Responses to Peer Review and Public Comments

Technical Support Document: Public Health Goals for Trihalomethanes in Drinking Water

February 2020

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Prepared by

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INTRODUCTION

This document contains responses to public comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for trihalomethanes (THMs) during the first and second public comment periods, and to comments from the external scientific peer reviewers.

OEHHA released the first draft of this PHG document for public comment on October 5, 2018, and held a public workshop on November 29, 2018 in Sacramento, California. The public comment period closed on January 4, 2019, after a 30-day extension. OEHHA received comments from the American Chemistry Council (ACC), the Association of California Water Agencies, the California Water Association, the California Municipal Utilities Association, the Southern California Water Coalition, the California – Nevada Section of the American Water Works Association, and the Environmental Working Group.

OEHHA released the second draft of the PHG technical support document for public comment on November 8, 2019. The public comment period closed on December 9, 2019. OEHHA received comments from the ACC.

Pursuant to Health and Safety Code section 116365(c)(3)(D), OEHHA submitted the THM PHG document for scientific peer review following the closure of the first comment period. Comments were received from the peer reviewers in May 2019.

The external scientific peer reviewers were:

Dale Hattis, PhD
Professor
The George Perkins Marsh Institute
Clark University
Worcester, MA

Matias S. Attene Ramos, PhD
Associate Professor
Department of Environmental and Occupational Health
Milken Institute School of Public Health
The George Washington University
Washington, D.C.

Cristina Villanueva Belmonte, PhD
Associate Research Professor
ISGlobal-Barcelona Institute of Global Health
Barcelona Biomedical Research Park (PRBB)
Barcelona, Spain

Ricard Marcos Dauder, PhD
Professor
Universitat Autonoma de Barcelona
Barcelona, Spain

OEHHA made changes in response to the public and peer review comments as appropriate, and incorporated them into the final version of the PHG technical support document posted on the OEHHA website.

The full public comments and peer review comment letters are posted on the OEHHA website along with this response document, and the final version of the PHG document.

In this document, comments appear in quotation marks where they are directly quoted from the submission. Note that for the public comments where the commenter included a footnote, we did not copy the footnote into the response document. Footnotes can be seen in the original public comment letters posted on the OEHHA website.

For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA web site at www.oehha.ca.gov.

OEHHA may also be contacted at:

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RESPONSES TO COMMENTS MADE DURING THE FIRST PUBLIC COMMENT PERIOD

RESPONSES TO COMMENTS RECEIVED FROM AMERICAN CHEMISTRY COUNCIL (ACC) (JANUARY 2019)

Comment 1. "OEHHA Has Not Assessed the Public Health Benefits of the Chlorination of Drinking Water that Can Result in Trihalomethane Formation"

"The gravity of public health risks addressed by drinking water disinfection necessitate a more thoughtful and balanced approached to THM risk assessment than is reflected in the TSD. OEHHA acknowledges the importance of disinfection relative to incremental cancer risk from exposure to THMs by reference to WHO and IARC findings (page 2), but then proceeds to evaluate THM cancer risks in isolation of the public health risks that could result from actions to reduce THM concentrations. This approach does not satisfy OEHHA's statutory requirements pertaining to the development of PHGs and is potentially harmful to public health."

"In establishing a PHG, Health and Safety Code §116365 directs OEHHA to estimate the level of the contaminant in drinking water "that is not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health" (emphasis added). While §116365 focuses on health effects associated with the drinking water contaminant, OEHHA also must consider "any significant risk to health." In other words, OEHHA must assess all potential health risks that directly relate to the action being contemplated. Due to the inseparable relationship between THM formation and life-saving disinfection of drinking water, and consistent with the WHO's cautionary language about controlling DBPs, any evaluation of THMs must take into account the significant public health benefits associated with chlorine disinfection of drinking water."

Response 1. We agree completely as to the utility and importance of drinking water disinfection, which the draft PHG document discussed on pg. 2 and pg. 268.

The commenter misinterprets the statute. In requiring OEHHA to prepare health risk assessments of drinking water contaminants, Health and Safety Code section 116365(c)(1) states:

> "The risk assessment shall contain an estimate of the level of the contaminant in drinking water that is not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health."

Thus, the statute is clear that OEHHA must look strictly at the adverse effects of the contaminant itself when developing a PHG. OEHHA's responsibility is to review the toxicity of chemicals in drinking water that are associated with disinfection, and assess risks from exposure to these byproducts. The risk assessment for THMs is based exclusively on risks from exposure to the THMs. Evaluation of the risks from microbial contaminants that might otherwise be present without disinfection of the drinking water, is outside the scope of this assessment.

The State Water Resources Control Board (SWRCB) establishes Maximum Contaminant Levels (MCL) in consideration of multiple factors. The risk-benefit tradeoff between residual disinfection byproducts in drinking water and exposure to microbial contaminants in drinking water is one of the factors that SWRCB will consider in updating the California MCL for THMs. OEHHA fully supports disinfection of drinking water, but this document is not, nor is it intended to be, an analysis of the health benefits of chlorine disinfection of drinking water.

Comment 2. "The Draft PHGs Are a Significant Departure from the Established Approach to Addressing Trihalomethanes"

"The California MCL for total THMs of 0.08 mg/L (80 µg/L) is the sum of the concentrations of chloroform, bromoform, BDCM, and DBCM. It was established by the California Department of Public Health in 2006. In 2010 OEHHA proposed a PHG for total THM based on an assessment of "the mean concentrations of each of the four chemicals in California drinking water" but never finalized the PHG. In the current proposal, OEHHA has replaced the single PHG for total THM with separate PHGs for each of the four THMs.

"OEHHA's proposal would appear to require that the SWRCB establish separate MCLs for each of the four THMs since there is no 'corresponding' PHG for total THM. If, on the other hand, the SWRCB determines that it can maintain the current approach of specifying a single MCL for total THMs, it would need to demonstrate how a single standard for total THM meets the requirement to be 'as close as feasible' to the four individual PHGs."

Response 2. Because the proportion of each THM found in drinking water varies regionally, it is not possible to identify a single PHG number for total THMs that represents the same *de minimis* risk level for all California drinking water, or to determine a single proportion of THMs that is representative of all California drinking water. Hence, OEHHA has proposed separate PHGs for the four THMs.

SWRCB has no objection to OEHHA's approach. Further, OEHHA's proposal does not require SWRCB to establish separate MCLs for each of the four THMs.

Comment 3. "A Recently Completed Analysis Finds no Association between Trihalomethane Exposure and Increased Bladder Cancer Risk"

"As OEHHA notes in the draft PHG document, USEPA, IARC, WHO, and Health Canada have found the epidemiological evidence for a weak association with bladder cancer to be inconclusive because of the large number of potentially confounding factors. Although bladder cancer incidence rates are a somewhat consistent result in some human studies, it is not possible to assess the potential impact of individual THMs or total THM because of smoking rates and other confounders and the long latency

period and high diagnosis age associated with the disease. However, a recently completed analysis of national trends in bladder cancer incidence provides important new insights into any potential association with THM exposure.

"The study found no discernible relationship between total THMs in drinking water and bladder cancer incidence in the U.S. over the more than 40 years since the passage of the federal SDWA. As noted in Figure 1, bladder cancer incidence rates in both males and females have remained relatively stable since the mid-1970s, while THM concentrations have been reduced by more than half over the same time period."

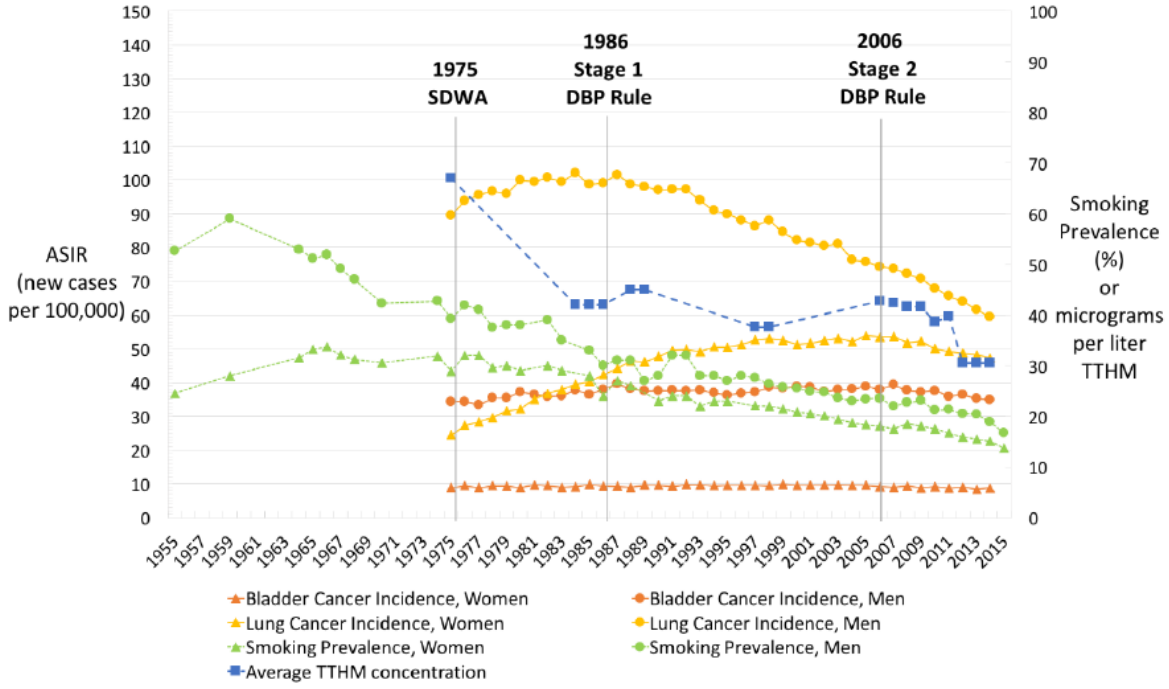


Figure 1. Annual age-adjusted smoking prevalence and bladder and lung cancer incidence, and total THM (TTHM) concentrations in drinking water systems in the United States, 1975-2015. (From Cortruvo and Amato, 2019)

Response 3. The information in Figure 1 of Cotruvo and Amato (2019) is based on an extremely broad ecologic assessment of both the exposure (national average total trihalomethane (TTHM) concentrations) and the outcome (national US bladder cancer incidence rates). There is no individual level information on whether those people who developed bladder cancer had higher or lower TTHM exposures than those who did not have bladder cancer. TTHM levels vary considerably from water source to water source across the US, so an ecologic assessment like this is not a valid way to evaluate causal associations. In addition, as discussed by Cotruvo and Amato (2019), there are a number of known factors that impact bladder cancer incidence rates. However, none of these is accounted for in the analysis shown in Figure 1. Any changes in the prevalence of any of these other factors over time could mask a potential relationship between TTHMs and bladder cancer in this type of very broad ecologic analysis.

National smoking rates are displayed, but smoking rates presented on a national level are not useful for evaluating the relationship between THMs and bladder cancer.

With regards to confounding, the most prevalent risk factors for bladder cancer are older age, smoking, race, and male sex, and most of the bladder cancer studies we reviewed in our draft PHG report (Appendix C) adjusted for or otherwise controlled for these factors. Several studies also adjusted or controlled for other potential risk factors including occupation or diet. Other risk factors like arsenic in drinking water, family history, or certain rare genetic conditions are likely not prevalent enough or not strongly related enough to THM levels to cause the increased relative risks identified in the studies we reviewed (Axelson, 1978).

Another important point regarding latency relates to the direction of the potential bias that might occur if a study did not appropriately account for latency. In all of the bladder cancer studies we reviewed, exposure was assessed using the same methods in all participants, regardless of whether they had bladder cancer or not. As such, any potential bias from errors in exposure assessment, such as those that might be related to latency, would most likely bias relative risk estimates towards the null, not towards the elevated relative risks identified in many of the studies we reviewed (Rothman and Greenland, 1998).

Most of the studies we reviewed were designed to consider the possibility of a long latency. For example, in the bladder cancer case-control study by Villanueva et al. (2007), THM exposure was based on residential history beginning at age 15 years old. Since the large majority of participants in this study were over 65 years old, this study accounted for a latency of up to 50 years in most subjects. In Cantor et al. (1998), exposure was based on lifetime residential history, ≥70% of which was known, and in King and Marrett (1996), only participants with at least 30 years of exposure data were included.

Comment 4. "There is Strong Evidence of Pharmacokinetic Differences between Exposure in Drinking Water and Dosing by Gavage"

"The proposed PHGs for the four THMs are based on cancer evidence from gavage studies using corn oil, despite the fact that drinking water and dietary studies have generally produced negative results. In a comparative study of gavage and drinking water exposures, THMs administered by gavage increased cell proliferation and decreased DNA methylation in mouse livers while dosing in drinking water produced a much smaller effect, particularly for chloroform.[13] These findings are consistent with the dose-response curves observed for the THMs, especially chloroform and BDCM, which suggest that THM levels must be sufficiently high to overcome natural detoxification mechanisms before they can exert a toxic effect. The weaker activity of THMs administered in drinking water likely results from its incremental delivery each time the mouse drinks – in contrast to bolus delivery by oral gavage. The slower rate of delivery by drinking water is expected to result in a lower liver concentration that increases the

opportunity for detoxification (Figure 2). Hence, the activity of the THM appears to be dependent on their rate of delivery (i.e., rapidly by oral gavage and more slowly in drinking water)."

"The pharmacokinetic differences between bolus-gavage and drinking-water (and dietary) dosing appear to play a significant role in explaining the disparity in the observed tumor incidence in the animal studies and need to be taken into consideration in assessing the toxicity of the THMs."

Response 4. OEHHA does not dispute that pharmacokinetic differences may exist among different routes and methods of exposure, though OEHHA disagrees that these pharmacokinetic differences ameliorate any cancer risk. It is not appropriate to assume that detoxification is complete following drinking water exposures; reactive metabolites generated at the cellular level can react with macromolecules including DNA.

In estimating the cancer slope factor for chloroform in the draft PHG document, OEHHA utilized studies that exposed animals via drinking water, drinking water plus inhalation, and gavage. The geometric mean was used as the basis for the PHG calculation. As indicated in the OEHHA draft document, "The geometric mean was calculated for the following reasons:

- the differences in species, strain, and sex of animals tested;
- the differences in route of administration, i.e., oral ingestion vs. inhalation;
- the differences in vehicle administration, i.e., gavage in corn oil or in toothpaste vs. drinking water;
- and the wide range of CSF_{human} estimates calculated, i.e., the forty-fold difference between the lowest value of 0.003 $(mg/kg\text{-}day)^{-1}$ and the highest value of 0.128 $(mg/kg\text{-}day)^{-1}$."

Not all drinking water studies of the THMs were negative. For example, among studies on BDCM, George et al. (2002) and Tumasonis et al. (1987) found significant increases in hepatic tumors in drinking water studies in rats. Tumasonis et al (1985) found increased incidence of liver tumors in rats treated with chloroform by the drinking water route. In Jorgenson et al. (1985), the incidences of renal tubular cell adenoma and combined renal tubular cell adenoma and carcinoma were significantly greater among high-dose Osborne-Mendel rats ($p < 0.01$, Peto Trend Test) treated with chloroform via drinking water, compared to matched controls. Further, in Nagano et al. (2006), the combined exposures to chloroform by inhalation and drinking water resulted in increased renal cell adenoma and carcinoma in Fisher 344 rats. Thus, THMs have been shown to induce cancer in animal models following drinking water and inhalation exposures, not just by gavage.

ACC's comments cited the Coffin et al. (2000) study as evidence that THMs administered by gavage increased cell proliferation and decreased DNA methylation in mouse livers while dosing in drinking water produced a much smaller effect. However, we note that toxicity as indicated by DNA methylation and increased cell proliferation

(labeling index) was still observed following drinking water exposures. For example, the proliferating cell nuclear antigenlabeling index (PCNA-LI) results in Figure 6 of the paper showed statistically significant increases for two of the four trihalomethanes administered in drinking water. The low-dose gavage exposures of the trihalomethanes increased the PCNA-LI to an extent similar to when the trihalomethanes were administered in drinking water. In the case of bromoform, administration in drinking water resulted in a higher level of labeling than a similar dose by gavage (Figures 5 and 6). Further, the results in Figure 10 in Coffin et al. (2000) clearly demonstrated there was no difference among routes of exposure in terms of decreasing DNA methylation for three out of four trihalomethanes examined (chloroform was the only exception). Thus, this paper does not support the notion that the trihalomethanes when given in drinking water cannot produce tumors.

Comment 5. "OEHHA Has Significantly Overestimated the Drinking Water Consumption Rate in Calculating the Public Health Goals"

"OEHHA's calculation of the drinking water concentration associated with a cancer risk of 10^{-6} for each of the four THMs is based on a susceptibility-weighted daily water intake (DWI). The weighted DWI, expressed in equivalent liters of water consumed per kilogram body weight per day, or Leq/kg-day, represents the product of the age sensitivity factor (ASF), the time spent in each life stage (expressed as a ratio), and the unweighted DWI for the life stage. Although this approach is consistent with OEHHA's method for accounting for early life-stage exposures, OEHHA proposes to add the weighted DWIs for each life stage to produce a lifetime DWI (DWIlife) as opposed to taking an average of the four life stage values.[16] The result is an inappropriately high estimate for lifetime daily water consumption, which also biases the calculated risk value to indicate and improbably high health risk.

"Using the DWIlife for chloroform of 0.180 Leq/kg-day, for example, a 70-kilogram adult would consume the equivalent of 12.6 liters of water per day – four times the total daily consumption rate of 3 Leq/day assumed in the 2010 draft PHG.[17] While ACC appreciates OEHHA's attempt to account for age sensitivity, an estimate that assumes a four-fold higher consumption rate for three-quarters of an individual's lifetime grossly overstates probable lifetime exposures for water use and consumption, even among outliers in the population. Using the average of the susceptibility weighted DWIs (equal to 0.045 Leq/kg-day), on the other hand, generates a total adult consumption rate of 3.15 Leq/day, which – while still quite high – is consistent with OEHHA's 2010 assumption."

"The disparity in assumptions about drinking water consumption rates is even more apparent when the proposed PHGs are compared to the no significant risk levels (NSRLs) developed by OEHHA under Proposition 65. For example, although OEHHA has lowered its cancer potency factor for chloroform by 60 percent (from 0.035 to 0.0137 per mg/kg-day) in the latest assessment, the estimate of drinking water risk for the proposed PHG is dramatically higher. Based on OEHHA's latest estimate, a 70-kg

individual would need to consume the equivalent of 50 liters of water daily to achieve the NSRL of 20 micrograms per day (µg/day) for chloroform (Table 1)."

Response 5. OEHHA disagrees with the assertion that drinking water intake rates have been overestimated. There are two components to OEHHA's susceptibility-weighted daily water intake rates that are independent of one another but appropriately applied. First, the age sensitivity factor (ASF) is a weighting factor applied to exposure at different lifestages to account for the increased susceptibility to carcinogens during early-in-life exposures. The ASFs reflect the available data and analyses indicating higher carcinogen potency when exposure occurs early in life (OEHHA, 2009). Since everyone goes through each lifestage, it is appropriate to add (not average as stated in the comment) the lifestage DWI to obtain a lifetime DWI. This is an approach consistent with US EPA's cancer risk assessment guidelines (US EPA, 2005a,b), which "view childhood as a sequence of lifestages rather than viewing children as a subpopulation" and states, "[F]or a susceptible lifestage, higher risks can be expected from exposures during only a portion of a lifetime, but everyone in the population may pass through those lifestages."

Second, liter equivalents (L_{eq}) account for the total amount of exposure to a chemical that one receives through the use of tap water. For volatile chemicals such as the THMs, exposure from tap water consists of oral ingestion as well as inhalation and dermal exposure while bathing, showering, and through other household uses. Thus, the L_{eq} value is not the amount of water an individual actually drinks but instead represents how much tap water one would have to drink to have the same amount of exposure to the chemical through the combined oral, inhalation, and dermal routes via typical household uses of tap water. Thus, one cannot compare the consumption rates multiplied by an ASF and inclusive of exposure via inhalation and dermal pathways to an unweighted estimate of drinking water ingestion rate for an adult.

Further, trying to compare the weighted exposure to how much water would need to be consumed by an adult to meet the NSRL from the Proposition 65 program is not appropriate. An NSRL is a general guidance number that identifies a level of exposure to a chemical from a product or environmental source that does not require a Proposition 65 warning. NSRLs are set for a higher risk level (10^{-5}) than PHGs (10^{-6}), and do not consider multipathway exposures. Further, neither age-specific drinking water ingestion rates nor age sensitivity factors are utilized in deriving NSRLs, which are not specific to drinking-water exposures.

Comments on carcinogenicity MOA for chloroform submitted by the American Chemistry Council, including their Attachment A analysis

"OEHHA Overstates the Potential Cancer Risk from Chloroform Exposure"

Comment 6. "…As noted by USEPA and WHO, however, there is compelling mechanistic evidence that both the hepatic and renal tumorigenic responses observed in previous carcinogenicity studies of chloroform are mediated by a non-genotoxic mechanism. As a consequence, USEPA concludes that 'chloroform is likely to be carcinogenic to humans only under high exposure conditions that lead to cytotoxicity and regenerative hyperplasia'.

"A state-of-the-science quantitative comparison of the available data for genotoxic and nongenotoxic cancer mechanism is included as Attachment A of these comments, based on a confidence scoring system developed by Becker et al. (2017). The results of this comparison indicate that there is strong counter evidence for several of the early diagnostic key events for a mutagenic mode of action (MoA), including three negative in vivo transgenic mouse datasets."

"In support of its conclusion, the draft PHG suggests 'a plausible genotoxic mechanism of chloroform carcinogenicity that involves covalent binding of chloroform-derived reactive metabolites to nucleic acid, nuclear protein or phospholipid.'

"Chloroform has produced generally negative results in tests for genotoxicity, however, both in vitro and in vivo. Using a published, comprehensive, quantitative weight-of-evidence (WoE) approach to evaluate large, heterogeneous genetic toxicology databases, chloroform's potential mutagenicity was assessed by an expert panel convened by the Health and Environmental Sciences Institute of the International Life Sciences Institute. On a scale of negative 100 (-100) to positive 100 (+100), chloroform scored negative 14.3 (-14.3), indicating that the WoE supports a non-genotoxic classification. Regarding conflicting data, the expert panel noted that --

> [T]he fact that a compound causes genotoxicity under some limited set of experimental conditions does not necessarily mean that carcinogenic effects of the compound would be related to mutagenicity.

"As a result, Boobis (2010) concluded that 'the weight of evidence is that genotoxicity is not the MoA for chloroform.' These findings are discussed in greater detail in Attachment A.

"OEHHA notes in the draft PHG that oxidative metabolism of chloroform to form phosgene via the P450 pathway is critical to its toxicity….Phosgene is a highly reactive electrophile that reacts rapidly to form covalent bonds with intracellular nucleophiles such as glutathione, proteins, lipids and other macromolecules. As a result, phosgene likely does not diffuse far from its site of production in mitochondria and the endoplasmic reticulum. This limits its potential molecular targets to those organelles and renders interaction with DNA in the nucleus highly unlikely, if not impossible. Conversion to phosgene as an obligate event in chloroform toxicity is thus consistent with the lack of evidence for chloroform-induced DNA damage *in vivo."*

Response 6. Carcinogens can have multiple modes of action (Guyton et al, 2018a, 2018b; Parfett and Desaulnier, 2017). Mode of action can depend on when during the lifestage exposure occurs or the level of exposure. The US Environmental Protection Agency (US EPA) says in its IRIS assessment of chloroform: "Thus, the weight-of-evidence of the genotoxicity data on chloroform supports a conclusion that chloroform is not strongly mutagenic, and the genotoxicity is not likely to be the predominant mode of action underlying the carcinogenic potential of chloroform." US EPA does not state that chloroform is NOT mutagenic, but that is not strongly so. Further, at higher levels of exposure US EPA does not say genotoxicity plays no role, but believes that cytotoxicity and subsequent tissue regeneration is active at higher exposures. In the case of chloroform, it is quite plausible that cytotoxicity and regeneration play a role at high levels of exposure, but that role is not evident at lower levels of exposure. In their discussion of uncertainties from their 2001 IRIS assessment of chloroform, US EPA notes that one of the uncertainties is that there are positive mutagenicity studies despite the overall negative results in the database. Note that US EPA used a threshold model to estimate a safe level of exposure for carcinogenicity by the oral route. However, they retain the linear model for inhalation exposure (noting in the IRIS file that they have not yet revisited the inhalation route of exposure). The available evidence is not, in our view, strong enough to deny any role for genotoxicity for chloroform-induced tumorigenicity. See responses below to Attachment A analysis for further discussion of the potential genotoxicity of chloroform.

OEHHA utilizes linearized extrapolation for dose-response characterization when there is insufficient evidence to rule out a non-threshold mechanism of action of a carcinogen. Although it is true that most studies of the genotoxicity of chloroform were negative, some were positive. Further, chloroform is metabolized to reactive metabolites including phosgene and dichloromethyl radical. Evidence for additional reactive metabolites is also in the literature. Several studies on chloroform demonstrated binding of phosgene to lipids and proteins, including the histones surrounding DNA; binding to histones means phosgene entered the nucleus and binding was not limited to macromolecules in the endoplasmic reticulum where it is produced. We agree with US EPA that chloroform is not strongly mutagenic. We disagree that it should, therefore, be treated solely as a threshold carcinogen and that low exposures can be ignored.

See below for more detailed responses to the analysis referred to in this comment as Attachment A.

Comment 7. "*In vitro* and *in vivo* evidence indicate that chloroform cytotoxicity and cell death exhibit a threshold in both liver and kidney. Together with the lack of chloroform toxicity in tissues that do not express CYP2E1, these results strongly indicate that sustained phosgene-induced cytotoxicity is a key event in chloroform-induced carcinogenesis."

Response 7. We agree that metabolism of chloroform by cytochrome P450 enzymes is important to the toxicity. Studies have shown chloroform is metabolized to phosgene

and other highly reactive chemicals *in vivo* and these metabolites may cause the range of toxicity effects observed, including carcinogenicity.

As noted in chapter 9 of the draft PHG document, the relationship between cytotoxicity, cell death and subsequent tissue regeneration, indicated by an increase in DNA synthesis as measured by labeling index (the proportion of labeled cells in S phase), has been extensively studied following chloroform administration. DNA synthesis is measured usually with either H^3-thymidine followed by autoradiography or with bromodeoxyuridine followed by methods to detect labeled DNA via monoclonal antibodies. DNA synthesis precedes cell division and thus is a good marker for tissue regeneration (Madhavan, 2007). However, increases in labeling index often did not reflect the observed pattern of toxicity, including carcinogenicity. Studies reported:

- toxicity occurring without an apparent increase in labeling index;
- toxicity increasing with continual exposure while the labeling index diminished;
- increased labeling index without apparent toxicity;
- cytotoxicity and increases in the labeling index without any tumorigenic response evident in the cancer bioassays at equivalent exposures.

A number of studies described in chapter 9 of the PHG draft evaluated hepatotoxicity and nephrotoxicity after single doses of choroform and attempted to relate these with labeling index. The studies were done as investigations into whether the mechanism of carcinogenicity might be cytotoxicity followed by tissue regeneration leading to expression of pre-existing mutations. The concept is that cytotoxicity and tissue regeneration can be measured via histopathology and DNA labeling, and that these should only occur at doses equivalent to those shown to induce tumor formation in the NCI studies. Not all the results of these studies were supportive of such a hypothesis.

In Larson et al. (1993), hepatotoxicity was observed in the male F344 rat at a dose below the high dose (set to mimic the high dose in the NCI 176 study), but increased labeling index was only observed at the high dose. Also, in Larson et al. (1993), no pathological lesions of the kidney in the female B6C3F1 mice were observed at doses up to 477 mg/kg-d, while increased labeling index was reported at 350 mg/kg-d. Neither nephrotoxicity nor increased labeling index should have been observed in the kidney according to the hypothesis, because tumors did not occur in female mouse kidney at the doses (238 and 477 mg/kg-day) used in the NCI (1976) study.

Similar investigations were conducted using repeat exposures to evaluate the relationship between toxicity and labelling index in relation to doses used in the NCI carcinogenicity bioassays. As in the single dose studies, some results are supportive of their hypothesis of cytotoxicity followed by tissue regeneration as the mechanism of carcinogenicity. However, some of the results are inconsistent with this hypothesis. Larson et al. (1994b) gave chloroform to male B6C3F1 mice at 0, 34, 90, 138 or 277 mg/kg-day, three to five mice/dose group, in corn oil by gavage for four or five days/week for three weeks. Histopathology and labeling index were determined in the

liver and kidney. In this study, the changes in labeling index in the liver appear to be consistent with histopathology in the liver. Renal lesions and increased labeling index were observed at the two higher doses, which are the same doses given in the NCI study. However, in the NCI (1976) study, chloroform did not induce renal tumors in B6C3F1 mice; thus, this is inconsistent with their hypothesis of tumor induction by chloroform.

Larson et al. (1994c) administered chloroform in corn oil (0, 3, 10, 34, 90, 238 or 477 mg/kg-day) or drinking water (0, 60, 200, 400, 900, 1,800 ppm) to groups of female mice for four days or three weeks (five mice/group). Hepatotoxicity and nephrotoxicity as well as changes in labeling index in these tissues were investigated. Drinking water exposures did not appear to result in elevated labeling index, although the animals had considerably lower doses the first four days because they did not want to drink the water. Over time as their water consumption increased, their exposures increased; this may have provided some protection against toxicity since lower initial doses being protective of subsequent higher doses has been observed in other studies. Nonetheless, this study provides some evidence that cytotoxicity and tissue regeneration may modulate the tumor response.

Hepatotoxicity increased with gavage doses in corn oil $\geq$ 34 mg/kg-day and became more severe after three weeks. Doses of 238 and 477 mg/kg-day markedly increased labeling index in the liver. Labeling index was increased at 3 weeks in the 90 mg/kg-day dose group, but not at the lower dose (34 mg/kg-day) even in animals that displayed hepatotoxicity at three weeks. While hepatotoxicity was more severe after 3 weeks than after 4 days at doses $\geq$ 34 mg/kg-day, the increase in labeling index was considerably greater at 4 days than at 3 weeks; this is counter to what one might expect if hepatotoxicity was followed by significant tissue regeneration. Mice receiving 34 mg/kg-day chloroform in corn oil should have displayed increased labeling index at three weeks, given the evidence of hepatotoxicity in these animals. The authors report a NOAEL for hepatotoxicity of 10 mg/kg-day and a NOAEL for increased labeling index of 34 mg/kg-day. This shows hepatotoxicity could occur at levels with no increase in labeling index.

Also, in Larson et al (1994c), increases in labeling index in the kidney were observed in mice receiving chloroform in corn oil or drinking water without evidence of renal toxicity. Yet, the administration of chloroform resulted in increased tumors in female mouse liver in the NCI (1976) study, but not in the kidney. Thus, increases in labeling index of an organ is not always associated with toxicity of or increased tumors in that organ.

A similar study in male Fisher 344 rats also led to some evidence supporting the hypothesis and some evidence inconsistent with that hypothesis. For example, while chloroform in drinking water did not appear to elevate labeling index, there was an inconsistent relationship between labeling index and cytotoxicity in rats when chloroform was given by gavage. Dose-dependent degenerative changes were observed in the kidneys of male rats receiving chloroform doses of ≥ 34 mg/kg-day in corn oil, which was more severe at three weeks. Administration of chloroform in corn oil resulted in an

increase in labeling index in the kidney only at the highest dose at four days, but not at three weeks. Thus cytotoxicity did not correlate well with regenerative response in the rat kidney in this study.

In Templin et al (1996), following inhalation exposure, hepatotoxicity observed in male and female rats exposed to 90 ppm (for 3 weeks, 7 days/week, or for 13 weeks 5 days/week, or for 13 weeks, 7 days per week) was not associated with an increase in labeling index in the liver; however, at 300 ppm extensive hepatotoxicity and increased labeling index were observed at all exposure times. In this same study, there were kidney lesions and increased labeling index in the kidney of female rats in Templin et al. (1996), but carcinogenicity bioassays did not find kidney tumors in the female rats. Thus, the hepatotoxicity did not correlate well with tissue regeneration in this study in rats, nor did the renal toxicity in female rats correlate with tumors in the carcinogenicity bioassays.

Groups of eight- to nine-week-old female B6C3F1 mice (ten/dose group) were gavaged by Melnick et al. (1998) with various doses of one of the four THMs in corn oil five days/week for three weeks. For chloroform, the equivalent doses were 55, 110, 238, and 477 mg/kg-day. Changes in labeling index in the liver were both dose- and compound-related with increased labeling index highest for chloroform followed by BDCM, DBCM, and bromoform. The dose-related change in labeling index did not correspond with the changes in indicators of liver toxicity (serum enzyme activity and increased liver weight) with dose. These findings suggest that the increase in labeling index is not directly related or necessarily proportional to the increase in hepatotoxicity.

The evidence that “sustained phosgene-induced cytotoxicity is a key event in chloroform-induced carcinogenesis” is not as clear as the commenters suggest. OEHHA has chosen as a matter of public health policy and prudence to utilize a linear model to estimate cancer potency based on:

- some evidence for genotoxicity of chloroform;
- evidence that reactive intermediates are formed during metabolism of chloroform and in fact at least one, phosgene, is a major metabolite;
- evidence that phosgene can bind to macromolecules including the histones surrounding DNA, and that other reactive intermediates have been found bound to cellular macromolecules;
- inconsistencies in the evidence for the hypothesis that cytotoxicity and tissue regeneration are solely responsible for chloroform tumorigenicity.

Comment 8. “Mutagenic mechanisms would be expected to produce DNA damage and increase tumor incidence in target organs at any level of chloroform that produces reactive metabolites (*i.e.*, at all doses) -- yet this is clearly not observed. Furthermore, since conversion of chloroform to reactive phosgene increases with increasing chloroform blood concentrations, a mutagenic mechanism cannot be reconciled with the observation that chloroform tumorigenesis occurs following bolus gavage

administration, but not with most doses administered in drinking water, which produce a greater area under the chloroform blood concentration curve."

Response 8. Mutagenic chemicals may indeed produce DNA damage at all exposures. That is one of the premises behind linearized cancer modeling and the concept of no threshold. However, as exposures get lower, it becomes much more difficult to observe tumors in animal studies because of sample size limitations. In other words, while there may be some DNA damage in animals at low exposure levels, one would need a large sample size to detect tumor effects.

Further, as described in Chapter 5 of the draft PHG document, a statistically significant dose-related increase in tumorigenesis was observed in the Jorgenson drinking water study (rat kidney adenoma and adenocarcinoma), as well as in studies via inhalation (Nagano et al, 1998; kidney adenomas and carcinomas in male mice) or a combination of inhalation and drinking water exposures (Nagano et al, 2006; renal adenomas and carcinomas in male rats). Thus, the implication in the comment that mutagenicity cannot be reconciled with the tumor incidences observed in animal studies with different routes of administration is inappropriate. While a greater area under the chloroform blood concentration-time curve may be associated with administration in drinking water, higher peak levels are likely obtained via bolus gavage which may influence the rate of tumor formation. The kinetic differences by route and method of administration and dosing level cannot be used to discount the role of genotoxicity in tumor formation or to discount the tumorigenicity of chloroform.

Comment 9. "Similarly, cytotoxic mechanisms that lack a threshold would be expected to increase tumor incidence at all chloroform doses at which cytotoxicity is measurable. This is also not observed. Instead, chloroform tumorigenesis requires dosing sufficient to sustain a cytotoxic effect in the kidney and/or liver. These observations argue strongly for a cytotoxic, threshold mechanism of chloroform carcinogenesis and preclude non-threshold mechanisms that might occur by either mutagenesis or cytotoxicity."

Response 9. The observations in animal studies do not preclude a role for mutagenicity in tumor induction. First, OEHHA's analysis is not based on a non-threshold mechanism related to cytotoxicity. Rather, we applied a linear model for estimating cancer potency using data from a number of studies and multiple exposure routes including gavage, drinking water and a combined drinking water and inhalation exposure. Second, even for strongly mutagenic compounds, it is often difficult to see tumors at very low exposures because it is logistically difficult to treat a large enough number of animals. Public health prudence dictates that OEHHA not ignore the genotoxicity of chloroform, albeit weak. Our analysis, therefore, assumes that the observed tumorigenicity in animals exposed to chloroform via bolus gavage, drinking water, inhalation, and inhalation plus drinking water may be at least in part due to genotoxic actions of chloroform.

Further, in the studies examining cytotoxicity, labeling index and the relationship to doses that produced tumors in the NCI studies, there are inconsistencies among dose-response, timing of measured effects, and tumorigenic doses that do not lend support to the notion that the only mechanism responsible for chloroform tumorigenicity is cytotoxicity followed by sustained tissue regeneration. (See our responses above and chapter 9 of the draft PHG).

Comments excerpted from the Executive Summary of Attachment A

Comment 10. "Using the quantitative MOA WOE confidence scoring approach described in Becker *et al*., 2017 and available data for CHCl3, the WOE for a mutagenic MOA (MOA#1) was compared to the WOE for a threshold cytotoxicity/regenerative proliferation MOA (MOA#2). The relevant dose-response and incidence data were summarized, and WOE confidence scores for both a mutagenic MOA and a threshold cytotoxicity/regenerative proliferation MOA were developed.

"This analysis indicates the following:

- It is highly unlikely that a mutagenic MOA is plausible for CHCl3-induced rodent liver tumors. Based on its negative MOA confidence score of -34.2, the WOE clearly does not support a mutagenic MOA for CHCl3-induced liver tumors. The negative score indicates there is strong counter-evidence for several of the early, diagnostic, key events (KEs) for a mutagenic MOA. In other words, the available data, including three negative in vivo transgenic mouse datasets, indicate it is highly unlikely that rodent liver tumors are induced by CHCL3 via a mutagenic MOA."

Response 10. Attachment A from the American Chemistry Council presents an analysis of mode of action of chloroform carcinogenicity comparing evidence for a mutagenic mode of action to that for a threshold mechanism dependent on cytotoxicity and resultant tissue regeneration. The analysis is based on a paper (Becker et al., 2017) that presents a method for reviewing evidence for mechanism of action. The method paper is described by the authors of the paper as an "early proof of concept", and as such is not fully developed nor accepted by the scientific community. The paper proposed an interesting approach but remains untested and has a number of problems. Of note, while the paper implies it is a quantitative method, it is really semi-quantitative at best given that it assigns numerical values to a qualitative evaluation of available evidence.

There are a number of problems with the method, as well as how it has been applied in Attachment A. As a result of methodological problems, some of which are described in the following paragraphs, different groups of experts would likely have very different semi-quantitative weighting of the evidence for mode of action. For example, in the analysis described in Attachment A of whether chloroform could be acting via a

mutagenic versus a cytotoxic/tissue regeneration mode of action, three out of five of the Key Events (KE 2, 3, and 4) weighed in the analysis all relate to potential mutagenicity. For a compound with weak mutagenicity, having 60% of the key events dependent on findings of mutagenicity automatically skews the analysis to a lower "score" as one in essence triple counts the same studies. Further, even for chemicals considered strongly mutagenic, there are rarely data clearly identifying pro-mutagenic DNA adducts in target tissue (KE2), insufficient repair or mis-repair of pro-mutagenic DNA adducts (KE3), or a specific early-induced mutation in cancer critical genes in the target tissue (KE4). By these measures, many known mutagens would not make the "cut" using this scheme to identify mode of action. The author of Attachment A in the end opines that there is "strong counter evidence" against KE2 and KE4, and moderately strong counter-evidence against KE 3. Much of this "counter-evidence" appears to be based on lack of study, and relates back to weak mutagenicity. This scoring method results in a very low score by the author for potential mutagenic mode of action. In contrast, there are three unique KEs for a cytotoxic/tissue regeneration mode of action, which do not rely on one underlying phenomenon. It is much less likely that there would be "double counting" of evidence for or counter evidence against an MOA in this scheme than there is in the scheme for a mutagenic MOA.

Further, the analysis lumps "limited evidence" equally with "inconsistent evidence" as "counter-evidence", which is obviously problematic. Limited evidence and inconsistent evidence are not the same, and should not be lumped together as "counter-evidence". Limited evidence generally means a lack of study – absence of evidence is not evidence of absence.

Other notable problems with the method include the way the semi-quantitative scores are characterized. For example, a semi-quantitative score of 0 to 25 is termed "low confidence" in the mode of action, while the next bin down, below zero is termed "highly unlikely" to be the mode of action. It also appears that the quality of individual studies cited as evidence for or against a particular MOA was not rated, as would be the case under a systematic review. Attachment A indicates that a systematic review of the literature was not conducted, but rather the author relied on existing reviews of the data. As stated on page 3 of Attachment A:

> "The intent of this case study is to illustrate the quantitative scoring methodology. It is not intended to be a complete discussion of all available and relevant studies. To that end, we did not conduct an in-depth systematic review of the available literature, but we based this evaluation in large part on data and lines of evidence from already published review articles, and those authors' evaluations of the quality of the empirical evidence."

Finally, the weighting scheme is presented as if there is only one possible mode of action. As noted above, there can be multiple modes of action by which a chemical induces formation of tumors.

Again, if the review were conducted by another party or group of scientists, and especially if there was an analysis of the quality of all studies, the scoring and conclusion would most likely be different.

Comments in the discussion of the Key Events (KE) for a mutagenic MOA described in Attachment A provided by the commenters

Comment 11. "The following KEs would be expected for a mutagenic MOA for induction of hepatocellular tumors by CHCl3:

• KE1 Oxidative metabolism of CHCl3 by CYP2E1 to highly reactive metabolites in target tissue, *e.g.*, phosgene
• KE2 Formation of pro-mutagenic DNA adducts in target tissue
• KE3 Insufficient repair or mis-repair of pro-mutagenic DNA adducts
• KE4 Early induced mutation in cancer critical genes in target tissue
• KE5 Cell proliferation, clonal expansion of mutant cells, additional mutations, and progression
• AO Development of liver tumors"

"KE #1: Good supporting data for the necessity of the metabolism step based on in vitro and in vivo data; extensive information on kinetics of CYP2E1 activity demonstrates CHCl3 is activated to form phosgene, and that this reaction is rapid and is saturable, with a maximum level reached rapidly; phosgene is very reactive thus it is rapidly removed either through further metabolism or through binding with nearby cellular macromolecules, likely highly compartmentalized to the CYP2E1-containing smooth endoplasmic reticulum, further supporting data generally showing no binding of activated CHCl3 metabolites with DNA. There do not seem to be data quantifying a dose-response for formation of phosgene over a range of exposures/doses; however, induction of CYP2E1 in vivo results in an increased metabolism of CHCl3 by liver microsomes from induced rats.

"There is strong support for the essentiality of this step provided by the CYP2E1 knock-out mice study demonstrating that CHCl3 treatment of these KO mice does not result in liver toxicity or other subsequent key events. Similar data with chemical inhibitors of CYP2E1 provide additional support for essentiality of this KE. This step initiates the sequence of KEs, thus fits temporally with an early KE."

Response 11. We agree that there are good data that chloroform is metabolized to phosgene, and that phosgene is a reactive metabolite of chloroform. We also agree that metabolism appears necessary for tumor induction. Existing evidence indicates that chloroform is metabolized by two major pathways: oxidative, producing phosgene (CCl_2O); and reductive, producing the dichloromethyl free radical ($\bullet CHCl_2$). In addition to metabolism in the liver, phosgene formation can occur in renal cortex and has been measured in a number of other organs. Studies described in chapter 4 of the draft PHG

document provide evidence that phosgene is formed via a high-affinity pathway via CYP2E1 and a low-affinity pathway via CYP2A6. There is also some evidence that CYP2B1/2 is active at higher chloroform exposures in the production of oxidative metabolites. Thus, there is evidence showing toxicity of chloroform can be mediated via multiple enzymes/enzyme systems and the importance of these enzymes/enzyme systems is dependent on the target organ and exposure levels.

We disagree that phosgene would be unable to bind to DNA. Phosgene can bind to cellular macromolecules, including histones in the nucleus, and since histones are in the nucleus and surround the DNA, binding of phosgene (or other reactive intermediates) to DNA is possible. Further, this indicates that phosgene can move from the site of formation in the endoplasmic reticulum into the nucleus. As well, binding to histones may result in changes in expression of genes, which may be related to tumor formation.

Further, as noted in chapter 4 of the draft document, phosgene is not the only reactive metabolite of chloroform. For example, Gemma et al (2004) observed that renal microsomes *in vitro* from two rat strains were unable to produce any oxidative metabolite, but adducts due to oxidative and reductive metabolites were detected *in vivo*. The results indicated the presence in the rat kidney of electrophilic metabolites other than phosgene, representing either oxidative metabolites formed elsewhere and sufficiently stable to be transported to the kidney or electrophilic metabolites secondary to the formation of reductive radicals.

Comment 12. "KE #2**:** There are no data demonstrating formation of pro-mutagenic DNA adducts from CHCl3 exposure. Reliable supporting data for DNA binding are very sparse, although binding to other macromolecules does occur. Other exposure biomarkers evaluated (e.g., sister chromatid exchanges) are not definitive evidence of DNA damage (no information is lost) and certainly not of pro-mutagenic DNA adduct formation. DNA strand break data are mixed, mostly negative, and again do not provide evidence of pro-mutagenic DNA adduct formation.

"This KE would need to occur very early in the sequence of KEs; with no supporting data, temporality cannot really be evaluated.

"KE #3**:** There are no data to demonstrate DNA repair of pro-mutagenic DNA adducts. There are a few datasets, both in vitro & in vivo, measuring UDS following CHCl3 exposure that mostly demonstrate no increase in unscheduled DNA synthesis, a hallmark of DNA repair. Temporality cannot be evaluated due to lack of data."

Response 12. The analysis presented in Attachment A has essentially three key events related to genotoxic effects. As noted above in response to comment 5, for KE 2, 3, and 4, very few mutagenic chemicals would have these specific types of data, namely evidence of formation of pro-mutagenic DNA adducts in the target tissue, evidence of insufficient repair or mis-repair of such pro-mutagenic DNA adducts, and evidence of early induced mutation in cancer-critical genes in target tissues. Requiring

layers of data on genotoxicity, which generally do not exist even for those chemicals that have plenty of evidence of genotoxicity, results in skewing the analysis of whether there is a mutagenic mode of action. When assessing a chemical such as chloroform, which has weak evidence of genotoxicity from generally older studies that did not have the specificity required to meet these KEs, the analysis essentially scored the weak evidence of genotoxicity three times in assessing whether there is any contribution to carcinogenicity via a mutagenic mode of action. As noted above in response to comment 5, the author of Attachment A essentially equated lack of evidence for these specific key events with strong counter-evidence. This is inappropriate, and a different reviewer may well have come up with a different "score."

Comment 13. **"**KE #4: At best, contradictory results exist for mutagenic effects of CHCl3, with most *in vitro* & *in vivo* mutagenicity data negative for mutation induction. The few datasets that have been published as demonstrating mutagenic effects from CHCl3 exposure mostly have significant flaws (*e.g*., inadequate numbers, no dose-response, not statistically or biologically significant positives) that affect reliability and validity. Perhaps the strongest counterevidence are the three negative transgenic studies; one that did not show any increase in *lacI* gene induced mutations in any tissue, including liver, and two transgenic mouse studies with no increase in liver tumors after 26 wks exposure to CHCl3; typically, increased tumors would be evident in the *p53-/-* and *ras*H2-Tg models following such an exposure to a mutagenic carcinogen. Temporality cannot be evaluated due to no data."

Response 13. We agree that the genotoxicity of chloroform is weak and that most studies were negative. There are a number of problems with the genotoxicity database for chloroform as discussed in Chapter 5 of the draft document. Because chloroform is volatile and activated by metabolism to a highly reactive intermediate, phosgene, the most relevant studies would be those that were conducted in a closed system to prevent loss of the chemical and would include activation, preferably by an endogenous system in which the metabolites are formed inside the test organism. Because chloroform genotoxicity studies have met these conditions to varying degrees, the possibility exists for false negative results. Although generally negative results were obtained, there are some positive studies indicating chloroform is capable of genotoxicity under appropriate experimental conditions. For example, Larson et al. (1994d) evaluated chloroform-induced DNA repair *in vitro* in female mouse hepatocytes and reported unscheduled DNA synthesis. Beddowes et al. (2003) found that 2-hour chloroform treatment of primary female rat hepatocytes resulted in statistically significant increases in DNA strand breaks (at 8 and 20 mM) and M_1dG adducts (at 4, 8 and 20 mM). Zhang et al. (2012) reported that incubation of human HepG2 hepatoma cells for 4 hours with chloroform induced DNA strand breakage at 10,000 µM (10 mM), but had no effect at concentrations from 1 to 1000 µM.

A few assays reported weakly positive results for mutation in mouse lymphoma cells (Mitchell et al., 1988) and sister chromatid exchange in rat leukemia cells (Fujie et al., 1993) and human lymphocytes (Morimoto and Koizumi, 1983; Sobti, 1984).

Morimoto and Koizumi (1983) found that a 72-hour exposure to chloroform (2, 10, or 50 mM) induced a concentration-dependent increase in the number of sister chromatid exchanges per cell in cultured human lymphocytes. This increase became statistically significant ($p < 0.05$) at chloroform concentrations equal to or greater than 10 mM. In parallel *in vivo* experiments, mice were fed 0 to 200 mg/kg-day of chloroform for four days. Bone marrow cells from these animals exhibited a dose-dependent increase in the number of sister chromatid exchanges per cell. The sister chromatid exchange frequency was significantly ($p < 0.05$) higher than in control animals at doses ≥ 50 mg/kg-day.

Teixidó et al. (2015), using zebrafish embryos, observed statistically significant induction of DNA damage following 72-hour exposure to chloroform (0.85 mM).

DiRenzo et al. (1982) found that chloroform bound to DNA at 0.46 ± 0.13 nmol/mg DNA/hour (mean of six experiments). Rosenthal (1987) interpreted the results of DiRenzo et al. (1982) to be evidence that metabolically activated chloroform can bind to DNA *in vitro* at very low levels although the assay did not utilize glutathione and thus may not be as indicative of the *in vivo* milieu. Another study measured low levels of DNA alkylation in the liver (0.0003 mol percent) or kidney (0.0001 mol percent) of male mice given an oral dose of 240 mg/kg ^{14}C-chloroform (Reitz et al., 1980).

Chloroform metabolites can bind to other cellular macromolecules. Fabrizi et al. (2003) reported that phosgene, the major active metabolite of chloroform, is able to form irreversible adducts with the N-terminus of human histone H2B *in vitro*. An earlier study showed that in the liver of rats treated *in vivo* with radiolabeled chloroform, histones and other nuclear proteins, but not DNA, became radiolabeled (Diaz Gomez and Castro, 1980a), suggesting that the metabolite phosgene is able to cross the nuclear membrane and make contact with histones to form adducts. Gemma et al. (2004) found covalent binding of chloroform metabolites to renal and hepatic microsomal phospholipids following *in vitro* and *in vivo* bioactivation.

These results indicate that chloroform may function at least partially through genotoxicity to produce tumors. We agree that cytotoxicity and resulting cellular proliferation may also play a role especially at high exposures. However, as noted in chapter 9 of the PHG draft and in response to comment 7 above, there are a number of discrepancies reported in the relationship among measures of cell proliferation, cytotoxicity and tumor formation. These discrepancies cast doubt that the purported threshold mechanism of carcinogenicity is the sole mechanism by which chloroform causes tumors.

It is unclear exactly which 3 negative transgenic studies are referred to in Comment 8. The p53 haploinsufficient or hemizygous (p53+/-, not p53-/- as stated by the commenters) transgenic animal studies discussed in the draft PHG evaluated effects of BDCM, not chloroform. Two transgenic animal studies employing chloroform are discussed in the draft PHG: the *ras*H2-Tg mouse model (Sehata et al., 2002) and the *lacI* transgenic mice (Butterworth et al., 1998). Sehata et al. (2002) exposed *ras*H2-Tg

mice to chloroform for 26 weeks instead of the standard two-year bioassay duration, which has produced positive findings of liver tumors in mice and kidney tumors in rats (Jorgensen et al., 1985; NCI, 1976). Given the observed growth of tumors in *ras*H2-Tg mice treated with MNU, a potent genotoxic carcinogen, which shows that the mice are responsive to carcinogens, the lack of chloroform carcinogenicity in this study may indicate perhaps that a longer chloroform exposure period is required for tumor formation or that the c-Ha-*ras* gene may not play a role in chloroform-induced carcinogenesis.

Butterworth et al. (1998) exposed female *lacI* transgenic mice by inhalation to chloroform at concentrations up to 90 ppm for up to 180 days. Whereas DMN, a DNA-reactive mutagen, induced a significant increase in *lacI* mutant frequency, there was no observed statistically significant increase in the frequency of *lacI* mutations in the liver, the only tissue examined, of chloroform-treated mice. As discussed by the authors, one possible reason for the observed lack of mutagenicity by chloroform is the low sensitivity of the *lacI* assay due to a high background mutation frequency. The relatively high baseline mutation frequency in this study compared to other studies employing this model limits the extent to which weak to modest mutagens may be able to induce increased mutation frequency to a level large enough to reach statistical significance above background levels. It is noteworthy that the *lacI* mutation frequency was slightly higher, although not statistically significant, for the chloroform-treated group than the control group at every exposure period evaluated. Another possible explanation provided for the observed lack of chloroform mutagenicity is that potential chloroform-induced DNA alterations may be of a type that is not detectable by the *lacI* assay. The *lacI* assay has been demonstrated to detect point mutations, but not other alterations to DNA such as large deletions or chromosomal aberrations, for example. Thus, the observed lack of chloroform mutagenicity under these particular experimental conditions does not rule out a possible role for genotoxicity in chloroform-induced carcinogenesis.

Comment 14. "KE #5: Clear evidence for induction of cell proliferation in liver is provided by several publications from different laboratories. Both gavage and inhalation repeated (13 or 26 wk) exposures to CHCl3 have induced dose-responsive increased labelling indices (LI) in mouse and rat liver, using BrdU labelling to quantitate cell proliferation. Two datasets with transgenic mice included BrdU LI in liver and clearly demonstrated dose-responsive increases in hepatocyte proliferation with CHCl3 exposure (a 26 wk inhalation and a 13 wk gavage).

"There is strong support for the essentiality of this step provided by the CYP2E1 knock-out mice study demonstrating that CHCl3 treatment of the KO mice does not result in any increased LI in liver, while the wild-type CYP2E1 mice demonstrated extensive hepatocyte proliferation. Pre-treatment with a chemical inhibitor of CYP2E1 also blocked the (regenerative) cell proliferation in liver of CHCl3-treated mice.

"The hepatic cell proliferation is a relatively early event, as some datasets show it has stopped by 13 wks of treatment, while other datasets show its occurrence following 13 or 26 wks of exposure.

"The temporal concordance of KE5 is maintained within the sequence of key events, as it occurs later than the expected timeframe for mutation induction."

Response 14. As noted in our more detailed response to Comment 7 above and in Chapter 9 of the draft PHG document, the evidence that "sustained phosgene-induced cytotoxicity is a key event in chloroform-induced carcinogenesis" is not as clear as the comments suggest. Increases in labeling index, reflecting tissue regeneration, often did not reflect the observed pattern of toxicity including carcinogenicity. The relationship between cytotoxicity and tissue regeneration, indicated by an increase in DNA synthesis as measured by labeling index (the proportion of labeled cells in S phase), has been extensively studied following chloroform administration. Studies reported:

- toxicity occurring without an apparent increase in labeling index;
- toxicity increasing with continual exposure while the labeling index diminished;
- increased labeling index without apparent toxicity;
- cytotoxicity and increases in the labeling index without any tumorigenic response evident in the cancer bioassays at equivalent exposures.

OEHHA has chosen as a matter of public health policy and prudence to utilize a linear model to estimate cancer potency based on:

- some evidence for genotoxicity of chloroform;
- evidence that reactive intermediates are formed during metabolism of chloroform and in fact at least one, phosgene, is a major metabolite;
- evidence that phosgene can bind to macromolecules including the histones surrounding DNA, and that other reactive intermediates have been found bound to cellular macromolecules;
- inconsistencies in the evidence for the hypothesis that cytotoxicity and tissue regeneration are solely responsible for chloroform tumorigenicity.

Comment 15. "AO: Strong evidence for induction of hepatocellular carcinoma/adenoma by high dose exposure to $CHCl_3$ in mice and rats, with several bioassays (gavage or inhalation) demonstrating increased incidence of these tumors. In addition, there are exposure levels that do not result in hepatic tumors, providing dose-response data. Tumors are identified only following ~50+ weeks of $CHCl_3$ treatment, supporting the temporal concordance of this AO."

Response 15. We agree that chloroform causes tumors at multiple sites in experimental animals through both oral and inhalation routes, as discussed extensively in Chapter 5 of the draft PHG.

Comments in the discussion of the Key Events (KE) for a cytotoxicity/regenerative proliferation MOA described in Attachment A

Comment 16. "...The following key events would be expected for a Cytotoxicity/Regenerative Cell Proliferation MOA for induction of liver tumors by CHCl3, which would have a threshold:

• KE1 Oxidative metabolism of CHCl3 by the P450 enzyme CYP2E1 to highly reactive phosgene.
• KE2 Sustained cytotoxicity to target cells, hepatocytes.
• KE3 Regenerative cell proliferation, clonal expansion of mutant cells, & progression in liver.
• AO Development of tumors in liver."

"KE1: Metabolic Activation of CHCl3 to Phosgene:
Good supporting data for the necessity of the metabolism step based on *in vitro* and *in vivo* data; extensive information on kinetics of CYP2E1 activity demonstrates CHCl3 is activated to form phosgene, and that this reaction is rapid and is saturable, therefore a maximum level is reached rapidly; phosgene is very reactive thus it is rapidly removed either through further metabolism or through binding with nearby cellular macromolecules; likely highly compartmentalized to the CYP2E1-containing smooth endoplasmic reticulum, further supporting data generally showing no binding of activated CHCl3 metabolites with DNA. There do not seem to be data quantifying a dose-response for formation of phosgene over a range of exposures/doses; however, induction of CYP2E1 *in vivo* results in an increased metabolism of CHCl3 by liver microsomes from the induced rats.

"There is strong support for the essentiality of this step provided by the CYP2E1 knock-out mice study demonstrating that CHCl3 treatment of these KO mice does not result in liver toxicity or other subsequent key events. Similar data with chemical inhibitors of CYP2E1 provide additional support for essentiality of this KE. This step initiates the sequence of KEs, thus fits temporally with an early KE."

Response 16. We agree that there are good data that chloroform is metabolized to phosgene, and that phosgene is a reactive metabolite of chloroform. We also agree that metabolism appears necessary for tumor induction. Chloroform is metabolized by two major pathways: oxidative, producing phosgene (CCl_2O); and reductive, producing the dichloromethyl free radical ($\bullet CHCl_2$). In addition to metabolism in the liver, phosgene formation can occur in renal cortex and has been measured in a number of other organs. Studies described in Chapter 4 of the draft PHG document provide evidence that phosgene is formed via a high-affinity pathway via CYP2E1 and a low-affinity pathway via CYP2A6. There is also some evidence that CYP2B1/2 is active at higher chloroform exposures in the production of oxidative metabolites.

We disagree that phosgene would be unable to bind to DNA. Phosgene can bind to cellular macromolecules, including histones in the nucleus, and since histones are in the nucleus and surround the DNA, binding of phosgene (or other reactive intermediates) to DNA is possible. As well, binding to histones may result in changes in expression of genes.

Further, as noted in Chapter 4 of the draft PHG document, phosgene is not the only reactive metabolite of chloroform. For example, Gemma et al (2004) observed that renal microsomes *in vitro* from two rat strains were unable to produce any oxidative metabolite, but adducts due to oxidative and reductive metabolites were detected *in vivo*. The results indicated the presence in the rat kidney of electrophilic metabolites other than phosgene, representing either oxidative metabolites formed elsewhere and sufficiently stable to be transported to the kidney or electrophilic metabolites secondary to the formation of reductive radicals.

Comment 17. "KE2: Phosgene induction of Hepatic Cytotoxicity/Necrosis:
Good supporting data from several published studies from different laboratories demonstrate a dose-response in rats and mice for hepatocellular cytotoxicity/necrosis following CHCl3 exposure by inhalation and by gavage. Histopathologic evaluation of liver tissue in mouse and rat following gavage or inhalation exposure to CHCl3 shows that there are doses that do not induce cytotoxicity/necrosis, but that higher exposures do cause liver toxicity. Two datasets with transgenic mice included liver histopathology and clearly demonstrated dose-responsive increases in hepatocyte degeneration and necrosis with increasing CHCl3 exposure (one inhalation and one gavage).

"There is strong support for the essentiality of this step provided by the CYP2E1 knock-out mice study demonstrating that CHCl3 treatment of these KO mice does not result in liver toxicity or other subsequent key events. In addition, pre-treatment with a chemical inhibitor of CYP2E1 also blocked the cytotoxicity/necrosis in liver of CHCl3-treated mice, providing additional supporting evidence of essentiality. The stop-exposure experiments also support essentiality of sustained cytotoxicity.

"The cytotoxicity/necrosis is an early event but can occur only after the metabolic activation of CHCl3, which is, in itself, relatively unreactive; this supports the temporal concordance of KE2 with the sequence of key events."

Response 17. OEHHA agrees that chloroform causes hepatotoxicity in a dose-dependent manner, and that chloroform is metabolized to compounds that cause tissue damage. We do not agree that cytotoxicity and tissue damage are the sole mode of action for chloroform carcinogenicity, although such damage likely influences the rate of tumor response. See more detailed responses above to Comment 7 regarding the relationship between cytotoxicity and subsequent tissue regeneration as it relates to tumorigenic doses.

Comment 18. **"**KE3: Induction of Regenerative Cell Proliferation in Liver (and hepatic foci of mutant cells, progression)

Strong evidence for induction of regenerative cell proliferation in liver is provided by several publications from different laboratories. Both gavage and inhalation exposure to CHCl3 have induced dose-responsive increased labelling indices (LI) in mouse and rat liver, using BrdU labelling to quantitate cell proliferation. Two datasets with transgenic mice included BrdU LI in liver and clearly demonstrated dose-responsive increases with CHCl3 exposure (one inhalation and one gavage); one transgenic study provides liver AHF.

"There is strong support for the essentiality of this step provided by the CYP2E1 knock-out mice study demonstrating that CHCl3 treatment of KO mice does not result in any increased LI in liver, while the wild-type mice demonstrated extensive hepatic regenerative cell proliferation. Pre-treatment with a chemical inhibitor of CYP2E1 also blocked the regenerative cell proliferation in liver of CHCl3-treated WT mice. The stop-exposure experiments also support essentiality of regenerative cell proliferation.

"The hepatic regenerative cell proliferation is a relatively early event, as some datasets show it has stopped by 13 wks of treatment; as it is induced by the cytotoxicity/necrosis, the temporal concordance of KE3 is maintained with the sequence of key events."

Response 18. We agree that metabolism of chloroform produces toxic metabolites that can induce dose-related tissue damage and that tissue regeneration follows at sufficiently toxic doses.

However, as noted above in the responses to Comment 7 regarding the data on measures of cytotoxicity and tissue regeneration, not all results are consistent with the idea that hepatic regeneration is a necessary step for tumor induction. There are inconsistent dose- and time-response patterns between measures of hepatotoxicity and tissue regeneration, and in comparing these two measures against doses that did or did not induce tumors. The data are not as clear as the commenters or their analysis imply.

Comment 19. **"**AO: Hepatocellular Carcinoma/Adenoma
Strong evidence for induction of hepatocellular carcinoma/adenoma by high dose exposure to CHCl3 in mice and rats, with several bioassays (gavage or inhalation) demonstrating increased incidence of these tumors. In addition, there are exposure levels that do not result in hepatic tumors, providing dose-response data. Tumors are identified only following ~50+ weeks of CHCl3 treatment, supporting the temporal concordance of this AO."

Response 19. We agree that chloroform causes tumors at multiple sites in experimental animals through both oral and inhalation routes, as discussed extensively in Chapter 5 of the draft PHG.

Comment Excerpted from Attachment A Conclusions

Comment 20. "Comparison of the MOA confidence scores for the two hypothesized MOAs is informative. Analysis of MOA#1, the mutagenic MOA, resulted in a confidence score of -34.2; a negative score indicates availability of data that provide counterevidence, thus contradict, a hypothesis. In the case of MOA#1, there are both significant counterevidence (contradictory evidence), such as the three negative *in vivo* transgenic mouse studies demonstrating that the influential KE4 does not occur, and several KEs that do not have reliable supporting data (KEs 2, 3, and 4), while all of these 3 KEs do have at least some negative (non-supporting) data. Altogether, the assessment results in a negative confidence score. Analysis of MOA#2, the cytotoxicity/regenerative proliferation MOA, presents a different picture, with a confidence score of +93.6 (out of a maximum of 100 possible). Such a high MOA confidence score indicates a wealth of strong supporting data, with little or no contradictory data. This is the case for MOA#2, with qualitative and quantitative supporting data available for most of the KEs."

"The MOA confidence scores developed here provide further support for the application of MOA#2 (cytotoxicity/regenerative proliferation MOA) in conducting human health risk assessment on CHCl3. This is particularly important as this MOA (MOA#2) supports a threshold approach to risk assessment, with evidence to indicate that there is an exposure/dose below that threshold where the MOA for cancer would not be triggered. If the KEs are not triggered, then there will be no subsequent CHCl3-related increased tumor incidence. Conduct of a MOA-based risk assessment on CHCl3 will allow determination of a threshold dose/exposure, below which no additional cancer risk is expected, in effect a Reference Concentration/Dose (RfC/RfD) for cancer. Indeed, USEPA has supported this idea with development of the RfD value as protective against cancer."

"…There is adequate evidence to establish the MOA#2 for CHCl3 induction of liver tumors in mice (and rats). Concordance of both dose-response and temporality have been established for the KEs along with essentiality, consistency, and analogy/coherence. Analysis of the alternative MOA (MOA#1, mutagenic MOA) resulted in a negative score for MOA confidence."

Response 20. For the reasons noted above in response to the general method of confidence scoring presented in Becker et al. (2017) and specific elements of the Attachment A analysis, OEHHA would not conclude that the sole mode of action for chloroform is through cytotoxicity and tissue regeneration. OEHHA has chosen as a matter of public health policy and prudence to utilize a linear model to estimate cancer potency based on:

- some evidence for genotoxicity of chloroform;

- evidence that reactive intermediates are formed during metabolism of chloroform and in fact at least one, phosgene, is a major metabolite;
- evidence that phosgene can bind to macromolecules including the histones surrounding DNA, and that other reactive intermediates have been found bound to cellular macromolecules;
- inconsistencies in the evidence for the hypothesis that cytotoxicity and tissue regeneration are solely responsible for chloroform tumorigenicity.

Comments on Other Trihalomethanes from ACC

Comment 21. "Bromoform Does Not Present a Carcinogenic Risk to Humans"

"The data from a variety of assays on the genotoxicity of bromoform are equivocal. There is some evidence to suggest that bromoform may be weakly mutagenic. Bromoform is largely positive in bacterial assays of mutagenicity conducted in closed systems and was positive in the Ames test in S. typhimurium strain, positive in TA98, and negative or equivocal in strains TA1535 or TA1937 (NTP, 1989). Bromoform yielded increased SCE and chromosomal aberrations in mouse and rat bone marrow cells but negative results in other mouse bone marrow tests, the rat liver UDS assay, and in the dominant lethal assay. There is no in vivo evidence of genotoxicity with bromoform.

"Despite these equivocal results, the draft PHG concludes that the weight of the available evidence indicates that bromoform is mutagenic and genotoxic and that the chemical is a genotoxic carcinogen – with no exposure threshold. This conclusion contradicts those reached by USEPA, IARC, WHO, and Health Canada. These authoritative bodies have rejected a cancer classification for bromoform based on evidence from a gavage study in only one species and equivocal evidence for genotoxicity."

Response 21. The commenter's statement, "There is no in vivo evidence of genotoxicity with bromoform" is incorrect. Positive genotoxicity results have been obtained for bromoform in both *in vitro* and *in vivo* assays such as micronuclei induction, sister chromatid exchange, chromosomal aberrations including DNA damage, sex-linked recessive lethal mutations, and aneuploidy. Both positive and negative results of *in vivo* genotoxicity tests on bromoform are summarized in Table 6.6 in the draft, as shown below. The weight of the available evidence indicates that bromoform is mutagenic and genotoxic, and thus presents a carcinogenic risk to humans.

Table 6.6 Summary of *In Vivo* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Result	References
Micronuclei induction	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Mouse, bone marrow cells	-	Stocker *et al*. (1997)
	Mouse, bone marrow cells	+	NTP (1989a)
	Newt, peripheral erythrocytes	+	Le Curieux *et al*. (1995)
	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
Chromosomal aberrations	Mouse, bone marrow cells	-	NTP (1989a)
	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
	Mouse, bone marrow cells	+	NTP (1989a)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)
Sex linked recessive lethal mutations	*Drosophila melanogaster*	+	Woodruff *et al*. (1985), NTP (1989a)
Heritable translocations	*Drosophila melanogaster*	-	
Aneuploidy	*Aspergillus nidulans*	+	Benigni *et al*. (1993)
Initiation	Rat, liver	-	Herren-Freund and Pereira (1986)

Geter et al. (2004c) exposed male F344/N rats to isomolar concentrations of the THMs at 0.5 g/L chloroform, 0.7 g/L BDCM, 0.9 g/L DBCM, or 1.1 g/L bromoform in drinking water for 26 weeks, with half the animals fed the normal 4.5% fat Purina 5001 diet and the other half receiving the feed supplemented with 19% animal fat. Rats administered bromoform and a high fat diet exhibited the most significant increase in aberrant crypt foci compared to other THMs.

There is adequate evidence that metabolism of bromoform can produce reactive metabolites, including the dihalomethyl radical •CHBr2. Tomasi et al. (1985) measured the production of a free radical intermediate in rat hepatocytes by electron spin resonance (ESR) spectroscopy. The intensity of the ESR signal was greatest for bromoform compared to other THMs. The signal was reduced by cytochrome P450 inhibitors. These data were interpreted as evidence that free radical formation depends on cytochrome P450-mediated reductive metabolism. Toxic effects caused by these reactive intermediates are expected to occur in the tissues in which they are formed.

The positive data for carcinogenicity and mutagenicity for bromoform, as well as the formation of mutagenic metabolites, justify the use of linear extrapolation of cancer risk, in accordance with cancer guidelines (US EPA, 2005; OEHHA, 2009). Cancer

guidelines require assumption of a linear dose response unless there is adequate evidence of a threshold mechanism of action, which is certainly not true in this case.

Comment 22. "The Proposed Public Health Goal Overstates the Potential Cancer Risk for Bromodichloromethane"

"While tumors have been reported in gavage studies with BDCM, studies in drinking water have been largely negative. NTP reported tumors of the large intestine and kidney in rats exposed to BDCM dissolved by gavage in corn oil. In its gavage study in mice, NTP reported kidney tumors in males and liver tumors in females. In a subsequent drinking water study, NTP did not observe a significant increase in tumors in either rats or mice. Although additional studies in drinking water studies have suggested liver tumors in rats, these studies have reported mixed results or provided only estimates of drinking water consumption. Notably, George *et al*. (2002) reported liver tumors in rats at the low and mid dose levels, but not at the highest dose, and no evidence of intestinal tumors at any dose level. In a diet study in rats, Aida *et al*. (1992) reported no significant differences in neoplasms between controls and treatment groups dosed for 24 months."

"As with the other THMs, OEHHA bases its draft PHG on its conclusion that BDCM is a genotoxic carcinogen with no exposure threshold. In vitro mutagenicity testing has produced mixed results, while the results from in vivo testing have been negative. Citing positive results in two studies conducted in closed systems to minimize loss of the test substance, OEHHA concludes that BDCM is genotoxic. Despite the evidence for genotoxicity, the disparate outcomes from gavage and drinking water bioassays strongly suggest that application of the default multistage cancer model is inappropriate. This conclusion is supported by an expert panel convened by Health Canada in 2008 to consider new data for BDCM. The panel noted that 'the combined data from the two [NTP] studies do not support a linear dose extrapolation.'

The draft PHG makes no attempt to reconcile the starkly different outcomes from the two studies by NTP. While it provides a summary of the results of the 2006 NTP bioassay, it appears to dismiss the findings by noting that '[w]ater consumption by the exposed mice was less than that of the controls throughout the study' because of 'poor palatability' of the water containing BDCM. OEHHA fails to note that NTP's conclusion that the 2006 study shows no evidence of carcinogenic activity in rats or mice is based on the actual water consumption levels, not on consumption compared to the control animals. OEHHA also does not mention NTP's conclusion that '[d]ifferences in organ dosimetry after gavage administration versus drinking water or dietary administration may be important in evaluating the carcinogenic activity of [BDCM].'

The conflicting results from the two NTP studies, combined with equivocal genotoxicity data, suggest a carcinogenic response for BDCM similar to that observed with chloroform. This is the conclusion of NTP, WHO, and Health Canada. Consistent with

the conclusion by the WHO, it is unlikely that a carcinogenic risk exists from BDCM exposures from finished drinking water."

Response 22. While there are a mix of results in the various carcinogenicity bioassays, it is common practice in risk assessment, and health protective to utilize study(ies) of sufficient quality that indicated sensitive responses in assessing cancer risk. Cancer risk assessment is generally based on the study showing the highest tumor incidence (US EPA, 2005; OEHHA, 2009), unless there is good rationale for using a central tendency estimate such as a geometric mean.

The results from the NTP drinking water study do not negate the effects in both mice and rats seen in the earlier NTP gavage studies. NTP (2006) indeed states that there was no evidence for carcinogenicity in the drinking water study, which examined male F344 rats and female B6C3F1 mice. In their earlier gavage studies, NTP notes clear evidence for carcinogenicity of BDCM in both male and female rats and mice. In their Report on Carcinogens (14th edition, 2016), NTP states, "Administration of bromodichloromethane by stomach tube caused benign and malignant kidney tumors (tubular-cell adenoma and adenocarcinoma) in male mice and in rats of both sexes, benign and malignant liver tumors (hepatocellular adenoma and carcinoma) in female mice, and benign and malignant colon tumors (adenomatous polyps and adenocarcinoma) in rats of both sexes". NTP describes BDCM as reasonably anticipated to be a human carcinogen (NTP, 2016).

Although differences in kinetics may factor into the response rates, comparison is somewhat complicated by the lower dose levels in the drinking water at least in the rat (highest dose 25mg/kg-day for rat) (NTP, 2006) versus gavage studies (lowest dose 50 mg/kg-day for rat) (NTP, 1987).

In a drinking water study, George et al. (2002) reported that hepatocellular adenomas were significantly increased at the low dose (7/45 in the 3.9 mg/kg-day group) and combined hepatocellular adenomas and carcinomas were increased in the low and mid-dose groups (8/45 at 3.9 mg/kg-day and 7/48 at 20.6 mg/kg-day) but not at the high dose. The lack of a statistically elevated response at the high dose decreases confidence but does not completely eliminate the biological significance of the responses at the two lower doses.

Health Canada (2006) classifies BDCM as a probable carcinogen to humans. They indicate BDCM probably acts via mutagenicity and used a linear model to estimate cancer slope. This is in contrast to their opinion that chloroform probably acts through a threshold mechanism. Health Canada stated in its document, "Among the four THMs [chloroform, BDCM, DBCM, bromoform] commonly found in drinking water, BDCM appears to be the most potent rodent carcinogen. BDCM caused tumours at lower doses and at more target sites than for any of the other THMs…" Health Canada's document indicates they would have a maximum acceptable concentration for BDCM of 16 µg/L. Their final number of 100 µg/L for total THMs, largely based on their analysis for chloroform, is a risk management number.

OEHHA does not agree that the genotoxicity data are equivocal. Although the overall data are mixed, a number of positive results were obtained for mutagenicity and genotoxicity assays in bacterial and mammalian systems. Thus, the weight of evidence suggests that BDCM is mutagenic and genotoxic. As discussed in our draft document, evidence *in vitro* suggests that a potential mechanism of carcinogenicity of BDCM may involve covalent binding to DNA of reactive intermediates generated by GSTT1-1-mediated metabolism of BDCM (Ross and Pegram, 2004). The authors suggest that this may occur in the kidney and large intestine (target organs of BDCM carcinogenicity in rats), which were demonstrated in this study to have a much lower detoxification rate to bioactivation rate (CYP/GST ratio) compared to the liver (a non-cancer target in rats). This may result in an enhanced relative production of reactive intermediates with the capacity to covalently modify DNA in target tissues, ultimately contributing to BDCM carcinogenicity. Also as discussed in our draft, several *in vivo* studies in rats suggest a plausible mechanism of carcinogenicity of brominated THMs in the colon involving formation of putative early preneoplastic lesions in the colon called aberrant crypt foci. DeAngelo et al. (2002) reported that the brominated THMs, particularly BDCM, administered in drinking water significantly induced the incidence of aberrant crypt foci in the colon of male F344/N rats. Geter et al. (2004b) then showed that formation of aberrant crypt foci in the colon of male F344/N rats was independent of the method of BDCM administration, with drinking water and corn oil gavage administration producing similar values of aberrant crypt foci per colon.

As discussed in the PHG draft, recent cancer risk estimates for exposure to the THMs in drinking water have assumed they do not operate through a threshold mechanism and have thus applied a linear dose-response model (Lee et al., 2004; Uyak, 2006; Wang et al., 2007a,b; Panyakapo et al., 2008; Viana et al., 2009; Pardakhti et al., 2011; Basu et al., 2011; Chowdhury et al., 2011; Legay et al., 2011; Yamamoto, 2011). OEHHA concurs with this approach in estimating cancer risks of the THMs. It is clear, as discussed in the draft document, that BDCM, DBCM, and bromoform have produced carcinogenic effects in animal studies. The data overall provide strong support for using a non-threshold model for the brominated trihalomethanes.

Comment 23. "Dibromochloromethane Does Not Present a Carcinogenic Risk to Humans"

"While DBCM was originally listed as a carcinogen under Proposition 65, it was delisted in 1999 following IARC's conclusion that DBCM was not classifiable as to its carcinogenicity to humans (Group 3). USEPA classified DBCM as a possible human carcinogen (Group C) in 1992, based on limited evidence for carcinogenicity in animals and structural similarity to other THMs. The 2008 WHO drinking water guidelines also consider DBCM as not classifiable based on the IARC classification. Health Canada classifies DBCM as possibly carcinogenic to humans (Group IIID) but concluded that there was insufficient information available to calculate a drinking water guideline for the substance.

Despite the clear consensus among other authoritative bodies, the draft PHG concludes that DBCM is carcinogenic noting that it is --

> structurally similar to the other THM species, which are classified as either probable or possible carcinogens; the liver is a common target for THM-related tumors; DBCM has not been as thoroughly studied as the other THM species, resulting in much less available data to assess; and the data from female mice in the critical study employed for the dose-response analysis show positive association with liver tumors.

In calculating the proposed PHG, OEHHA concludes that DBCM causes cancer via a genotoxic mechanism citing results in two assays conducted in a closed system. Similar to the other brominated THMs, the genotoxicity data for DBCM are equivocal. The cancer evidence is limited with no evidence in the rat studies and only one study in mice reporting a tumor increase. As with the other THMs, moreover, positive cancer results were only noted in the gavage study where pharmacokinetic difference in dosing likely impacted the results. Given these circumstances, OEHHA's conclusion is at odds with current cancer hazard and risk assessment guidelines which prescribe a weight-of-evidence-based approach. In this case, a risk assessment that employs "the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology" as required by Health and Safety Code §116365, would lead to the conclusion that DBCM is not carcinogenic to humans. Any cancer risk that may exist, moreover, is likely to exhibit a threshold. This was the conclusion of USEPA when it established an MCLG of 60 µg/L for DBCM."

Response 23. The most current principles, practices, and methods do not mandate a consensus among disparate agencies. It should be noted that many studies from around the world have conducted risk assessments of exposure to THMs in drinking water that include the assumption that all four of the THMs provide a carcinogenic risk to humans, as discussed in the PHG document.

OEHHA disagrees that the genotoxicity data are equivocal. As indicated in our draft document, "Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of S. typhimurium; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosomal aberrations *in vivo* and *in vitro*; DNA damage in bacteria; and aneuploidy in mammalian cells and Aspergillus nidulans. Negative results have also been obtained *in vitro* and *in vivo*. For some *in vitro* tests, negative findings may have occurred because measures were not taken to prevent volatilization of the test compound." The overall weight of evidence suggests that DBCM is mutagenic and genotoxic.

OEHHA cites the NTP (1985) cancer bioassay in Fisher 344/N rats and B6C3F1 mice, where DBCM was administered by gavage in corn oil. This is the only available cancer bioassay for DBCM. No tumors were found in rats. In male mice, there was a significant increase in hepatocellular carcinomas in the high dose males but not

combined hepatocellular adenomas and carcinomas compared to controls. NTP considered the evidence equivocal for male mice. Note that the study in male mice was compromised due to high mortality from a dosing error in the low dose male mice. The NTP gavage study reported statistically significant elevated incidence of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in female B6C3F1 mice. NTP states that there is some evidence of carcinogenicity in the female mice. It is not uncommon that a carcinogen induces tumors in one rodent species and not in another. While there are a mix of results in the various carcinogenicity bioassays, it is common practice in risk assessment, and health protective to utilize the study(ies) that indicated the most sensitive response in assessing cancer risk.

Comment 24. "OEHHA Should Adopt a Single Public Health Goal Based on the Most Relevant Animal Studies"

"In proposing that all four THMs are genotoxic carcinogens, OEHHA ignores the weight of the scientific evidence and the consensus reached by other authoritative bodies and, in the case of DBCM, its own previous conclusions. The available evidence does not support a conclusion that bromoform and DBCM are carcinogenic. Substantial evidence indicates, moreover, that chloroform and BDCM are threshold carcinogens unlikely to present a cancer risk at levels found in finished drinking water.

ACC urges OEHHA to develop a single PHG for total THM, based on its evaluation of non-cancer effects, that will provide adequate protection for exposure to any of the individual THMs. In establishing such a PHG, OEHHA can also ensure that exposure levels are below the thresholds for potential risks of cancer presented by chloroform and BDCM. Although OEHHA will still need to consider the health effects information for the individual substances, those data should be considered in the context of a single goal rather than individual goals."

Response 24. See OEHHA's responses to Comment 2 and Comments 6-9.

RESPONSES TO COMMENTS JOINTLY SUBMITTED BY THE ASSOCIATION OF CALIFORNIA WATER AGENCIES, THE CALIFORNIA WATER ASSOCIATION, THE CALIFORNIA MUNICIPAL UTILITIES ASSOCIATION, THE SOUTHERN CALIFORNIA WATER COALITION, AND THE CALIFORNIA – NEVADA SECTION OF THE AMERICAN WATER WORKS ASSOCIATION (JANUARY 2019)

Comment 1. "The Draft PHGs for Individual THMs are a Significant Departure from the Established Approach to Addressing THM"

"…the establishment of individual PHGs for each of the four regulated THM constituents would be a major departure from the current risk management approach in California involving regulation of total THMs. OEHHA's proposed PHGs place too much emphasis on reducing incremental cancer risks from exposure to THMs that other authoritative bodies have established are minor in comparison to the health risks posed by inadequate drinking water disinfection. To the extent they result in new drinking water standards that force changes to effective drinking water disinfection practices, the proposed PHGs could have unintended consequences for public health."

"Health and Safety Code (H&SC) §Section 116365(c)(1) states: "The Office of Environmental Health Hazard Assessment shall prepare and publish an assessment of the risks to public health posed by each contaminant ***for which the state board proposes a primary drinking water standard***.". We emphasize the close linkage of PHGs with MCLs to maintain that OEHHA should not operate in a vacuum when publishing a PHG for any contaminant but should consider the full public health context from readily available sources… It is highly significant that there are no existing state or federal MCLs for any of the four individual THMs.

"Under OEHHA's proposal, water utilities would be required to report exceedances of substances for which no regulatory standard exists. As OEHHA knows, published PHGs impose specific obligations on public water systems, including periodic public disclosures where water contains concentrations of contaminants above corresponding PHGs. Therefore, while they may not be enforceable regulatory standards, PHGs that reach beyond the authority of the statute are inappropriate and can cause confusion and concern about drinking water safety and confound the intent of the Health and Safety Code provisions that seek to provide effective public right-to-know."

Response 1. We agree completely as to the utility and importance of water disinfection. However, during disinfection with chlorine or chloramine, halogenated disinfection byproducts are formed in the drinking water. Health and Safety Code section 116365(c)(1) requires OEHHA to look strictly at the adverse effects of a contaminant on public health and not to weigh the relative benefits and risks of water treatment and the resulting disinfection byproducts. After completion of the PHGs, the State Water Resources Control Board (SWRCB) determines the appropriate regulatory

standard(s) while taking into consideration the technological and economic feasibility, among other factors. This would include the risk-benefit tradeoff between residual disinfection byproducts in drinking water and exposure to microbial contaminants in untreated water.

Health and Safety Code section 116470(a) requires public water systems in their annual consumer confidence reports to disclose whether a detected contaminant exceeds the corresponding PHG or MCL. Section 116470(b) requires public water systems serving more than 10,000 service connections that detect one or more contaminants in drinking water that exceed the applicable PHG to prepare a written report identifying the contaminant(s). The report is required to provide other pertinent information, including the numerical public health risk associated with the MCL and the PHG for each contaminant. We believe the commenters are correct that, upon finalization of the PHG document, these requirements would apply to the PHGs for each of the four THMs. This would be consistent with the statute's intent that a public water system's customers be informed of contaminants exceeding the PHG, even if the system is in compliance with the MCLs for those contaminants. The statute also makes clear that a public water system is not required "to take any action to reduce or eliminate any exceedance of a public health goal."

Comment 2. "**OEHHA Must Evaluate the Public Health Benefits of Chlorination Relative to the Public Health Risks of THMs**"

"Almost all community water systems in California use some type of chlorine-based disinfection method—either alone or in combination with other chlorine and non-chlorine disinfectants. …In addition to controlling disease-causing organisms, chlorination offers additional benefits, including:

• Eliminating slime bacteria, molds and algae that commonly grow in water supply reservoirs;

• Controlling and reducing microorganism-containing biofilms;

• Removing chemical compounds that hinder disinfection; and

• Reducing disagreeable tastes and odors.

"Perhaps most importantly, only chlorine-based chemicals provide residual disinfection capacity in the distribution system to control and reduce microbial re-growth. OEHHA needs to recognize that surface water systems are ***required as a matter of federal regulation*** to ensure a disinfectant residual is present throughout the distribution system. …It is therefore critical, in assessing the risks associated with the ingestion of THMs in drinking water, to also consider the substantial benefits to public health associated with disinfection by chlorination. The use of chlorine for disinfection has virtually eliminated waterborne microbial diseases because of its ability to kill or

inactivate essentially all enteric pathogenic microorganisms, including viruses and bacteria from the human intestinal tract.…Numerous public health organizations, including the World Health Organization (WHO), have consistently described the

profound historical and continuing public health benefits that chlorination provides and strongly caution that:

[I]n attempting to control DBP [disinfection byproduct] concentrations, it is of

paramount importance that the efficiency of disinfection is not compromised and that a suitable residual level of disinfectant is maintained throughout the distribution system." (Commenter cites WHO. 2017. Guidelines for Drinking-water Quality. Fourth Edition Incorporating the First Addendum, at p. 173.)

"In establishing a public health goal, Health and Safety Code section 116365(c)(1) directs OEHHA to estimate the level of the contaminant in drinking water 'that is not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health.' As part of this assessment, OEHHA is required to take into account several factors including '[s]ynergistic effects resulting from exposure to, or interaction between, the contaminant and one or more other substances or contaminants.' While the authorizing language does not define "synergistic effects," this term has a common definition in the public health literature that considers the combined biological effect of exposure to two or more substances. A plain reading of this language suggests that OEHHA's assessment must include an evaluation of the effects that may result from the creation of THMs as well as the effects that may result from efforts to reduce THM concentrations in drinking water.''

"As the WHO and other entities charged with protection of public health have indicated, the public health benefits of drinking water disinfection are indispensable and far greater than the public health risks associated with incremental exposures to DBPs. As a result, it is inappropriate and potentially harmful to public health to consider the public health impacts of the THMs without also considering whether further efforts to reduce THM exposures would sacrifice the more important public health benefits. Since the statute requires the State Water Resources Control Board (SWRCB) to set the enforceable MCL 'as close as feasible to the corresponding public health goal' this evaluation should not be left to a subsequent decision which would presume only public health benefits with the proposed PHGs. Moreover, it [would] be inappropriate for staff in the SWRCB's Division of Drinking Water to make that evaluation because they do not possess the necessary breadth or depth of expertise in epidemiology, risk assessment, and toxicology.

"This is an unusual instance where actions taken to reduce exposure to a chemical contaminant could significantly *increase* overall health risks from drinking water consumption. To avoid this outcome, OEHHA must exercise its statutory discretion and scientific expertise to evaluate this potential risk tradeoff and ensure that any proposed PHG will not inadvertently create a bigger public health problem than it seeks to solve."

Response 2. We agree completely as to the utility and importance of disinfectants. As the comment notes, the State Water Resources Control Board (SWRCB) is the entity that promulgates the MCL. SWRCB is fully aware of the need and importance of disinfecting California's drinking water. We disagree with the commenters that SWRCB lacks the expertise to promulgate an MCL or MCLs that are feasible to achieve and that adequately balance the risks and benefits of drinking water disinfection and the creation of THMs. The risk-benefit tradeoff between residual disinfection byproducts in drinking water and exposure to microbial contaminants in drinking water is the kind of risk-management analysis that a regulatory entity such as SWRCB is supposed to undertake. As the name implies, a Public Health Goal is a non-regulatory number that identifies a target for SWRCB to consider as it also weighs treatment costs and technologies, and the importance of drinking water disinfection. OEHHA acknowledged the importance of drinking water disinfection on pgs. 2 and 268 of the draft document. However, this document is not, nor is it intended to be, an analysis of the health benefits of chlorine disinfection of drinking water.

Comment 3. Comments on Derivation of Proposed PHG

"In developing the proposed PHGs for THMs, OEHHA has ignored the conclusions reached by various authoritative bodies, including the U.S. Environmental Protection Agency (USEPA), International Agency for Research on Cancer (IARC), WHO, and Health Canada. OEHHA based each of its four proposed PHGs on cancer evidence in laboratory animals exposed by oral gavage – despite the fact that studies evaluating dietary and drinking water exposures failed to find carcinogenic effects. OEHHA also has overestimated daily water consumption rates, reporting values approximately four times higher than indicated by available data, even after adjusting for life-stage variability and age-sensitivity. Taken together, these anomalies significantly overstate the risks associated with exposure to the four THMs that have led to the proposed PHGs that are not supported by the scientific evidence.

- "Chloroform is a Threshold Carcinogen

"Contrary to OEHHA's analysis, USEPA, WHO, and Health Canada have concluded that cancer risk from chloroform is defined by a non-genotoxic, or threshold, mechanism. Their independent conclusions are based on substantial evidence that cancers observed in laboratory animals ***only*** result from sustained exposure to levels of chloroform that overwhelm the animal's natural defense mechanisms. Consistent with this conclusion, USEPA has adopted a maximum contaminant level goal (MCLG) – analogous to the PHG – of 70 parts per billion (ppb) for chloroform, which is more stringent than corresponding values developed by Health Canada (80 ppb) and the WHO (300 ppb). Despite the weight of the evidence and expert opinion supporting the threshold mechanism for chloroform, and the safety factors included in USEPA's MCLG calculation to protect all populations, including potentially sensitive subpopulations, OEHHA's proposed PHG is still 175 times lower (more stringent) than USEPA's MCLG."

Response 3. Carcinogens can have multiple modes of action (Guyton et al., 2018a, 2018b; Parfett and Desaulnier, 2017). Mode of action can depend on when during the lifestage exposure occurs or the level of exposure. US EPA says in their 2001 assessment of chloroform: "Thus, the weight-of-evidence of the genotoxicity data on chloroform supports a conclusion that chloroform is not strongly mutagenic, and the genotoxicity is not likely to be the predominant mode of action underlying the carcinogenic potential of chloroform." US EPA does not state that chloroform is NOT mutagenic, but that is not strongly so. Further, at higher levels of exposure, US EPA does not say genotoxicity plays no role, but that they believe that cytotoxicity and subsequent tissue regeneration is active at higher exposures. In the case of chloroform, it is quite plausible that cytotoxicity and regeneration play a role at high levels of exposure, but that role is not evident at lower levels of exposure. In their discussion of uncertainties from its 2001 IRIS assessment of chloroform, US EPA (2001) notes that one of the uncertainties is that there are positive mutagenicity studies despite the overall negative results in the database. The available evidence is not, in our view, strong enough to rule out any role for genotoxicity for chloroform-induced tumorigenicity.

See responses above (Responses 11-20) to the American Chemistry Council's Attachment A analysis for further discussion of potential genotoxicity of chloroform.

OEHHA utilizes linearized extrapolation when there is potential for mutagenic action of a carcinogen. Although it is true that most studies of genotoxicity were negative, some were positive. Further, chloroform is metabolized to reactive metabolites including phosgene and dichloromethyl radical. Evidence for additional reactive metabolites is also in the literature. Reactive metabolites can bind to cellular macromolecules. Several studies have demonstrated binding of phosgene to lipids and proteins, including the histones surrounding DNA; binding to histones means phosgene entered the nucleus and binding was not limited to macromolecules in the endoplasmic reticulum where it is produced. We agree with US EPA that chloroform is not strongly mutagenic. We disagree that it should, therefore, be treated solely as a threshold carcinogen and that low exposures can be ignored.

Comment 4. "Bromoform in [sic] Not Carcinogenic"

"While bromoform is listed as a carcinogen under Proposition 65 and by the US EPA, more recent assessments by IARC (1999) and Health Canada (2006) have concluded that the substance is unlikely to be carcinogenic. IARC designated bromoform as "not classifiable" as to its carcinogenicity (Group 3) while Health Canada considers it "possibly carcinogenic to humans" (Group IIID). In its most recent review, Health Canada concluded there was insufficient information available to support a drinking water guideline for bromoform. The WHO adopted the IARC Group 3 designation, establishing a drinking water guideline of 100 ppb."

Response 4. Positive genotoxicity results have been obtained for bromoform in both *in vitro* and *in vivo* assays such as micronuclei induction, sister chromatid exchange, chromosomal aberrations including DNA damage, sex-linked recessive lethal mutations, and aneuploidy. Both positive and negative results of *in vivo* genotoxicity tests on bromoform are summarized in Table 6.6 in the draft, as shown below. The weight of the available evidence indicates that bromoform is mutagenic and genotoxic, and thus presents a carcinogenic risk to humans.

Table 6.6 Summary of *In Vivo* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Result	References
Micronuclei induction	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Mouse, bone marrow cells	-	Stocker *et al*. (1997)
	Mouse, bone marrow cells	+	NTP (1989a)
	Newt, peripheral erythrocytes	+	Le Curieux *et al*. (1995)
	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
Chromosomal aberrations	Mouse, bone marrow cells	-	NTP (1989a)
	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
	Mouse, bone marrow cells	+	NTP (1989a)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)
Sex linked recessive lethal mutations	*Drosophila melanogaster*	+	Woodruff *et al*. (1985), NTP (1989a)
Heritable translocations	*Drosophila melanogaster*	-	
Aneuploidy	*Aspergillus nidulans*	+	Benigni *et al*. (1993)
Initiation	Rat, liver	-	Herren-Freund and Pereira (1986)

Geter et al. (2004c) exposed male F344/N rats to isomolar concentrations of the THMs at 0.5 g/L chloroform, 0.7 g/L BDCM, 0.9 g/L DBCM, or 1.1 g/L bromoform in drinking water for 26 weeks, with half the animals fed the normal 4.5% fat Purina 5001 diet and the other half receiving the feed supplemented with 19% animal fat. Rats administered bromoform and a high fat diet exhibited the most significant increase in aberrant crypt foci compared to other THMs.

There is adequate evidence that metabolism of bromoform can produce reactive metabolites, including the dihalomethyl radical •CHBr2. Tomasi et al. (1985) measured the production of a free radical intermediate in rat hepatocytes by electron spin resonance (ESR) spectroscopy. The intensity of the ESR signal was greatest for

bromoform compared to other THMs. The signal was reduced by cytochrome P450 inhibitors. These data were interpreted as evidence that free radical formation depends on cytochrome P450-mediated reductive metabolism. Toxic effects caused by these reactive intermediates are expected to occur in the tissues in which they are formed.

The positive data for carcinogenicity and mutagenicity for bromoform, as well as the formation of mutagenic metabolites, justify the use of linear extrapolation of cancer risk, in accordance with cancer guidelines (US EPA, 2005; OEHHA, 2009). Cancer guidelines require assumption of a linear dose response unless there is adequate evidence of a threshold mechanism of action, which is certainly not true in this case.

Comment 5. "Bromodichloromethane Cancer Risk is Overstated"

"The WHO concluded in 2008 that its drinking water guideline value of 60 ppb for bromodichloromethane (BDCM) protects against any potential cancer risk from this substance, based on conflicting results from two cancer studies conducted by the National Toxicology Program (NTP, 2016). A panel of experts convened by Health Canada in 2008 concluded that the combined data from the two NTP studies do not support the default non-threshold cancer model employed by OEHHA in its draft Technical Support Document (TSD). Based on this analysis, the panel concluded that retention of Health Canada's previous guideline of 16 ppb is 'unlikely to be necessary.'"

Response 5. While there are a mix of results in the various carcinogenicity bioassays, it is a common and health-protective practice in risk assessment to utilize the study(ies) that indicate the most sensitive response in assessing cancer risk. Cancer risk assessment is generally based on the study showing the highest tumor incidence (US EPA, 2005; OEHHA, 2009).

The results from the NTP drinking water study do not negate the effects in both mice and rats seen in the earlier NTP gavage studies. NTP (2006) indeed states that there was no evidence for carcinogenicity in the drinking water study, which examined male F344 rats and female B6C3F1 mice. In their earlier gavage studies, NTP notes clear evidence for carcinogenicity of BDCM in both male and female rats and mice. In its Report on Carcinogens (14th edition, 2016), NTP states "Administration of bromodichloromethane by stomach tube caused benign and malignant kidney tumors (tubular-cell adenoma and adenocarcinoma) in male mice and in rats of both sexes, benign and malignant liver tumors (hepatocellular adenoma and carcinoma) in female mice, and benign and malignant colon tumors (adenomatous polyps and adenocarcinoma) in rats of both sexes". NTP describes BDCM as reasonably anticipated to be a human carcinogen (NTP, 2016).

Although differences in kinetics may factor into the response rates, comparison is somewhat complicated by the lower dose levels in the drinking water at least in the rat (highest dose 25 mg/kg-day for rat) (NTP, 2006) versus gavage studies (lowest dose 50 mg/kg-day for rat) (NTP, 1987).

In a drinking water study, George et al. (2002) reported that hepatocellular adenomas were significantly increased at the low dose (7/45 in the 3.9 mg/kg-day group) and combined hepatocellular adenomas and carcinomas were increased in the low and mid-dose groups (8/45 at 3.9 mg/kg-day and 7/48 at 20.6 mg/kg-day) but not at the high dose. The lack of a statistically elevated response at the high dose decreases confidence but does not completely eliminate the biological significance of the responses at the two lower doses.

Health Canada (2006) classifies BDCM as a probable carcinogen to humans. They indicate BDCM probably acts via mutagenicity and used a linear model to estimate cancer slope. This is in contrast to their opinion that chloroform probably acts through a threshold mechanism. Health Canada stated in its document that "Among the four THMs [chloroform, BDCM, DBCM, bromoform] commonly found in drinking water, BDCM appears to be the most potent rodent carcinogen. BDCM caused tumours at lower doses and at more target sites than for any of the other THMs…" Health Canada's document indicates they would have a maximum acceptable concentration for BDCM of 16 µg/L. Their final number of 100 µg/L for total THMs, largely based on their analysis for chloroform, is a risk management number.

Although the overall data are mixed, a number of positive results were obtained for mutagenicity and genotoxicity assays in bacterial and mammalian systems. Thus, the weight of evidence suggests that BDCM is mutagenic and genotoxic. As discussed in our draft document, evidence *in vitro* suggests that a potential mechanism of carcinogenicity of BDCM may involve covalent binding to DNA of reactive intermediates generated by GSTT1-1-mediated metabolism of BDCM (Ross and Pegram, 2004). The authors suggest that this may occur in the kidney and large intestine (target organs of BDCM carcinogenicity in rats), which were demonstrated in this study to have a much lower detoxification rate to bioactivation rate (CYP/GST ratio) compared to the liver (a non-cancer target in rats). This may result in an enhanced relative production of reactive intermediates with the capacity to covalently modify DNA in target tissues, ultimately contributing to BDCM carcinogenicity. Also as discussed in our draft, several *in vivo* studies in rats suggest a plausible mechanism of carcinogenicity of brominated THMs in the colon involving formation of putative early preneoplastic lesions in the colon called aberrant crypt foci. DeAngelo et al. (2002) reported that the brominated THMs, particularly BDCM, administered in drinking water significantly induced the incidence of aberrant crypt foci in the colon of male F344/N rats. Geter et al. (2004b) then showed that formation of aberrant crypt foci in the colon of male F344/N rats was independent of the method of BDCM administration, with drinking water and corn oil gavage administration producing similar values of aberrant crypt foci per colon.

As discussed in the PHG draft, recent cancer-risk estimates for exposure to the THMs in drinking water have assumed they do not operate through a threshold mechanism and have thus applied a linear dose-response model (Lee et al., 2004; Uyak, 2006; Wang et al., 2007a,b; Panyakapo et al., 2008; Viana et al., 2009; Pardakhti et al., 2011; Basu et al., 2011; Chowdhury et al., 2011; Legay et al., 2011; Yamamoto, 2011). OEHHA concurs with this approach in estimating cancer risks of the THMs. It is clear,

as discussed in the draft document, that BDCM, DBCM, and bromoform have produced carcinogenic effects in animal studies. The data overall provide strong support for using a non-threshold model for the brominated trihalomethanes.

Comment 6. "Dibromochloromethane is Not Carcinogenic"

"OEHHA delisted dibromochloromethane (DBCM) from Proposition 65 in 1999, following the decision by IARC that it was "not classifiable" (Group 3) as to its carcinogenicity. Based on the IARC decision, the WHO set its DBCM guideline value at 100 ppb. Health Canada concluded in 2003 that the there was insufficient information to calculate a drinking water value. In 1992, US EPA classified DBCM as a "possible human carcinogen" (Group C), based on inadequate human data, limited evidence for carcinogenicity in animals, and consideration of structural similarity to other THMs which are known animal carcinogens. In the 1998, USEPA set an MCLG for DBCM at 60 ppb – 600 times higher than OEHHA's proposed PHG for the substance."

Response 6. Many assessments of exposures to THMs in drinking water conducted by authorities from around the world include the assumption that all four of the THMs provide a carcinogenic risk to humans, as discussed in the PHG document.

The overall weight of evidence suggests that DBCM is mutagenic and genotoxic. As indicated in our draft document, "Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of S. typhimurium; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosomal aberrations *in vivo* and *in vitro*; DNA damage in bacteria; and aneuploidy in mammalian cells and Aspergillus nidulans. Negative results have also been obtained *in vitro* and *in vivo*. For some *in vitro* tests, negative findings may have occurred because measures were not taken to prevent volatilization of the test compound."

OEHHA cites the NTP (1985) cancer bioassay in Fisher 344/N rats and B6C3F1 mice, where DBCM was administered by gavage in corn oil. This is the only available cancer bioassay for DBCM. No tumors were found in rats. In male mice, there was a significant increase in hepatocellular carcinomas in the high dose males but not combined hepatocellular adenomas and carcinomas compared to controls. NTP considered the evidence equivocal for male mice. Note that the study in male mice was compromised due to high mortality from a dosing error in the low dose male mice. The NTP gavage study reported statistically significant elevated incidence of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in female B6C3F1 mice. NTP states that there is some evidence of carcinogenicity in the female mice. It is not uncommon that a carcinogen induces tumors in one rodent species and not in another. While there are a mix of results in the various carcinogenicity bioassays, it is a common and health-protective practice in risk assessment to utilize the study(ies) that indicated the most sensitive response in assessing cancer risk.

Comment 7. "OEHHA Significantly Overstates Drinking Water Consumption Rate"

"In addition to our concerns about OEHHA's proposal to consider all four THMs as genotoxic carcinogens and to use default assumptions to calculate the potential cancer risks, we are troubled by OEHHA's overestimate of water consumption rates used to generate the public health goal. As part of its PHG derivation, OEHHA uses a susceptibility-weighted daily water intake of 0.180 to 0.185 equivalent liters per kilogram body weight per day (Leq/kg-day). This lifetime consumption rate is much higher than the weighted intake for any of the four life stages identified by OEHHA. For example, it appears to assume that an individual will consume 12.6 liters of water – 3.3 gallons – or more on a daily basis for the entirety of their time as a 70-kg adult. This rate is more than four times the total daily consumption rate of 3 L*eq*/day assumed in the 2010 draft PHG and much higher than the average consumption rate recommended by authoritative bodies. Using the average of the susceptibility weighted DWIs (equal to 0.045 Leq/kgday), on the other hand, generates a total adult consumption rate of 3.15 Leq/day which is far more realistic."

Response 7. OEHHA disagrees with the assertion that drinking water intake rates have been overestimated. There are two components to OEHHA's susceptibility-weighted daily water intake rates that are independent of one another but appropriately applied. First, the age sensitivity factor (ASF) is a weighting factor applied to exposure at different lifestages to account for the increased susceptibility to carcinogens during early-in-life exposures. The ASFs reflect the available data and analyses indicating higher carcinogen potency when exposure occurs early in life (OEHHA, 2009). Since everyone goes through each lifestage, it is appropriate to add (not average as stated in the comment) the lifestage DWI to obtain a lifetime DWI. This is an approach consistent with US EPA's cancer risk assessment guidelines (US EPA, 2005a,b), which "view childhood as a sequence of lifestages rather than viewing children as a subpopulation" and states, "[F]or a susceptible lifestage, higher risks can be expected from exposures during only a portion of a lifetime, but everyone in the population may pass through those lifestages."

Second, liter equivalents (L_{eq}) account for the total amount of exposure to a chemical that one receives through the use of tap water. For volatile chemicals such as the THMs, exposure from tap water consists of oral ingestion as well as inhalation and dermal exposure while bathing, showering, and through other household uses. Thus, the L_{eq} value is not the amount of water an individual actually drinks but instead represents how much tap water one would have to drink to have the same amount of exposure to the chemical through the combined oral, inhalation, and dermal routes via typical household uses of tap water. Thus, one cannot compare the consumption rates multiplied by an ASF and inclusive of exposure via inhalation and dermal pathways to an unweighted estimate of drinking water ingestion rate for an adult.

Further, trying to compare the weighted exposure to how much water would need to be consumed by an adult to meet the NSRL from the Proposition 65 program is not

appropriate. An NSRL is a general guidance number that identifies a level of exposure to a chemical from a product or environmental source that does not require a Proposition 65 warning. NSRLs are set for a higher risk level (10^{-5}) than PHGs (10^{-6}), and do not consider multipathway exposures. Further, neither age-specific drinking water ingestion rates nor age sensitivity factors are utilized in deriving NSRLs, which are not specific to drinking-water exposures.

RESPONSES TO COMMENTS RECEIVED FROM ENVIRONMENTAL WORKING GROUP (EWG) (NOVEMBER 2018)

Comment 1. "EWG strongly agrees with OEHHA's proposed public health goals. …cancer based public health goals for trihalomethanes are supported by the findings of human epidemiological studies." The commentor notes the associations in the epidemiological literature with colorectal cancer and bladder cancer in humans.

"Second, EWG applauds OEHHA's approach of using Age Sensitivity Factors for different life stages." The commentor notes that findings from the peer-reviewed literature support this approach.

Response 1. OEHHA acknowledges the comments.

Comment 2. "Third, the one-in a million cancer risk levels for four trihalomethanes published by OEHHA are fully consistent with the estimated cancer risk levels for trihalomethanes presented in a risk assessment published in 2015 by the U.S.EPA and academic researchers. Specifically, a study S. Regli et al 2015 analyzed the lifetime risk from exposure to trihalomethanes in drinking water and reported that each 1 ug/L increase in trihalomethane concentration corresponded to 10^{-4} added lifetime risk of bladder cancer.…this study provides strong supportive information for OEHHA's assessment. EWG notes that OEHHA did not use this study to develop the PHGs and urges OEHHA to describe this study in more detail."

Response 2. OEHHA reviewed Regli et al., 2015, but did not, as the commenter notes, describe the study in detail. As noted in Appendix C, page 389, Table C3. "Epidemiologic studies not used in the review of disinfection byproduct exposure and cancer", we did not include it in the Appendix C epidemiology review primarily because it is a risk assessment rather than an original epidemiological study. We also did not describe several other studies that attempted to use epidemiological studies in risk assessment. The primary reason is that these studies look at exposures either to total THM or to categories of disinfection by-products. Thus, while important, these do not describe risks to specific THMs, which we are estimating in our cancer risk assessment. Nonetheless, it is interesting to compare the results of such assessments to our estimates based on the animal studies, as done by the commenter.

Comment 3. "Specifically, Regli et al. analyzed bladder cancer risk, from six epidemiological studies previously published as a meta-analysis by Villanueva et al. (2003), as a function of trihalomethane exposure from tap water. As the study reported, this experimentally observed dose-response relationship between cancer risk and exposure is complex, since it is nearly linear at higher exposure levels; convex in the mid-dose exposure range; and concave, or supralinear at lower doses. This potential

supralinearity at lower doses would indicate that the dose-response relationship might be steeper at lower concentrations and that a linear relationship may be conservative."

Response 3. Regli et al. (2015) hypothesize, based on data demonstrating increased levels of trihalomethanes in drinking water with increased levels of bromide ion in the water source, that bladder cancer risk would increase due to contamination of drinking water sources with bromide ion occurring from industrial processes. They first estimate potential increased bladder cancer risk as a function of increased THMs in the finished water. They then estimate increased THM in the finished water as a function of bromide ion concentrations, and, finally, they estimate potential increase in bladder cancer as a result of bromide ion contamination of source water.

In reviewing US EPA's assessment conducted for its 2006 drinking water disinfection byproduct rule, Regli et al. note that the cancer risk dose-response was constructed by fitting a spline function to ORs derived from a pooled analysis of six case-control studies. As noted in the comment, Regli et al. describe the curve as "complex; concave near the origin, convex in the middle and nearly linear at higher exposure levels." Regli et al. also note that given the wide confidence bands about the curve, there is considerable uncertainty about the actual dose-response relationship. In the end, Regli et al. re-weight the data used by US EPA to obtain the dose-response curve by using the log transformed variance in order to better account for uncertainty of the exposure assessment in the epidemiology studies. Regli et al. thus obtained a linear slope, and estimated attributable risk for bladder cancer per µg/l increase in THM, as approximately 10^{-4}, or one in ten thousand. Note also that US EPA used a linear slope in their cost-benefit assessment for the Economic Analysis of the DBP rule.

We agree that if the dose-response curve is supralinear at low dose, then a linear model of cancer risk from THM exposure could be an underestimate.

Comment 4. "Starting with the Regli et al. finding that a 1 ppb increase in trihalomethane concentration corresponds to 10^{-4} added lifetime risk, and assuming the linearity of dose-response relationship, we calculate that 0.001 ppb concentration of total trihalomethanes would correspond to 10^{-6} added lifetime cancer risk. This 0.01 ppb concentration for one-in-a-million cancer risk is below OEHHA's proposed public health goals for individual trihalomethanes, which are in the 0.06 – 0.5 ppb range. EWG finds that the public health goals published by OEHHA are conservative and consistent with human data."

"EWG strongly supports OEHHA's proposed public health goals for trihalomethanes and the methodology used to derive the cancer risk values for these chemicals."

Response 4. OEHHA acknowledges the commenter's comparison of the analysis by Regli et al. (2015) with our cancer-based proposed public health goals. We agree that the comparison is helpful and provides support for the proposed PHGs for trihalomethanes.

REFERENCES CITED IN RESPONSES

Axelson O (1978). Aspects on confounding in occupational health epidemiology. Scand J Work Environ Health 4:85-89.

Basu M, Gupta SK, Singh G, Mukhopadhyay (2011). Multi-route risk assessment from trihalomethanes in drinking water supplies. Environ Monit Assess. 178 (1-4):121-34.

Becker RA et al., 2017. Quantitative weight of evidence to assess confidence in potential modes of action. Regul Toxicol Pharmacol. 86: 205-220.

Beddowes EJ, Faux SP, Chipman JK (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. Toxicology 187(2-3):101-15.

Benigni R, Andreoli C, Conti L, Tafani P, Cotta-Ramusino M, Carere A, Crebelli R (1993). Quantitative structure-activity relationship models correctly predict the toxic and aneuploidizing properties of six halogenated methanes in *Aspergillus nidulans*. Mutagenesis 8(4):301-5.

Butterworth BE, Templin MV, Constan AA, Sprankle CS, Wong BA, Pluta LJ, Everitt JI, Recio L. (1998). Long-term mutagenicity studies with chloroform and dimethylnitrosamine in female *lacI* transgenic B6C3F1 mice. Environ Mol Mutagen 31(3):248–256.

Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, et al. (1998). Drinking water source and chlorination byproducts. I. Risk of bladder cancer. Epidemiol 9(1):21-28.

Chowdhury S, Rodriguez MJ, Sadiq R (2011). Disinfection byproducts in Canadian provinces: Associated cancer risks and medical expenses. J Haz Mat 187: 574–584.

Coffin JC, Ge R, Yang S, Kramer PM, Tao L, Pereira MA (2000). Effect of trihalomethanes on cell proliferation and DNA methylation in female B6C3F1 mouse liver. Toxicol Sci 58(2):243-252.

Cotruvo JA, Amato H (2019). Trihalomethanes: concentrations, cancer risks, and regulation. J Am Water Works Assoc 111:1, 12-20.

DeAngelo AB, Geter DR, Rosenberg DW, Crary CK, George MH (2002). The induction of aberrant crypt foci (ACF) in the colons of rats by trihalomethanes administered in the drinking water. Cancer Lett 187(12):25-31.

Diaz Gomez MI, Castro JA (1980a). Covalent binding of chloroform metabolites to nuclear proteins: no evidence for binding to nucleic acids. Cancer Lett 9:213-8.

DiRenzo AB, Gandolfi AJ, Sipes IG (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA. Toxicol Lett 11:243-52.

Fabrizi L, Taylor GW, Cañas B, Boobis AR, Edwards RJ (2003). Adduction of the chloroform metabolite phosgene to lysine residues of human histone H2B. Chem Res Toxicol 16(3):266-75.

Fujie K, Aoki T, Wada M (1990). Acute and subacute cytogenetic effects of the trihalomethanes on rat bone marrow cells *in vivo*. Mutat Res 242(2):111-9.

George MH, Olson GR, Doerfler D, Moore T, Kilburn S, DeAngelo AB (2002). Carcinogenicity of bromodichloromethane administered in drinking water to Male F344/N Rats and B6C3F1 mice. Int J Toxicol 21(3):219-30.

Gemma S, Testai E, Chieco P, Vittozzi L (2004). Bioactivation, toxicokinetics and acute effects of chloroform in Fisher 344 and Osborne Mendel male rats. J Appl Toxicol 24(3):203-10.

Geter DR, George MH, Moore TM, Kilburn S, Huggins-Clark G, DeAngelo AB (2004b). Vehicle and mode of administration effects on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to bromodichloromethane. J Toxicol Environ Health A 67(1):23-9.

Geter DR, George MH, Moore TM, Kilburn SR, Huggins-Clark G, DeAngelo AB (2004c). The effects of a high animal fat diet on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to trihalomethanes in the drinking water. Toxicol Lett 147(3):245-52.

Guyton KZ, Rieswijk L, Wang A, Chiu WA, Smith MT (2018) Key characteristics approach to carcinogen hazard identification. Chem Res Toxicol 31(12):1290-1292.

Guyton KZ, Rusyn I, Chiu WA, Corpet DE, van den Berg M, Ross MK, Christiani DC, Beland FA, Smith MT (2018). Application of key characteristics of carcinogens in cancer hazard identification.

Hayashi M, Kishi M, Sofuni T, Ishidate Jr M (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem Toxicol 26:487-500.

Health Canada (2006). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Trihalomethanes. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. Prepared by the Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment. May 2006, with April 2009 addendum. Accessed at: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecssesc/pdf/pubs/water-eau/trihalomethanes/trihalomethanes-eng.pdf.

Herren-Freund SL, Pereira MA (1986). Carcinogenicity of by-products of disinfection in mouse and in rat liver. Environ Health Perspect 69:59-66.

Jorgenson TA, Meierhenry EF, Rushbrook CJ, Bull RJ, Robinson M (1985). Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fund Appl Toxicol 5:760-9.

King WD, Marrett LD (1996). Case-control study of bladder cancer and chlorination by-products in treated water (Ontario, Canada). Cancer Causes Control 7(6):596-604.

Kogevinas M, Villanueva CM, Font-Ribera L, Liviac D, et al. (2010). Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environ Health Perspect 118(11):1531-7.

Larson JL, Wolf DC, Butterworth BE (1993). Acute hepatotoxic and nephrotoxic effects of chloroform in male F-344 rats and female B6C3F1 mice. Fund Appl Toxicol 20: 302-15.

Larson JL, Wolf DC, Butterworth BE (1994b). Induced cytolethality and regenerative cell proliferation in the livers and kidneys of male B6C3F$_1$ mice given chloroform by gavage. Fund Appl Toxicol 23:537-43.

Larson JL, Wolf DC, Butterworth BE (1994c). Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F$_1$ mice: comparison of administration by gavage in corn oil vs. *ad libitum* in drinking water. Fund Appl Toxicol 22:90-102.

Larson JL, Sprankle CS, Butterworth BE (1994d). Lack of chloroform-induced DNA repair *in vitro* and *in vivo* in hepatocytes of female B6C3F$_1$ mice. Environ Mol Mutagen 23:132-6.

Le Curieux F, Gauthier L, Erb F, Marzin D (1995). Use of the SOS chromotest, the Ames fluctuation test and the newt micronucleus test to study the genotoxicity of four trihalomethanes. Mutagenesis 10(4):333-41.

Lee SC, Guo H, Lam SMJ, Lau SLA (2004). Multipathway risk assessment on disinfection by-products of drinking water in Hong Kong. Environ Res 94:47–56. Comment in: Science-based risk assessments for drinking water disinfection by-products. Butterworth BE, Chlorine Chemistry Council. Environ Res 98(2):276-8.

Legay C, Rodriguez MJ, Sadiq R, Sérodes JB, Levallois P, Proulx F (2011). Spatial variations of human health risk associated with exposure to chlorination by-products occurring in drinking water. J Environ Manage 92(3):892-901.

Madhavan HN (2007). Simple Laboratory methods to measure cell proliferation using DNA synthesis property. J Stem Cells Regen Med 3(1): 12–14.

Melnick RL, Kohn MC, Dunnick JK, Leininger JR (1998). Regenerative hyperplasia is not required for liver tumor induction in female B6C3F$_1$ mice exposed to trihalomethanes. Toxicol Appl Pharmacol 148(1):137-47.

Mitchell A, Rudd C, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. Environ Mol Mutag 12(Suppl 13):37-101.

Morimoto K, Koizumi A (1983). Trihalomethanes induce sister chromatid exchanges in human lymphocytes *in vitro* and mouse bone marrow cells *in vivo*. Environ Res 32:72-9.

Nagano K, Nishizawa T, Yamamoto S, Matsushima T (1998). Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Advances in the Prevention of Occupational Respiratory Diseases. Proc. of the 9th International Conf. on

Occupational Respiratory Diseases, Kyoto, Japan, October 13 – 16, 1997. Chiyotani K, Hosoda Y, Aizawa Y, eds. Elsevier Science, New York, NY, pp. 741-6.

Nagano K, Kano H, Arito H, Yamamoto S, Matsushima T (2006). Enhancement of renal carcinogenicity by combined inhalation and oral exposures to chloroform in male rats. Toxicol Environ Health A. 69(20):1827-42.

NCI (1976). Report on Carcinogenesis Bioassay of Chloroform. NTIS PB-264018. National Cancer Institute (NCI), National Institute of Health, Bethesda, MD. Accessed at: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/trchloroform.pdf.

NTP (1985). Toxicology and carcinogenesis studies of chlorodibromomethane (CAS No. 124-48-1) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report Series No. 282, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (1987). Toxicology and carcinogenesis studies of bromodichloromethane (CAS No. 75-27-4) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report Series No. 321, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (1989a). Toxicology and carcinogenesis studies of tribromomethane (bromoform) (CAS No. 75-25-2) in F344/N rats and B6C3F1 mice (gavage studies). NTP Technical Report Series No. 350, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (2006). Toxicology and carcinogenesis studies of bromodichloromethane (CAS No. 75-27-4) in male F344/N rats and female B6C3F1 mice (Drinking Water Studies). National Toxicol Program Tech Rep Ser 532:1-248.

NTP (2016). Chloroform. In: Report on Carcinogens, Fourteenth Edition. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

OEHHA (2008). Air toxics hot spots risk assessment guidelines: technical support document for the derivation of noncancer reference exposure levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

OEHHA (2009). Air Toxics Hot Spots Program Risk Assessment Guidelines, Part II. Technical Support Document for Describing Available Cancer Potency Factors. May 2009. Office of Environmental Health Hazard Assessment, Oakland and Sacramento, CA.

OEHHA (2012) Air Toxics Hot Spots Risk Assessment Guidelines, Part IV Technical Support Document for Exposure Assessment and Stochastic Analysis. August, 2012. Office of Environmental Health Hazard Assessment, Oakland and Sacramento, CA.

Panyakapo M, Soontornchai S, Paopuree P (2008). Cancer risk assessment from exposure to trihalomethanes in tap water and swimming pool water. J Environ Sci (China). 20(3):372-8.

Pardakhti AR, Bidhendi GR, Torabian A, Karbassi A, Yunesian M (2011). Comparative cancer risk assessment of THMs in drinking water from well water sources and surface water sources. Environ Monit Assess 179(1-4):499-507.

Parfett CL, Desaulniers D (2017). A Tox21 approach to altered epigenetic landscapes: Assessing epigenetic toxicity pathways leading to altered gene expression and oncogenic transformation in vitro. Int J Mol Sci 18:1179-1255.

Potter CL, Chang LW, DeAngelo AB, Daniel FB (1996). Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats. Cancer Lett 106(2):235-42.

Regli S, Chen J, Messner M, Elovitz MS, Letkiewicz FJ, Pegram RA, Pepping TJ, Richardson SD, Wright JM (2015). Estimating Potential Increased Bladder Cancer Risk Due to Increased Bromide Concentrations in Sources of Disinfected Drinking Waters. Environ Sci Technol. 2015 Nov 17;49(22):13094-102.

Reitz RH, Quast JF, Stott WT, Watanabe PG, Gehring PJ (1980). Pharmacokinetics and macromolecular effects of chloroform in rats and mice: implications for carcinogenic risk estimation. Water Chlorinat 3:983-93.

Rosenthal SL (1987). A review of the mutagenicity of chloroform. Environ Mol Mutagen 10:211-26.

Ross MK, Pegram RA (2004). In vitro biotransformation and genotoxicity of the drinking water disinfection byproduct bromodichloromethane: DNA binding mediated by glutathione transferase theta 1-1. Toxicol Appl Pharmacol 195(2):166-81.

Rothman K, Greenland S (1998). Precision and validity of studies. In: Modern Epidemiology. Rothman K, Greenland S, eds. Philadelphia, Lippincott Raven, 115–134.

Sehata S, Maejima T, Watanabe M, Ogata S, et al. (2002). Twenty-six-week carcinogenicity study of chloroform in CB6F1 *ras*H2-transgenic mice. Toxicol Pathol 30(3):328-38.

Sobti RC (1984). Sister chromatid exchange induction potential of the halogenated hydrocarbons produced during water chlorination. Chromosome Information Service No. 37, pp. 17-9.

Stocker KJ, Statham J, Howard WR, Proudlock RJ (1997). Assessment of the potential *in vivo* genotoxicity of three trihalomethanes: chlorodibromomethane, bromodichloromethane, and bromoform. Mutag 12:169-73.

Teixidó E, Piqué E, Gonzalez-Linares J, Llobet JM, Gómez-Catalán J (2015). Developmental effects and genotoxicity of 10 water disinfection by-products in zebrafish. J Water Health 13(1):54-66.

Templin MV, Larson JL, Butterworth BE, Jamison KC, et al. (1996a). A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32(1):109-25.

Tomasi A, Albano E, Biasi F, Slater TF, Vannini V, Dianzani MU (1985). Activation of chloroform and related trihalomethanes to free radical intermediates in isolated

hepatocytes and in the rat in vivo as detected by the ESR-SPIN trapping technique. Chem-Biol Inter 55:303-316.

Tumasonis CF, McMartin DN, Bush B (1985). Lifetime toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. Ecotoxicol Environ Saf 9:233-240.

Tumasonis CF, McMartin DN, Bush B (1987). Toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. J Environ Pathol Toxicol Oncol 7:55-64.

US EPA (2001). Toxicological Review of Chloroform (CAS No. 67-66-3) in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-01/001. US Environmental Protection Agency, Washington, DC.

US EPA (2005a). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum US Environmental Protection Agency Washington, DC. EPA/630/P-03/001F, March 2005.

US EPA (2005b). Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC. EPA/630/R-03/003F, March 2005.

Uyak V (2006). Multi-pathway risk assessment of trihalomethanes exposure in Istanbul drinking water supplies. Environ Int 32:12–21.

Viana RB, Cavalcante RM, Braga FMG, Viana AB, et al. (2009). Risk assessment of trihalomethanes from tap water in Fortaleza, Brazil. Environ Monit Assess 151:317–325.

Villanueva CM, Cantor KP, Grimalt JO, Malats N, et al. (2007). Bladder cancer and exposure to water disinfection byproducts through ingestion, bathing, showering, and swimming in pools. Am J Epidemiol 165(2):148-156.

Wang GS, Deng YC, Lin TF (2007a). Cancer risk assessment from trihalomethanes in drinking water. Sci Total Environ 387(1-3):86-95.

Wang W, Ye B, Yang L, Li Y, Wang Y (2007b). Risk assessment on disinfection by-products of drinking water of different water sources and disinfection processes. Environ Int 33(2):219-25. (Erratum 33(5):7167.)

Woodruff RC, Mason JM, Valencia R, Zimmering S (1985). Chemical mutagenesis testing in *Drosophila*: V. Results of 53 coded compounds tested for the National Toxicology Program. Environ Mutag 7:677-702.

Yamamoto K (2011). Is advanced treated water truly high quality?: the answer from a viewpoint of cancer risk induced by trihalomethane class. Bull Environ Contam Toxicol 87:101–105.

Zhang L, Xu L, Zeng Q, Zhang SH, Xie H, Liu AL, Lu WQ (2012). Comparison of DNA damage in human-derived hepatoma line (HepG2) exposed to the fifteen drinking water disinfection byproducts using the single cell gel electrophoresis assay. Mutat Res 741(1-2):89-94.

RESPONSES TO EXTERNAL SCIENTIFIC PEER REVIEW COMMENTS

RESPONSES TO COMMENTS RECEIVED FROM DR. DALE HATTIS

General comments

"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."

Detailed comments

Comment 1. "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."

The Commenter quotes page 2 of the draft document: "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." The commenter states: "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."

Response 1. We added text indicating the PHG is based on the one per million risk level calculated from the 95% upper confidence limit of the extrapolated slope of the dose-response curve.

Comment 2. The commenter quotes pg. 3 of the draft document: "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 $(mg/kg\text{-}day)^{-1}$. 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."

The commenter states: "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.

"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.)"

Response 2. In the case of chloroform, as noted in the draft PHG document, there were a number of cancer bioassays in animals for which the route and method of administration differed. Chloroform was administered by gavage in corn oil, by gavage in toothpaste, through drinking water, and via inhalation or through both inhalation and drinking water. The resulting slope factors differed by about forty fold. Rather than use the highest value as the slope factor, OEHHA tried to account for the variable slope factors, acknowledging the differences in pharmacokinetics between a gavage dose in corn oil or in toothpaste, administration through drinking water, and exposure via inhalation, by using a geometric mean to include data from all available studies. Applying a probabilistic method would be another more complicated way to approach this problem but would require a good deal of resource investment to complete, would not answer the questions around pharmacokinetic influences on the potency factor, and would not necessarily add more certainty to the resulting slope estimate.

Comment 3. The commenter quotes pg. 7: "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."

The commenter says: "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."

Response 3. The quote from the OEHHA document is referring to the process by which the state Water Resources Control Board sets the Maximum Contaminant Level. The Board needs to consider other factors including technical feasibility and cost. In that process, the Board considers whether attempting to reduce the formation of disinfection byproducts compromises the efficacy of disinfection. We did not delete the sentence but reworded it slightly so that there is no confusion about where in the process this step is taken.

Comment 4. The commenter refers to pg. 21 in commenting on chloroform: "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 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.'

The commenter states: "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."

Response 4. The estimates of multi-route exposures are made using the CalTox model. The model indicates that compared to other routes of exposure for infants, the inhalation route is negligible. While it is true, as the comment points out, that infants breathe more per body weight than other age groups, the exposure via inhalation does not contribute much in this case relative to oral exposures. Thus, for simplicity, the value of zero is embedded in the model.

Comment 5. The commenter quotes pg. 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."

The commenter says: "…To the best of my recollection CalTox is not a diffusion-based model, but a model that assumes dynamic equilibrium among phases."

Response 5. CalTox is a fugacity model and the diffusion from one medium to another is part of the modeling of the equilibrium among phases.

Comment 6. The commenter quotes pg. 31: "Chloroform metabolism 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 umol 14CO2 expired/kg-hour."

The commenter says: "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."

Response 6. We agree that the statement appears contradictory. We replaced the word "fully" in the sentence in question with "highly."

Comment 7. The commenter quotes pg. 32: "There is some evidence that the dichloromethyl radical, •CHC1 2 , 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."

The commenter says: "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."

Response 7. We agree that all metabolic enzymes would be operative across the range of concentrations, but for different enzymes with very different Michaelis constant (Km) for a specific substrate, the relative importance of each enzyme will be different across the range of concentrations. That is what is implied by the wording of the sentence: "… the reductive pathway seems less relevant at low environmental exposures". Note we did not say the reductive pathway is irrelevant.

Comment 8. The commenter quotes pg. 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."

The commenter says: “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.”

Response 8. We reworded the introductory paragraph to indicate that species differences exist in the extent of metabolism of chloroform to CO_2. While we agree that extent of metabolism depends on relative rates by different pathways, the available studies indicate different pathways of metabolism are operating at different rates across species.

Comment 9. The commenter quotes pg. 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.”

The commenter says: “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.”

Response 9. We reworded the sentence as follows: **“**Oxidation of DBCM to carbonyl halogenides, which are electrophilic and very unstable intermediates that readily react with nucleophiles in tissues, may be involved in its toxic action”. We also added the following from a previous section on oxidative metabolism of the trihalomethanes: “As noted above, the initial, rate-limiting reaction of oxidative metabolism is insertion of oxygen at the C–H bond of THMs to produce a trihalomethanol (CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (CX_2O), a structural analogue of phosgene. The dihalocarbonyl may form adducts with various cellular nucleophiles, hydrolyze to yield carbon dioxide, or undergo a glutathione-dependent reduction to yield carbon monoxide.” Thus, the reader is reminded of material in an earlier section describing the production of reactive metabolites that may bind to cellular macromolecules.

Comment 10. The commenter quotes pg. 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).

The commenter says: “’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 Vmax and Km values.”

Response 10. We have reworded the sentence to reflect the comment’s point that the contribution to metabolism may be modest at lower doses.

Comment 11. The comment quotes pg. 43: "The large confidence limits reflect the wide variability and small number of subjects involved."

The commenter says: "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."

Response 11. We replaced the word "variability" with "uncertainty".

Comment 12. The commenter quotes pg. 43: "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)."

The commenter says: "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."

Response 12. We deleted the entire sentence as it is not relevant to the risk assessment, and there is apparently an issue with the citation.

Comment 13. The commenter quotes pg. 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."

The commenter says: "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)."

Response 13. We agree that the sentence is poorly worded. We re-worded the sentence to read: “Greater renal toxicity was observed when chloroform was administered in corn oil in male rodents.” As the comment points out, there is no data supporting a direct chemical interaction between chloroform and corn oil.

Comment 14. The commenter quotes pg. 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.”

The commenter says: “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.”

Response 14. The clinical chemistry changes in the water–intake matched controls were observations made from interim sacrifices at months 6, 12, and 18, and at study termination. These changes were similar to those in the treatment groups that drank less water, hence suggesting that the changes in clinical chemistry were related to low water intake and not the chloroform exposure.

Comment 15. Referring to the term “distilled pesticide-analysis quality chloroform” on pg. 86, the commenter says: “This is highly unusual terminology. Perhaps a footnote is in order to explain why the chloroform is described in this way.”

Response 15. We added a footnote to this sentence as follows: “The chloroform used in the study was pesticide-quality chloroform. The chloroform was distilled at 61°C using a steam bath twice a week and the distillate was used to prepare drinking water solutions. The purpose of the distillation was to separate chloroform from diethylcarbonate which had been found in chloroform preserved with ethanol.”

Comment 16. Referring to pg. 85, the commenter says: “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.”

Response 16. The dose-response analyses are conducted in Chapter 10, not in the sections describing the results of the cancer bioassays. The section on carcinogenicity for each THM lays out the data available for the analyses described in Chapter 10.

Comment 17. The commenter quotes pg. 101: "Selected studies on the subchronic toxicity of bromoform are summarized in Several published studies have addressed the subchronic oral toxicity of bromoform." [sic]

The commenter says: "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.'"

Response 17. We have fixed this formatting problem, and the sentence and table are now in their proper place.

Comment 18. The commenter quotes pg. 105: "An increase was reported in micronuclei in peripheral blood lymphocytes one hour after swimming for 40 minutes in an indoor chlorinated pool."

The commenter says: "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?"

Response 18. Indeed, Kogevinas et al. (2010) reported statistically significant increases in micronuclei (MN) in peripheral blood lymphocytes (PBL) associated with brominated THMs one hour after swimming for 40 minutes in an indoor chlorinated pool (1.92 MN/1,000 cells, 95% CI of 0.21-3.63 for BDCM, p = 0.03; 5.04 MN/1,000 cells, 95% CI of 1.23-8.84 for bromoform, p = 0.01).

As noted by the authors, timing is critical for the collection of samples for biomarker analyses. Given the lack of precedence to follow (i.e., similar studies of genotoxic effects in swimmers) and the time constraints of their study, the authors were limited in the timing of collection of blood for extraction of PBL, thus the 2-hour window following swimming for blood draws. They did not report any other time point for collection of blood for extraction of PBL; however, they did collect urine 2 hours after swimming for mutagenicity analysis and 2 weeks after swimming for analysis of micronuclei formation (to allow urothelial cells time to reach the epithelial surface and exfoliate, according to the authors).

That said, MN can be observed in PBL *in vivo* or *ex vivo* following exposure to genotoxins *in vivo*. PBL precursors (in bone marrow, spleen or thymus) can express MN *in vivo* but it can take up to weeks for their maturation and entry into the peripheral blood stream to occur; thus, MN would not be evident in PBL sampled within a few hours following acute genotoxin exposure (Fenech et al., 2016). However, there is an exception to this: the cytokinesis-block micronucleus (CBMN) assay can be used to induce MN expression in PBL immediately following acute *in vivo* genotoxin exposure; MN are scored in PBL that have been stimulated in a test tube *ex vivo* to divide for one mitotic division, as identified as binucleated cells, by blocking cytokinesis using

cytochalasin-B. Hence, this assay, which was used by Kogevinas et al. (2010), allows for the selective analysis of MN in binucleated cells that have undergone one round of mitosis. Therefore, it is plausible that, in individuals acutely exposed (e.g., for one hour) to genotoxins, DNA lesions induced in circulating lymphocytes that lead to structural or numerical chromosomal aberrations can manifest as MN in culture *ex vivo* using the CBMN assay (Fenech et al., 2011; 2016). As a result, a one-hour exposure is considered sufficient to achieve a positive result in the CBMN assay.

It is worth mentioning that the authors minimized potential confounding by comparing subjects with themselves before and after exposure over a limited period of time. Furthermore, a recent study conducted in the same swimming pool with double the number of subjects (having similar population characteristics) and similar protocol for study design and sample collection and analyses found no genotoxic effects, including MN in PBL (Font-Ribera et al., 2019). Presumably, this may be due to lower levels of brominated THMs in the municipal water supply: the 2010 study had a mean of 34 µg/L while the 2019 study had a median of 9.5 µg/L. Although both studies have similar total THM median levels (45.5 µg/L in 2010 vs. 48.5 µg/L in 2019), the composition changed such that brominated THM were predominant in the 2010 study while chloroform was predominant in the 2019 study.

Comment 19. Referring to pg. 111, the commenter says: "Some basic description of the 'colony probe hybridization method' would be helpful. This is not a very common assay."

Response 19. We added text to explain in brief what this method is designed to do.

Comment 20. The commenter quotes pg. 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 commenter says: "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."

Response 20. The incidences given by the study authors (Ruddick et al., 1983) are number of fetuses affected per number of affected litters, not number of affected litters out of total litters. There were 14 to 15 litters in each dosing group.

Comment 21. The commenter quotes pg. 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 commenter says: “The second sentence flatly contradicts the first. If there are ’no data’, how can there be “clinical observations”? Clinical observations are data.”

Response 21. We have corrected the text to show that no data on neurotoxicity in controlled human exposure studies were available. We agree that clinical observations are data.

Comment 22. The commenter quotes pg. 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.”

The commenter says: “This run-on sentence needs to be broken up into at least two and perhaps 3 parts to be intelligible.”

Response 22. We have broken the sentence into two.

Comment 23. Referring to pg. 3 of the summary and the section on bromodichloromethane beginning on pg. 125, the commenter says: “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.”

Response 23. Comment noted.

Comment 24. Referring to a description of mutagenesis assays on BDCM, the commenter quotes from the OEHHA document, pg. 143: “The minimum amount required to elicit a mutagenic response was 600 µmol.”

The commenter states: “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.”

Response 24. We agree that the wording of the study results regarding mutagenic responses implies a threshold and agree that there is unlikely a threshold for mutagenesis. However, there may be a practical laboratory threshold for measuring mutagenesis. We reworded the sentence substituting the word “measure” for “elicit”.

Comment 25. The commenter quotes pg. 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."

The commenter says: "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."

Response 25. We evaluated the incidence data with the Cochran-Armitage trend test and found a dose-related increased trend in sternebral aberrations for the BDCM-treated animals. This information has been added to the text.

Comment 26. The commenter quotes pg. 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 commenter says: "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."

Response 26. Comment noted.

Comment 27. Referring to the paragraph on pg. 150 that begins with "NTP (1998)", the commenter says: "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.

Response 27. The paragraphs describe measurements of reproductive toxicity in both males and females. Note that the text describes the results of the study as negative: "BDCM exposure did not alter any reproductive parameter investigated, except for a non-dose-related increase in live fetuses per birth at 100 ppm in Group C females, and a slight decrease in corpora lutea in Group A females at 700 ppm. NTP (1998) concluded that BDCM was not a short-term developmental or reproductive toxicant at any of the doses tested".

Comment 28. The commenter quotes pg. 156: "BDCM treatment resulted in decreased antibody-forming cells in serum and decreased hemagglutination titers."

The commenter says: " How could there be antibody-forming cells in serum? Serum is necessarily free of cells of any kind. This makes no sense."

Response 28. This sentence should read: "BDCM treatment resulted in decreased antibody-forming cells in the spleen and decreased hemagglutination titers (plasma antibody titers)." We have fixed the sentence in the text.

Comment 29. The commenter quotes pg. 180: "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)."

The commenter says: "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."

Response 29. OEHHA conducted a trend test and the result is a $p<0.1$ for the males. Our statement that the hepatic lesions were weakly dose-related is correct. We have added the result of the trend test to the text.

Comment 30. The commenter quotes pg. 185: "The minimum amount required to elicit a mutagenic response was 57 µmol."

The commenter says: "This phrasing incorrectly implies a threshold for the mutagenic response."

Response 30. Although we agree that mutagenicity likely does not have a threshold, the practicalities of measuring mutagenicity in the laboratory (particularly in these older studies) have resulted in the phrasing in the text. We substituted the word "measure" for "elicit" in the text so that the sentence reads: **"**The minimum amount required to measure a mutagenic response was 57 µmol in these studies."

Comment 31. The commenter quotes pg. 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)".

The commenter says: "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??)"

Response 31. The study by Geter et al. (2004c) was meant to evaluate whether a high fat diet influenced the formation of aberrant crypt foci (ACF) in the intestine in animals dosed with trihalomethanes. Animals were given one of 4 trihalomethanes in drinking water or just water, and either a regular or a high fat diet. The authors reported the formation of 4 total ACF and 0.67 per colon in chloroform treated animals fed a regular

diet. In the study, there were zero ACF formed in the water control animals fed a regular diet, and 4 total ACF, 0.83 per colon, in water control animals fed a high fat diet. We also thought that compared to the regular diet water-only animals, it looked like chloroform treatment resulted in induction of aberrant crypt foci. We ran a test of significance (without raw data, and estimating the pooled variance from the reported SD) and obtained a p value of 0.7. We decided to just note in the text what the incidence of ACF was reported by the authors.

Comment 32. The commenter quotes 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."

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

Response 32. We disagree that the comparison of tumorigenicity and liver toxicity is invalid because the Hill model is used. While the Hill model is not typically used to assess cancer dose-response, it can be useful in comparing dose-response across endpoints, as is done in Melnick et al. (1998). We are keeping this text in the document, and are reporting what the authors of the study concluded.

Comment 33. The commenter quotes pg. 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."

The commenter says: "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."

Response 33. While we agree that tissue toxicity and subsequent cellular regeneration is not the underlying mechanism for tumor formation, we are reporting papers that discuss this hypothesis in our document. OEHHA notes the formation of highly reactive metabolites in several places in the document and discuss the potential role of these metabolites in interacting with cellular macromolecules including DNA. We also provide a critique of the hypothesis in our responses to public comments.

RESPONSES TO COMMENTS RECEIVED FROM DR. MATIAS ATTENE RAMOS

Re: Chloroform

Comment 1. "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 PubChem. 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."

Response 1. We agree that the data from the high-throughput system assays may help describe possible mechanisms by which a chemical induces toxicity. We also agree that the results are more difficult to interpret than traditional toxicity tests. This particular case poses special challenges for interpretation because these chemicals are all relatively low molecular weight and volatile. There are no special procedures to ensure that the chemicals stay in solution either in the well plates or in storage or get into the cells in the cell-based assays. Thus, there is great uncertainty in this case with regard to the results of the assays. The US Environmental Protection Agency (US EPA) did not have these chemicals in its standard ToxCast library for this reason.

The Tox21 collaboration did screen the chemicals in many assays, and OEHHA accessed the ToxCast/Tox21 data[1] on the THMs assessed using high throughput assays. However, the analytical quality control results (at https://tripod.nih.gov/tox21) for the Tox21 samples used for these compounds indicated serious concerns with the results.

Chloroform was reported to be active in 14 out of 235 assays per the criteria used by US EPA as noted in the ToxCast Chemistry Dashboard. However the quality control findings on the federal government website (https://tripod.nih.gov/tox21) indicated "CAUTION, No Sample Detected" and "Biological Activity Unreliable."

Similarly, DBCM was reported active in five of 235 assays, and carried the quality control flags "CAUTION, Low Concentration" and "Concentration 5-30% of expected

[1] https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data; accessed October 1, 2019

value." The five assays were all done in the same system evaluating cell viability in a human kidney cell line but at different time points.

Bromoform was reported active in three of 239 assays. In contrast to the other THMs, the AC_{50} values were well below the cytotoxicity limit. The three assays measured regulation of transcription factor activity in human cell lines. In a breast cell line, bromoform increased reporter gene activity related to the androgen receptor gene. In a kidney cell line, bromoform induced an increase in GAL4 β-lactamase reporter gene activity related to the PGR (progesterone receptor) gene. However, it is unclear how much weight to give these findings. The quality control flag for the test sample noted "CAUTION, Low Concentration" and "Concentration 5-30% of expected value."

Similarly, the analytic quality control flags for BDCM were "CAUTION, Low Concentration," "Concentration 5-30% of expected value," "CAUTION, Concentration <5% of expected value," and "Biological Activity Unreliable."

OEHHA stresses that given the volatile nature of these compounds, and the quality control flags reported on the Tox21 website, the assay results are highly uncertain and therefore, were not reported in the final PHG document.

Comment 2. "The discussion of the available carcinogenicity data is sound and complete."

Response 2. Comment noted.

Re: Bromoform

Comment 3. "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."

Response 3. See response to comment 1 above.

Comment 4. "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) form 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)."

Response 4. We have edited the document as suggested by changing the title of the table to "Summary of *In Vitro* Mutagenicity and Genotoxicity Studies on Bromoform."

We also added the specific assay used to detect DNA damage into the table so that the reader understands which assays were used for this endpoint.

Comment 5. "The carcinogenicity literature is complete and well discussed."

Response 5. Comment noted.

Re: Bromodichloromethane

Comment 6. "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."

Response 6. See the response to comment 1 above.

Comment 7. "The genotoxicity and mutagenicity data seems to be mostly discussed correctly. The Robbianno et al. (2004) 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."

Response 7. We changed the headings to reflect the concern expressed in the comment. We have a separate section for Robbiano et al. (2004) as it used human cells but have re-titled the headers to indicate whether the data are from *in vivo* or *in vitro* exposures. We edited Table 7.5 to change the title to indicate the table summarizes both mutagenicity assay results and genotoxicity assay results. We also added the specific assay used to detect DNA damage into the table. We also are making sure to use the terms mutagenicity and genotoxicity properly in the text.

Comment 8. "The discussion of the available carcinogenicity data is sound and complete."

Response 8. Comment noted.

Re: Dibromochloromethane

Comment 9. "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."

Response 9. See the response to comment 1 above.

Comment 10. "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."

Response 10. We edited Table 8.4 to change the title to indicate the table summarizes both mutagenicity assay results and genotoxicity assay results. We also added in the specific assay used to detect DNA damage into the table.

Comment 11. "The discussion of the carcinogenicity classification of the compound is accurate and reflects the limited information available for this chemical."

Response 11. Comment noted.

Re: Mechanisms of action of carcinogenicity

Comment 12. "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 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."

Response 12. Comments noted. We agree that there is likely more than one mechanism involved in the development of tumors and that this impacts the choice of model used to assess the cancer potency of the compounds.

Re: Final conclusions

Comment 13. "The reports gives a comprehensive description of the literature covering the toxicological studies for the four trihalomethanes of interest. It clearly discusses the known cancer and non-cancer effects for the 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 appropriate. 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."

Response 13. Comments noted.

RESPONSES TO COMMENTS RECEIVED FROM DR. CRISTINA VILLANUEVA BELMONTE

General comments

"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."

Response. We appreciate the comments. We responded to specific comments on the CalTOX models below.

Regarding the comments on setting separate PHGs for the trihalomethanes, we recognize this inherently assumes independence of effects. This is common in setting any standard whether for drinking water or air. There are no data that we are aware of on the interactions between THMs. Further, even if we had adequate data, there are not consistent ratios of the four THMs across sources of drinking water such that we could combine the information into one PHG. Instead, we focus on providing a health protective value for each chemical.

We appreciate the insight into the validity of the PHGs based on the Costet et al. (2011) meta-analysis of bladder cancer. That is an interesting way of viewing the reasonableness of the PHGs.

Detailed comments

Re: SUMMARY

Comment 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."

Response 1. We changed the text to indicate that more than 600 DBPs have been identified and added the reference to the bibliography.

Re: INTRODUCTION/ PURPOSE

Comment 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."

Response 2. We consider use of chlorine dioxide a form of chlorination.

Re: PRODUCTION, USE AND ENVIRONMENTAL OCCURRENCE

Comment 3. "Page 9, second sentence. '… occurrence and exposure are provided Table 2.1' typographic error, 'in' is missing between 'provided' and 'Table 2.1.'"

Response 3. We have fixed the typographic error.

Re: ENVIRONMENTAL OCCURRENCE

Comment 4. "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).

"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.

"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."

Response 4. Comments noted.

Re: EXPOSURE TO THMS VIA TAP WATER

Comment 5. "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."

Response 5. The draft PHG document cites a previous analysis of intake rates by OEHHA for its risk assessment guidelines (OEHHA, 2012). The source document is the Technical Support Document for Exposure Assessment and Stochastic Analysis and is available here: https://oehha.ca.gov/air/air-toxics-hot-spots

Comment 6. "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?"

Response 6. The draft PHG document cites a previous analysis of intake rates by OEHHA for its risk assessment guidelines (OEHHA, 2012). The value assigned is the one given in the table (0.047 L/kg-d). The source document is the Technical Support Document for Exposure Assessment and Stochastic Analysis and is available here: https://oehha.ca.gov/air/air-toxics-hot-spots

Re: MULTI-ROUTE EXPOSURE ESTIMATES FROM TAP WATER USE

Comment 7. "Page 21. 'OEHHA 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 table 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."

Response 7. Human exposure to chemical contaminants in tap water can occur via oral ingestion, as well as inhalation or dermal contact while performing common household activities, such as bathing, showering, and flushing toilets. Exposure estimates differ across life stages (fetus, infant, child, and adult) due to physiological and activity pattern changes. CalTOX equations are used to calculate how much each route (oral, inhalation, and dermal) contributes to total daily exposure to a contaminant in tap water. The relative contributions of the different routes are then used to estimate a daily drinking water intake equivalent (DWI, in L_{eq}/kg-day) of multiroute exposure to tap water for each life stage. The liter equivalent (L_{eq}/kg-day) value represents the equivalent of how much water a person would have to drink to account for exposures via ingestion, inhalation and dermal uptake. The lifetime daily multiroute intake rate of tap water in L_{eq}/kg-day is the time-weighted average of these life-stage specific tap water intake rates. OEHHA has developed an appendix with the equations used in CalTOX that we are appending to all the PHG documents going forward.

OEHHA uses CalTox to estimate contributions from different routes of exposure. CalTox is a complex mathematical model, and may differ from other models used. The references mentioned by the commenter (Jo et. al. 1990a, b) utilize different parameters to calculate the internal dose. Differences in breathing rates, absorption rates and efficiencies, and body surface areas will affect the estimated contributions from each route.

The equation used to calculate ingestion multiplies the concentration of chloroform in tap water times the water intake rate at each lifestage. Ingestion is relatively simple to calculate, whereas the equations used to calculate inhalation and dermal exposures are much more complex. To calculate inhalation exposures, concentrations in both indoor air and bathroom air are calculated separately, then used to calculate a total inhalation exposure. It should be noted that multiple breathing rates are used for each lifestage, accounting for time spent during various activities (i.e. active, resting, showering), and thus include exposure scenarios beyond time spent showering.

Dermal uptake is dependent on exposure time and each chemical's specific parameters. These values are supplied in the CalTox program. As a result, the dermal uptake of chemicals in the tap water during shower or bath are derived from one of three equations, depending on the chemical's diffusion lag time. Additional parameters, such as skin permeability coefficients and body surface, play a role in dermal absorption.

CalTox calculates relative contributions for each route of exposure. A detailed summary can be found in the CalTox manual's Executive Summary at https://www.uta.edu/faculty/shinh/assets/CalTOX_Technical%20reports_part%20I.pdf.

Table A3 shows CalTOX results for relative contributions of multiple routes of exposure to chloroform in tap water for various life stages. These values are derived using the OEHHA-derived input parameters listed in Table 3.2, along with physical-chemical specific values for chloroform. The physical-chemical input parameters used for chloroform, taken from the CalTox program, are:

Chemical Properties

Compound	Chloroform		Value used	Mean value	Coeff. Var.	Adjustment	Notes
Molecular weight (g/mol)		MW	1.19 E+02	1.19E+02	0.01	1	
Octanol-water partition coefficient		Kow	9.00 E+01	9.00E+01	0.07	1	
Melting point (K)		Tm	2.10 E+02	2.10E+02	0.03	1	
Vapor Pressure in (Pa)		VP	2.74 E+04	2.74E+04	0.09	1	
Solubility in mol/m3		S	6.68 E+01	6.68E+01	0.04	1	Kaw
Henry's law constant (Pa-m^3/mol)		H -	4.34 E+02	4.34E+02	0.46	1	0.1805406
Diffusion coefficient in pure air (m2/d)		Dair	8.99 E-01	8.99E-01	0.08	1	1.04 E-05
Diffusion coefficient; pure water (m2/d)		Dwater	9.88 E-05	9.88E-05	0.25	1	1.14 E-09
Organic carbon partition coefficient Koc		**Koc -**	6.00 E+01	6.00E+01	0.33	1	m^2/s
Ocatanol/air partition coefficient		**Koa -**	4.99 E+02	-9.90E+01	0.10	1	
Partition coefficient in ground/root soil layer		**Kd_s -**	9.34 E-01	-9.90E+01	0.10	1	
Partition coefficient in vadose-zone soil layer		**Kd_v -**	1.83 E-01	-9.90E+01	0.10	1	
Partition coefficient in aquifer layer		**Kd_q -**	1.83 E-01	-9.90E+01	0.10	1	
Partition coeffic. in surface wtr sediments		**Kd_d -**	1.20 E+00	-9.90E+01	0.10	1	
NOT USED		Kps -	5.71 E-01	-9.90E+01	4.00	1	
Leaves/phlm wtr prtn cff.(wet kg/m3 per wet kg/m3)		**Kl_phl -**	5.51 E-01	-9.90E+01	0.10	1	
Stem/xylem-fluid prtn cff (m^3[xylem]/m^3[stem])		**Ks_x -**	1.21 E+00	-9.90E+01	0.10	1	
Transpiration stream cncntrtn fctr (m^3[wtr]/m^3[ts])		**TSCF -**	4.47 E-01	-9.90E+01	0.10	1	
Biotrnsfr fctr, plant/air (m3[a]/kg[pFM])		**Kpa -**	4.81 E-03	-9.90E+01	14.00	1	
Biotransfer factor; cattle-diet/milk (d/kg[milk])		**Bk -**	7.15 E-07	-9.90E+01	11.00	1	
Biotransfer factor; cattle-diet/meat (d/L)		**Bt -**	2.26 E-06	-9.90E+01	13.00	1	
Biotransfer fctr; hen-diet/eggs (d/kg[egg contents])		**Be -**	1.43 E-05	-9.90E+01	14.00	1	
Biotrnsfr fctr; brst mlk/mthr intake (d/kg)		**Bbmk -**	1.80 E-05	-9.90E+01	10.00	1	
Bioconcentration factor; fish/water		**BCF -**	4.32 E+00	-9.90E+01	0.60	1	
Particle scavenging ratio of rain drops		**Psr_rain -**	5.00 E+04	5.00E+04	2.40	1	
Skin permeability coefficient; cm/h		**Kp_w -**	2.50 E-02	-9.90E+01	2.40	1	
Skin-water/soil partition coefficient (L/kg)		**Km -**	1.00 E+00	-9.90E+01	1.30	1	
Fraction dermal uptake from soil		**dfct_sl-**	2.01 E-01	2.01E-01	1.00	1	

A parameter with a "-" symbol after it, indicates a parameter that can be calculated by a default algoritm when **the value of mean for this parameter is <0**. Otherwise the liste value is used.

Chemical Properties (continued)

Reaction half-life in air (d)	Thalf_a	1.43 E+02	1.43E+02	1.00	1	
Reaction half-life in surface soil (d)	Thalf_g	6.10 E+01	6.10E+01	1.10	1	
Reaction half-life in root-zone soil (d)	Thalf_s	6.10 E+01	6.10E+01	1.20	1	
Reaction half-life in vadose-zone soil (d)	Thalf_v	1.28 E+03	1.28E+03	1.00	1	
Reaction half-life in ground water (d)	Thalf_q	1.31 E+03	1.31E+03	1.30	1	
Reaction half-life in surface water (d)	Thalf_w	1.04 E+02	1.04E+02	1.20	1	
Reaction half-life in sediments (d)	Thalf_d	9.35 E+01	9.35E+01	1.40	1	
Reaction half-life in the leaf surface (d)	Thalf_ls	1.43 E+02	-9.90E+01	1.00	1	

Re: PHARMACOKINETICS/ABSORPTION

Comment 8. "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% to 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.

"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)."

Response 8. The daily dose from bathing calculated by Xu et. al. (2002) uses US EPA's recommended methodologies for calculating dermal dose from bathing activities. This differs from OEHHA's approach of using the CalTox model to estimate relative contributions from each source (ingestion, inhalation, and dermal). Parameter values used in CalTox calculations may differ than those used by US EPA. In addition, OEHHA specific values for breathing rate, inhalation rate, and body surface area are used (see Table 3.2) when calculating relative route-specific contributions. Please see response to comment 7 for additional information.

Re: DISTRIBUTION

Comment 9. "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)."

Response 9. We have added the paper to the section on dermal absorption.

Re: IMMUNOTOXICITY (CHLOROFORM)

Comment 10. "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."

Response 10. Although this paper is interesting, we did not add it as the authors admitted they could not attribute the changes in measured cytokines, which were significant, after swimming in a chlorinated pool uniquely to DBPs.

Re: NEUROTOXICITY (CHLOROFORM)

Comment 11. "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."

Response 11. We did not add the Grandjean paper because it is a review. Villanueva et al. (2018) evaluated the association between DBP exposure during pregnancy and child neuropsychological outcomes at 1 and 4–5 years of age using a cohort of Mother-Child pairs in Spain (INMA project 2003-2008). Tap water concentrations of trihalomethanes were modeled for each month of pregnancy, based on measurements at the tap and reports from water agencies. These concentrations were then combined with ingestion, showering and bathing habits to estimate multi-route exposures. Modeled concentrations were multiplied by daily use and uptake factors for the different routes of exposure to estimate a daily blood concentration of the trihalomethanes. The investigators separately analyzed the associations between chloroform, combined brominated trihalomethanes, and total trihalomethanes and measures of

neuropsychological development based on the Bayley Scales of Infant Development in 1 year olds and the McCarthey Scales of Children's Abilities in 4 to 5 year olds. Linear regression was used to estimate associations adjusting for a number of covariables (e.g., maternal age, height, weight, intelligence, SES, smoking and alcohol consumption, etc) in 1855 subjects at 1 year, and 1453 subjects at 4-5 years of age. Most of the evaluated associations were null. However, the association between a doubling of all-route total THM exposure and cognitive score was significant ($p<0.05$) with a decrease in cognitive score of -0.54 (95%CI -1.03 to -0.05). The investigators also found a statistically significant decrease in cognitive score of −0.64 (−1.16 to −0.12) points associated with a doubling of all route total brominated THMs. The investigators acknowledge that the results should be cautiously interpreted and that chance cannot be ruled out given the small magnitude of the association and the large number of tests performed. Although this is a well-conducted study and one of the first to look at these types of associations, we cannot attribute the observed effect to any one THM. We did however add it to the section on neurotoxicity of BDCM.

Comment 12. "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."

Response 12. We did not add this reference because of the co-exposure to perchloroethylene.

Re: CARCINOGENICITY (CHLOROFORM)

Comment 13. "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."

Response 13. Both papers involve the same populations but present different results. For example, in Villaneuva et al., 2006, bladder cancer ORs are presented both by strata of average TTHM exposure and by strata of total tap water intake (Villaneuva et al., 2004 does not). This is interesting because it potentially allows the reader to examine the possible influences of each of these factors individually and examine possible interaction between the two. Additionally, Villaneuva et al., 2004 presents a number of interesting results that are not in Villaneuva et al., 2006. Overall, for completeness sake we have cited both papers.

Re: NEUROTOXICITY (BROMOFORM)

Comment 14. "Page 115. See comment [12], and also consider the citation Villanueva et al. 2018 in comment [13]."

Response 14. As noted above, we did not include the Guariglia et al. citation because of co-exposure to perchloroethylene. See also response 11 above regarding Villanueva et al. (2018).

Re: IMMUNOTOXICITY (BROMODICHLOROMETHANE)

Comment 15. "Page 156. See comment [10]"

Response 15. Although interesting, we did not add Vlaanderen et al., 2017 as the authors admitted they could not attribute the changes in measured cytokines, which were significant, after swimming in a chlorinated pool uniquely to DBPs.

Re: NEUROTOXICITY (BROMODICHLOROMETHANE)

Comment 16. "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)."

Response 16. See response 11 above regarding Villanueva et al. (2018). Moser et al. (2007) has been added into the section on BDCM neurotoxicity.

Re: IMMUNOTOXICITY (DIBROMOCHLOROMETHANE)

Comment 17. "Page 192. See comment [10]."

Response 17. Although interesting, we did not add Vlaanderen et al. 2017 as the authors admitted they could not attribute the changes in measured cytokines, which were significant, after swimming in a chlorinated pool uniquely to DBPs.

Re: NEUROTOXICITY (DIBROMOCHLOROMETHANE)

Comment 18. "Page 192. Consider the citation Villanueva et al. 2018 in comment [13]."

Response 18. See response 11 above regarding Villanueva et al. (2018).

Re: MECHANISMS OF ACTION OF CARCINOGENICITY

Comment 19. "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."

Response 19. We have added text about epigenetic mechanisms of carcinogenicity, including some of the citations listed above and citations cited in the peer-review comments from Dr. Attene Ramos.

Re: DOSE-RESPONSE ASSESSMENT

Comment 20. **"**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."

Response 20. The comments are noted. We provided responses to the specific comments below.

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

Comment 21. "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."

Response 21. We appreciate the commenter's identification of the error in the text, which we have corrected. The best-fit model was the Hill model with a BMDL of 12.9 mg/kg-d, which is what we used to derive the ADD.

Re: CANCER DOSE-RESPONSE ANALYSES AND ACCEPTABLE DAILY DOSE CALCULATIONS

Comment 22. "The rationale behind the formula of $CSF_{animal} = 0.05/BMDL05$ is not clear. An explanation would be appreciated to understand it."

Response 22. The cancer Slope Factor or CSF is the slope of the modeled dose-response curve. Thus, the slope is the benchmark response rate (5 percent response) divided by the modeled dose associated with that percent response, in this case the $BMDL_{05}$.

Re: NON-CANCER HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS

Comment 23. "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."

Response 23. These DWI are derived in Chapter 3 and represents multi-route exposure in Liter equivalents/kg-day. The numbers differ slightly because the physico-chemical characteristics of the four trihalomethanes differ slightly and thus the intake via inhalation and dermal routes differ slightly. This is explained in chapter 3.

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

Comment 24. **"**Page 303. Check the webpage https://energyanalysis.lbl.gov/tool/caltox. From my computer, the requested page "/tool/caltox" could not be found."

Response 24. Unfortunately, the link is no longer available. The CalTox model is now available, along with relevant documentation, at https://www.uta.edu/faculty/shinh/ . OEHHA has developed an appendix with the equations used in CalTOX that we are appending to all the PHG documents going forward.

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

Comment 25. "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."

Response 25. Cancer type is specified in the second row of the table, and table rows are sorted by cancer type. This is now stated in the title of the table.

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

Comment 26. "Page 389. This table should be organized alphabetically by author and year of publication to facilitate the reading."

Response 26. We appreciate the comment and reorganized the table as suggested

RESPONSES TO COMMENTS RECEIVED FROM DR. RICARD MARCOS DAUDER

Comment 1. "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."

Response 1. We added a list of abbreviations.

Comment 2. "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."

Response 2. We have added the paper to this section.

Comment 3. "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, showing 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."

Response 3. OEHHA utilizes the CalTox model for our standard risk assessments. We are aware that other models may have different results. There are data that support dermal absorption of trihalomethanes. The issue is the proportional amount of absorption by the different routes, which differs model to model. As we move forward in the program, we will be evaluating data and models on multi-route exposure for different chemicals.

Comment 4. "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."

Response 4. We have added this paper to the section.

Comment 5. "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."

Response 5. We have added this paper to the section.

Comment 6. "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."

Response 6. We have added this paper into the section on genetic toxicity of chloroform.

Comment 7. "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."

Response 7. We did not add this paper to the section as it is a review paper.

Comment 8. "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."

Response 8. We present the results of the re-analysis by Hard et al. (2000) of the Jorgenson data in Table 5.5, and discuss the paper in the second to last paragraph of this section.

Comment 9. "Section 6. Toxicological profile of Bromoform

"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."

Response 9. Comment noted.

Comment 10. "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."

Response 10. We cite deAngelo et al. (2007) in Chapter 9 Mechanisms of Action of Carciogenicity. The paper is mentioned under the heading "Development of Preneoplastic Lesions".

Comment 11. "The title of the section: *Effects in animals –in vitro assays* should be modified to *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."

Response 11. We changed the overarching section headings from "Effects in Humans" to Effects in humans *in vivo*" and from "Effects in Animals" to "Effects in *in vitro* and *in vivo* assays". That takes care of most of the confusion noted by the comment.

Comment 12. "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."

Response 12. We have added this paper to the section on genetic toxicity of bromoform.

Comment 13. "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."

Response 13. Comment noted. This paper did not figure significantly into the Public Health Goal, but rather was included in our description of the acute effects of BDCM administration. We are simply reporting the results of the study.

Comment 14. "Second paragraph of page 138. I do not know if it is adequate to include here the effects of the other brominated THMs."

Response 14. Comment noted. We included the reported information on aberrant crypt foci formation in the rat intestine from DeAngelo et al. (2002) following administration of different THMs and the positive and negative controls for completeness.

Comment 15. "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 se 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."

Response 15. We have edited the tables so that they are responsive to the comment. We moved aneuploidy citations to chromosomal aberrations. We also edited the section headings so that they break out *in vitro* and *in vivo* and do not break them out by human versus animal. We believe that the revised tables are clearer.

Comment 16. "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."

Response 16. We have added the paper to this section.

Comment 17. "In the section of Immunotoxicity lacks the study of Alhasson et al., 2016.

"Alhasson F, Dattaroy D, Das S, Chandrashekaran 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."

Response 17. We appreciate the comment pointing to this study. This complex study is quite interesting from a mechanistic point of view in evaluating the kidney disease associated with non-alcoholic fatty liver disease. However, we did not feel it was necessary to add this paper as it used intraperitoneal administration of one concentration of BDCM to induce lipid peroxidation primarily for the purpose of evaluating the association of NAFLD to kidney toxicity. The paper does not add to the knowledge about dose-response of BDCM.

Comment 18. "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."

Response 18. We have added Moser et al. (2007) to the neurotoxicity section.

Villanueva et al. (2018) evaluated the association between DBP exposure during pregnancy and child neuropsychological outcomes at 1 and 4–5 years of age using a cohort of Mother-Child pairs in Spain (INMA project 2003-2008). Tap water concentrations of trihalomethanes were modeled for each month of pregnancy, based on measurements at the tap and reports from water agencies. These concentrations were then combined with ingestion, showering and bathing habits to estimate multi-route exposures. Modeled concentrations were multiplied by daily use and uptake factors for the different routes of exposure to estimate a daily blood concentration of the trihalomethanes. The investigators separately analyzed the associations between chloroform, combined brominated trihalomethanes, and total trihalomethanes and measures of neuropsychological development based on the Bayley Scales of Infant Development in 1 year olds and the McCarthey Scales of Children's Abilities in 4 to 5 year olds. Linear regression was used to estimate associations adjusting for a number of covariables (e.g., maternal age, height, weight, intelligence, SES, smoking and alcohol consumption, etc) in 1855 subjects at 1 year, and 1453 subjects at 4-5 years of age. Most of the evaluated associations were null. However, the association between a doubling of all-route total THM exposure and cognitive score was significant ($p<0.05$) with a decrease in cognitive score of -0.54 (95%CI -1.03 to -0.05). The investigators also found a statistically significant decrease in cognitive score of −0.64 (−1.16 to −0.12) points associated with a doubling of all route total brominated THMs.

The investigators acknowledge that the results should be cautiously interpreted and that chance cannot be ruled out given the small magnitude of the association and the large number of tests performed. Although this is a well-conducted study and one of the first to look at these types of associations, we cannot attribute the observed effect to any one THM. We did, however include this paper in the PHG section on neurotoxicity of DBCM.

Comment 19. "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."

Response 19. Although the paper is interesting, we are not adding it because it is not particularly informative as to the carcinogenicity of any single trihalomethane and because it has an ecologic study design.

Comment 20. "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 Biomonitoing 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."

Response 20. We have edited the section headings and edited the tables according to the comment. We appreciate the commenter's suggestions.

We added the Sekihashi et al. study to the table. It was discussed in the text but inadvertently left out of the table.

Landi et al. (1999) is discussed in the sections on genotoxicity of the trihalomethanes.

Comment 21. "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 included 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 ≥ 613 µmol/kg/day, but not micro-/anophthalmia.”

Response 21. We have added the paper to this section. However, that study used a mixture of THMs and thus it is not possible to attribute the observed effect to any particular THM.

Comment 22. “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.”

Response 22. See response 18 above.

Comment 23. “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.

Response 23. We have added a discussion of epigenetic mechanism of action for carcinogenicity to the chapter, and have cited the papers referenced in the comment.

Comment 24. “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 stablished.

“As indicated, to calculate the ADD it is necessary to include certain *uncertainty factors (UF)*. This would means 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 expertise. 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 fate 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.”

Response 24. Comment noted. We are only able to use the data that are available, and recognize the uncertainties with limited data. Indeed, that is one reason to use uncertainty factors.

Comment 25. “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.”

Response 25. Comment noted. There is not an expectation of tissue or site concordance for tumors from chemical carcinogens across species. While that is sometimes the case, more often it is not.

Comment 26. “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.”

Response 26. Comment noted. This problem is obvious to risk assessors, but we appreciate that not everyone will think of this issue in reading a risk assessment.

Comment 27. “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 meanly 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."

Response 27. Comment noted.

Comment 28. "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 $1x10^{-6}$ as cancer risk level?"

Response 28. The risk level is set at $1x10^{-6}$ for the Public Health Goal. But the Maximum Contaminant Level can be set at a higher risk level, based on economic, technical or other factors affecting feasibility. Thus, the State Water Resources Control Board would consider the benefits of disinfection along with the risks from disinfection by-products in setting the Maximum Contaminant Level.

Comment 29. "In the subsection of *Risk Characterization*, different mechanisms and uncertainties are indicated. For i.e. genotoxicity, not 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."

Response 29. Comment noted. We agree that assessing mixtures such as DBPs in drinking water is complex and difficult. It is one of the reasons we opted to propose a separate PHG for each of the trihalomethanes.

REFERENCES CITED IN RESPONSES TO PEER REVIEW COMMENTS (NOT OTHERWISE CITED ABOVE):

DeAngelo AB, Geter DR, Rosenberg DW, Crary CK, George MH (2002). The induction of aberrant crypt foci (ACF) in the colons of rats by trihalomethanes administered in the drinking water. Cancer Lett 187(1-2):25-31.

Fenech M, Lirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Narppa H, Eastmond DA, Tucker JD, Thomas P (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26:125-132.

Fenech M, Knasmueller S, Bolognesi C, Bonassi S, Holland N, Nigliore L, Palitti F, Natarajan AT, Kirsch-Volder M, (2016). Molecular mechanisms by which *in vivo* exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes *in vivo* and *ex vivo* in humans. Mutatuin Res 770:12-25.

Font-Biera L, Marco E, Grimalt JD, Pastor S, Marcos R, Abramsson-Zetterberg L, Pedersen M, Grummt T, Junek R, Barreiro E, Heederik D, Spithoven J, Critelli R, Naccarati A, Schmalz C, Zwener C, Liu J, Zhang X, Mitch W, Gacia-Lavedan E, Arjona L, de Bont J, Taris L, Vineis P, Kogevinas M, Villanueva CM (2019). Exposure to disinfection by-products in swimming pools and biomarkers of genotoxicity and respiratory damage – The PISCINA2 Study. Env Int 131:104988.

Geter DR, George MH, Moore TM, Kilburn SR, Huggins-Clark G, DeAngelo AB (2004c). The effects of a high animal fat diet on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to trihalomethanes in the drinking water. Toxicol Lett 147(3):245-52.

Jo WK, Weisel CP, Lioy PJ (1990a). Chloroform exposure and the health risk associated with multiple uses of chlorinated tap water. Risk Anal 10(4):581-5.

Jo WK, Weisel CP, Lioy PJ (1990b). Routes of chloroform exposure and body burden from showering with chlorinated tap water. Risk Anal 10:575-80.

Judson R, Houck K, Martin M, Richard AM, Knudsen TB, Shah I, Little S, Wambaugh J, Setzer JW, Kothya P, Phuong J, Filer D, Smith D, Reif D, Rotroff D, Leinstreuer N, Sipes N, Xia M, Huang R, Crofton K, Thomas RS (2016), Analysis of the effects of cell stress and sytotoxicity on *in vitro* assay activity across a diverse chemical assay space. Tox Sci 152:323-339.

Kogevinas M, Villanueva CM, Font-Ribera L, Liviac D, et al. (2010). Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environ Health Perspect 118(11):1531-7.

Melnick RL, Kohn MC, Dunnick JK, Leininger JR (1998). Regenerative hyperplasia is not required for liver tumor induction in female $B6C3F_1$ mice exposed to trihalomethanes. Toxicol Appl Pharmacol 148(1):137-47.

Moser VC, Phillips PM, McDaniel KL, Sills RC (2007).Neirotoxicological evaluation of two disinfection by-products, bromoduchloromethane and dibromoacetonitrile, in rats. Toxicol 230:137-144

NTP (1998). Final Report on the short-term reproductive and developmental toxicity of bromodichloromethane (CAS No. 75-27-4) administered in drinking water to Sprague-Dawley rats. Pub no. NTIS/PB99-111262. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

OEHHA (2012). Air toxics hot spots program risk assessment guidelines: technical support document for exposure assessment and stochastic analysis. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

Robbiano L, Baroni D, Carrozzino R, Mereto E, Brambilla G (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney. Toxicology 204(2-3):187-95.

Ruddick JA, Villeneuve DC, Chu I (1983). A teratological assessment of four trihalomethanes in the rat. J Environ Sci Health 1318(3):333-49.

Villanueva CM, Cantor KP, Cordier S, Jaakkola JJ, et al. (2004). DBPs and bladder cancer: a pooled analysis. Epidemiology 15(3):357-67.

Villanueva CM, Cantor KP, King WD, Jaakkola JJ, et al. (2006). Total and specific fluid consumption as determinants of bladder cancer risk. Int J Cancer 118(8):2040-7.

Villanueva CM, Gracia-Lavedan E, Julvez J, Santa-Marina L, Lertxundi N, Ibarluzea J, Llop S, Ballester F, Fernandez-Somoano A, Tardon S, Vrijheid M, Guxens M, Sunjer J (2015). Drinking water disinfection by-products during pregnancy and child neuropsycholofical development in the INMA Spanish cohort study. Env Int 130:113-122.

Xu X, Mariano TM, Laskin JD, Weisel CP (2002). Percutaneous absorption of trihalomethanes, haloacetic acids, and haloketones. Toxicol Appl Pharmacol 184:19-26.

RESPONSES TO COMMENTS MADE DURING THE SECOND PUBLIC COMMENT PERIOD

RESPONSES TO COMMENTS RECEIVED FROM AMERICAN CHEMISTRY COUNCIL (DECEMBER 2019)

Comment 1. "OEHHA's revised draft continues to retain the PHGs for individual THMs proposed in the October 2018 draft, disregarding the preponderance of available data and the conclusions of multiple regulatory and public health organizations regarding the weight of evidence for the carcinogenic potential of THMs."

"In its current proposal, OEHHA would establish a PHG for each of the four THMs that is two orders of magnitude below levels that are currently reported by water utilities. Developing separate goals for each THM, moreover, creates significant inconsistencies with current state and federal THM policy and raises challenges in meeting California's mandate under state Safe Drinking Water Act."

Response 1. OEHHA has not disregarded the weight of the evidence on the carcinogenicity of THMs. See responses to comments below.

Because the proportion of each THM found in drinking water varies regionally, it is not possible to determine a single proportion of THMs that is representative of all California drinking water. Hence, OEHHA has proposed separate PHGs for the four THMs.

The California State Water Resource Control Board (SWRCB) has no objection to OEHHA's approach. Further, OEHHA's proposal does not require the SWRCB to establish separate MCLs for each of the four THMs.

Comment 2. "Primary and secondary disinfection of drinking water supplies with chlorine is one of the most significant public health achievements of the twentieth century. It has saved millions of lives and spared countless illnesses. Because of the inevitable presence of organic matter in source water and distribution systems, disinfection byproducts, such as the THMs, are produced in the application of this vital, life-saving public health practice."

"As emphasized by the World Health Organization (WHO) and the International Programme on Chemical Safety (IPCS), it is critical that efforts to control THMs and other disinfection byproducts do not compromise the effectiveness of drinking water disinfection technologies and practices. The point of the WHO and IARC statements is that the integral connection between disinfecting drinking water and THM formation requires that any evaluation of THM toxicity must also explicitly consider the public health benefits associated with the disinfection process. This is a risk assessment issue that must not be deferred to the State Water Resources Control Board, as suggested by OEHHA, which lacks both the statutory mandate and the subject matter expertise to do such analyses."

Response 2. OEHHA agrees completely with the utility and importance of drinking water disinfection, as noted in the Technical Support Document (see page 2, *Necessity*

of Disinfection, and page 270, *Disinfection Benefits versus THM Risk*). The commenter misinterprets the statute. In requiring OEHHA to prepare health risk assessments of drinking water contaminants, Health and Safety Code section 116365(c)(1) states:

> "The risk assessment shall contain an estimate of the level of the contaminant in drinking water that is not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health."

Thus, the statute is clear that OEHHA must look strictly at the adverse effects of the contaminant itself when developing a PHG. As such, we are obligated to report on the toxicity of chemicals based on the available data. OEHHA's responsibility is to review the toxicity of the residual levels of chemicals in drinking water that are associated with disinfection, and assess risks from exposure to these byproducts. The risk assessment for THMs is based exclusively on risks from exposure to the THMs. Evaluation of the risks from microbial contaminants that might otherwise be present without disinfection of drinking water, is outside the scope of this assessment.

We disagree with the commenter that SWRCB lacks the expertise to promulgate an MCL or MCLs that are feasible to achieve and that adequately balance the risks and benefits of drinking water disinfection and the creation of THMs. The risk-benefit tradeoff between residual disinfection byproducts in drinking water and exposure to microbial contaminants in drinking water is the kind of risk-management analysis that a regulatory entity such as SWRCB is supposed to undertake. As the name implies, a Public Health Goal is a non-regulatory number that identifies a target for SWRCB to consider as it also weighs treatment costs and technologies, and the importance of drinking water disinfection. OEHHA acknowledged the importance of drinking water disinfection on pgs. 2 and 270 of the second public-review draft document. However, this document is not, nor is it intended to be, an analysis of the health benefits of chlorine disinfection of drinking water.

Comment 3. "Numerous epidemiology studies have attempted to evaluate potential links between THM levels resulting from drinking water chlorination and human cancer risks, but interpretation of these data is complicated by weak cancer response and multiple confounding factors. A recent, multi-nation analysis, moreover, failed to find an association between THMs and bladder cancer, which is the most consistently reported adverse health effect in other studies."

Response 3. Cotruvo and Amato (2019) is based on an extremely broad ecologic assessment of both the exposure (national average total trihalomethane (TTHM) concentrations) and the outcome (national US bladder cancer incidence rates). There is no individual-level information on whether those people who developed bladder cancer had higher or lower TTHM exposures than those who did not have bladder cancer. TTHM levels vary considerably from water source to water source across the US, so an ecologic assessment like this is not a valid way to evaluate causal associations. In addition, as discussed by Cotruvo and Amato (2019), there are a

number of known factors that impact bladder cancer incidence rates. However, none of these is accounted for in the analysis shown in Figure 1 of that publication. Any changes in the prevalence of any of these other factors over time could mask a potential relationship between TTHMs and bladder cancer in this type of very broad ecologic analysis. National smoking rates are displayed, but smoking rates presented on a national level are not useful for evaluating the relationship between THMs and bladder cancer.

With regards to confounding, the most prevalent risk factors for bladder cancer are increasing age, smoking, race, and male sex, and most of the bladder cancer studies we reviewed in our draft PHG report (Appendix C) adjusted for or otherwise controlled for these factors. Several studies also adjusted or controlled for other potential risk factors including occupation or diet. Other risk factors like arsenic in drinking water, family history, or certain rare genetic conditions are likely not prevalent enough or not strongly related enough to THM levels to cause the increased relative risks identified in the studies we reviewed (Axelson, 1978).

Another important point regarding latency relates to the direction of the potential bias that might occur if a study did not appropriately account for latency. In all of the bladder cancer studies we reviewed, exposure was assessed using the same methods in all participants, regardless of whether they had bladder cancer or not. As such, any potential bias from errors in exposure assessment, such as those that might be related to latency, would most likely bias relative risk estimates towards the null, not towards the elevated relative risks identified in many of the studies we reviewed (Rothman and Greenland, 1998).

Most of the studies we reviewed were designed to consider the possibility of a long latency. For example, in the bladder cancer case-control study by Villanueva et al. (2007), THM exposure was based on residential history beginning at age 15 years. Since the large majority of participants in this study were over 65 years old, this study accounted for a latency of up to 50 years in most subjects. In Cantor et al. (1998), exposure was based on lifetime residential history, ≥70% of which was known, and in King and Marrett (1996), only participants with at least 30 years of exposure data were included.

Comment 4. "The THMs Are not Genotoxic Carcinogens"

"In lieu of the available evidence for a threshold effect, OEHHA continues to argue that all four THMs are genotoxic carcinogens to support its default linear extrapolation of cancer risk, even though the evidence for genotoxicity is equivocal.

"The THMs are readily metabolized by two major pathways, oxidative and reactive, predominantly in the liver and kidney. Both pathways are catalyzed by cytochrome P450 enzymes. Although some metabolism of THMs may occur via the reductive pathway, metabolism by the oxidative pathway predominates—particularly at lower,

environmentally relevant doses. As a result, chloroform is metabolized to carbon dioxide via the reactive dihalocarbonyl intermediate phosgene ($COCl_2$). For the brominated THMs, oxidative metabolism produces a trihalomethanol (CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (CX_2O), a structural analogue of phosgene."

"Once formed, phosgene and its dihalocarbonyl counterparts react rapidly to bind with intracellular nucleophiles such as glutathione, proteins, lipids, and other macromolecules. As a result, these metabolites do not diffuse far from the site of production in mitochondria and the endoplasmic reticulum. This hyper reactivity limits their potential molecular targets to these organelles and renders interaction with DNA in the nucleus highly unlikely, if not impossible."

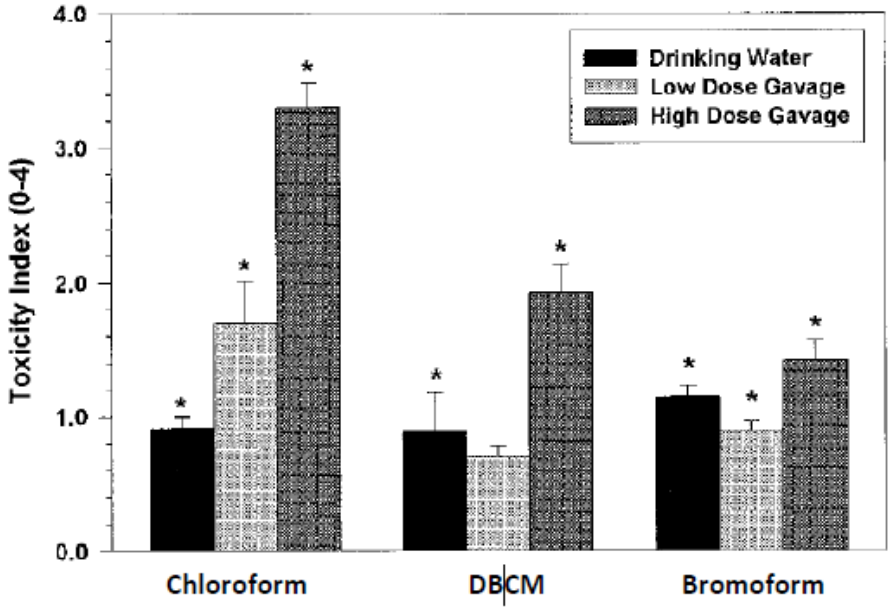
"Based on the significant body of data supporting the importance of oxidative metabolism, it is generally agreed that conversion to the reactive metabolites phosgene or dihalocarbonyl is a key event in THM toxicity. The ability of cells to repair damage caused by low levels of these metabolites, moreover, is consistent with the lack of evidence for THM-induced DNA damage *in vivo*. Yet, the draft PHG concludes, based on the results of *in vitro* testing in rats that cancer results from "the presence in the rat kidney of electrophilic metabolites [of chloroform] other than phosgene, representing either oxidative metabolites formed elsewhere and sufficiently stable to be transported to the kidney or electrophilic metabolites secondary to the formation of reductive radicals."

Response 4. We agree that metabolism of THMs by cytochrome P450 enzymes is important to the toxicity of these chemicals. Studies have shown THMs are metabolized to phosgene and other highly reactive chemicals including halocarbonyls *in vivo* and these metabolites may cause the range of toxicity effects observed, including carcinogenicity. The assertion that cells can completely repair damage caused by the metabolites of THMs and thus eliminate the risk of DNA damage and subsequent cancer is without merit. As well, the idea that the reactive metabolites cannot gain access to the nucleus is questionable as reactive metabolites have been found bound to histones surrounding the DNA inside the nucleus, as discussed in Chapter 9. The comment is incorrect in stating there is no evidence for THM-induced DNA damage *in vivo*. See response to comment 6 below.

Comment 5. Regarding chloroform, the comment states: "Despite acknowledging that genotoxicity testing with chloroform has produced results that are 'typically mild and occurred at high or cytotoxic concentrations,' the draft PHG asserts that chloroform is capable of inducing genetic toxicity under various experimental conditions. Critically, however, standard *in vivo* testing has failed to produce evidence of genotoxicity. The experimental conditions required to produce positive results, moreover, required significant manipulation of the test systems. For example, metabolism of chloroform by the reductive pathway has only been observed following induction of P450 enzymes

with the addition of phenobarbital. In contrast, negligible reductive metabolism has been observed in non-induced animals."

"The characteristics of chloroform-induced tumors are inconsistent with OEHHA's genotoxic, no-threshold hypotheses of carcinogenic action. Mutagenic mechanisms would be expected to produce DNA damage and increase tumor incidence in target organs at any level of chloroform that produces reactive metabolites (*i.e.*, at all doses)—yet this is clearly not observed. Further, since conversion of chloroform to reactive phosgene increases with increasing chloroform blood concentrations, a mutagenic mechanism cannot be reconciled with the observation that chloroform tumorigenesis occurs following bolus gavage administration, but not with most doses administered in drinking water – particularly since drinking water exposures produce greater overall blood concentrations of chloroform (Figure 1)."



"Figure 1. Ability of THMs to induce liver toxicity; * indicates significant difference from the vehicle control group, p-value <0.05"

Response 5. Carcinogens can have multiple modes of action (Guyton et al., 2018a, 2018b; Parfett and Desaulnier, 2017). The mode of action can depend on when during the lifestage exposure occurs or the level of exposure. As discussed in the document and in previous responses to public comments, the available evidence is not, in our view, strong enough to deny any role for genotoxicity for chloroform-induced tumorigenicity.

OEHHA utilizes linearized extrapolation for dose-response characterization when there is insufficient evidence to rule out a non-threshold mechanism of action of a carcinogen. Although it is true that most studies of genotoxicity of chloroform were negative, some

were positive. Further, chloroform is metabolized to reactive metabolites including phosgene and dichloromethyl radical. Evidence for additional reactive metabolites is also in the literature. Several studies on chloroform have demonstrated binding of phosgene to lipids and proteins, including the histones surrounding DNA; binding to histones means phosgene entered the nucleus and binding was not limited to macromolecules in the endoplasmic reticulum where it is produced.

Mutagenic chemicals may indeed produce DNA damage at all exposures. That is one of the premises behind linearized cancer modeling and the concept of no threshold. However, as exposures get lower, it becomes much more difficult to observe tumors in animal studies because of sample size limitations. In other words, while there may be some DNA damage in animals at low exposure levels, one would need a large sample size to detect tumor effects.

Further, as described in Chapter 5 of the draft PHG document, a statistically significant dose-related increase in tumorigenesis was observed in the Jorgenson drinking water study (rat kidney adenoma and adenocarcinoma), as well as in studies via inhalation (Nagano et al., 1998; kidney adenomas and carcinomas in male mice) or a combination of inhalation and drinking water exposures (Nagano et al., 2006; renal adenomas and carcinomas in male rats). Thus, the implication in the comment that mutagenicity cannot be reconciled with the tumor incidences observed in animal studies with different routes of administration is inappropriate. While a greater area under the blood concentration-time curve may be associated with administration in drinking water, higher peak levels are likely obtained via bolus gavage which may influence the rate of tumor formation. The kinetic differences by route and method of administration and dosing level cannot be used to discount the role of genotoxicity in tumor formation or to discount the tumorigenicity of chloroform.

The observations in animal studies of different rates of tumor formation with different routes and methods of administration do not preclude a role for mutagenicity in tumor induction. First, OEHHA's analysis is not based on a non-threshold mechanism related to cytotoxicity. Rather, we applied a linear model for estimating cancer potency using data from a number of studies and multiple exposure routes including gavage, drinking water and a combined drinking water and inhalation exposure. Second, even for strongly mutagenic compounds, it is often difficult to see tumors at very low exposures because it is logistically difficult to treat a large enough number of animals. Public health prudence dictates that OEHHA not ignore the genotoxicity of chloroform, albeit weak. Our analysis, therefore, assumes that the observed tumorigenicity in animals exposed to chloroform via bolus gavage, drinking water, inhalation, and inhalation plus drinking water may be at least in part due to genotoxic actions of chloroform.

As indicated in our draft document, "Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of *S. typhimurium*; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosomal aberrations *in vivo* and *in vitro*; DNA damage in bacteria; and aneuploidy in mammalian cells and *Aspergillus nidulans*. Negative results have also

been obtained *in vitro* and *in vivo*. For some *in vitro* tests, negative findings may have occurred because measures were not taken to prevent volatilization of the test compound."

Finally, a number of studies investigated whether the mechanism of carcinogenicity might be cytotoxicity followed by tissue regeneration leading to expression of pre-existing mutations. The concept is that cytotoxicity and tissue regeneration can be measured via histopathology and DNA labeling, and that these should only occur at doses equivalent to those shown to induce tumor formation in the NCI studies. As noted in Chapter 9, the relationship between cytotoxicity, cell death and subsequent tissue regeneration, indicated by an increase in DNA synthesis as measured by labeling index (the proportion of labeled cells in S phase), has been extensively studied following chloroform administration. However, increases in labeling index often did not reflect the observed pattern of toxicity, including carcinogenicity. This is discussed at length in Chapter 9 of the Technical Support Document. Studies reported:

- toxicity occurring without an apparent increase in labeling index;
- toxicity increasing with continual exposure while the labeling index diminished;
- increased labeling index without apparent toxicity;
- cytotoxicity and increases in the labeling index without any tumorigenic response evident in the cancer bioassays at equivalent exposures.

The evidence that sustained phosgene-induced cytotoxicity is the only mechanism of carcinogenicity for chloroform is not as clear as the commenters suggest. OEHHA has chosen as a matter of public health policy and prudence to utilize a linear model to estimate cancer potency based on:

- some evidence for the genotoxicity of chloroform;
- evidence that reactive intermediates are formed during metabolism of chloroform and in fact at least one, phosgene, is a major metabolite;
- evidence that phosgene can bind to macromolecules including the histones surrounding DNA, and that other reactive intermediates have been found bound to cellular macromolecules;
- inconsistencies in the evidence for the hypothesis that cytotoxicity and tissue regeneration are solely responsible for chloroform tumorigenicity.

Comment 6. "There are data from a variety of assays on the genotoxicity of the brominated THMs. Although there is some evidence to suggest that they may be weakly mutagenic, there is no *in vivo* evidence of genotoxicity."

"Although the metabolism of BDCM and the other brominated THMs via glutathione conjugation is quantitatively minor pathway, it has been suggested that the reactive metabolites formed may be toxicologically significant. Because the metabolites are

unstable, however, they will react with molecules near the site of generation and – as with the metabolites of the P450 pathway – unlikely to interact with DNA in the nucleus."

Response 6. Many positive genotoxicity studies have been published for the brominated THMs. These are discussed in the chapters on the toxicity of the individual THMs. The comment implies there are no studies showing genotoxicity *in vivo* for the brominated THMs. This is incorrect. Positive genotoxicity results have been obtained for bromoform in both *in vitro* and *in vivo* assays such as micronuclei induction, sister chromatid exchange, chromosomal aberrations including DNA damage, sex-linked recessive lethal mutations, and aneuploidy. Both positive and negative results of *in vivo* genotoxicity tests on bromoform are summarized in Table 6.6 in the draft PHG document, as shown below. The weight of the available evidence indicates that bromoform is mutagenic and genotoxic, and thus presents a carcinogenic risk to humans.

Table 6.6 Summary of *In Vivo* Genotoxicity Studies on Bromoform

Endpoint	Assay system	Result	References
Micronuclei induction	Mouse, bone marrow cells	-	Hayashi *et al*. (1988)
	Mouse, bone marrow cells	-	Stocker *et al*. (1997)
	Mouse, bone marrow cells	+	NTP (1989a)
	Newt, peripheral erythrocytes	+	Le Curieux *et al*. (1995)
	Human, peripheral lymphocytes	+	Kogevinas *et al.* (2010)
Chromosomal aberrations	Mouse, bone marrow cells	-	NTP (1989a)
	Rat, bone marrow cells (oral) Rat, bone marrow cells (i.p.)	+ +	Fujie *et al*. (1990)
Sister chromatid exchange	Mouse, bone marrow cells	+	Morimoto and Koizumi (1983)
	Mouse, bone marrow cells	+	NTP (1989a)
DNA damage	Rat, renal cells	-	Potter *et al*. (1996)
Unscheduled DNA synthesis	Rat, hepatocytes	-	Stocker *et al*. (1997)
Sex linked recessive lethal mutations	*Drosophila melanogaster*	+	Woodruff *et al*. (1985), NTP (1989a)
Heritable translocations	*Drosophila melanogaster*	-	
Aneuploidy	*Aspergillus nidulans*	+	Benigni *et al*. (1993)
Initiation	Rat, liver	-	Herren-Freund and Pereira (1986)

The positive data for carcinogenicity and mutagenicity for bromoform, as well as the formation of mutagenic metabolites, justify the use of linear extrapolation of cancer risk, in accordance with cancer guidelines (US EPA, 2005a; OEHHA, 2009). Cancer guidelines require assumption of a linear dose response unless there is adequate

evidence of a threshold mechanism of action and no evidence of genotoxicity, which is certainly not true in this case.

The weight of evidence suggests that brominated THMs like BDCM are mutagenic and genotoxic. Although the overall data are mixed, a number of positive results were obtained for mutagenicity and genotoxicity assays in bacterial and mammalian systems. *In vivo* data for BDCM genotoxicity are available for chromosomal aberrations, sister chromatid exchange, induction of micronuclei, and DNA damage and repair, as discussed in Chapter 7 of the draft PHG document.

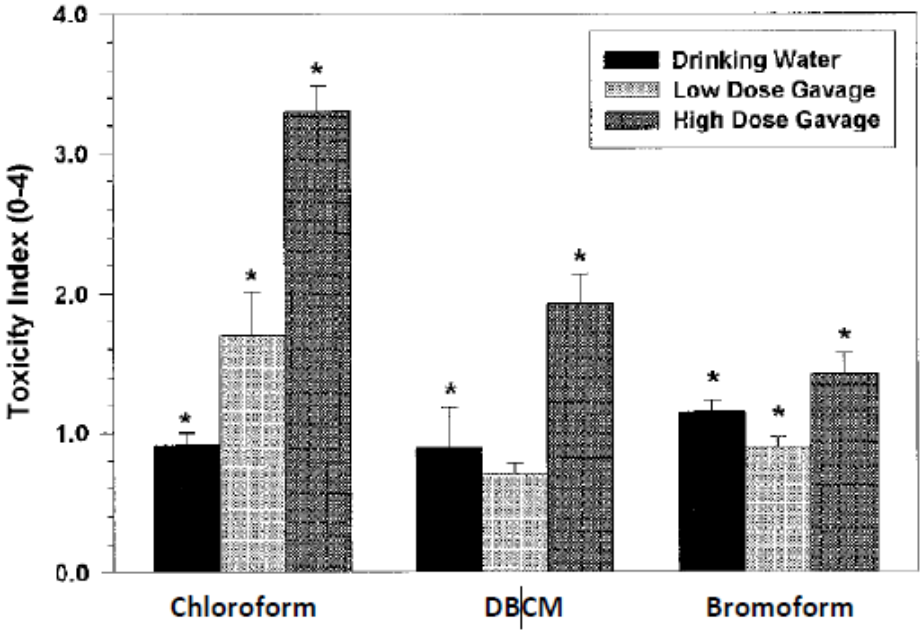
For DBCM, as indicated in our draft document, "Although the overall data are mixed, positive results have been obtained for mutagenicity in several test strains of *S. typhimurium*; mutagenicity in cultured mouse lymphoma cells; induction of sister chromatid exchange and chromosomal aberrations *in vivo* and *in vitro*; DNA damage in bacteria; and aneuploidy in mammalian cells and *Aspergillus nidulans*. Negative results have also been obtained *in vitro* and *in vivo*. For some *in vitro* tests, negative findings may have occurred because measures were not taken to prevent volatilization of the test compound." The overall weight of evidence suggests that DBCM is mutagenic and genotoxic.

Finally, the comment suggests that glutathione conjugated metabolites cannot be responsible for carcinogenicity of brominated THMs because they are too reactive to reach the DNA. This assertion is not substantiated. As discussed in our draft document, evidence *in vitro* suggests that a potential mechanism of carcinogenicity of BDCM may involve covalent binding to DNA of reactive intermediates generated by GSTT1-1-mediated metabolism (glutathione conjugation) of BDCM (Ross and Pegram, 2004). The authors suggest that this may occur in the kidney and large intestine (target organs of BDCM carcinogenicity in rats), which were demonstrated in this study to have a much lower detoxification rate to bioactivation rate (CYP/GST ratio) compared to the liver (a non-cancer target in rats). This may result in an enhanced relative production of reactive intermediates with the capacity to covalently modify DNA in target tissues, ultimately contributing to BDCM carcinogenicity. Also as discussed in our draft, several *in vivo* studies in rats suggest a plausible mechanism of carcinogenicity of brominated THMs in the colon involving formation of putative early preneoplastic lesions in the colon called aberrant crypt foci. DeAngelo et al. (2002) reported that the brominated THMs, particularly BDCM, administered in drinking water significantly induced the incidence of aberrant crypt foci in the colon of male F344/N rats. Geter et al. (2004b) then showed that formation of aberrant crypt foci in the colon of male F344/N rats was independent of the method of BDCM administration, with drinking water and corn oil gavage administration producing similar values of aberrant crypt foci per colon.

Comment 7. "There Is Strong Evidence of Pharmacokinetic Differences between Exposure in Drinking Water and Dosing by Gavage"

"The unchanged, proposed PHGs for the four THMs are based on cancer evidence from gavage studies using corn oil, despite the fact that drinking water and dietary studies have generally produced negative results. The toxicological targets of the THMs (*i.e.,* liver and kidney) are the same regardless of the route of exposure. However, drinking water exposures fail to induce tumors at daily doses greater than the doses that produce tumors by oral gavage. Because both dosing methods involve absorption via the gastrointestinal tract, the possible differences would relate only to how the test doses are administered—corn oil gavage versus water consumption—and the time-frame of administration.

"In a comparative study of gavage and drinking water exposures, THMs administered by gavage increased cell proliferation and decreased DNA methylation in mouse livers; dosing in drinking water produced a much smaller effect, particularly for chloroform.

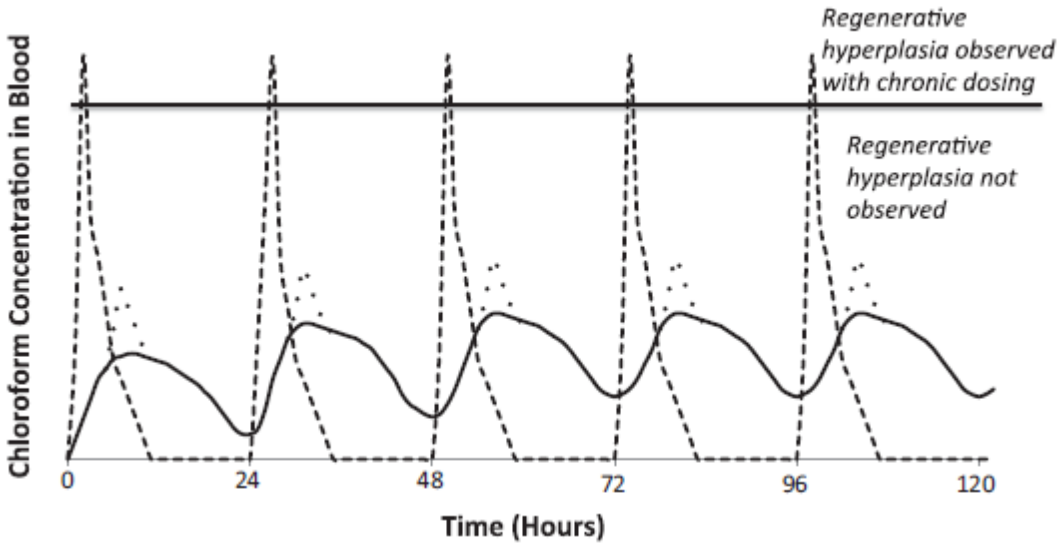


"Figure 1. Ability of THMs to induce liver toxicity; * indicates significant difference from the vehicle control group, p-value <0.05"

"These findings are consistent with the dose-response curves observed for the THMs, especially chloroform and BDCM, which suggest that exposure to THM levels must be sufficiently high to overcome natural detoxification mechanisms before they can exert a toxic effect. The weaker activity of THMs administered in drinking water likely results from incremental exposure each time the mouse drinks, in contrast to bolus delivery by oral gavage. The slower rate of delivery by drinking water consumption is much more

relevant to potential human exposures and is expected to result in a lower liver concentration that increases the opportunity for detoxification (Figure 2). Hence, the toxicity of the THM is dependent on the rate of delivery (*i.e.*, rapidly by oral gavage and more slowly in drinking water).

”The slower rate of delivery by drinking water results in lower metabolite concentrations that reduce the likelihood that concentrations will overwhelm reduced glutathione (GSH) and other detoxification mechanisms. This appears to be true even though the doses administered in drinking water produce a greater average concentration than with bolus delivery (Figure 2).



“Figure 2. Illustration of pharmacokinetics of chloroform following administration by oral gavage (- - - -), drinking water (_____), and inhalation (. . . .) routes. Area under the concentration curve: drinking water > gavage > inhalation”

”The available evidence support a conclusion that the pharmacokinetic differences between bolus-gavage and drinking-water (and dietary) dosing play a significant role in explaining the disparity in the observed tumor incidence in the animal studies and should be explicitly taken into consideration in assessing the toxicity of the THMs. Unless a threshold existed for bioactivation of a THM to a mutagenic metabolite, there would be no reason that a genotoxic MoA would exhibit this profound difference with time-frame of administration. Nor is there any known mechanism by which corn oil would alter metabolism of chloroform. Hence, the absorption and distribution kinetics of the THMs strongly support an MoA that relies on a threshold. Furthermore, the profound difference in tumorigenicity favors a mechanism that does not rely on damage to DNA. These conclusions are consistent with independent assessments by the U.S. EPA, IARC, WHO and Health Canada. OEHHA is the only entity proposing a different interpretation of the science but, as noted above, that interpretation is grounded more in speculation than in science.”

Response 7. OEHHA does not dispute that pharmacokinetic differences may exist among different routes and methods of exposure, though OEHHA disagrees that these pharmacokinetic differences ameliorate any cancer risk. It is not appropriate to assume that detoxification is complete following drinking water exposures; reactive metabolites generated at the cellular level can react with macromolecules including DNA.

OEHHA did not estimate the cancer slope factor using only corn oil gavage studies for chloroform. In estimating the cancer slope factor for chloroform in the draft PHG document, OEHHA utilized studies that exposed animals via drinking water, drinking water plus inhalation, and gavage. The geometric mean was used as the basis for the PHG calculation. As indicated in the OEHHA draft document, "The geometric mean was calculated for the following reasons:

- the differences in species, strain, and sex of animals tested;
- the differences in route of administration, i.e., oral ingestion vs. inhalation;
- the differences in vehicle administration, i.e., gavage in corn oil or in toothpaste vs. drinking water;
- and the wide range of CSF_{human} estimates calculated, i.e., the forty-fold difference between the lowest value of 0.003 $(mg/kg\text{-}day)^{-1}$ and the highest value of 0.128 $(mg/kg\text{-}day)^{-1}$."

In contrast to the comment, not all drinking water studies of the THMs were negative. For example, among studies on BDCM, George et al. (2002) and Tumasonis et al. (1987) found significant increases in hepatic tumors in drinking water studies in rats. Tumasonis et al. (1985) found increased incidence of liver tumors in rats treated with chloroform by the drinking water route. In Jorgenson et al. (1985), the incidences of renal tubular cell adenoma and combined renal tubular cell adenoma and carcinoma were significantly greater among high-dose Osborne-Mendel rats ($p < 0.01$, Peto Trend Test) treated with chloroform via drinking water, compared to matched controls. Further, in Nagano et al. (2006), the combined exposures to chloroform by inhalation and drinking water resulted in increased renal cell adenoma and carcinoma in Fisher 344 rats. Thus, THMs have been shown to induce cancer in animal models following drinking water and inhalation exposures, not just by gavage.

ACC comments cited the Coffin et al. (2000) study as evidence that THMs administered by gavage increased cell proliferation and decreased DNA methylation in mouse livers while dosing in drinking water produced a much smaller effect. However, we note that toxicity as indicated by DNA methylation and increased cell proliferation (labeling index) was still observed following drinking water exposures. For example, the PCNA-labeling index (PCNA-LI) results in Figure 6 of the paper showed statistically significant increases for two of the four THMs administered in drinking water. The low-dose gavage exposures of the THMs increased the PCNA-LI to an extent similar to when the THMs were administered in drinking water. In the case of bromoform, administration in drinking water resulted in a higher level of labeling than a similar dose by gavage

(Figures 5 and 6 of Coffin et al., 2000). Further, the results in Figure 10 in Coffin et al. (2000) clearly demonstrated there was no difference among routes of exposure in terms of decreasing DNA methylation for three out of four THMs examined (chloroform was the only exception). Thus, this paper does not support the notion that the THMs when given in drinking water cannot produce tumors.

Comment 8. "OEHHA Significantly Overestimates the Drinking Water Consumption Rate in Calculating PHGs"

"OEHHA's calculation of the drinking water concentration associated with a cancer risk of 10^{-6} for each of the four THMs is based on a susceptibility-weighted daily water intake (DWI). The weighted DWI (DWIlife), expressed in equivalent liters of water consumed per kilogram body weight per day (or Leq/kg-day), represents the product of the age sensitivity factor (ASF), the time spent in each life stage (expressed as a ratio), and the unweighted DWI for the life stage. Although this approach is consistent with OEHHA's method for accounting for early life-stage exposures, the draft PHGs do not take into consideration the chemical-specific data available for the THMs.

"All of the bioassays considered by OEHHA in developing the draft PHGs began exposing the test animals when they were only a few weeks old to better approximate lifetime exposures. In the National Cancer Institute (NCI) study of chloroform, for example, the rats were 5 weeks old at the beginning of the study, while the mice were only 3.5 weeks old. Although there are undoubtedly differences between the development of young rodents and human children, those differences are already accounted for in the calculation of the human cancer slope factor. As a result, there is no need to include an ASF to account for the sensitivity of children from aged 2–16 years.

"In addition, while the draft PHGs suggest the possibility of an alternative MoA to explain the toxicity of the individual THMs, OEHHA's analysis is largely based on the generally accepted metabolism via the P450-mediated pathway. However, as noted by OEHHA in the Second Public Review Draft, many of the P450 enzymes are not expressed, or are expressed at very low levels, in the fetus. It is inappropriate, therefore, to include an ASF for the 3rd trimester in the susceptibility-weighted daily water intake.

"While we continue to believe that the DWIlife used in developing the proposed PHGs is unrealistically high, we urge OEHHA to use assumptions that explicitly consider what is known about the THM studies and metabolism to develop a more plausible estimate of lifetime water consumption in its derivation of PHGs for THMs."

Response 8. OEHHA disagrees with the assertion that drinking water intake rates have been overestimated. There are two components to OEHHA's susceptibility-weighted daily water intake rates that are independent of one another but appropriately applied. First, the age sensitivity factor (ASF) is a weighting factor applied to exposure

at different lifestages to account for the increased susceptibility to carcinogens during early-in-life exposures. The ASFs reflect the available data and analyses indicating higher carcinogen potency when exposure occurs early in life (OEHHA, 2009). Since everyone goes through each lifestage, it is appropriate to add the lifestage DWI to obtain a lifetime DWI. This is an approach consistent with US EPA's cancer risk assessment guidelines (US EPA, 2005a,b), which "view childhood as a sequence of lifestages rather than viewing children as a subpopulation" and states, "[F]or a susceptible lifestage, higher risks can be expected from exposures during only a portion of a lifetime, but everyone in the population may pass through those lifestages."

None of the cancer bioassays for the THMs include in utero exposure nor exposures prior to weaning. In the NCI study, rats were started at 52 days of age, or almost 7.5 weeks. Rats at 52 days of age are young adults, and thus this bioassay cannot account for effects of chloroform on carcinogenicity from pre-or postnatal exposures. Similarly, in the NCI study mice were started at 35 days of age, or 5 weeks old. Mice at 5 weeks of age are already sexually mature, although they could be considered "teens." For all the other chloroform bioassays, animals ranged from 5 to 8 weeks of age, all young adults. We see no reason to discard the age-sensitivity factors for ages 2 to 16, based on the age of the mice in the NCI study.

The data available to OEHHA for determining the Age Sensitivity Factors indicates that early-in-life exposures in rodents, and particularly perinatally to weaning, resulted in higher carcinogen potencies across different structures of carcinogens than exposures later in life (OEHHA 2009). While one might expect that, for metabolically activated carcinogens, the development of the CYP 450 enzymes would coincide with increased potency, this does not appear to be the case for the chemicals OEHHA (2009) and US EPA (US EPA, 2005a,b) evaluated, which were all the chemicals for which there were data to conduct an evaluation. Many factors are at play, including low levels of detoxifying enzymes relative to adults, and high cell division and differentiation rates compared to adults. Thus, the relationship is more complex than the commenter assumes. Given that rodents are born at a stage approximating human development in the third trimester, it is appropriate to apply the ASF derived from the perinatal exposures in rodents to human exposures occurring in the third trimester. This is discussed in detail in OEHHA (2009), our peer reviewed guidelines on assessing cancer risk.

Second, liter equivalents (L_{eq}) account for the total amount of exposure to a chemical that one receives through the use of tap water. For volatile chemicals such as the THMs, exposure from tap water consists of oral ingestion as well as inhalation and dermal exposure while bathing, showering, and through other household uses. Thus, the L_{eq} value is not the amount of water an individual actually drinks but instead represents how much tap water one would have to drink to have the same amount of exposure to the chemical through the combined oral, inhalation, and dermal routes via typical household uses of tap water. Additionally, OEHHA utilizes age-specific drinking water ingestion rates (OEHHA, 2012), which account for the higher drinking water intake per body weight for infants and children relative to adults. Thus, one cannot

compare the consumption rates multiplied by an ASF and inclusive of exposure via inhalation and dermal pathways to an unweighted estimate of drinking water ingestion rate for an adult.

Comment 9. "OEHHA has not Considered Additional Information Provided Well in Advance of the Second Public Comment Period"

"As part of its comments on the first draft of the PHGs for THMs, ACC submitted the following list of relevant references that have not been incorporated into the latest draft.

• Boobis AR *et al.* IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol* 36(10):781–792 (2006). https://doi.org/10.1080/10408440600977677

• Boobis AR. Mode of action considerations in the quantitative assessment of tumour responses in the liver. *Basic Clin Pharmacol Toxicol* 106(3):173–179 (2010). https://doi.org/10.1111/j.1742-7843.2009.00505.x

• Boobis AR *et al.* Application of key events analysis to chemical carcinogens and noncarcinogens. *Crit Rev Food Sci Nutr* 49(8):690–707 (2009). https://doi.org/10.1080/10408390903098673

• Borgert *et al.* Modernizing problem formulation for risk assessment necessitates articulation of mode of action. *Reg Toxicol Pharma* 72:538-551 (2015). http://dx.doi.org/10.1016/j.yrtph.2015.04.018

• Butterworth BE *et al.* A comprehensive approach for integration of toxicity and cancer risk assessments. *Regul Toxicol Pharmacol* 29(1):23–36 (1999). https://doi.org/10.1006/rtph.1998.1273

• Butterworth BE *et al.* A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancer Lett* 93(1):129–146 (1995). https://doi.org/10.1016/0304-3835(95)03794-W

• Butterworth BE *et al.* The role of regenerative cell proliferation in chloroform-induced cancer. *Toxicol Lett* 82–83:23–26 (1995). https://doi.org/10.1016/0378-4274(95)03543-5

• Butterworth BE *et al.* Long-term mutagenicity studies with chloroform and dimethylnitrosamine in female *lacI* transgenic B6C3F1 mice. *Environ Mol Mutagen* 31(3):248–256 (1998). https://doi.org/10.1002/(SICI)1098-2280(1998)31:3<248::AID-EM6>3.0.CO;2-G

• Conolly RB Butterworth BE. Biologically based dose response model for hepatic toxicity: a mechanistically based replacement for traditional estimates of

noncancer risk. *Toxicol Lett* 82–83:901–906 (1995). https://doi.org/10.1016/0378-4274(95)03528-1

• Cotruvo JA Amato H. National trends of bladder cancer and trihalomethanes in drinking water: a review and multicountry ecological study. *Dose Response* 17:1(2019). https://doi.org/10.1177/1559325818807781

• Meek ME *et al.* Chloroform: exposure estimation, hazard characterization, and exposure– response analysis. *J Toxicol Environ Health B Crit Rev* 5(3):283–334 (2002). https://doi.org/10.1080/10937400290070080

• Tomati L. Cell proliferation and carcinogenesis: A brief history and current view based on an IARC workshop report. *Environ Health Perspect* 101(suppl. 5):149–152 (1993). https://doi.org/10.1289/ehp.93101s5149

• Tyson CA *et al.* Correlations of in vitro and in vivo hepatotoxicity for five haloalkanes. *Toxicol Appl Pharmacol* 70(2):289–302 (1983). https://doi.org/10.1016/0041-008X(83)90105-9

• Uehleke H Werner T. A comparative study on the irreversible binding of labeled halothane trichlorofluoromethane, chloroform, and carbon tetrachloride to hepatic protein and lipids in vitro and in vivo. *Arch Toxicol* 34(4):289–308 (1975). https://doi.org/10.1007/BF00353849

"We are resubmitting these citations to ensure that they, and the additional articles cited in this comment, are considered in further revisions to the PHG document."

Response 9. It is OEHHA's goal to present a full picture of the relevant information to the readers in our PHG draft, though we do not discuss all studies that are not particularly relevant to the assessment in question. For example, most of the references cited above discuss different considerations and methods for risk assessment. OEHHA has established guidelines for risk assessment that have been peer-reviewed and are consistent with other government agencies including the US Environmental Protection Agency. We would not spend time discussing these other methods and considerations (some of which are already incorporated into our risk assessment methods guidelines) in a technical support document for a specific chemical Public Health Goal. As well, few of the studies listed above are original research studies of the THMs. This technical support document focuses on our analysis of original research on the four THMs. OEHHA acknowledges the comment and has revisited the above-mentioned studies that are original research studies (Butterworth et al., 1998; Cortruvo and Amato, 2019; Tyson et al., 1983; Uehleke and Werner, 1975), but not the papers related to risk assessment methodologies.

The paper by Butterworth et al. (1998) is already in the draft (Chapter 5, chloroform under Genotoxicity *In Vivo* studies), although we unfortunately left the reference out of the bibliography. We apologize for that confusion.

We addressed the Cortruvo and Amato (2019) paper here and in the responses to public comment from the first public review period. This paper is an epidemiological study with ecological design and cannot be used to make any connection between THM exposure and bladder cancer (see responses to comment 3 above).

Tyson et al. (1983) was essentially a methods paper to evaluate whether one could use isolated hepatocytes to rank the potency of chemicals as hepatotoxicants. The investigators compared the release of liver enzymes *in vitro* to similar parameters *in vivo*. The study was meant to determine if an *in vitro* system could mimic an *in vivo* system. As such, we did not feel it added to the discussion of hepatotoxicity for the trihalomethanes, which is already extensively discussed in the document.

Ueheleke et al. (1975) is an older German paper with English translation that reports almost no detail. The investigators were evaluating the irreversibility of binding of ^{14}C label from four halogenated compounds including chloroform. They note irreversible binding to endoplasmic reticulum extracts. This paper is not very informative and is not added to the technical support document.

REFERENCES CITED IN RESPONSES (NOT OTHERWISE CITED ABOVE)

Axelson O (1978). Aspects on confounding in occupational health epidemiology. Scand J Work Environ Health 4: 85-9.

Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, et al. (1998). Drinking water source and chlorination byproducts. I. risk of bladder cancer. Epidemiol 9(1):21-8. Comment by Lang et al., 9(1):7-8, 1998.

Coffin JC, Ge R, Yang S, Kramer PM, Tao L, Pereira MA (2000). Effect of trihalomethanes on cell proliferation and DNA methylation in female B6C3F1 mouse liver. Toxicol Sci 58(2):243-252. Accessed at: https://doi.org/10.1093/toxsci/58.2.243

DeAngelo AB, Geter DR, Rosenberg DW, Crary CK, George MH (2002). The induction of aberrant crypt foci (ACF) in the colons of rats by trihalomethanes administered in the drinking water. Cancer Lett 187(12):25-31.

George MH, Olson GR, Doerfler D, Moore T, Kilburn S, DeAngelo AB (2002). Carcinogenicity of bromodichloromethane administered in drinking water to Male F344/N Rats and B6C3F1 mice. Int J Toxicol 21(3):219-30.

Geter DR, George MH, Moore TM, Kilburn S, Huggins-Clark G, DeAngelo AB (2004b). Vehicle and mode of administration effects on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to bromodichloromethane. J Toxicol Environ Health A 67(1):23-9.

Guyton KZ, Rieswijk L, Wang A, Chiu WA, Smith MT (2018a). Key characteristics approach to carcinogen hazard identification. Chem Res Toxicol 31(12):1290-1292.

Guyton KZ, Rusyn I, Chiu WA, Corpet DE, van den Berg M, Ross MK, Christiani DC, Beland FA, Smith MT (2018b). Application of key characteristics of carcinogens in cancer hazard identification.

Jorgenson TA, Meierhenry EF, Rushbrook CJ, Bull RJ, Robinson M (1985). Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fund Appl Toxicol 5:760-9.

King WD, Marrett LD (1996). Case-control study of bladder cancer and chlorination by-products in treated water (Ontario, Canada). Cancer Causes Control 7(6):596-604.

Nagano K, Nishizawa T, Yamamoto S, Matsushima T (1998). Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Advances in the Prevention of Occupational Respiratory Diseases. Proc. of the 9th International Conf. on Occupational Respiratory Diseases, Kyoto, Japan, October 13 – 16, 1997. Chiyotani K, Hosoda Y, Aizawa Y, eds. Elsevier Science, New York, NY, pp. 741-6.

Nagano K, Kano H, Arito H, Yamamoto S, Matsushima T (2006). Enhancement of renal carcinogenicity by combined inhalation and oral exposures to chloroform in male rats. Toxicol Environ Health A. 69(20):1827-42.

OEHHA (2009). Air Toxics Hot Spots Program Risk Assessment Guidelines, Part II. Technical Support Document for Describing Available Cancer Potency Factors. May 2009. Office of Environmental Health Hazard Assessment, Oakland and Sacramento, CA. Accessed at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html.

OEHHA (2012). Air Toxics Hot Spots Risk Assessment Guidelines, Part IV Technical Support Document for Exposure Assessment and Stochastic Analysis. August, 2012. Office of Environmental Health Hazard Assessment, Oakland and Sacramento, CA. Accessed at: https://oehha.ca.gov/air/crnr/notice-adoption-technical-support-document-exposure-assessment-and-stochastic-analysis-aug

Parfett CL, Desaulniers D (2017). A Tox21 approach to altered epigenetic landscapes: Assessing epigenetic toxicity pathways leading to altered gene expression and oncogenic transformation in vitro. Int J Mol Sci 18:1179-1255.

Ross MK, Pegram RA (2004). In vitro biotransformation and genotoxicity of the drinking water disinfection byproduct bromodichloromethane: DNA binding mediated by glutathione transferase theta 1-1. Toxicol Appl Pharmacol 195(2):166-81.

Rothman K, Greenland S (1998). Precision and validity of studies. In: Modern Epidemiology. Rothman K, Greenland S, eds. Philadelphia, Lippincott Raven, 115–134.Tumasonis CF, McMartin DN, Bush B (1985). Lifetime toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. Ecotoxicol Environ Saf 9:233-240.

Tumasonis CF, McMartin DN, Bush B (1987). Toxicity of chloroform and bromodichloromethane when administered over a lifetime in rats. J Environ Pathol Toxicol Oncol 7:55-64.

US EPA (2005a). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, US Environmental Protection Agency Washington, DC. EPA/630/P-03/001F, March 2005.

US EPA (2005b). Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC. EPA/630/R-03/003F, March 2005.

Villanueva CM, Cantor KP, Grimalt JO, Malats N, et al. (2007). Bladder cancer and exposure to water disinfection byproducts through ingestion, bathing, showering, and swimming in pools. Am J Epidemiol 165(2):148-56.