



December 9, 2019

Lauren Zeise, Ph.D.
Director
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Post Office Box 4010
Sacramento, CA 95812-4010

Re: Public Health Goals for Trihalomethanes in Drinking Water (Second Public Review Draft, November 2019)

Dear Dr. Zeise:

The Chlorine Chemistry Division (CCD)¹ of the American Chemistry Council submitted extensive comments on the Office of Environmental Health Hazard Assessment's (OEHHA) first draft of Public Health Goals (PHG) for the trihalomethanes (THMs²) released in October 2018. 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 organizations³ regarding the weight of evidence for the carcinogenic potential of THMs. Instead, the draft PHG postulates vague and largely unsubstantiated suggestions of carcinogenic modes of action (MoA) to fit an antiquated approach to risk assessment that could potentially compromise the health of Californians.

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. The potential health effects of the THMs have been well studied and regulations to reduce their concentrations in and exposure from finished drinking water have been in place since the late 1970s.

¹ CCD represents major producers and users of chlorine in North America and works to promote and protect the sustainability of chlorine chemistry processes, products and applications in accordance with the principles of Responsible Care®.

² Chloroform, bromoform, bromodichloromethane (BDCM), and dibromochloromethane (DBCM).

³ Including the U.S. Environmental Protection Agency, the International Agency for Research on Cancer (IARC), the World Health Organization (WHO), and Health Canada.



As emphasized by the World Health Organization (WHO)⁴ and the International Programme on Chemical Safety (IPCS)⁵, 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,⁶ which lacks both the statutory mandate and the subject matter expertise to do such analyses.

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. In addition to the practical challenges of implementing the draft PHGs, ACC has the following concerns about the scientific basis for the proposed PHGs –

- THMs are not genotoxic carcinogens;
- The proposed PHGs do not account for well-established and significant pharmacokinetic differences between drinking water and gavage exposure in test animals;
- OEHHA greatly overestimates lifetime drinking water exposures to the THMs; and
- OEHHA has not considered additional peer reviewed information provided well in advance of the second public comment period.

The THMs Are not Genotoxic Carcinogens

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

⁴ WHO. Guidelines for Drinking-water Quality, 4th ed. World Health Organization, Geneva, Switzerland (2011). https://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/

⁵ IPCS (2000). Disinfectants and Disinfection By-Products. Environmental Health Criteria 216. World Health Organization, Geneva, Switzerland. https://www.who.int/ipcs/publications/ehc/ehc_216/en/

⁶ OEHHA PHG Second Review Draft, at 271.



studies.⁷ In the absence of consistent human data suggesting adverse health effects, OEHHA must base its assessment of cancer risks on the results of animal bioassays for the individual THMs.

While animal data may be considered relevant to potential cancer risks in humans, IARC stresses the importance of considering the availability of additional scientific information including data demonstrating that “the mechanism of carcinogenicity in experimental animals does not operate in humans”⁸ or that “the suppression of key mechanistic processes leads to the suppression of tumour development.”⁹ Consideration of the MoA by which a substance causes health effects in animals can not only assist in assessing the relevance of those effects in humans, but it also can inform regulatory decisions about potential risks to humans at environmentally relevant exposure levels.

In evaluating potential cancer risks from THM exposure, OEHHA assumes a default linear extrapolation of the dose response information available from animal studies for each individual THM – despite clear evidence that cancer in rodents occurs only after sustained exposure to elevated levels of the substances. 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.¹⁰

Studies in mice lacking an ability to metabolize chloroform to phosgene via the oxidative pathway did not exhibit the hepatic, renal, and nasal toxicity observed in the wild-type animals

⁷ 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>

⁸ IARC. IARC Monographs on the Identification of Carcinogenic Hazards to Humans – Preamble. World Health Organization. Lyon (January 2019), at 35. <https://monographs.iarc.fr/wp-content/uploads/2019/07/Preamble-2019.pdf>

⁹ IARC Preamble, at 33.

¹⁰ Unlike chloroform, brominated THMs may undergo a subsequent glutathione-dependent reduction to yield carbon monoxide.



exposed to chloroform in the same experiment.¹¹ Similarly, pretreatment of female rats and mice with inhibitors of oxidative metabolism reduced the acute renal and hepatic toxicity of BDCM.¹² Thus, the authors concluded that metabolism by the oxidative pathway is critical to the toxicity observed in mice.

Once formed, phosgene and its dihalocarbonyl counterparts react rapidly to bind with intracellular nucleophiles such as glutathione, proteins, lipids, and other macromolecules.¹³ 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.¹⁴ While mitochondria can tolerate some level of toxicological insult caused by the binding to the reactive metabolites without any change in membrane permeability,¹⁵ toxicity would be expected to occur only after metabolite production is sufficient to overwhelm cellular repair capability. This resilience and repair capability protects the animal from toxic effects at low doses of THM, which is consistent with the results of the animal studies involving exposure via contaminated drinking water described later in this comment letter.

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.”¹⁶

¹¹ Constan AA *et al.* Metabolism of chloroform by cytochrome P450 2E1 is required for induction of toxicity in the liver, kidney, and nose of male mice. *Toxicol Appl Pharmacol* 160(2):120-126 (1999). <https://doi.org/10.1006/taap.1999.8756>

¹² Thornton-Manning JR *et al.* Toxicity of bromodichloromethane in female rats and mice after repeated oral dosing. *Toxicol* 94(1-3):3-18 (1994). [https://doi.org/10.1016/0300-483X\(94\)90024-8](https://doi.org/10.1016/0300-483X(94)90024-8)

¹³ Microsomal studies indicate that about 75 percent of covalent binding following treatment with chloroform is to phospholipids. OEHHA. Public Health Goals for Trihalomethanes in Drinking Water: Second Review Draft (November 2019), at 32.

¹⁴ Borgert CJ *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>

¹⁵ 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>

¹⁶ OEHHA PHG Second Review Draft, at 30.



The California Safe Drinking Water Act requires OEHHA to base its PHG risk assessments on “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.”¹⁷ This language suggests strongly a risk assessment based on a threshold mechanism of cancer which is supported by the weight of evidence and widely recognized by “public health professionals who are experienced practitioners” in the relevant scientific fields. Moreover, it argues against a risk assessment based on speculation about hypothetical MOAs that are not supported by available scientific information.

Chloroform

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.¹⁸ 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.¹⁹

In addition to suggesting that chloroform-derived metabolites other than phosgene could form as a result of reductive metabolism, which has been rarely observed in standard *in vivo* testing, the draft PHG posits that stable oxidative metabolites might form elsewhere and be subsequently transported to the site of the cancer. No such metabolites have been observed in the significant testing that has occurred to date. Moreover, if these postulated stable metabolites were able to induce genotoxicity, one would expect to observe tumors where they were formed as well as in the kidney.

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

¹⁷ Health and Safety Code §116365(c)(1).

¹⁸ *In vitro* test systems can provide mechanistic insights, but important considerations include the limitations of the test system (e.g. in metabolic capabilities) as well as the suitability of a particular test article (*i.e.*, because of physical and chemical characteristics). IARC Preamble, at 28.

¹⁹ Fawell J. Risk assessment case study – chloroform and related substances. *Food Chem Toxicol* 38(Supp 1): s91-s95 (2000). [https://doi.org/10.1016/S0278-6915\(99\)00129-5](https://doi.org/10.1016/S0278-6915(99)00129-5)



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).

Brominated THMs

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. For BDCM, cytochrome P450 oxidation accounts for 99% of the metabolism in the liver and 84–88% of the metabolism in the kidney. Several studies have shown that as the BDCM exposure level increases, the percentage of metabolism due to oxidation decreases.²⁰

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.²¹ 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.

Using physiologically based pharmacokinetic (PBPK) modeling, NTP (2006) analyzed the relative contribution of cytochrome P450 and GST metabolism in the liver and kidney in rats following oral exposure to BDCM. The ratios of cytochrome P450 to GST metabolism were observed to change with increasing dose. The dose-related changes are likely due to saturation of the P450 metabolic pathway resulting in higher levels of BDCM in the blood and availability to other tissues.²²

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

²⁰ Allis JW Zhao G. Quantification evaluation of bromodichloromethane metabolism by recombinant rat and human cytochrome P450s. *Chem Biol Interact* 140(2):137-153 (2002). [https://doi.org/10.1016/S0009-2797\(02\)00022-4](https://doi.org/10.1016/S0009-2797(02)00022-4)

²¹ Ross MK Pegram RA. 2003. Glutathione transferase theta 1-1-dependent metabolism of the water disinfection byproduct BDCM. *Chem Res Toxicol* 16:216-226 (2003). <https://doi.org/10.1021/tx0200820>

²² NTP. 2006. Toxicology and carcinogenesis studies of bromodichloromethane in male F344/N rats and female B6C3F1 mice (drinking water studies). National Toxicology Program Tech Rep Ser 532 (2006). https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr532.pdf



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.²³

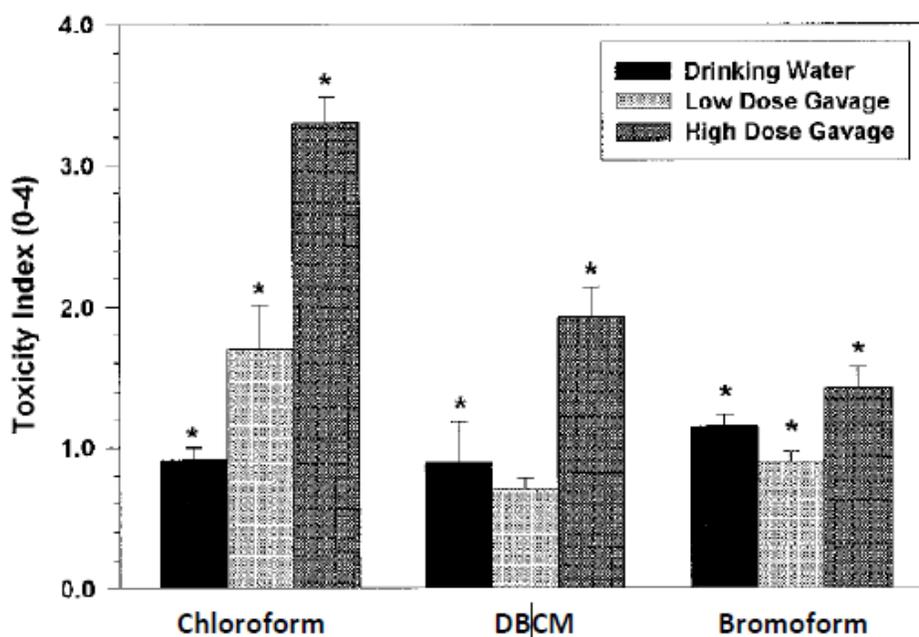


Figure 1. Ability of THMs to induce liver toxicity; * indicates significant difference from the vehicle control group, p-value <0.05²⁴

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

²³ Coffin JC *et al.* Effect of trihalomethanes on cell proliferation and DNA methylation in female B6C3F1 mouse liver. *Toxicol Sci* 58(2):243-252 (2000). <https://doi.org/10.1093/toxsci/58.2.243>

²⁴ *Ibid.*



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).

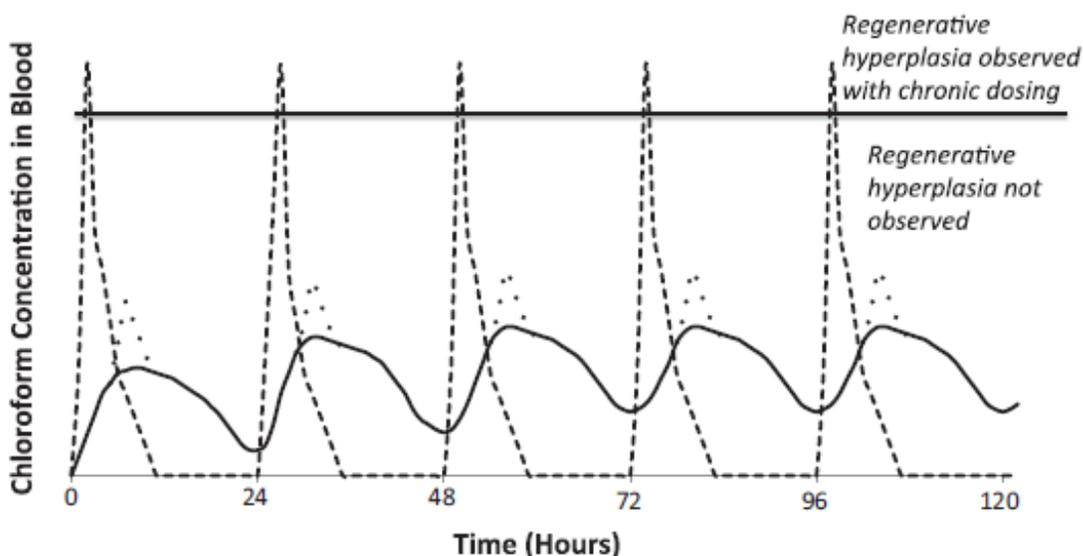


Figure 2. Illustration of pharmacokinetics of chloroform following administration by oral gavage (---), drinking water (____), and inhalation (...) routes. Area under the concentration curve: drinking water > gavage > inhalation²⁵

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

²⁵ Borgert *et al.* 2015.

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.

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 (DWI_{life}), expressed in equivalent liters of water consumed per kilogram body weight per day (or $L_{eq}/kg\text{-day}$), 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.²⁶

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.²⁷ 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 DWI_{life} 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.

²⁶ OEHHA. Technical Support Document for Cancer Potency Factors: Methodologies for Derivation, Listing of Available Values, And Adjustments to Allow For Early Life Stage Exposures. (May 2009).

²⁷ NCI. Report on Carcinogenesis Bioassay of Chloroform. NTIS PB-264018. National Institute of Health, Bethesda, MD. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/trchloroform.pdf.



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>
- 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>
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- 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>
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- 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](https://doi.org/10.1016/0378-4274(95)03543-5)
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- 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](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.

Please feel free to contact me if you have any questions about the information provided above, or would like to discuss this information in greater detail.

Sincerely,



Judith Nordgren
Managing Director
Chlorine Chemistry Division

