidney. *Toxicol Sci* **87**, 344–352 (2005).
- Salas, L. A. *et al.* DNA methylation levels and long-term trihalomethane exposure in drinking water: An epigenome-wide association study. *Epigenetics* **10**, 650–661 (2015).
- Salas, L. A. *et al.* Gene expression changes in blood RNA after swimming in a chlorinated pool. *J Environ Sci* **58**, 250–261 (2017).
- Salas, L. A. *et al.* LINE1 methylation in granulocyte DNA and trihalomethane exposure is associated with bladder cancer risk. *Epigenetics* **9**, 1532–1539 (2014).
- Yang P, *et al.* Prenatal exposure to drinking water disinfection by-products and DNA methylation in cord blood. *Sci Total Environ* **586**, 313-318 (2017).

In addition, Nieuwenhuijsen *et al.* 2009 [Nieuwenhuijsen, M. J. *et al.* The epidemiology and possible mechanisms of disinfection by-products in drinking water. *Philos Trans A Math Phys Eng Sci* **367**, 4043–4076 (2009).] reviewed mechanisms of action of disinfection by-products including THMs. The authors of the report could consider this manuscript to verify the completeness of the section about mechanisms of action.

10. DOSE-RESPONSE ASSESSMENT

25) The procedure to estimate the ADD, cancer slopes and PHG is quite complex, with multiple steps using formulae that are given, probably based on similar procedures previously conducted for other chemicals. The rationale behind some of the formulae are not evident, although the process seems to be established and accepted as it is. Despite the complexity of the procedures, the explanations are generally clear.

NON-CANCER DOSE-RESPONSE ANALYSES AND ACCEPTABLE DAILY DOSE CALCULATIONS

26) Page 238. Last paragraph. The text says that the best model fit was the Polynomial for continuous data, and BMDL_{1SD} for rats is 12.9. However, Table 10.8 below shows that the value for this model is 12.7 for females (39.7 for males). According to the Table 10.8, the value of 12.7 for females and 32.5 for males corresponds to the Hill model, not the polynomial. This apparent mismatch is confusing and should be checked or clarified.

CANCER DOSE-RESPONSE ANALYSES AND ACCEPTABLE DAILY DOSE CALCULATIONS

27) The rationale behind the formula of $CSF_{\text{animal}}=0.05/\text{BMDL}_{05}$ is not clear. An explanation would be appreciated to understand it.

*11. HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS
NON-CANCER HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS*

28) Page 258. Table 11.1. It is not clear how DWI here are calculated and some explanations are warranted. In addition, the reader would expect the same value for the different THMs. It is not clear why it slightly varies.

APPENDIX A. ESTIMATING DERMAL AND INHALATION EXPOSURES VIA TAP WATER USING CALTOX

29) Page 303. Check the webpage <https://energyanalysis.lbl.gov/tool/caltox>. From my computer, The requested page "/tool/caltox" could not be found.

APPENDIX C. EPIDEMIOLOGIC STUDIES OF DISINFECTION BYPRODUCTS AND CANCER STUDIES AVAILABLE

TABLE C1. EPIDEMIOLOGIC STUDIES OF DISINFECTION BYPRODUCTS AND CANCER PUBLISHED SINCE 1985

30) Page 333. This is a comprehensive review summarizing the state-of the art of epidemiological literature. The table is large table with a lot of information. In order to facilitate the reading, it may be helpful to include sub-captions, or intermediate rows in the table specifying the cancer type that follows.

TABLE C3. EPIDEMIOLOGIC STUDIES NOT USED IN THE REVIEW OF DISINFECTION BYPRODUCT EXPOSURE AND CANCER

31) Page 389. This table should be organized alphabetically by author and year of publication to facilitate the reading.

Dale Hattis
Clark University
5/16/19

Gerald W. Bowes, Ph.D.
Manager, Cal/EPA Scientific Peer Review Program
Office of Research, Planning and Performance
State Water Resources Control Board
1001 "I" Street, 13th Floor
Sacramento, California 95814

Via email GBowes@waterboards.ca.gov

Dear Dr. Bowes:

Here are my responses to the various issues you posed to me on the trihalomethane hazard review documents. Please contact me if any parts of the discussion need further clarification.

The Big Picture

Reviewers are not limited to addressing only the specific topics presented above, and are asked to consider the following:

- (a) For each proposed PHG, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in deriving the PHG based on cancer effects.

OEHHA appears to have addressed the major issues relevant to assessing the cancer hazard of the trihalomethanes covered in their document.

- (b) For each proposed health protective concentration, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to each chemical and to the methods applied in deriving the health protective concentration based on non-cancer health effects.

OEHHA has addressed the usual scientific issues raised in the context of setting public health protection goals for drinking water standards. There are broader risk management issues that have likely been considered beyond the scope of the scientific discussion.

- (c) For each chemical reviewed, please comment on whether a relevant study useful for assessing dose-response relationship or otherwise informing the PHG development was missed.

I did my own literature search and I did not come across any significant papers that were missed.

- (d) PHGs must be protective of known sensitive subpopulations. Please comment on whether each PHG is health protective.

It is difficult to adequately address this question as the degree of health protectiveness desired is not defined. What can be said is that the proposed "Public Health Goals" seem to reflect a degree of protectiveness that is broadly in line with similar goals derived for other carcinogens.

The document at present reports deriving the standards to meet a goal of limiting extra risk from each drinking water contaminant to one per million over a lifetime of exposure. Not mentioned in the discussion is that this conventional number is not a central estimate of risk, but a statistical upper 95% confidence limit, considering only some specific statistical uncertainties. Among the uncertainties, for example are the uncertainties inherent in interspecies projection of cancer risks, and the uncertainties inherent in projecting from less-than-lifetime observations of cancer risk in animals to the risk of full lifetime exposure of people. (This is aside from the additional risk in people from exposures in early life, which is included in the calculation). In these areas the document has followed conventional practice for risk assessments for putative genetically acting carcinogens in California, but that does not mean there are no uncertainties beyond the specific considerations included in the calculations.

My detailed comments on specific sections of the document are given below:

Attachment 1, p. 2--"The PHG for each THM is set at a level where the cancer risk is one per one million persons exposed over a 70-year lifetime."

Any mention of the one per million risk number should be accompanied by an uncertainty statement—that this is an 95% upper confidence limit on the purely statistical portion of the uncertainties—omitting many other sources of uncertainty in the calculation, including, for example interspecies projection of the cancer risk.

Reviewers are asked to determine whether the scientific work product is "based upon sound scientific knowledge, methods, and practices."

Generally the document is based on sound methods and practices. However there are some details that should be improved or clarified before finalization.

1. Chloroform

I agree that cancer is primary adverse effect of concern.

For both the PHG and non-cancer health protective concentration, “OEHHA is using the benchmark dose (BMD) approach for cancer potency and point of departure (POD) determination, respectively, from animal toxicology studies.”

The benchmark dose method for analyzing dose response relationship is standard.

“To determine the health protective concentration for cancer, that is, the concentration of chloroform in drinking water that is associated with a one-in-one-million risk of cancer for people exposed over a lifetime, OEHHA first derived a cancer potency for chloroform of 0.014 milligrams per kilogram of bodyweight per day (mg/kg-day) ⁻¹. This number is the geometric mean of potency estimates derived from several datasets on liver and kidney tumors in rodents. The cancer potency was then used to derive the proposed PHG for chloroform of 0.4 ppb.”

Using a geometric mean of results from four studies is not standard. Unfortunately my knowledge of past practices in California risk assessments for putative genetically acting agents is not extensive enough for me to be able to report with complete confidence what past standard practices have been in this area. My impression is that it is likely that past practice would have been to resolve this uncertainty by taking the highest of the four potency estimates.¹

However even that is not entirely satisfactory as I see it. The four estimates are evidently each derived from observations at a single cancer site, whereas every human has a full set of potential cancer sites all over his or her body. Logically, it would be desirable to sum the risks expected for all the sites with statistically significant elevations of tumor risks. Moreover, with four independent estimates for the interspecies projection, it would be better to develop a probabilistic combination of the data and use probabilistic techniques to pick a desired quantile of the uncertainty distribution for decision-making, rather than take a simple geometric mean. Some work I have done in the past could point the way to assembling relevant information for the probabilistic components for this type of analysis (Hattis, D. and Lynch, M. K. “Empirically Observed Distributions of Pharmacokinetic and Pharmacodynamic Variability in Humans—Implications for the Derivation of Single Point Component Uncertainty Factors Providing Equivalent Protection as Existing RfDs.” In *Toxicokinetics in Risk Assessment*, J. C. Lipscomb and E. V. Ohanian, eds., Informa Healthcare USA, Inc., 2007, pp. 69-93.)

p. 3

“The PHGs for the THMs are based on the cancer endpoint because it is the most sensitive effect of the THMs. The PHG for each THM is set at a level where the cancer risk is one per one-million persons exposed over a 70-year lifetime.”

No mention of upper confidence limit status of the 1/million estimate or presence or absence of other conservative assumptions or treatment of multiple cancer data sets or sites of action.

“An overview of the toxicity and the calculation of PHGs and the non-cancer health-protective concentrations for the four THMs follows.”

“To determine the health protective concentration for cancer, that is, the concentration of chloroform in drinking water that is associated with a one-in-one-million risk of cancer for people exposed over a lifetime, OEHHA first derived a cancer potency for chloroform of 0.014 milligrams per kilogram of bodyweight per day (mg/kg-day) ⁻¹. This number is the geometric mean of potency estimates derived from several datasets on liver and kidney tumors in rodents. The cancer potency was then used to derive the proposed PHG for chloroform of 0.4 ppb.”

p. 7 “The purpose of this document is to estimate health-protective concentrations for the four major regulated trihalomethanes (THMs) found in drinking water as a result of disinfection methods chloroform (CHCl₃), bromoform (CHBr₃), bromodichloromethane (BDCM; CHBrCl₂), and dibromochloromethane (DBCm; CHBr₂Cl) – and to develop public health goals (PHGs) for each individual THM. These assessments are based on comprehensive analyses of information on the toxicology of each compound. PHGs are based solely on protection of public health without regard to cost impacts or other factors. PHGs for carcinogens are set at a de minimis risk level of one in a million (10⁻⁶) for exposures over a 70-year lifetime. In these assessments, when estimating lifetime cancer risks, OEHHA accounts for the early-life sensitivity to carcinogens and enhanced water intake relative to bodyweight of the young.”

These are standard practices.

“The US government and the State of California have adopted drinking water standards in the form of maximum contaminants levels (MCLs) for chemical contaminants that are created during drinking water disinfection. Both the state and federal MCLs are set at 80 micrograms per liter (80 µg/L) for the total concentration of THMs in drinking water. The determination of the MCL explicitly balances the important benefits of water disinfection against the risks of exposure to residual toxic byproducts in the drinking water, as well as technical feasibility.”

¹ On further reading I found some support for my impression that the highest of alternative potency estimates would usually have been adopted as the regulatory value. On page 254, the cancer potency for BDCM is discussed as follows:

“... Converting the CSF animal for the large intestine tumor incidence in male rats to the human equivalent cancer potency resulted in a CSF human estimate of 0.0255 (mg/kg-day) ⁻¹. Analysis of the liver tumor incidence in female mice produced a CSF human estimate of 0.087 (mg/kg-day) ⁻¹. Because the mouse data yielded a higher cancer slope factor, it will be used as the basis for the PHG calculation.”

I doubt that an explicit balancing has been done. Where in the document can the balancing and comparison with marginally increased or decreased target levels from the 80 Mcg/L be found? I suggest that this claim of explicit balancing be deleted or the reader should be referred to the place in the document where the balancing calculation is detailed.

Bromoform-Specific comment

p. 14—"According to the US Environmental Protection Agency (US EPA) Toxics Release Inventory, 136,266 pounds of bromoform were disposed of or released into the environment by industrial facilities in the United States in 2015 (US EPA, 2017)."

Surely six significant figures are not warranted by the likely accuracy of the toxic release inventory data for bromoform, or of interest to the reader. It will suffice to communicate 136,000 pounds to the reader.

Return to Chloroform comment

p. 21 It seems highly dubious to assume a value of "0" for the infant inhalation rate in Table 3.2 The reasoning given in the document (note c) is that

"they typically do not shower or flush toilets. These are the dominant inhalation exposure scenarios; therefore the inhalation pathway is excluded for infants."

I would counter that even though the infants do not contribute much in terms of aerosolizing material, they nevertheless breathe quite a lot as they are very active and require inhalation of air to support their muscular activity, as well as growth and development and basic metabolism to support life. The stated inhalation rate of 0 is a clear error and must be replaced with a sensible finite value in the dosimetry calculations.

p. 22: "...much higher air concentrations in an equilibrium state, CalTOX also considers diffusion in water and air in the water-to-air mass transfer modeling. In the CalTOX exposure model, water-to-air transfer for the THMs is limited by their diffusion in water, resulting in relatively comparable indoor and bathroom air concentrations and exposures via the inhalation route."

You can check with Tom McKone, but to the best of my recollection CalTox is not a diffusion-based model, but a model that assumes dynamic equilibrium among phases.

p. 22—as discussed above in connection with Table 3.2, the "zero" in the inhalation column for infants must be replaced by sensible finite values. The babies do breathe. It exposes the analysis to ridicule if this is not recognized in the document by a non-zero breathing rate.

p. 29—"Pegram et al. (1997) exposed the standard mutagenicity tester strain TA1535 and strain transfected with the rat Gstt1-1 gene to BDCM. They found evidence that mutagenicity of BDCM is enhanced by GSTT-mediated conjugation with glutathione; they also noted that the comparatively low affinity of the GSTT-mediated pathway for chloroform offers a possible mechanistic explanation for the differences observed in mutagenic potential of the brominated THMs compared to chloroform."

Typos: The first line should have "a" inserted between "and" and "strain". The second line should also have an "a" inserted between "by" and "GSTT".

p. 30—Figure 4.2—the repeated use of "N²" in this figure is not standard. Perhaps this is meant to signify a specific nitrogen atom in the molecule. If so this should be explained in clear text. If not, then it nevertheless needs explanation. The figure is attributed to "DeMarini et al. (1997)-- DeMarini DM, Shelton ML, Warren SH, Ross TM, et al. (1997). Glutathione S-transferase-mediated induction of GC→AT transitions by halomethanes in Salmonella. Environ Mol Mutagen 30(4):440-7." The figure is present there with the nonstandard use of "N²".

p. 31. "Chloroform saturation was fully saturated in the Osborne-Mendel rat at doses of 90 and 180 mg/kg, working at a maximal rate of 40 and 50 μmol ¹⁴CO₂ expired/kg-hour."

This statement is self-contradictory. Clearly, if the metabolism were "fully saturated" then the metabolism rates at the two doses would be the same. In fact the most that should be expected with higher doses is that metabolism should approach saturation. Saturation can never be fully reached no matter how high the concentration of substrate.

p. 32 "There is some evidence that the dichloromethyl radical, •CHCl₂, is formed by reductive dehalogenation of chloroform (Tomasi et al., 1985). Production of dichloromethyl radical was significant at a chloroform concentration greater than or equal to 1 mM, increasing linearly with substrate concentration. CYP2E1 was the primary enzyme involved in the reductive reaction. Based on these in vitro studies, the reductive pathway seems to be less relevant at low environmental exposures, since it is active at high substrate concentrations."

I would suggest deleting the last sentence. The share of each enzymatic pathway at different concentrations is determined by Michaelis constants of the respective enzymes. There is no concentration of substrate at which only a single enzyme is operative

p. 33 "Species differences exist in the extent of chloroform metabolism (Brown et al., 1974, Taylor et al., 1974; Reynolds et al., 1984; Mink et al., 1986; Corley et al., 1990). Brown et al. (1974) reported that mice metabolized chloroform to carbon dioxide to the greatest extent (about 85 percent) and rats to a lesser degree (67 percent); only a small amount (18 percent) of chloroform was metabolized by monkeys.

These “extent of metabolism” results depend on the relative rates of metabolism by different pathways. They are of little basic interest and do not appreciably illuminate species differences in metabolism as claimed.

Dibromochloromethane comment:

p. 35 “Oxidation of DBCM to carbonyl halogenides, which are electrophilic and very unstable intermediates that readily react with nucleophiles in tissues, is a key step in its toxic action.”

It is not apparent what support exists for this conclusion. The conclusion does not seem to follow logically from the previous sentences in the paragraph.

Return to chloroform comment

p. 37—The attempts to infer ranges of activity for CYP2E1 from observations with other substrates are unconvincing. Enzyme activities are often substrate specific and it is speculative to use interindividual variability observations for one substrate to say anything about human interindividual variability for metabolism of another substrate.

p. 41 “The closely related CYP isoforms CYP2B1 and CYP2B2 are also believed to participate in the metabolism of chloroform in rats, though generally only at higher doses (ILSI, 1997; US EPA, 1997, 1998c).

“only at higher doses” seems to imply some cutoff at high dose which needs to be exceeded for metabolism by these enzymes to begin. This is wrong. If the enzyme is present it is active at all doses, although the contribution to overall metabolism may be modest in comparison to other enzymes that are also present, depending on the respective V_{max} and K_m values.

p. 43 “The large confidence limits reflect the wide variability and small number of subjects involved.”

I would substitute “uncertainty” for variability in this case. The range largely results from great uncertainty in the estimate of the odds ratio rather than variability among cases.

“Unlike other CYPs that are mainly regulated at the transcriptional level, CYP2E1 activity appears to be primarily influenced at the post-transcriptional and post-translational levels, specifically by substrate binding and stabilization of the mRNA or protein (Bolt et al., 2003).”

Binding to mRNA seems highly unlikely as RNA does not ordinarily have the binding sites manifested on the translated protein. I would delete the reference to binding to mRNA.

The Bolt et al. 2003 reference does not appear to be in the list of citations. At the end of the chapter it is listed as:

Bolt HM, Roos PH, Thier R (2003). The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: Consequences for occupational and environmental medicine. *Int Arch Occup Environ Health* 76:174–185 (as cited in Neafsey et al., 2009).

I was able to find the abstract on line. It says nothing about the dubious claim of control via binding of the substrate to mRNA

p. 50 “There is some evidence that the greater renal toxicity of chloroform when administered in corn oil in male rodents is due to an interaction between chloroform and corn oil.”

I disagree. The fact that the toxic action of chloroform is altered by administration in corn oil is not likely to be due to a direct chemical interaction between chloroform and corn oil. The authors do not advance any evidence of a chemical reaction between corn oil and chloroform. Much more likely is that some physiological change resulting from chloroform exposure alters the animals’ response to corn oil (or, alternatively, some change resulting from corn oil exposure changes the animals’ response to chloroform).

p. 83 “The matched control group displayed many of the same hematology and clinical chemistry changes as the treated groups, suggesting that the observed changes were secondary to reduced water intake and body weight, rather than a direct effect of chloroform.”

On what basis are the effects in the control groups considered “changes”? Changes over time? Clearly temporal changes in parameters cannot be the results of the chloroform administered to experimental groups and should be mentioned only as a puzzling anomaly of the experimental results.

p. 86 “distilled pesticide-analysis quality chloroform.” This is highly unusual terminology. Perhaps a footnote is in order to explain why the chloroform is described in this way.

p. 85—The cancer section continues with the seemingly endless repetition of raw dose response results. There is no analysis (at least in this section of the document) of slope factors and confidence limits. Techniques for such analyses are well established. I would have expected some such analytical results because that is where derivation of regulatory standards will inevitably go. The result is an immensely unilluminating set of raw findings that cannot be compared to get a sense of how potent the THMs are in customary units used for other carcinogens.

Bromoform comment

p. 101—"Selected studies on the subchronic toxicity of bromoform are summarized in Several published studies have addressed the subchronic oral toxicity of bromoform." (sic)

Evidently the reference to a missing table has been omitted from the text. The table summarizing the results and the text reference should be restored. The missing text probably has been inadvertently transferred to the top of page 102—"Table 6.4 below."

p. 105—"An increase was reported in micronuclei in peripheral blood lymphocytes one hour after swimming for 40 minutes in an indoor chlorinated pool."

This seems very quick after exposure. Were there observations at any other time after exposure? What is known about the time course of micronucleus appearance and disappearance after known mutagenic exposures?

p. 111—Some basic description of the "colony probe hybridization method" would be helpful. This is not a very common assay.

p. 113 "The incidence of affected fetuses per number of affected litters in the 0, 50, 100, and 200 mg/kg-day groups, respectively, was 3/3, 4/3, 4/3 and 7/5 for a 14th rib; 1/1, 5/3, 6/5, and 13/8 for sternebral aberrations; 1/1, 1/1, 6/3, and 6/4 for interparietal variations; and 1/1, 0/0, 0/0, and 6/4 for wavy ribs.

The fact that 3/3 litters were evidently affected in the control group (0 mg/kg-day) precludes the possibility that a significantly increased incidence of aberrations could be observed. So these results are unhelpful.

p. 115 "No data on the neurotoxicity of bromoform in humans were available. Clinical observations are consistent with central nervous system depression (summarized in US EPA, 1994a)."

The second sentence flatly contradicts the first. If there are "no data", how can there be "clinical observations"? Clinical observations are data.

p. 116 "The experiments examined acute dose effects (described in the next paragraph), 14- and 90-day treatments at 300 or 3,000 times the estimated average human daily intake of bromoform in disinfected tap water (0.9 and 9.2 mg/kg-day, respectively), 30 days of treatment at 100 mg/kg-day, and 60 days of treatment at 100 or 400 mg/kg-day."

This run-on sentence needs to be broken up into at least two and perhaps 3 parts to be intelligible.

"The minimum amount required to elicit a mutagenic response was 600 μmol ."

Use of the "minimum amount" language implies a threshold for the mutagenic response. This is inappropriate and should be changed. Mutagenesis is almost never caused by processes that are expected to have thresholds.

Bromodichloromethane—p. 3 of the summary comment and the section beginning p. 125 of the main document. I agree that for this trihalomethane as well, cancer is the primary health effect of concern because of both the carcinogenesis observations and the evidence for a genetic mode of action. Moreover, of all the THMs, the dose response analysis indicates that it merits the most protective (lowest) value for the public health goal.

p. 148 "The numbers of affected litters out of total litters were 2/9, 4/14, 7/13, and 6/10 for control, low-, mid-, and high-dose groups; our analysis using the Fisher exact test indicates that none of these increases differs significantly from control."

It may well be that none of the treated groups, evaluated individually, differs significantly from control. However it seems likely that there could be a positive trend in these data that would be statistically significant. This kind of trend test should be done and reported.

p. 150 "For the corn oil vehicle, the ED 05 and BMDL were 48.4 and 39.3 mg/kg-day, respectively. For the aqueous vehicle, the ED 05 and BMDL were 33.3 and 11.3 mg/kg-day, respectively. Thus the corn oil vehicle yielded a higher BMDL than the aqueous vehicle, reflecting the different CIs around the estimated five percent response levels."

The marginally higher BMDL for the corn oil vehicle hardly seems meaningful. In any event the result is most likely attributable to somewhat faster delivery from the water vehicle to the systemic circulation.

p. 150 (paragraph beginning "NTP (1998)"—reproductive findings are given for males but not females. Either reproductive parameters for females should be given, or there should be a statement that there were no comparable results for females.

p. 156 "BDCM treatment resulted in decreased antibody-forming cells in serum and decreased hemagglutination titers." How could there be antibody-forming cells in serum? Serum is necessarily free of cells of any kind. This makes no sense.

p. 180-1 "The incidence and severity of hepatic lesions (increased cytoplasmic volume and vacuolation due to fatty infiltration) were increased in exposed animals compared to the vehicle control. The response was weakly dose-related in males (incidence: vehicle control, 5/9; 5 ppm, 3/10; 50 ppm, 4/10; 500 ppm, 5/10; 2500 ppm, 6/9),

I don't believe a significant increase with dose is indicated by these data. The author should report the results of a trend test and, if it is in fact negative as I suspect, delete the claim that the response is dose related.

p. 185 "The minimum amount required to elicit a mutagenic response was 57 μmol ."

This phrasing incorrectly implies a threshold for the mutagenic response.

p. 201 “whereas no statistically significant increase was observed with chloroform treatment (although there were 4 total ACF in chloroform treated animals and 0.67 ± 0.33 ACF per colon with regular diet versus zero in water vehicle controls).

It is hard for me to believe that the chloroform result is really not statistically significant. This should be re-checked. Perhaps it is somehow not stated clearly (4 total ACF vs 0.67 in controls??)

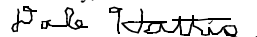
p. 203 “For both compounds, the study authors compared the dose-response for liver toxicity (enzyme and labeling index data) and tumorigenicity (data from previous NTP bioassays) using the Hill equation model, finding that the shape of the dose-response as well as the Hill exponents were different for liver toxicity and tumorigenicity. The authors therefore concluded that their results do not support a causal relationship between liver toxicity with subsequent reparative hyperplasia and tumor development.”

I think this should be cut. The Hill equation with its nonlinearity is not a recognized cancer dose response model.

p. 207 “Larson et al. (1993) suggested that their findings support the hypothesis that tumors occurred in the kidney of male rats and the liver of female mice in the NCI (1976) study because of toxicity and regeneration of the injured tissues that resulted from the high doses.”

In my view it is not helpful to resurrect crackpot toxicity and regeneration theories of carcinogenesis. Cancer is well recognized to be the result of a series of somatic mutations that often result from reactions with DNA. Once it is clear that highly reactive metabolites such as phosgene result from chloroform metabolism, and this must occur at all doses of chloroform, no further evidence of likely low dose carcinogenesis via genetic mechanisms is needed for reasonable people.

Sincerely,



Dale Hattis

**Trihalomethanes in drinking water: Chloroform, Bromoform,
Bromodichloromethane and Dibromochloromethane**

Public Health Goals

Review

Ricard Marcos

May 2019

SUMMARY

This is a very exhaustive document reporting data on the potential health effects associated with the exposure of four trihalomethanes (THMs) namely chloroform, bromoform, bromodichloromethane and dibromochloromethane.

In addition to reporting data on environmental occurrence in drinking water and multi-route exposure, the document point out relevant aspects on their pharmacokinetics (absorption, distribution, metabolism and excretion).

Four different sections are dedicated to the toxicological profile of each one of the four studied trihalomethanes.

Another section is dedicated to the known/potential mechanisms of carcinogenic action of chloroform and the three brominated THMs.

The dose-response assessment for both cancer and non-cancer effects is also evaluated. Finally, non-cancer/cancer health protective concentrations in drinking water are proposed.

The document also includes five appendices reporting data i) estimating exposures using the CALTOX model, ii) based on PBPK models, iii) on epidemiological studies on cancer incidence, iv-v) benchmark dose analysis for non-cancer and cancer endpoints.

Missing information

Since throughout the entire document many different abbreviations are used, it is necessary to include an exhaustive relation of all the abbreviations used in the document. Ideally, the inclusion of definitions (when it is advisable) also would help potential readers.

Sections 1 and 2.

Section 1 correspond to a brief introduction on the purpose of the document, chemical identity, and organization of the document.

Section 2 includes sections such as production, use and environmental occurrence.

I have no comments on the content/distribution of these two sections.

Section 3. Exposure to THMs via tap water

Multi-route exposure considerations.

In addition to the reported studies, there is another one (Prah et al., 2002) with interesting information on this topic. Authors constructed a dermal exposure system constructed of inert and impervious materials. The interface between the glass and Teflon exposure tank and the subject was custom-made of clear Tedlar (polyvinylfluoride) so that the depth of the arm in the media could be monitored. Blood concentrations taken from 14 human subjects before, during, and after the 1-h exposure demonstrated that

measurable DBPs were absorbed. The DBPs measured in the water and blood of the subjects were chloroform, bromodichloromethane, and dibromochloromethane

Prah JD, Blount B, Cardinali FL, Ashley DL, Leavens T, Case MW. The development and testing of a dermal exposure system for pharmacokinetic studies of administered and ambient water contaminants. *J Pharmacol Toxicol Methods*. 2002, 47(3): 189-195.

From Table 3.3 is a bit surprising that CalTOX model provide data indicating that exposure via dermal route is quite similar to inhalation, taking into account that THMs are volatile, mainly when hot water is used (cooking, showering and bathing). I do not know if there are strong evidences supporting this statement.

In fact, in a recent study (Zhang et al., 2018) the concentrations of THMs in human tissues were predicted based on a physiologically based pharmacokinetic (PBPK) models, and the health risk of THMs for participants were estimated. Furthermore, the carcinogenic risk of mixtures, according to the method proposed by USEPA and PBPK model based method, was calculated and compared. TCM and BDCM were the major risk factors, and inhalation was the main exposure route of THMs.

Zhang Y, Zhang N, Niu Z. Health risk assessment of trihalomethanes mixtures from daily water-related activities via multi-pathway exposure based on PBPK model. *Ecotoxicol Environ Saf*. 2018, 163: 427-435.

Section 5. Toxicological profile of Chloroform

Acute toxicity.

- Effects in humans

In addition to the reported information, there is a report on poisoning due to chloroform ingestion. In that case, a 30-year-old female ingested 20-30 mL of 99% chloroform solution, which caused rapid loss of consciousness, transient hypotension and severe respiratory depression requiring endotracheal intubation and ventilation. In addition to early CNS depression, and delayed hepatotoxicity, severe gastrointestinal injury and dermatitis with chloroform ingestion was reported.

Jayaweera D, Islam S, Gunja N, Cowie C, Broska J, Poojara L, Roberts MS, Isbister GK. Chloroform ingestion causing severe gastrointestinal injury, hepatotoxicity and dermatitis confirmed with plasma chloroform concentrations. *Clin Toxicol (Phila)*. 2017, 55(2): 147-150.

Subchronic toxicity.

- Effects in humans

There is a report of two cases of hepatotoxicity in cleanroom workers due to high retained chloroform air concentrations. Two women, aged 34 and 41 years, who were working in a medical endoscopic device manufacturer as cleanroom workers for approximately 40-

45 days suffered severe liver damage. Two measured time-weighted averages of the chloroform concentration in the air in the cleanroom were 82.74 and 64.24 ppm, which are more than 6 times the legal occupational exposure limit in Korea.

Kang YJ, Ahn J, Hwang YI. Acute liver injury in two workers exposed to chloroform in cleanrooms: a case report. *Ann Occup Environ Med.* 2014, 26(1): 49.

Genetic toxicity

- In vitro assays

There is a recent study carried out in bacteria that is not included (Khallef et al., 2018)

In that study, *Salmonella typhimurium* TA98 and TA100 strains were employed. Chloroform showed a direct mutagenic effect since the number of revertant colonies gradually increase in dose-dependent manner at all concentrations tested. These positive findings were observed both in the absence and presence of S9 metabolic activation.

Khallef M, Cenkci S, Akyil D, Özkara A, Konuk M, Benouareth DE. Ames and random amplified polymorphic DNA tests for the validation of the mutagenic and/or genotoxic potential of the drinking water disinfection by-products chloroform and bromoform. *J Environ Sci Health A Tox Hazard Subst Environ Eng.* 2018, 53(2): 154-159.

Developmental and Reproductive Toxicity

In this section, there is a recent and interesting review that perhaps should be included (Williams et al., 2018)

Williams AL, Bates CA, Pace ND, Leonhard MJ, Chang ET, DeSesso JM. Impact of chloroform exposures on reproductive and developmental outcomes: A systematic review of the scientific literature. *Birth Defects Res.* 2018, 110(17): 1267-1313.

Carcinogenicity

Although the reference of Hard et al. (2000) is indicated in the References section, I have not found this reference in the discussion of this section.

The results of this reevaluation should be included:

Hard GC, Boorman GA, Wolf DC. Re-evaluation of the 2-year chloroform drinking water carcinogenicity bioassay in Osborne-Mendel rats supports chronic renal tubule injury as the mode of action underlying the renal tumor response. *Toxicol Sci.* 2000, 53(2): 237-244.

Section 6. Toxicological profile of Bromoform

Toxicity

Although the study of Lodhi et al. (2017) was carried out *in vitro*, analyzing the effects on human blood samples, the obtained results are interesting enough to be indicated elsewhere. Hemoglobin (HGB) and mean corpuscular hemoglobin concentration levels lowered as they were significantly affected ($p < 0.05$) by bromoform at all administered doses.

Lodhi A, Hashmi I, Nasir H, Khan R. Effect of trihalomethanes (chloroform and bromoform) on human haematological count. J Water Health. 2017, 15(3):367-373.

Genetic Toxicity

Although I have not been able to access to the complete version of this document (DeAngelo et al., 2007), effects on human colon cells are reported. If it is possible, it should be mentioned.

DeAngelo AB, Jones CP, Moyer MP. Development of normal human colon cell cultures to identify priority unregulated disinfection by-products with a carcinogenic potential. Water Sci Technol. 2007, 56(12): 51-55.

The title of the section: *Effects in animals –in vitro assays* should be modified by **In vitro assays**. I do not think that studies with bacteria can be included under the “animal” heading.

Since the study of Landi et al. (1999a) is already indicated in the section of human cells, it should be deleted from Table 6.5.

In a similar way, the study of Morimoto and Koizumi (1983), also carried out in human lymphocytes, should be moved from the table and discussed in the section of human cells

Similarly, the title of the section: *Effects in animals –in vivo assays*, should be modified by **In vivo assays**. I do not think that studies with bacteria can be included under the “animal” heading.

From the data included in the Table 6.6, it is not clear to me if data on *Aspergillus* must be included here or in the previous table, just as occurs with bacteria data. Perhaps a new reference (Khallef et al., 2015) should also be included. In that study, authors use the plant *Allium* as a model to detect genotoxic effects in root cells. Exposure to bromoform significantly decreased mitotic index, increased the total of chromosomal aberration, and increased the levels of primary DNA damage as detected by the comet assay.

Khallef M, Liman R, Konuk M, Ciğerci İH, Benouareth D, Tabet M, Abda A. Genotoxicity of drinking water disinfection by-products (bromoform and chloroform) by using both *Allium* anaphase-telophase and comet tests. Cytotechnology. 2015, 67(2): 207-213.

Section 7. Toxicological profile of Bromodichloromethane

Second paragraph of page 128. I do not think that the body weight decrease after 24 h of exposure is a relevant value. In fact, authors (Keegan et al., 1998) did not mention this in their abstract.

Second paragraph of page 138. I do not know if it is adequate to include here the effects of the other brominated THMs.

Genetic toxicity section.

The structure of this section is a bit confusing. Humans and animals studies must refer to whole organism *in vivo* studies, not to the *in vitro* use of human/mammalian cells.

Thus, the first sub-section **effects in humans** must contain only the epidemiological data, that I would rename as **biomonitoring** data. The experimental data must move to an *in vitro* data section

A second sub-section will constitute *in vivo* studies, according to the relevance of these studies, regarding the *in vitro* data. In this new section, the sequence used in the text and in the table must match. If the studies are explained according to their increasing relevance, first data must correspond to primary DNA damage (no DNA damage). The comment on the result obtained in the study of Teixido et al. must indicate that DNA damage was evaluated using the comet assay, detecting DNA strand breaks.

The results of Benigni et al. (1993) measuring aneuploidy in *Aspergillus* should be discussed after the micronucleus discussion. It should be remembered that micronuclei can be originated by aneuploidy (in addition to chromosome breakage).

In Table 7.6, the study of Kogevinas et al. (2010) should be eliminated because this is the study indicated in the Biomonitoring studies with humans. As suggested, human biomonitoring studies must constitute a different subsection.

The third sub-section would be constituted by the *in vitro* studies. As previously indicated, the sequence in the text must match with the sequence in the Table. This means that both contents should be revised.

In Table 7.5 the study of Merch-Sundermann et al. (1989) was carried out only with *E. coli*. This means that its inclusion in the chromosome alterations part must be removed to the DNA damage part. In this part, the reference of Merch-Sundermann et al., in human lymphoblastic cells must be removed.

The detection of aneuploidy reported by Matsuoka et al. (1996) was carried out using the chromosome aberration assay. Consequently, it must be removed from the sister-chromatid endpoint towards the chromosomal aberrations endpoint.

In the section of **Developmental and Reproductive Toxicity** lacks the study of Bielmeier et al. (2007)

Bielmeier SR, Murr AS, Best DS, Harrison RA, Pegram RA, Goldman JM, Narotsky MG. Effects of bromodichloromethane on ex vivo and in vitro luteal function and bromodichloromethane tissue dosimetry in the pregnant F344 rat. *Toxicol In Vitro*. 2007 21(5): 919-928.

In this section, a previous study of these authors is indicated (Bielmeier et al. 2001). The new results suggest that BDCM disrupts pregnancy in F344 rats via two modes: disruption of luteinizing hormone (LH) secretion, and disruption of the corpore lutea's ability to respond to LH.

In the **section of Immunotoxicity** lacks the study of Alhasson et al., 2016.

Alhasson F, Dattaroy D, Das S, Chandrashekar V, Seth RK, Schnellmann RG, Chatterjee S. NKT cell modulates NAFLD potentiation of metabolic oxidative stress-induced mesangial cell activation and proximal tubular toxicity. *Am J Physiol Renal Physiol*. 2016, 310(1): F85-F101.

In that study, authors indicate that obesity and nonalcoholic fatty liver disease (NAFLD) are associated with the development and progression of chronic kidney disease. In addition, NAFLD induces liver-specific cytochrome P-450 (CYP)2E1-mediated metabolic oxidative stress after administration of bromodichloromethane (BDCM), acting as a substrate of CYP2E1 enzyme. In addition, NAFLD CD1D knockout mice treated with BDCM exhibited increased tubular cell death and cytokine release, as consequence of exposure.

In the **section of Neurotoxicity** are missing the studies of Moser et al. (2007) and Villanueva et al., (2018).

Moser VC, Phillips PM, McDaniel KL, Sills RC. Neurotoxicological evaluation of two disinfection by-products, bromodichloromethane and dibromoacetonitrile, in rats. *Toxicology*, 2007, 230(2-3): 137-144.

Villanueva CM, Gracia-Lavedan E, Julvez J, Santa-Marina L, Lertxundi N, Ibarluzea J, Llop S, Ballester F, Fernández-Somoano A, Tardón A, Vrijheid M, Guxens M, Sunyer J. Drinking water disinfection by-products during pregnancy and child neuropsychological development in the INMA Spanish cohort study. *Environ. Int.*, 2018, 110: 113-122.

In the first study, bromodichloromethane (BDCM) was administered to male and female F-344 rats via drinking water for 6 months. Average intakes were approximately: 9, 27, and 72 mg/(kg day). Results indicated few neurobehavioral changes, but these were not considered as toxicologically relevant.

The second study is a population-based mother-child cohort study in Spain. Neuropsychological development was measured at 1 year of age using the Bayley Scales of Infant Development, and at 4-5 years with the McCarthy Scales of Children's Abilities. Minor associations were observed between DBP exposure during gestation and child neuropsychological development at 1 year, but disappeared at 4-5 years. Although a suggestive association was identified for exposure to brominated THMs and the cognitive score at 4-5 years, according to the authors chance cannot be ruled out.

In the **section of Carcinogenesis (Effects in Humans)**. It is true that from the epidemiological studies it is not possible to assign a potential risk to individual THM compounds. Nevertheless, there is a relative new paper that could be indicated, because the reported study was carried out in the US population: Min and Min (2016).

Min JY, Min KB. Blood trihalomethane levels and the risk of total cancer mortality in US adults. Environ. Pollut., 2016, 212: 90-96.

This study analyzed data from the 1999-2004 Third National Health and Nutrition Examination Survey and the Linked Mortality File of the United States. A total of 933 adults (20-59 years of age) with available blood THM levels, and no missing data for other variables, were included. Four different THM species (chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform) were included. Results indicate that the baseline blood THM species, particularly brominated THMs, were significantly associated with total cancer mortality in adults. Although this study should be confirm by other studies, findings suggest a possible link between THM exposures and cancer.

Section 8. Toxicological profile of Dibromochloromethane

Genetic toxicity section (page 183).

As occurs in the other cases, the structure of this section is a bit confusing.

In the Effects in Animals section, studies using *Aspergillus* are included! I would prefer two sections referring to those studies using whole organisms (in vivo studies), and to studies using human/mammalian cells and bacteria, yeast and fungus (in vitro studies). Consequently, the already existing sections in vitro assays and in vivo assays need to gain relevance in the content of this section.

If studies are presented/discussed according to their relevance, after the studies in humans those *in vivo* studies should be placed, before the *in vitro* studies.

Tables and text should follow the same rational. If text starts explaining data of primary DNA damage, and moving toward a higher complexity, tables should not start with i.e. micronuclei induction, that refers to fixed damage with special relevance as biomarker of cancer risk.

In Table 8.5, the study of Kogevinas et al. (2010) should be eliminated because this is the study already indicated in the Biomonitoring studies with humans (Effects in humans).

I have detected that there is a study (Sekihashi et al., 2002) lacking in Table 8.5.

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, Knasmüller S, Tsuda S, Sasaki YF. Comparative investigation of multiple organs of mice and rats in the comet assay. Mutat Res. 2002, 517(1-2): 53-75.

In that study, rats and mice were orally exposed to DBCM and the effects on DNA were evaluated using the comet assay (measuring DNA strand breaks) in different organs. Rats resulted more sensitive since positive genotoxic effects were detected in stomach, colon, liver, kidney, blood and lungs. In mice, positive induction of DNA damage was observed in colon, liver and brain.

In the *in vivo* studies (Table 8.5), results with zebrafish are included. Nevertheless, the studies with *Drosophila* are included in the *in vitro* studies (Table 8.4). *Drosophila* is a classical *in vivo* model; consequently, this information must move from the *in vitro* to the *in vivo* section

In the *in vitro* data, the results of Benigni et al. (1993) measuring aneuploidy in *Aspergillus*, and those of Matsuoka et al. (1996) measuring aneuploidy in Chinese hamster lung fibroblasts should be placed/discussed after the chromosomal aberrations discussion. It must be remembered that aneuploidy is a chromosome numerical aberration.

Regarding the genotoxic mechanism of action, and the consequent risk for humans, the paper of Landi et al. (1999) could be quoted:

Landi S, Hanley NM, Warren SH, Pegram RA, DeMarini DM. Induction of genetic damage in human lymphocytes and mutations in *Salmonella* by trihalomethanes: role of red blood cells and *GSTT1-1* polymorphism. *Mutagenesis*, 1999, 14(5): 479-482.

In that study, authors exposed *Salmonella* strains -expressing or not the *TPT100* gene- to the most mutagenic brominated THM detected in *Salmonella*, dibromochloromethane (DBCM). This study was carried out either in the presence or absence of S9 or red blood cells from *GSTT1-1(+)* or *GSTT1-1(-)* individuals. S9 did not activate DBCM in the non-expressing strain, and it did not affect the ability of the expressing strain to activate DBCM. As with S9, red cells from either genotypic group were unable to activate DBCM in the TPT100 strain. However, red cells (whole or lysed) from both genotypic groups completely repressed the ability of the expressing strain RSJ100 to activate DBCM to a mutagenic compound. Such results suggest a model in which exposure to brominated THMs may pose an excess genotoxic risk in *GSTT1-1(+)* individuals, to those organs and tissues that both express this gene and come into direct contact with the brominated THMs, such as is the case of colon tissue.

In the **section of Developmental and Reproductive Toxicity** perhaps the study of Narotsky et al. (2011) should be included:

Narotsky MG, Best DS, McDonald A, Godin EA, Hunter ES 3rd, Simmons JE. Pregnancy loss and eye malformations in offspring of F344 rats following gestational exposure to mixtures of regulated trihalomethanes and haloacetic acids. *Reprod Toxicol*. 2011, 31(1): 59-65

In that study, F344 rats were treated with mixtures of the four THMs (chloroform, bromodichloromethane, chlorodibromomethane and bromoform). The mixtures were administered daily by gavage on gestation days 6-20. Litters were examined postnatally. This approach does not include visceral or skeletal examinations and, therefore, would not be able to detect some anatomical changes potentially caused by THM. However, in conjunction with assessing growth and viability, this approach readily detects pregnancy loss and micro-/anophthalmia, two endpoints of particular interest for THMs exposure. Results indicated that THM mixture caused pregnancy loss at $\geq 613 \mu\text{mol/kg/day}$, but not micro-/anophthalmia.

In the **section of Neurotoxicity**, as indicated for the other THMs perhaps a reference on the study of Villanueva et al., (2018) should also be indicated.

Villanueva CM, Gracia-Lavedan E, Julvez J, Santa-Marina L, Lertxundi N, Ibarluzea J, Llop S, Ballester F, Fernández-Somoano A, Tardón A, Vrijheid M, Guxens M, Sunyer J. Drinking water disinfection by-products during pregnancy and child neuropsychological development in the INMA Spanish cohort study. *Environ. Int.*, 2018, 110: 113-122.

This is a population-based mother-child cohort study in Spain. Neuropsychological development was measured at 1 year of age using the Bayley Scales of Infant Development and at 4-5 years with the McCarthy Scales of Children's Abilities. Minor associations were observed between DBP exposure during gestation and child neuropsychological development at 1 year disappeared at 4-5 years. Although a suggestive association was identified for exposure to brominated THMs and the cognitive score at 4-5 years, chance cannot be ruled out.

Section 9. Mechanisms of action of carcinogenicity

I do not know if the revision of Komulainen (2004) on this topic should be incorporated elsewhere.

Komulainen H. Experimental cancer studies of chlorinated by-products. *Toxicology*. 2004, 198(1-3): 239-248. Review

When explaining the mechanism of action of THMs inducing carcinogenicity, nothing is indicated about the potential role of epigenetic changes induced by THMs exposure and their relationship with cancer incidence.

Enclosed there are four papers dealing with this topics that can help to understand this potential mechanism of action.

Salas LA, Villanueva CM, Tajuddin SM, Amaral AF, Fernandez AF, Moore LE, Carrato A, Tardón A, Serra C, García-Closas R, Basagaña X, Rothman N, Silverman DT, Cantor KP, Kogevinas M, Real FX, Fraga MF, Malats N. LINE-1 methylation in granulocyte DNA and trihalomethane exposure is associated with bladder cancer risk. *Epigenetics*. 2014 9(11): 1532-1539.

- Donà F, Houseley J. Unexpected DNA loss mediated by the DNA binding activity of ribonuclease A. *PLoS One*. 2014 9(12): e115008.
- Salas LA, Bustamante M, Gonzalez JR, Gracia-Lavedan E, Moreno V, Kogevinas M, Villanueva CM. DNA methylation levels and long-term trihalomethane exposure in drinking water: an epigenome-wide association study. *Epigenetics*. 2015, 10(7): 650-661.
- Kuppusamy SP, Kaiser JP, Wesselkamper SC. Epigenetic Regulation in Environmental Chemical Carcinogenesis and its Applicability in Human Health Risk Assessment. *Int J Toxicol*. 2015 34(5): 384-392

It is obvious that most of the studies of carcinogenesis carried out using THMs (and by extension DBPs) were carried time ago and, and at such moments the role of epigenetic mechanisms was underdeveloped. At present, this mechanism cannot be ignored, and less in an updated revision like the present.

Section 10. Dose-response assessment

This section explains very well the methods used to calculate the acceptable daily dose (ADD). In the same way, the concept and sources to obtain the point of departure (POD) values are clearly established.

As indicated, to calculate the ADD it is necessary to include certain *uncertainty factors (UF)*. This would mean that calculated ADD values are an estimate more than a real and unquestionable value. This should be clearly stated for any reader of the document, mainly for those who are not experts. Thus, slight changes in the denominator of the formula ($ADD=POD/UF$) can produce important changes in the estimated ADD values.

When considering the ADD estimation for the respective THMs, it is clear that the more robust is the experimental background more confident are the obtained data. In addition, although a chronic study looks robust, in most of the cases there is one only experiment, without the possibility to contrast the obtained results with other equivalent studies. In the case of i.e. chloroform, this occurs with the study of Heywood et al. (1979) using dog Beagle, being this study the only one using this mammalian model organism in cancer studies.

Although this general criticism is applicable to both non-cancer and cancer dose-response analyses, for the non-cancer analysis the included studies usually evaluate different targets related with the effects on the same organ. For i.e. chloroform, effects on liver move from “increased fat cysts plus increased markers of liver damage” (Heywood et al., 1979) to “hepatic necrosis” (Hard et al., 2000). Obviously, it can be argued that both end-point represent different aspects of “liver lesions”. In addition, the number of studies evaluating the effects of the different THMs is also an important variable. It is obvious that there are more studies evaluating the effects of chloroform and bromoform than for BDCM and DBCM, which can suppose a potential bias.

For the cancer dose-response analysis, perhaps some of the objections above indicated are not applicable since all the studies used the same target: neoplastic lesions. Nevertheless, there is an important point that I do not find reflected in the document. This is related with the evaluated target. From the epidemiological data in humans, it seems the most of the studies agree that bladder cancer is the tumor more frequently associated to THMs exposure. Nevertheless, this is not a target evaluated/found in the studies using mammalian models, where usually liver and kidney are the organs giving positive in animal studies. Again, it could be argued that kidneys and bladder form part of the same genitourinary system.

It would be nice if this target-discrepancies are indicated/discussed elsewhere.

In the **cancer dose-response analyses** and **cancer potency derivation**, it should be stated the difficulties of establish robust dose-response curves when only two, or maximum three doses have been evaluated in the animal cancer study.

Section 11. Health protective drinking water concentrations

This last section starts evaluating the non-cancer (and cancer) health-protective water concentrations. Since the concentrations for non-cancer are higher than for cancer effects, the section focus mainly in cancer as a target.

It is an interesting approach to differentiate between life stages, because the sensitivity can be different, and the exposure levels also. General considerations about habits can be dangerous. Perhaps it is true that infants do not get used to showering as adults (avoiding exposure to volatile forms) but, alternatively, they possibly take more and longer baths.

At the end of this section, there is an interesting reflection about the benefits of disinfection *versus* THM risk. This reflection, may change the use of 1×10^{-6} as cancer risk level?

In the subsection of *Risk Characterization*, different mechanisms and uncertainties are indicated. For i.e. genotoxicity, no all the authors found positives results, what leave some doubts about their relevance. In addition, the fact that the most “potent” data were obtained in bacteria reduces a bit its relevance when risk characterization approaches are used.

The point about potential interactions between THMs, and by extension between DBPs, is also interesting. As indicated, some studies reported synergistic effects between compounds. Unfortunately, the complexity of the problem with many DBPs in water samples, make difficult to get sound answers on this topic.