Responses to Major Comments on Technical Support Document

Public Health Goal
For
1,2,3-Trichloropropane
In Drinking Water

Prepared by

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INTRODUCTION

The following are the combined responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for 1,2,3-trichloropropane (TCP). The first four sets of comments are based on the first released review draft, posted for public comment on September 14, 2007. Changes were made in response to these comments, and the updated document was posted again for public comment on February 6, 2009. Two more sets of comments were received in response to this second posting. Additional changes have been incorporated into the final version of the PHG document posted on the OEHHA website.

For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. 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 MAJOR COMMENTS RECEIVED

Comments from David Eastmond, University of California, Riverside

Comment 1: "For a number of sections, there appears to be a strong reliance on secondary sources (ATSDR, WHO, etc.) and it was not clear if the primary sources had been checked. The reliance on secondary sources is a particular problem when using the HSBD which generally just copies and pastes things from other sources. In cases such as in Table 1 where the secondary sources provide somewhat differing values, it is not clear why one value was selected and not the other (see technical comments below). Unless there is a compelling reason to choose one over the other, I would recommend providing both values."

Response 1: The PHG document does rely on secondary sources for information on subjects such as physical constants, production, use data and levels detected in the environment. These can be found in readily available reference sources, or are reported as data but not used for quantitative assessment for deriving the PHG. Table 1 was revised and multiple values were provided.

Comment 2: "There seems to be inconsistencies in the Production and Uses section. ... Additional and informative details on the production and use of TCP are provided in the WHO CICAD document (WHO, 2002). … I would suggest that additional details be provided such as those described in WHO (2002) [e.g. TCP presence within Telone at 0.17 % by weight; its presence within epichlorohydrin, etc.]."

Response 2: Some additional information was added to this section. OEHHA decided not to add much detailed information about levels of TCP in products, because production methods can change and these data may quickly become outdated. Generally, information in the PHG document regarding sources, levels in the environment, etc., is meant to be more descriptive, providing the reader with a little overview about the chemical. The document is not intended to be authoritative on these subjects.

Comment 3: "I find the metabolism data to be somewhat confusing. The abbreviations in Figure 1 should be defined."

Response 3: Figure 1 was revised for clarity.

Comment 4: "The data appear to indicate the cytochrome P450 monooxygenase-mediated activation can result in metabolites that primarily bind to proteins whereas bioactivation involving glutathione results in metabolites that preferentially binding to DNA. If this is correct interpretation I would suggest that this be included in the summary, and that the Mechanism section on pages 20-21 be revised to be more informative."

Response 4: There are relatively few studies regarding the roles of different metabolic pathways in TCP activation and deactivation and the consequences of metabolism (binding to proteins and DNA). As discussed in the metabolism section of the PHG document, the administration of phenobarbital, which induces certain forms of cytochrome P450, reduced TCP binding to both hepatic protein and DNA *in vivo* (Weber and Sipes, 1990). Another inducer of P450 did not have the same effect. Pretreatment of rats with SKF 525-A, a P450 inhibitor, increased binding to both proteins and DNA in the study. Reducing cell glutathione levels by pretreatment with 1-buthionine-(R,S)sulfoximine resulted in less DNA binding and a marked increase in binding to protein.

The findings of Weber and Sipes (1992) in an *in vitro* study complicate the picture. As discussed in the Metabolism section of the PHG document, the pretreatment of rats with phenobarbital resulted in a marked increase in the binding of TCP to microsomal proteins *in vitro* compared to untreated rats. The *in vitro* addition of glutathione reduced the binding of TCP to microsomal protein, suggesting that glutathione also scavenges reactive metabolites generated by cytochrome P450.

The metabolism of TCP is complicated and the findings of the few available studies provided some ambiguous information. Both cytochrome P450 and a glutathione pathways appear to be involved in TCP metabolism and the reactions are probably competitive. OEHHA reviewed the studies in the PHG document and determined that the data precluded any definitive conclusions regarding how different pathways influence bioactivation and the carcinogenesis of TCP *in vivo*.

Comment 5: "With regard to the mode of action, there is clear evidence that TCP is mutagenic and genotoxic in *in vitro* systems. … In my opinion, the high incidence and specificity of mutations reported by Ito *et al.* (1996) should be emphasized rather than the observation that the detected transversions were not consistent with the authors' predicted miscoding properties of the adduct."

Response 5: OEHHA agrees that TCP is clearly mutagenic in *in vitro* systems and the observation of specific point mutation in the *k* and *h*-ras oncogenes is strong evidence of mutagenic activity *in vivo*. The text was revised to emphasize this finding. However, the focus of Ito *et al*. (1996) was an investigation of a possible mechanism of TCP carcinogenicity. Their interesting finding was that the transversions detected in forestomach tumors (tissues obtained from the NTP study) were not consistent with the identified major DNA adduct (which doesn't result in conversions). This suggests that other adducts or additional mechanism(s) were involved in the pathogenesis of the tumors. We felt that this finding needed to be discussed to provide a more complete picture of the lack of understanding of mechanism of action of TCP**.** These data buttress the idea that we have a very incomplete understanding of the process of carcinogenesis.

Comment 6: "I think the mechanistic aspects of the document would be strengthened by adding a short section describing the results seen with other short-chain halogenated alkanes."

Response 6: OEHHA agrees. Information regarding the structurally related halogenated propane DBCP was added to the document.

Comment 7: *Immunotoxicity and neurotoxicity information*. "There is some information on immunotoxicity in the ATSDR monograph (ATSDR, 1992). In addition, in a PubMed search, I was able to identify an article [Albrecht WN (1987)] … which according to the abstract, may provide some information on the neurotoxicity of TCP."

Response 7: ATSDR (1992) discussed one study that indicated TCP is a very mild skin sensitizer in guinea pig but also mentioned in another study the vehicle employed was itself a mild skin sensitizer. Discussion of the Albrecht study was added to the PHG document. Albrecht (1987) evaluated several environment chemicals for effects on CNS excitability. TCP was administered by i.p. injection and its effect on general CNS excitability produced by the subcutaneous injection of pentylenetetrazol (PTZ) was evaluated. TCP had no apparent effect on CNS stimulation (induction of convulsions) by PTZ.

Comment 8: "For the non-cancer effects, I was a bit surprised that the newer benchmark dose (BMD) approach was not used, but the NOAEL is certainly acceptable."

Response 8: While significant changes in several hematological parameters (erythrocytes, leukocytes, lymphocytes levels) were observed, a consistent pattern of dose-response was not observed across these endpoints and often was not observed within a given hematological parameter. Thus while OEHHA could identify a dose where adverse effects associated with exposure to TCP appeared to be occurring, the development of a dose-response relationship based on one or more of these endpoints was problematic. Therefore, the NOAEL approach was employed to develop the health protective concentration for non-carcinogenic effects.

Comment 9: "For assessing the non-cancer effects, OEHHA chose hematological changes occurring in the 17-week NTP study as the critical endpoint and study. While this seems to be an acceptable choice, it is not clear why they chose this study and endpoint rather than other changes such as the renal or hepatic changes that appeared in the 2-year study. The statement on page 21 that "no toxic effects that were not related to the occurrence of tumors were identified in the subsequent two-year bioassay" seems questionable to me, given some changes were reported (e.g. a high incidence of hyperplasia in the kidney without an increase in kidney tumors and an increase in eosinophilic foci in the liver, etc)."

Response 9: NTP discussed non-neoplastic adverse effects observed in the 2-year study. For rats NTP reported: "Of the clinical findings, none were considered to be directly related to organ toxicity other than those associated with chemical-induced neoplasms of the oral mucosa, forestomach or mammary glands." For mice NTP reported "no clinical findings were considered to be directly related to organ toxicity other than those associated with chemical-induced neoplasms." These findings were added to the PHG document.

In the interim sacrifice groups, eosinophilic foci were observed in 60 mg/kg female mice. Eosinophilic foci were considered by NTP to be "a possible precursor of adenoma." In rats in the 15 month interim sacrifice, 2 of 10 males and 0 of 8 females receiving 10 mg/kg-day exhibited hyperplasia in the kidney, and 6 of 10 males and 2 of 8 females receiving 30 mg/kg-day exhibited hyperplasia in the kidney.

The non-neoplasia effects in the NTP study at 15 months (which are questionable) were observed at a dose higher than the NOAEL for hematological changes that we used to develop a health protective concentration for non-carcinogenic effects.

Comment 10: "I do have a recommendation for the presentation of the animal tumor data. … The data from both the main and interim studies should be included in Tables 4 and 5. … When feasible, I would suggest presenting the data in three categories: papillomas, carcinomas, and papillomas or carcinomas. Since many of the mice had more than one tumor, it is not clear to me how this was handled when modeling the data."

Response 10: Changes were made to Tables 4 and 5 and data from the interim sacrifice groups were provided in two new tables, Tables 6 and 7.

Mice in the NTP (1993) study often had both forestomach carcinomas and papillomas. When this occurred in a given animal, the modeling was based on the occurrence of a carcinoma (which was considered to be cause of death to the animals in the modeling used to generate the cancer slope factor). Text in the PHG document was modified to indicate this.

Comment 11: "OEHHA also mentions that animals from the interim sacrifice group were censored (removed from the analysis) if they did not display a tumor because they did not live until the end of the animals' natural lifetime."

Response 11: The text in the draft PHG document was confusing and now has been revised. All animals were included in the analysis (none were removed). How the time to tumor modeling considered animals in the interim sacrifice group is important. Tumors observed at the time of interim sacrifice were judged not responsible for the animal's deaths (the sacrifice was responsible for the deaths). Animals without tumors at the time of interim sacrifice were scored as not having a tumor. The modeling accounts for the shortened lifespan of these animals because of the interim sacrifice. The individual animal data for animals in the interim sacrifice groups are not in the NTP technical report but can be found on Excel spreadsheets provided on the NTP Web site (http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=longtermbioassaydata.datasearch&study_no=C60220B&study_length=2%20Years). A reference to this site is added to the document.

Comment 12: "The choice of the 17-week study for the non-cancer effects seems to be an acceptable choice but should be justified as other studies such as the 2-year bioassay or the reproductive studies could have been chosen."

Response 12: The text has been modified to explain the selection of the 17 week study. Basically the findings from the subchronic studies were selected because no adverse effects unrelated to the tumors were observed in the two-year study and the reproductive studies yielded higher NOAELs.

Comment 13: "However, while standard health-protective methods have been used to estimate the risk in the low dose region, the resulting proposed PHG is so low (>1 ppt) that one can't help but wonder if the approach being used isn't overly conservative. The observation that seemingly minor assumptions can change the potency estimates in the time to tumor model by more that 100-fold is a concern. Indeed, the fact that so many of the major effects seen in the 2-year cancer bioassay were cancer-related would suggest to me that the assumption that the carcinomas and papillomas were the cause of death (footnote 4) might be a more likely option for the time-to-tumor modeling."

Response 13: The findings of the NTP cancer bioassay were internally consistent, very high incidences of tumors in multiple organs in male and female rats and mice. The data indicate that TCP is a potent carcinogen. The results of the dose-response modeling, a high cancer potency (and therefore a low PHG), reflect that TCP is a potent carcinogen.

OEHHA conducted a sensitivity analysis in mice on 1) how the assumptions regarding papillomas/carcinomas being incidental or responsible for the animal's death influenced the estimates of cancer potency, and 2) how including the interim sacrifice group influenced the estimates of cancer potency. The results are shown in Table 8.

Assuming all forestomach tumors (papillomas or carcinomas) were responsible for the animals' death (footnote 5) had a relatively small effect on cancer potency compared to the assumption that carcinomas were fatal and papillomas were incidental. Assuming that all forestomach tumors were incidental to the cause of death had a major effect on potency (footnote 4). Given that NTP reported that "neoplasm of the forestomach in rats and mice … were the principal cause of death of most animals dying or killed moribund before the end of the studies," the assumption that all tumors were incidental (the one assumption that did have a major effect on the estimated potency) to the cause of death appears to be problematic, and therefore this approach was not used. Including the interim sacrifice group had little effect on the cancer potency except if all tumors were assumed to be incidental (the problematic assumption).

Comment 14: "Because the CSF derived from the LED_{10}, R on page 26 is really an upper estimate of the individual cancer risk. This should be presented as such here and throughout the document".

Response 14: OEHHA agrees. The text in the PHG document was modified.

Comment 15: "However, there is DNA adduct data that suggest that the relationship between dose and response may be influenced by the route of administration and be more related to peak dose rather than cumulative dose. To me this implies that the dose response relationship may have a non-linear component."

Response 15: The dose-response relationship used to develop the PHG is strictly based on tumor incidence, not on adduct data. While one DNA adduct predominated in the tissues of animals treated with TCP (La *et al*., 1995, 1996), other adducts have also been identified in another studies (La and Swenberg, 1997). Studies of La and coworkers revealed that the pattern of increase of the major adduct (S-[1-(hydroxymethyl)-2-(N7-guanyl)ethyl]glutathione) in tissues was not concordant with the site of tumors in the 1993 NTP cancer bioassay (La *et al*., 1995, 1996). Given the lack of concordance between tumor sites and sites with increased adduct levels, the use of dose-adduct level as a surrogate for dose-tumor response is problematic.

Comment 16: "In their studies, La *et al.* (1996) demonstrated that DNA adduct levels and cell proliferation in selected TCP target tissues were significantly higher when a 6 mg/kg dose of TCP was administered orally by gavage as compared to when it was administered via drinking water. This suggests that risk estimates based on the NTP studies, which were conducted by oral gavage, may overestimate the cancer risk of TCP present at low concentrations in drinking water."

Response 16: As indicated in the text of the PHG document, neither sites with increased adduct levels nor sites with increased cell proliferation were concordant with the tumor sites in the NTP 1993 bioassay. The levels of other adducts in the tissues or other factors such as the ability of a tissue to repair DNA may also govern where tumors occur and the dose-response relationship at a given site. Therefore, the dose-response relationship was based on the incidence of tumors and not on the incidence of DNA adducts.

The vehicle used to administer an agent will often influence pharmacological and toxicological activity of the agent, as do the stomach contents with an oral administration. For example, the toxicity to the stomach of non-steroidal anti-inflammatory drugs, alcohol and certain antibiotics is reduced when taken with meals. How the corn oil vehicle employed in the NTP (1993) study affected the tumorigenic response and if the risk estimate is too high or too low, compared to the drinking water route, is unclear.

Comment 17: "While there isn't a specific section that describes the uncertainties.…"

Response 17: Uncertainty is discussed in the Risk Characterization section of the PHG document.

Comments from Mark Nicas, University of California, Berkeley

Comment 1: "Overall the approach used in developing the PHG document with regard to toxicology data was appropriate. However, the approach used to estimate non-drinking water exposure was entirely opaque. On page 6, the document states that the CALTOX multimedia exposure model "to determine if inhalation and dermal exposure to 1,2,3-trichloropropane, mainly during showering, would be expected to substantially add to the daily exposure.…" Given that the general structure of the model as applied to residential TCP exposure was not described, and that all model inputs were not identified, one

cannot judge the reliability of the conclusion drawn from the model output, namely, that inhalation exposure is essentially equivalent to ingesting another 2 L/day of water containing TCP. … Because human health risk depends on dose, a more transparent estimation of exposure (and dose) is required."

Response 1: We agree that the CalTOX model is not transparent and that the complexity of the inputs and the calculation makes it difficult for a reviewer to assess its validity. We have continually tried to improve on how to present and describe this calculation in our PHG documents. The discussion in this PHG document has been expanded for clarification. Basically, volatilization and exposure are predicted from a chemical's physical properties according to fugacity principles and a multipathway exposure model (see http://eetd.lbl.gov/ie/ERA/caltox/index.html). In the few cases in which multi-route exposure to a similar chemical has been measured in a household situation, such as for chloroform (Jo *et al*., 2005), results similar to those from CalTOX have been obtained, so it is clear that the principles and assumptions incorporated into the model at least approximately represent a real-world scenario. The CalTOX model and a user manual are available for download (http://www.dtsc.ca.gov/AssessingRisk/ctox_dwn.cfm) for those who might wish to check its results.

Comment 2: "Estimating TCP Carcinogenic Potency: The document needs to better justify the reliance on forestomach carcinoma data because: (1) humans do not have a forestomach, and (2) in Table 6 which reports the time–to-tumor modeling results, the q_1^* estimates based on other tissue sites … are 14- to 125-fold lower than the estimate $q_1^* = 25\ (\text{mg/kg-day})^{-1}$ based on the female mouse forestomach. It is certainly more health conservative to base the analysis on "the most sensitive cancer endpoint" (forestomach carcinoma induction), but at face value it does not seem biologically appropriate."

Response 2: IARC (2003) provided a thorough discussion of the relevance of forestomach tumors in evaluating carcinogenicity to humans (note underline by OEHHA to highlight a pertinent conclusion):

> "The precise underlying mechanism of action for any forestomach carcinogen is at present not fully known. Nevertheless, most genotoxic forestomach carcinogens appear to act through a mode of action involving genetic changes in oncogenes and tumour suppressor genes. Non-DNA reactive agents such as butylated hydroxyanisole appear to cause forestomach tumours primarily through initial cytotoxicity and subsequent sustained cell proliferation and hyperplasia.
>
> While humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. Thus, in principle, carcinogens targeting the forestomach squamous epithelium in rodents are relevant for humans. Also, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodents may target a different tissue in humans. Furthermore, tumorigenic effects in the forestomach are usually accompanied by similar effects in other tissues, indicating that there may be either general (e.g., genotoxic or receptor interactive) or multiple modes of action. However, the relevance for humans is probably limited for agents that have no demonstrable genotoxicity and that are solely

carcinogenic for the forestomach squamous epithelium in rodents after oral administration, since the exposure conditions are quite different between the experimental animals and humans. Consequently, for these agents, the mode of carcinogenic action could be specific to the experimental animals.

There are considerable gaps in knowledge regarding factors that may be of importance for forestomach carcinogenesis. The role of physiological factors such as absorption and transit time on forestomach carcinogenesis is not well understood. Identification of genetic alterations in tumours induced by genotoxic and putative non-genotoxic compounds should give a clearer understanding of underlying mechanisms. Furthermore, the role of biotransformation of xenobiotics, either by the forestomach epithelium or by the luminal contents, in the induction of forestomach tumours needs to be studied on a case-by-case basis. The influence of pH on the carcinogenic process warrants attention. Also, the possible influence of neuroendocrine factors such as gastrin and other neuropeptides on the squamous epithelium of the forestomach may require further study.

In evaluating the relevance of the induction of forestomach tumours in rodents for human cancer the exposure conditions in the experiments have to be considered. The exposure conditions during oral administration are unusual (particularly if gavage dosing is employed) in that physical effects may result in high local concentrations of test substances in the forestomach and prolonged exposure of the epithelial tissue. Such factors may contribute to responses that may be unique for the forestomach. Nevertheless, carcinogens that are DNA-reactive and cause forestomach tumours in rodents — even if they only caused tumours at this site — should be evaluated as if they presented a carcinogenic hazard to humans**.** DNA-reactive agents with a high organ-specificity may be rare, however, because a carcinogen acting through a genotoxic mechanism would be expected to induce tumours at a number of sites. The anomaly of diethyl sulfate (for which genotoxicity has been demonstrated *in vivo*) is probably an artefact of its high chemical reactivity whereby local damage is produced and only low concentrations are available for distribution to other tissues. Agents that only produce tumours in the forestomach in rodents after prolonged treatment through non-DNA reactive mechanisms may be of less relevance to humans, since human exposure to such agents would need to surpass time-integrated dose thresholds in order to elicit the carcinogenic response. As has been summarised by Dybing & Sanner (this volume) very few of those agents that have been associated with rodent forestomach tumours are without some form of genotoxicity and approximately 85% of the total list of agents also induce tumours in other organs. The problem to be solved with any evaluation of a single agent is whether the genotoxicity is an essential property for the induction of the observed tumorigenicity. A solution to the problem is only possible after careful evaluation of all the toxicological and metabolic evidence specific to the individual agent."

Given there is strong evidence of TCP genotoxicity, and, that tumors occurred at a number of sites in male and female mice, OEHHA believes the occurrence of forestomach tumors in the mouse is relevant to humans and therefore we have used it as the basis of the PHG.

Comment 3: "Related to the appropriate tissue site is the 1996 La, *et al.*, study finding … that the gavage bolus dose method (used in the NTP study) versus the drinking water dose method in mice produced higher concentrations of DNA adducts and also caused increased cell proliferation not observed with the drinking water dosing route…. As stated in the 2006 CICAD 56 for TCP: 'It appears that the high local concentrations to be expected from the gavage bolus dose led to significant adduct formation and cell proliferation in contrast to the continuous but lower local concentrations resulting from drinking-water exposure. Consequently, it has to be expected that gavage exposure will overestimate the carcinogenic potency of 1,2,3-trichloropropane.'"

Response 3: The effect of vehicle is complex. Use of a corn oil vehicle may or may not have resulted in higher localized concentration of TCP and it is difficult to predict what would have occurred if TCP was delivered in drinking water. TCP was administered by NTP in a corn oil vehicle to reach the high doses needed for their study (not possible with a water vehicle because of the low TCP solubility). La and associates (1996) selected the lowest dose of TCP used by NTP for their study. But because of solubility issues, they still could not deliver TCP in water alone, and used a 0.5 percent solution of Emulphor 620L (not typically found in drinking water). So La and associates (1996) compared two different vehicles, neither of which was plain drinking water.

The effect of the corn oil vehicle on the amount of TCP that partitions directly into the cells that line the gut (and the resulting concentration) is unclear; it may be higher or lower than from the 0.5 percent Emulphor vehicle, depending on the affinity of TCP for lipids in the cell membranes relative to the corn oil/food/ water mixture in the gut or Emulphor/food/water mixture in the gut. Less TCP may partition from a more hydrophobic vehicle such as corn oil and enter into cells lining the forestomach and stomach of rodents.

The administration of TCP in corn oil would be expected to reduce the rate of gastric emptying and this probably results in a longer residency time in the stomach. How this affects the amount of TCP that partitioned into either part of the stomach or into the intestine is unclear. It is also unclear if tumors observed in the forestomach were due to the direct movement of TCP into cells that line the forestomach or due to absorption in the intestine and then movement to the forestomach, because the intestine is usually the major site of absorption. Also delayed gastric emptying may result in more detoxification by the liver and less TCP being available for distal tissues including the forestomach (first pass clearance). Less TCP available to distal tissues could result in an underestimate of risk relative to use of an aqueous vehicle.

If the use of a corn oil vehicle resulted in a localized high concentration of TCP, irritation, necrosis, and cytotoxicity in the forestomach could have occurred, which theoretically could be responsible for tumors. At 8 and 17 weeks, 15 months and at final sacrifice in the NTP study, samples of various tissues including the stomach and forestomach were examined for histopathology (see Tables 3, 15, A5, B5 and C5 in the NTP 1993 report). No irritation or necrosis was observed/reported in the stomach or forestomach of male or female rats or mice at 8 or 17 weeks. No hyperplasia was observed/reported in the stomach or forestomach of male or female rats at 8 or 17 weeks.

NTP reported "focal hyperplasia of the stratified squamous epithelium of the forestomach" in some dosed rats at 15 months and at sacrifice in the two year study. No notable irritation, inflammation or necrosis was reported in male or female rats at 15 months or two years.

Mild hyperplasia in the forestomach was observed in the male mouse in the second highest dose group at 8 and 17 weeks, in female mouse at 8 weeks in the high dose group, and in the three highest dose groups after 17 weeks. Focal hyperplasia of the forestomach epithelium was observed in all dose groups of female mice and in almost all dose groups of male mice at 15 months. Dose-related increases in the incidence of hyperplasia were observed in male mice, while the incidence of hyperplasia was markedly increased only in high-dose female mice at final sacrifice. No notable irritation, inflammation or necrosis was reported at 15 months or final sacrifice in male or female mice.

In conclusion, no evidence of localized irritation, inflammation or necrosis was observed in the forestomach of rats or mice administered TCP. Hyperplasia was observed in the forestomach, but this was not surprising, given that hyperplasia is generally considered a precursor of tumors.

Comment 4: *As quoted from the 2006 CICAD 56 for TCP*, "A number of chemicals, including the structurally related 1,2-dibromo-3-chloropropane [DBCP] are also known to induce high incidences of forestomach tumors, but only when administered via gavage."

Response 4: Cancer bioassays employ high doses of chemicals because of sensitivity issues, the limited ability to detect statistically significant changes in tumor incidence in small groups of rodents. When chemicals are of low water solubility, corn oil is often used as a vehicle to administer the high doses needed, which is then generally administered by gavage. Chemicals structurally related to TCP such as DBCP are also of low water solubility, and therefore have often been tested in cancer bioassays using a corn oil vehicle by gavage. Lack of a positive study in drinking water would not be surprising. However, with DBCP, there are positive studies by gavage in corn oil, incorporation in the diet, and by inhalation (OEHHA, 1999). All three modes of administration resulted in tumors of the forestomach or stomach. Forestomach tumor potency in these studies was greatest when DBCP was administered in the diet, not by gavage.

Comment 5: "One would expect that the high local concentration effect due to gavage dosing would be much diluted in other tissues (liver, mammary gland, pancreas, uterus) compared to the forestomach; in turn, neoplasms at these other tissue sites may be better cancer endpoints."

Response 5: Gastric emptying would be expected to be retarded by the use of a corn oil vehicle. This would probably result in more time for the liver to metabolize TCP (first pass clearance), thereby reducing the amount of TCP reaching organs such as the kidney, mammary gland, heart and perhaps the forestomach. Whether using a corn oil vehicle

that is administered by gavage improves or provides better estimates of cancer potency is unclear.

Comment 6: "Curiously, the La, *et al.*, study found that gavage dosing caused more DNA adduct formation and cell proliferation in the mouse liver and kidney than in the forestomach. In addition, incidence of carcinomas in the NTP study was highest in the mouse forestomach, not significantly increased in the mouse liver, and not observed in the mouse kidney. This circumstance likely led the authors of the PHG document to dismiss the La, *et al*., findings."

Response 6: PHGs are developed to protect public health from the adverse effects associated with chemicals. Not all effects are considered adverse. Adduct formation and increased cell proliferation are not, in themselves, necessarily considered to be adverse, but are a concern if they lead to tumor formation. So this effect was not selected as the basis for development of a PHG for TCP.

However, the La *et al*. (1996) study is important. Its findings indicate that increased adduct formation and increased cell proliferation are not necessarily predictive of increases in tumors in an organ following exposure to TCP. The increase in tumors in a number of organs in rats and mice, the basis of the proposed PHG, is the major concern. Because increases in cell proliferation and increases in adduct formation were not predictive of tumor occurrence, this endpoint was judged not useful in estimating the risk associated with the increases in tumors in mice or rats.

Comment 7: "In light of the issues concerning the gavage dose method and the lack of a human forestomach, the PHG document should more fully justify its reliance on the forestomach data. For example, perhaps it is logical to consider the tissue site at which the highest chemical concentration would exist. For the gavage bolus delivery, in rodents that tissue is the forestomach, whereas in humans it might be the stomach. If there is no unique feature to the rodent forestomach (as opposed to the human stomach) that somehow makes the forestomach exquisitely more sensitive to carcinogenesis, then it is appropriate to consider the forestomach carcinoma incidence."

Response 7: It is difficult to know which organ is subjected to the highest concentration of an agent when tissue levels were not measured. However, following oral exposure the highest toxicant levels might be expected in the intestine or the liver, given that little absorption would be expected to occur in the stomach. In addition to exposure concentration, other factors such as the ability of a tissue to metabolize TCP to active metabolite(s), inactivate or eliminate the active metabolite(s), and repair damage (DNA) that is responsible for the occurrence of tumors may determine the ability of the agent to cause tumors in a given tissue. Because of these and other uncertainties, OEHHA selects the most sensitive site to develop a dose-response relationship to fulfill its mandate of protecting public health.

Comment 8: "The PHG document estimated that inhalation exposure is essentially equivalent to ingesting another 2 L/day of water containing TCP. However, two items

indicate that, in general, daily inhalation of TCP vapor would contribute substantially less than ingesting 2 L of water containing TCP.

First, on Page 6, the PHG document stated that "the vadose soil compartment was loaded with various concentrations of 1,2,3-trichloropropane…" The assumption seems to have been that if drinking water contains TCP, then so must the soil water surrounding a single-family residence. The two media need not be linked."

Response 8: The CalTOX model contains many modules. Estimation of residential exposure to TCP due to ingestion of drinking water, inhalation and dermal contact with TCP that originated from the domestic water supply is accomplished by inputting TCP into the vadose zone in the model. From documentation of CalTOX: "Contaminants in this layer are assumed to move downward to the ground-water zone primarily by capillary motion of water and leaching. … In the current version of CalTOX, we do not explicitly model the flow and dilution of contaminants in ground water. Instead, we consider the concentration of a contaminant in the water leaching from the vadose-zone soil as an input to the ground-water zone. This concentration is used to make calculations of potential doses of contaminants in ground water" (see http://www.dtsc.ca.gov/AssessingRisk/upload/techman2.pdf).

As used by OEHHA, CalTOX estimates human exposure based on the use of groundwater as the source of domestic water in a residence. CalTOX estimates the doses associated with exposure due to ingestion of water and due to inhalation and dermal contact in the shower and other activities elsewhere in the residence.

Comment 9: "Second, on page 6, the PHG document stated that showering would be the primary source (and perhaps the only meaningful source) of TCP exposure other than drinking water. In this regard, the document seems to have ignored the 2003 Tancrede *et al.*, study.… That study found that the fraction of TCP volatilized from shower water … was 20 percent, and that only 5% to 17% of the vaporized TCP could be recovered from air. The implication of the latter finding was that there was at least one mechanism other than ventilation that removed vaporized TCP from shower stall air. The net result is that only a small percent of TCP (1 to 4%) was both lost from shower water and available for inhalation."

Response 9: The Tancrede *et al*. (1992) study cited in INCHEM, 2003 evaluated five volatile organic compounds in a shower stall constructed in the laboratory. A known amount of TCP was added to the water coming into the shower. The investigators measured the amount of TCP in the water leaving the shower (drain water) and also sampled TCP levels in air in the breathing zone after the shower ran for 10 minutes. The difference in the amount of TCP entering and leaving the shower indicated that 20 percent of TCP that entered the shower was lost and was assumed to have volatilized. Of that 20 percent, roughly 5 to 15 percent was accounted for in shower stall air (or 85 to 95 percent of the 20 percent was not accounted for).

Because so little of the "missing mass" was accounted for, it is difficult to know if there is a problem with the methodology in the study. It is problematic to rely on the finding that only 5 to 15 percent of the missing mass is available for inhalation exposure without

knowing where the missing mass went to. Perhaps the missing TCP that volatilized into the air was sorbing onto plastic materials of the shower enclosure that was used in this study and which would eventually degas and be available for exposure. Such sorption into the enclosure would probably not occur in a ceramic/glass enclosed shower. Vapor migrating into the rest of the bathroom is also a significant exposure source. The "loss or lack of accounting" for a large mass of TCP makes the Tancrede *et al.* (1992) study not very useful for estimating inhalation exposure.

Comment 10: "The Tancrede, *et al*., study did not report airborne TCP levels during showering. I made the following estimate… [*0.14 to 0.27 L*]. Thus, whereas the PHG document assumed consumption of 4 Leq/day of drinking water, the more appropriate value would be approximately 2.2 Leq/day." Additionally, "other residential sources of emission from drinking water were not discussed. … [W]ashing laundry in hot water and boiling water for cooking would be expected to release a greater fraction of the TCP as compared to showering. Depending on the rooms and the ventilation conditions in which these activities are performed, and the extent of these activities, TCP inhalation might assume equal if not greater importance than ingestion."

Response 10: The commenter's estimate of exposure from the shower uses some problematic parameters and assumptions. The calculation is based on a showering time of 12 minutes. EPA (1997) recommends an average showering duration of 10 minutes, with a 50th percentile value of 15 minutes and 95th percentile value of 35 minutes. The exposure estimate did not account for the time of exposure in the shower room after showering. EPA (1997), citing Tsang and Kelpeis (1996), estimates post-showering exposure duration of 5 minutes (50th percentile of the distribution) for males and females, 20 minutes (90th percentile), and 30 minutes (95th percentile). A total inhalation exposure of 12 minutes is therefore relatively low. An upper bound estimate would be 30 minutes or more. Also the CalTOX estimates have no "missing mass."

CalTOX considers exposure throughout the house related to dispersion of the shower vapors throughout the house, as well as from the many other uses of water. Our discussion has been updated to note this, in agreement with the comment. It should be noted that the Leq/day has a rather modest effect on the calculation, compared to other variables and choices.

Comment 11: **"**I suggest the document include a short generic appendix (which might be used in other PHG documents) describing the general multi-stage model and meaning of q_1*. … Absent this explanation, the document is unintelligible to a reader not versed in the standard way that OEHHA estimates carcinogenic potency."

Response 11: We agree that the cancer potency calculation is difficult for an inexperienced reader to follow. We strive to make the method descriptions as clear as possible, and to provide adequate reference to the voluminous supporting literature. With all due respect to this important observation, we have resisted adding extensive appendices on methods which are more cogently described in the original literature. We

also should note that the cancer potency calculation in this document uses a time-to-tumor model and not the simpler multistage model.

Comment 12: "On page 23 the quantity LED_{10} was described as the "lower-bound of the dose associated with a 10% cancer risk," and on page 24 it is described as "the lowest estimate of the lower bound on the dose causing a 10 percent tumor incidence…." My impression is that the LED_{10} is a lower confidence limit (perhaps the lower 95% confidence limit) on the daily dose causing an excess cancer risk of 10%. Because the LED_{10} is a statistical estimate it should be precisely defined."

Response 12: We agree. The text was modified and the LED_{10} is now more precisely and consistently defined.

Comment 13: "However, I question why the LED_{10} quantity was used in the first place… [T]he PHG document should explain why the cancer slope factor is based on the LED_{10} value rather than the q_1* estimate."

Response 13: In accordance with the U.S. EPA (2005a) Cancer Guidelines, the default procedure used by OEHHA (and U.S. EPA) was changed several years ago to use models to generate dose-response relationships within the observable range of the study (generally 5 to 10 percent tumor incidence). From a point of departure, generally the lower 95 percent confidence limit on the dose associated with a extra tumor incidence of 5 or 10 percent (at the lower end of the observable range), the dose associated with 10^{-6} extra risk is derived by drawing a straight line to the origin (no risk at zero dose). This method is believed to produce slightly less model-dependent potency estimates than a direct extrapolation from q_1* (without a fixed point of departure).

Comment 14: "[I]t would be more useful for Table 1 to list K_{aw} = 0.013 rather than H = 3.17×10^{-4} m^3·atm/mol."

Response 14: Both forms of the Henry's law constant are now provided in Table 1.

Comments from I.H. Suffet, University of California, Los Angeles

Comment 1: "The pesticides that produce 1,2,3-Trichloropropane should be named."

Response 1: The name of the pesticide was added.

Comment 2: "Why the calculation of PHG should be based on 4L per day should be explained. It is not intuitive."

Response 2: Additional explanation has been added.

Comment 3: P. 6/7, para 1. "Sentence 2 makes no sense. Clarify – If you sacrifice an animal, how can you collect samples of excreta at various times."

Response 3: Sentence was rewritten for clarification.

Comment 4: "If I interpret this correctly, the second sentence should read – TCP, (upon injection) was …….. in the liver was > 70% in the initial tissues. Instead of larger the aforementioned tissues."

Response 4: Sentence was rewritten to clarify. Actually, the comparison between amounts in liver and other tissues was at four hours. Basically, TCP redistributes to the liver.

Comment 5: "P. 6/7, Para 3 end of paragraph should have a reference or is it also from Vope *et al*., 1984?"

Response 5: The end of the paragraph is still referring to Volp *et al*., 1984. This has been clarified by repeating the citation.

Comment 6: *Minor modifications were suggested to the metabolism description on pp. 7-9.*

Response 6. The text was modified correspondingly.

Comment 7: *Acute toxicity.* "Is not sentence 2 a subacute toxicity and does it belong in the section below?"

Response 7: The sentence was moved to the subchronic toxicity section.

Comment 8: *Acute toxicity.* "Pg 11, Para 2. Male and female.… Isn't this about acute effects?"

Response 8: Acute study generally means one dose or short exposure duration (e.g., four hours). The animals described here were exposed for four weeks.

Comment 9: "Toxicological Effect on Humans. P. 10 Acute toxicity. Are these subacute effects? Acute effects means death to me? Is this correct?"

Response 9: Acute describes exposure duration, not effects. Acute = 1 dose or very short (4 hours) exposure period. The changes resulting from acute exposures may be immediate or delayed (respiratory, neurotoxicity, etc.).

Comment 10: *Evidence for Carcinogenicity – Animal studies.* "A reference or a Figure that shows the increase in tumors relationship to dose is recommended to be included for clarity."

Response 10: Reference added.

Comment 11: "The only other major critical information that might affect public health is a better understanding of residence time in groundwater that would indicate site cleanup of the sources of TCP."

Response 11: While the introductory sections of the PHG documents provide a short summary of how exposure to an agent can occur, the documents are focused on developing health-based criteria for toxicants found in domestic water supplies. PHGs can be useful in focusing environmental cleanups of soil and groundwater to target levels of toxicants in groundwater that when used as a source of domestic water will not present a significant risk to human health. How the cleanups should be engineered (which may involve site-specific environmental half-life estimates) is beyond the scope of PHG documents.

Comments from Sapphire Group and Murray & Associates (Nov 24, 2007)

Comment 1: *Point of contact tumors.* "Because many of these rodent tumor sites lack human tissue homologues, they are not relevant to humans and hence are not useful for quantitative dose-response assessment. … Although the forestomach was one of the only identified target organs identified in both rats and mice, forestomach tumors are not relevant to human health for two reasons pertaining to (1) biology and (2) mode of action."

Response 1: The relevance of forestomach tumors is discussed in detail by IARC (2003):

> "The precise underlying mechanism of action for any forestomach carcinogen is at present not fully known. Nevertheless, most genotoxic forestomach carcinogens appear to act through a mode of action involving genetic changes in oncogenes and tumour suppressor genes. Non-DNA reactive agents such as butylated hydroxyanisole appear to cause forestomach tumours primarily through initial cytotoxicity and subsequent sustained cell proliferation and hyperplasia.
>
> While humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and the upper two-thirds of the oesophagus. Thus, in principle, carcinogens targeting the forestomach squamous epithelium in rodents are relevant for humans. Also, the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodents may target a different tissue in humans. Furthermore, tumorigenic effects in the forestomach are usually accompanied by similar effects in other tissues, indicating that there may be either general (e.g., genotoxic or receptor interactive) or multiple modes of action. However, the relevance for humans is probably limited for agents that have no demonstrable genotoxicity and that are solely

carcinogenic for the forestomach squamous epithelium in rodents after oral administration, since the exposure conditions are quite different between the experimental animals and humans. Consequently, for these agents, the mode of carcinogenic action could be specific to the experimental animals."

The clear genotoxicity of this chemical in multiple organs means that tumors in all organs should be considered. Concordance (increased tumors at the same site in different species) is not a critical factor in determining that a chemical is a carcinogen. In this case, the strength of evidence of carcinogenicity is enhanced by the observation of statistically significant increases in tumors in a number of organs in mice and rats, many of which were not concordant between the two species.

Comment 2: "With respect to mode-of-action, the forestomach typically achieves high concentrations of the chemical following gavage, which occurs over an extended period of exposure time since it serves as a storage organ – and no such condition occurs in humans. Indeed, the contact time of tissues in the upper digestive tract is so brief as to have no impact on the tumor formation or incidence."

Response 2: The stomachs in humans and rodents serve similar functions, to temporarily store food and to continue digestion. The rate that food passes from the stomach into the intestine in humans is often quite slow, depending on the content and volume of the meal. The rate of gastric emptying in both humans and rodents is retarded by fats in the meal. The strong association of rates of gastric cancer in humans with dietary constituents (Liu and Russell, 2008) does not support the above inference that dietary carcinogens would "have no impact on the tumor formation or incidence."

Comment 3: "In several cases these two factors [*unique anatomy and increased contact time*] combine to result in forestomach hyperplasia and inflammation (as observed in TCP-exposed rodents), which in turn contribute to the carcinogenic process through a tumor promotional mechanism."

Response 3: In the NTP (1993) bioassay, inflammation, irritation or necrosis were minimal or absent. Hyperplasia can result from direct genotoxic action, rather than be secondary to inflammation, and is generally considered a precursor to tumors. TCP is active as a genotoxic agent *in vivo* and *in vitro*.

Comment 4: "Reliance on mouse forestomach tumors (rather than systemic tumors in rats), as did OEHHA, in the derivation of its PHG results in an overstatement of cancer potency of about 1,000-fold, indicating that an equally protective PHG could be about 700 ppt or 1000-fold higher than at 0.7 ppt proposed PHG."

Response 4: TCP administration resulted in tumors in multiple organs in both male and female mice and rats. When developing health-based criteria, public health agencies routinely select the data set from the most sensitive species and sex if multiple data sets (of sufficient quality) are available. In addition, when tumors are observed at more than one site, the site with the highest incidence of tumors or which yields the highest cancer

potency is generally selected. This is because of the variability of effects in humans and the mandate to protect sensitive human populations.

Selection of the most sensitive species, study, and tumor site is recommended in the OEHHA Air Toxics Hot Spots Program Risk Assessment Guidelines (OEHHA, 2002) and in California's Guideline for Chemical Carcinogen Risk Assessments and Their Scientific Rationale (CDHS, 1985). It is mandated in the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) and is the default approach of the U.S. EPA (2004, 2005a). For this case, the high potency and lethality of this multiple-site, genotoxic carcinogen indicates to us that it would be prudent to choose the tissue with the greatest response. The U.S. EPA (2007) cancer potency factor derived from combined rat tumors was 4 $(mg/kg\text{-}day)^{-1}$, whereas the OEHHA potency was 24 $(mg/kg\text{-}day)^{-1}$ in the reviewed draft, so there appears to be only about a 6-fold difference, not a 1000-fold potency difference by the two approaches.

Comment 5: "Similarly, the World Health Organization (2003) reported, in the context of TCP, that 'it is not known how relevant forestomach tumours after gavage administration in the rats are for human risk.' In addition, the U.S. Presidential/ Congressional Commission on Risk Assessment and Risk Management (1997) also stated that 'Another tumor response that is believed to be irrelevant to humans is that which occurs only in the rodent forestomach after administration of a chemical by gavage.' Furthermore, USEPA in its recently released supporting documentation for its IRIS values for TCP (USEPA, 2007) rejected reliance on the mouse tumors for the quantification of risk and of safe levels of exposure."

Response 5: IARC, the agency within the WHO specifically mandated to evaluate cancer research, summarized the relevance of rodent forestomach tumors as follows (IARC, 2003): "In evaluating the relevance of the induction of forestomach tumours in rodents for human cancer the exposure conditions in the experiments have to be considered. The exposure conditions during oral administration are unusual (particularly if gavage dosing is employed) in that physical effects may result in high local concentrations of test substances in the forestomach and prolonged exposure of the epithelial tissue. Such factors may contribute to responses that may be unique for the forestomach. Nevertheless, carcinogens that are DNA-reactive and cause forestomach tumours in rodents — even if they only caused tumours at this site — should be evaluated as if they presented a carcinogenic hazard to humans." (Underline added for emphasis.) We think it is clear that TCP meets the IARC criterion.

The U.S. EPA (2007) draft review of TCP did not use the mouse forestomach tumor data for the potency extrapolation because 100 percent tumor incidence makes it difficult to do dose-response, not because they thought the tumors were irrelevant. However, the time-to-tumor information within the mouse data set does provide an indication of relative potency across the dose range, which has been incorporated into the derivation we presented. U.S. EPA in choosing to use the rat data acknowledged the uncertainty introduced by choosing a response with an apparent lower tumor potency.

Comment 6: "Tumors of the oral cavity were the only other point-of-contact tumors from TCP administered by corn oil gavage, and they were reported in rats and to lesser degree in mice (NTP, 1991). … Furthermore, the residence time and concentration of TCP on the oral mucosa of humans would likely be too brief to achieve a carcinogenic impact on this tissue; therefore, these tumors may not be relevant to humans."

Response 6: A number of well-known carcinogens in human and animal studies cause point of contact tumors. Cigarette smoke, asbestos, formaldehyde, soot, n-nitroso-dimethylamine, n-nitroso-diethylamine and benzo(a)pyrene produce point of contact tumors. The occurrence of these tumors at the point of contact is a big concern. Estimates of the potencies of these agents absolutely consider tumors at the point of contact, the lung or GI tract.

Given that the dose in the 1993 NTP study was administered by gavage, little point of contact exposure would be expected in the oral cavity. Any contact in the oral cavity would also be expected to have been very brief in the rodent, but they did develop oral tumors. Brief exposures in the rodent that resulted in tumors of the oral cavity in the NTP 1993 bioassay should be a concern for human risk.

It is unclear whether systemically absorbed TCP could have been responsible for the tumorigenic response observed in the oral cavity or forestomach. Tumors occurred at a number of sites distal from the point of absorption, some in tissues that were in direct contact with ingested food and water, and some that were not.

Comment 7: *Systemic Tumors.* "Many of the systemic tumor sites also lack human tissue homologues, and, therefore, are not useful for quantitative dose-response assessment because they are not considered relevant to humans. Concordance of target tissues is important because of tissue specificity for both metabolism of TCP to a proximate carcinogen … and sensitivity of tissues to the concentration of the carcinogen."

Response 7: Although there are many differences between rodents and humans, there is nothing to suggest that TCP is acting via a species-specific or organ-specific mechanism that does not operate in humans. As indicated in the preamble to the IARC monographs:

> "All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (e.g., aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio *et al.*, 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals … also present a carcinogenic hazard to humans. **Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in**

animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (IARC, 2006).**"** **[**Bold added.]

Concerning tissue site concordance, we agree with the U.S. EPA (2005a) guidance, which states:

> "Site concordance of tumor effects between animals and humans should be considered in each case. Thus far, there is evidence that growth control mechanisms at the level of the cell are homologous among mammals, but there is no evidence that these mechanisms are site concordant. Moreover, agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans. On the other hand, certain modes of action with consequences for particular tissue sites (e.g., disruption of thyroid function) may lead to an anticipation of site concordance."

Comment 8: *Corn oil gavage exposures to TCP are likely to overstate the PHG based on cancer risk when compared to drinking water exposures. In the cases of chloroform and 1,2-dichloroethane,* "...high dose administration in corn oil produced tumors, whereas administration in tap water produced no compound-related tumors."

Response 8: As indicated in the response to the second commenter's third comment, the vehicle often can influence toxicity, and it is unclear how the vehicle affected the tumor incidence in the 1993 NTP study. It is also unclear whether a corn oil vehicle is inappropriate. In any regard, administration of water-insoluble chemicals at high doses requires a vehicle such as corn oil that can solubilize the chemical. In some cases, apparent vehicle-related differences in response are likely to be due to differences in the amount of compound that was administered (a dose-related effect).

Comment 9: "The role of exposure vehicle (drinking water vs. oil gavage) in producing DNA adducts and cell proliferation in B6C3F1 mice has also been addressed for TCP (La et al., 1996). …Corn oil gavage exposures to TCP increased DNA adduct formation in the kidney and liver but not via drinking water exposure. Whereas drinking water exposures to 6 mg/kg-day TCP failed to increase cell proliferation in forestomach, glandular stomach, kidney and liver, corn oil gavage exposures to the same dose level produced significant increases in all four tissues."

Response 9: As mentioned earlier (see first commenter, response to comments 15 and 16), tissue adduct levels or the occurrence of tissue hyperplasia were not parallel to the occurrence of tumors in those tissues. As indicated in the PHG text, some other mechanism appears to be at work.

Comment 10: "Consistent with these data, WHO (2003) concluded for TCP: 'Marked differences in toxicity have been reported between rats administered 1,2,3-trichloropropane in drinking water ... and rats exposed by gavage.'"

Response 10: Other than adverse effects associated with the occurrence of tumors, there was little toxicity observed in the animals in the 1993 NTP study. In general, the vehicle can have an important role in the pharmacological and toxicological actions of agents, sometime increasing toxicity and at other times decreasing toxicity. A number of medications (nonsteroidal anti-inflammatory agents, iron, certain antibiotics, AIDS medications) that are directly irritating to the stomach are taken with food (which also retards gastric emptying) to prevent stomach irritation. Thus the use of corn oil may be protective of the stomach and forestomach. Irritation in the forestomach was not observed in the 1993 NTP study.

Comment 11: "In the PHG documentation, information regarding the mode-of-action by which TCP produces tumors in rodents is limited to a very brief paragraph … with emphasis placed on genotoxicity … [*implying that*] TCP caused cancer in animals by first most likely producing adducts to DNA… [*However*] OEHHA's PHG … recognizes that adduct formation appeared to <u>not</u> be "predictive of tumor formation." Regrettably, in other parts of the PHG documentation OEHHA [*leaves out*] this important caveat. That omission may well lead to a misunderstanding by DHS and other readers."

Response 11: Very little is known about the mechanism of action of TCP. From the available genotoxicity studies; *in vitro* mutagenicity studies and *in vivo* studies where DNA adducts were detected, OEHHA found compelling evidence that TCP is genotoxic. However, we acknowledged that understanding of the causation of cancer is incomplete, pointing out the interesting finding that the pattern of adducts and cell proliferation in tissues was not consistent with the sites with statistically significant increases in tumors. Further advances in cancer research may eventually provide a better understanding of the pathogenesis of the TCP-induced cancer and hopefully allow a more complete and accurate discussion of the findings. In the meantime, we have attempted to provide a clear discussion of the available data.

Comment 12: "[I]f OEHHA's underlying assumption that DNA adduct formation is a necessary and obligatory step in TCP-induced cancer is incorrect, then the methods used by OEHHA to estimate a PHG for TCP (*e.g.*, linear low-dose extrapolation model) will likely produce a PHG that is overly stringent, a situation that should be communicated to DHS in the PHG documentation."

Response 12: The Risk Characterization section of the PHG acknowledges the uncertainties in the cancer risk assessment, which are basically the same (with regard to low-dose extrapolation) as in every other cancer potency calculation.

Comment 13: "No empirical genotoxicity data in any *in vivo* studies have been shown to be either necessary steps in the formation of tumors or correlated with the presence of tumors. … OEHHA correctly noted that, for TCP, factors other than adduct formation

appear to be involved in tumorigenesis. Nonetheless OEHHA did not attempt to describe a PHG based on a non-genotoxic mode of action as a means of demonstrating to DHS a PHG based on an equally plausible assumption of nonlinearity." *Later*, "…a non-linear low-dose extrapolation procedure … as the most scientifically justified, needs to be applied to correctly define the very low-end of the dose-response range at which PHGs are set."

Response 13: We strongly disagree that nonlinearity is equally plausible or in any way justified in this risk assessment. Our statement about other factors involved in tumorigenesis was meant to imply that there appear to be *additional* factors, not to negate the relevance of the observed DNA adducts and other genotoxicity endpoints. Because of the positive genotoxicity studies and the high incidences of tumors observed without precursor tissue damage indicative of cytotoxicity and repair, TCP appears to play the role of a genotoxic carcinogen. Cancer guidelines require assessing such a chemical by the default linear extrapolation method. We see no useful purpose in making a calculation based on a mechanism or assumption that is not supported by the available evidence or any reasonable presumption.

Comment 14: "Inclusion of TCP metabolism and kinetics in estimating cancer potency provides a more scientifically defensible basis to establish a PHG for TCP. … In liver homogenates from rats and humans, TCP is metabolized by microsomal enzymes (P-450 family) to 1,3-dichloroacetone, a reactive substance which is suspected by OEHHA and others of being the intermediate responsible for carcinogenicity in rodents.… According to IARC (1995) the same studies demonstrate that 1,3-dichloroacetone is generated at a rate ten times faster by rat microsomes than by human microsomes (IARC, 1995) suggesting that humans may well be less susceptible, by as much as 10-fold, to the carcinogenic influence of TCP than rats."

Response 14: As indicated in the PHG document, TCP metabolism is complicated, involving several pathways and the microsomal mono-oxygenases and glutathione mediated metabolism. Which metabolite is the proximate carcinogen is unclear. Microsomal metabolism rates *in vitro* in humans and rodents may have little to do with rates of metabolism *in vivo*.

How differences in TCP metabolism in rodents and humans could affect estimates of risk is purely speculative. As discussed in the PHG text, inducers of P450 resulted in reduced adduct levels in rat and, therefore, more TCP metabolism by the monooxygenases in the rat could translate into an underestimate of human risk.

Comment 15: "Another consideration not incorporated into the PHG is derived from the observation that as TCP is metabolized by the microsomal enzymes of humans and rats, the reactive metabolites were covalently bound to microsomal proteins. This type of binding is expected to reduce the bioavailability of the reactive species in the cell, in effect becoming a form of detoxification."

Response 15: We agree that increased binding to microsomal proteins in the rat and mouse could result in less binding of reactive metabolites to DNA. However, there are

too many uncertainties regarding macromolecular binding to support a risk estimate based on or corrected for binding in the rat and mouse, compared to humans.

Comment 16: "The proposed PHG is based on allometric scaling of administered dose (body weight raised to the ¾ power) as the dose measure used to estimate the cancer potency of TCP. However, this practice is inconsistent with the mode-of-action described. … Consequently, the appropriate interspecies (rat to human) adjustment of TCP dose and metabolites is one, not the 0.75 power used by OEHHA, because for both rat and humans, metabolism is essentially complete."

Response 15: The mechanism by which TCP administration produced statistically significant increases in tumors in a number of organs in male and female rats and mice is unclear. TCP does not appear to be the proximate carcinogen; carcinogenicity appears to require metabolism to an active form. The complex metabolism of TCP (as shown in Figure 1 in the PHG document) involves the monooxygenase, cytochrome P450s and glutathione mediated metabolic pathways. Studies in the liver have shown that inducers of cytochrome P450 increase *in vitro* metabolism rates with a corresponding increase in binding to microsomal proteins. However, induction reduced the amount TCP binding to DNA. No information is available concerning TCP metabolism in other target tissues in the rat (tissues where tumors also occurred) and none in the mouse.

Given the uncertainty as to the proximate carcinogen(s) and how metabolism by various pathways affects the formation and disappearance of the proximate carcinogen(s), it is difficult to draw a conclusion regarding the most appropriate approach for dose scaling. OEHHA chose the conventional approach of scaling the dose in animals to humans based on the ratio of body weight to the ¾ power.

Comment 16: *In support of the OEHHA decision to use a time-to-tumor model,* "OEHHA states on page 22, 'Unfortunately the cause of death of individual animals was not reported.' This statement is misleading since NTP clearly states that for rats 'In most of these rats, the clinical findings and moribund condition were attributed to chemical-induced neoplasms of the oral mucosa or forestomach,'" *with a similar statement for mice.*

Response 16: The PHG document clearly indicates what NTP stated, that early deaths were primarily due to tumors in the forestomach. However, this information is not inconsistent with the statement in the PHG text that the cause of death of individual animals was not reported. The time-to-tumor model requires a determination (or assumption) of the cause of death of each animal, which was not reported by the NTP.

Comment 17: "There remains, however, a need to account for competing causes of death when assessing tumors other than forestomach. If the PHG document uses the time-to-response model from ToxRisk …, it should also assess all data sets using the best fitting model from USEPA's Benchmark Dose Software (BMDS, version 1.4.1) and applying the poly-3 adjustment of Portier and Bailer (1989) to address early mortality."

Response 17: As indicated in the PHG text, the time-to-tumor model was employed because both the time that the tumors were observed and the incidence of tumors can be used to establish a dose-response relationship. The models in the BMDS package do not consider the time that the tumors were observed in developing a dose-response relationship, and we are not familiar enough with the Portier and Bailer approach to adequately assess its applicability in this case. However, we did double-check the calculations with another time-to-tumor package (developed at OEHHA) which gave essentially identical responses.

Comment 18: "Consistent with the use of the non-linear Benchmark model, the NAS/NRC in its latest report on dioxin (NRC, 2006) recommended to USEPA the presentation of both linear and non-linear low-dose extrapolations for a carcinogen which has mutagenic properties but is unlikely to be eliciting tumors via that genotoxic mode-of-action as is the case for TCP."

Response 18: There is very strong evidence that TCP is genotoxic and appears to act via a genotoxic mechanism of action.

Comment 19: "Moreover, the point of departure (POD) selected by OEHHA for its low-dose extrapolation is inappropriate for many of the data sets assessed. OEHHA adopted a response rate of 10% in identifying a point-of-departure for all tumor types. Although the use of the default ED10 and its 95% lower confidence limit might appear to be appropriate for some tumor types (e.g., tumors of the pancreas and mammary gland), it is inappropriate for several of the tumor types used in the calculations because of the 100 % response provides no information as to the dose-response."

Response 19: Point of departure is defined by U.S. EPA (2005a) as "an estimated dose (expressed in human equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses." Also from U.S. EPA (2005b), "An excess risk of 10% is the default BMR, since the 10% response is at or near the limit of sensitivity in most cancer bioassays and in some noncancer bioassays as well. If a study has greater than usual sensitivity, then a lower BMR can be used, although the ED10 and LED10 should always be presented for comparison purposes." The POD of 10 percent incidence of forestomach or liver tumors was well within the observable range in the 2003 NTP study, when including the earlier sacrifice times, for the time-to-tumor model.

Comment 20: "OEHHA provided no data to justify its assumption [*of a 4 L/day total equivalent intake*], and merely cited the use of the CALTOX model simulations to support this assumption. However, neither are the details of the assumptions and parameter values used in the CALTOX simulations presented in the PHG document nor are TCP parameter values included in the latest version of CALTOX available from OEHHA's website. Without these details, the validity and accuracy of OEHHA's calculations cannot be assessed.…"

Response 20: The parameters used to run the CalTOX model are now presented in the text. No added assumptions were involved in running the model. Documentation on CalTOX is available at: http://www.dtsc.ca.gov/AssessingRisk/ctox_model.cfm.

Comment 21: "The Poor Quality of The TCP Cancer Studies for Estimating Safe Levels of Exposure (PHG) in Tap Water Needs to be Fully Articulated - The cancers studies of TCP in rats and mice have an admittedly high (in some cases 100%) incidence of early mortality, much of which is due to point-of-contact tumors. While such finding may be helpful at screening substances for the presence of carcinogenic ability regardless of dose, they are generally regarded as of little of no use to characterize the cancer potency in the test species, much less estimate dose-response relationships at far lower dose ranges which humans may experience."

Response 21: Production of a very high incidence of tumors in a cancer bioassay in both sexes of both species and at multiple sites does not indicate poor quality of the bioassay. It indicates that the agent is a highly potent carcinogen. OEHHA acknowledges and describes in the discussion the uncertainty that this high incidence brings to the potency calculation. As previously discussed, point-of-contact tumors associated with other chemicals or agents, such as cigarette smoking, soot, asbestos, benzo(a)pyrene, and nitrosamines, are a major concern, and have been used in deriving health-protective levels.

Comment 22: "A major limitation of these and similar studies is that the experimental doses exceed the "maximum tolerated doses" (MTD). For decades, authoritative bodies have recognized that to be of any value in characterizing cancer dose-response, lifetime studies need to be conducted at doses that do not overwhelm the physiological (including defense systems) capacity of the test subjects. … The NTP cancer studies do not meet this criterion."

Response 22: There is no indication that the MTD was exceeded in this study. The animals tolerated exposure to TCP quite well, with little indication of toxicity that was unrelated to the occurrence of tumors. Only when tumors occurred did the animals became ill and die (at high doses, quite prematurely). The appearance of the tumors is a major concern and provides the basis for the proposed PHG.

Comment 23: "The Cumulative Impact of Changing Multiple Parts of PHG Derivation" *together could raise the PHG by as much as 1,000-fold* "and still achieve full protection of public health."

Response 23. OEHHA agrees that if multiple changes were made to the approach used to derive the PHG that the changes would have a large cumulative impact. However, we do not feel it is appropriate to display a matrix of criteria based on various combinations of possible changes rather than using the standard risk assessment assumptions to derive a health-protective PHG. Other approaches would not meet OEHHA's mandates: "If the contaminant is a carcinogen or other substance that may cause chronic disease, the public health goal shall be set at the level that, based on currently available data, does not pose

any significant risk to health," and "If [OEHHA] finds that currently available scientific data are insufficient to determine the level of a contaminant at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety, or the level that poses no significant risk to public health, the public health goal shall be set at a level that is protective of public health, with an adequate margin of safety" (California Health and Safety Code Section 116365(c) (B) and (D).

Comments from Sapphire Group and Murray & Associates (March 9, 2009)

Comment 1: "*CRITERION 1: PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.*

OEHHA is not proposing to estimate a PHG for TCP based on acute toxicity, but only on toxicity from chronic (*i.e.*, repeated and prolonged) exposure. Hence, this criterion is not applicable to this estimated PHG. Consequently, the section "Toxicology: Acute Toxicity" and other sections related to acute toxicity should be removed as that information provides no substantive value to the derivation of the chronic PHG for TCP."

Response 1: The PHG document for TCP contains one sentence describing the acute toxicity of TCP in order to address the cited section of the California Health and Safety Code. These data indicate that TCP is acutely toxic but only at high exposure levels. Therefore the PHG is not based on an acute toxic endpoint but is based on protection from an effect that occurs at much lower levels of exposure, namely cancer. It also means that the PHG provides protection from acute toxicity.

Comment 2: "Tumors, particularly those of the rodent forestomach, attributed to TCP exposure are not all relevant to humans. We note that Dr. David Eastmond (one of OEHHA's peer reviewers) also concluded that reliance on forestomach tumors "does not seem biologically appropriate." In response, OEHHA has argued that the mode-of-action of rodent forestomach carcinogenesis is unknown but likely to be genotoxic. OEHHA fails to recognize that forestomach rodent (point-of-contact) tumors are the consequence of concentration and contact time, are associated with the corn oil vehicle, and as a result have no bearing on modes-of-action at human doses thousands of times lower than those experienced in rodent screening bioassays."

Response 2: The relevance of forestomach tumors has been well addressed by IARC, as discussed above in several responses to comments. IARC concluded that forestomach tumors are "biologically appropriate" to use in evaluation of genotoxic carcinogens. Given that conclusion, the default exposure and risk extrapolation methods are necessary, because more specific data on parameters such as concentration versus effect and contact time are not available for a more specific comparison between humans and the test species.

Comment 3: "Consideration of the mode-of-cancer-action of TCP justifies scientifically defensible increases in OEHHA's proposed PHG. OEHHA argues that TCP must be acting via a genotoxic mode-of-action. While we agree that the "mechanism-of action" is unknown ["mode" and "mechanism" are significantly different, as OEHHA must be aware], OEHHA provides no evidence to demonstrate that genotoxicity must be the sole or major mode-of-action. No empirical genotoxicity data in any *in vivo* studies have been shown to be either necessary steps in the formation of tumors or correlated with the presence of tumors. The evidence for genotoxicity is restricted to *in vitro* studies of high TCP concentrations, mostly of non-mammalian cells exposed to nonphysiological concentrations, and where the findings were at times inconsistent. By contrast, non-genotoxic mode(s)-of-action for TCP carcinogenic effects in rodents is important to consider for the relevant systemic tumors. The information supporting this interpretation is that: TCP, when administered by corn oil gavage to male B6C3F1 mice, has been shown to increase cell proliferation rates (BrdU Labeling Index, LI), while the same daily dose given in drinking water produced no such effect. Mice were given TCP by gavage or drinking water (6 mg/kg-d for 10 days over two weeks). LI in forestomach of gavage-treated mice were roughly 3-fold higher than controls at both 18 and 30 hours after the last gavage dose of TCP (La *et al.*, 1996). These findings are consistent with the greater cytotoxicity observed for TCP when administered by gavage (NTP, 1993) vs. drinking water (Villeneuve *et al.*, 1985)."

Response 3: We reiterate, as stated in the PHG document, that TCP tested positive in both *in vivo* and *in vitro* genotoxicity studies. *In vivo* studies revealed single strand chromosome breaks in rat liver and kidney and formation of DNA adducts in both rats and mice. Given the genotoxicity of TCP, a linear dose-response relationship (no threshold assumption) is appropriate to assume for PHG development. OEHHA did not state and does not agree that "genotoxicity must be the sole or major mode-of-action" for TCP or for evaluation of any carcinogen by the default linear extrapolation.

OEHHA discussed the finding of the La *et al.* (1996) study and concluded that the proliferation response did not appear to be consistent with the occurrence of tumors in a tissue. As stated in the TCP PHG document: "Statistically significant increases in cell proliferation were not observed in any of the four tissues when 1,2,3-TCP was administered in drinking water. In contrast, statistically significant increased cell proliferation was observed in all four tissues when 1,2,3-TCP was administered in corn oil. The changes in adduct formation and cell proliferation occurred in tissues with a tumorigenic response in the NTP bioassay (liver and forestomach) as well as tissues where no response was detected (glandular stomach and kidney). In this study, neither adduct formation nor cell proliferation appeared to be predictive of the tumorigenic response observed in the NTP bioassay."

No irritation or necrosis was observed/reported in the stomach or forestomach of male or female rats or mice at 8 or 17 weeks. No hyperplasia was observed/reported in the stomach or forestomach of male or female rats at 8 or 17 weeks. NTP reported "focal hyperplasia of the stratified squamous epithelium of the forestomach" in some dosed rats at 15 months and at sacrifice in the two year study. Dose-related increases in the incidence of hyperplasia were observed in male mice, while the incidence of hyperplasia was only markedly increased in high-dose female mice at final sacrifice. No notable

irritation, inflammation or necrosis was reported at 15 months or final sacrifice in male or female rats or mice.

Comment 4: "Because of evidence for a non-genotoxic mode-of-action, a non-linear low-dose extrapolation procedure (described elsewhere in these comments), as the most scientifically justified, needs to be applied to correctly define the very low-end of the dose-response range at which PHGs are set. The dose-response model that should be used for TCP is the Benchmark Dose approach employing uncertainty factors and not the linear time-to-response model employed in OEHHA's draft. OEHHA's current draft continues to use the time-to-tumor model for low-dose extrapolation without having compared the applicability and fits of other models."

Response 4: OEHHA does not agree with the statement that "a non-linear low-dose extrapolation procedure … as the most scientifically justified, needs to be applied…." OEHHA follows standard risk assessment guidelines in applying low-dose linear extrapolation to genotoxic carcinogens. 1,2,3-TCP was positive in a number of *in vivo* and *in vitro* genotoxicity studies, and it would be inappropriate to treat this chemical with methods developed for non-genotoxic chemicals. A linear approach (no assumed threshold for effect) is the proper method for developing a dose-response relationship for carcinogenic effects for TCP.

Comment 5: "OEHHA's current draft continues to use the time-to-tumor model for low-dose extrapolation without having compared the applicability and fits of other models. Dr. David Eastmond (one of OEHHA's peer reviewers) is strongly concerned about the suitability of the time to-tumor model because of its relative instability (*i.e.*, minor assumptions can change potency estimates by 100-fold). We found no indication of any attempts by OEHHA to test alternate models through conventional curve-fitting. OEHHA's draft expresses strong certainty (little or no uncertainty) that its model produces correct estimates without the benefit of a full qualitative (biological) and quantitative uncertainty assessment. Consequently, the resulting proposed PHG is unreliable and needlessly conservative."

Response 5: OEHHA explored in the PHG document how various assumptions in the time-to-tumor model affected the estimates of risk. Only one assumption, assuming all tumors were incidental to the death of the animals, had a major impact on the potency estimate. Given that NTP reported that "neoplasm of the forestomach in rats and mice, … were the principal cause of death of most animals dying or killed moribund before the end of the studies," the assumption that all tumors were incidental to the cause of death (the one assumption that did have a major effect on the estimated potency) appears to be problematic, and therefore this approach was not used. No other assumption within this model had a major effect on the potency estimate.

OEHHA employed a time-to-tumor model because of the high early mortality in the study which resulted from the very high incidence of tumors in certain tissues. This is discussed in the PHG. A potency estimate based on the multistage model was provided in the PHG document for comparative purposes. This model was considered not very

satisfactory because the 100 percent tumor incidence in several tissues by the end of the study limited the dose-response characterization.

Comment 6: *Consideration of additive effects.* "Our analysis found no evidence to indicate that TCP in food or air would add to the body burdens of TCP from drinking water largely because of its relatively rapid elimination from the body particularly in the dose range found in drinking water. OEHHA's draft does not mention the presence of any potential for additive effects of exposure to TCP in media other than drinking water and resulting body burden. However, the OEHHA draft seeks to define a relative source contribution (RSC) for TCP in water, and speculates that the contribution from drinking water is 20% of the total without providing evidence for the presence of TCP in foods or air in the breathing zone. Such a situation supports an RSC no less than 80%, which would increase the PHG by a factor of 4."

Response 6: We agree with the basic premise of this comment. Levels of TCP in air appear to be very low and based on Henry's constant, TCP in food would be expected to be fleeting. For this reason, the relative source contribution (RSC) used for non-cancer risk estimation has been set at 0.8 for all drafts. The cancer risk estimate does not use an RSC because it is based on extra risk from drinking water alone.

Comment 7: "It is noteworthy that the OEHHA draft fails to present relevant drinking water data to substantiate its case for human exposure to TCP in drinking water. Missing are (1) average values (related to degree of safety unlike peak values which are not related to chronic toxicity), (2) prevalence of positive findings (which are related body burdens; the lower the prevalence, the lower the body burdens), and (3) distributions of average daily doses across the population (needed to estimate safe levels of exposure)."

Response 7. OEHHA presented the available data regarding the level of TCP in sources of drinking water in California. Average levels, prevalence, and distribution of TCP in water supplies are not needed to develop a PHG, a value that is protective of the human population.

Comment 8: "Our detailed analysis indicates that the evidence is sufficient to estimate a safe level of TCP in drinking water; and our evaluation concludes that the safe concentration in drinking water should be in the range of 0.7 and 1.0 ppb. However, our analysis stipulates that some evidence relied upon in the OEHHA draft is not suitable to estimate safe levels in drinking water (see our comments of 24 November 2007) of exposure. Specifically, in the cancer studies of TCP in rats and mice, the experimental doses exceed the "maximum tolerated dose" (MTD), rendering them of no practical value in estimating safety or risks to humans health"

Response 8: Yes, this is comment 22 in the previous set of comments from Sapphire Group and Murray & Associates. Our response remains the same. MTD refers to frank toxic effects other than development of tumors. The NTP report makes clear that effects other than tumors were minor. NTP reported for the rat: "Of the clinical findings, none were considered to be directly related to organ toxicity other than those associated with

chemical-induced neoplasms of the oral mucosa, forestomach or mammary glands." For mice NTP reported "no clinical findings were considered to be directly related to organ toxicity other than those associated with chemical-induced neoplasms."

Comment 9: "Our comprehensive analysis of the relevant data on TCP indicates that a nonlinear dose-response consistent with a threshold region is compatible with the data on the chronic toxicity and carcinogenicity of TCP, and that recognition should lead to the application of the Benchmark Dose methodology with the use of justified uncertainty factors to identify the threshold region for human exposure to TCP. OEHHA's draft treats TCP as a no-threshold toxicant and employs a linear extrapolation method to estimate risks at low doses. Our analysis shows that this approach is supported only weakly. However, the OEHHA documentation should at least present both perspectives to permit those who set drinking water standards to understand to the merits of each."

Response 9: Cancer risk assessment guidelines require the use of linear extrapolation for genotoxic carcinogens. The evidence is very strong that 1,2-3-TCP is a genotoxic carcinogen. While the commenters had a clear rationale for arguing that the forestomach tumors are point-of-contact tumors, that is not equivalent to evidence that the effects therefore occur by a threshold mechanism. OEHHA is not aware of any evidence that this is the case.

OEHHA may show a range of potencies based on various credible choices, but does not provide calculations for all possible interpretations of the data. Given the strong evidence of genotoxicity, a linear dose-response relationship (i.e., no threshold of effect) was employed to calculate the PHG for TCP, and OEHHA recommends that this value and approach be used in developing regulatory values.

Comments from John Peter Wargo, Yale University (March 7, 2009)

Comment 1: "The methods OEHHA used to estimate the cancer potency factor or "q*" for TCP, appear to be reasonable, given the available data. OEHHA might have used a model would weight early-life exposures higher than laterlife exposures, particularly given the findings of genotoxicity, mutagenicity, and carcinogenicity. This consideration would have increased the Office's estimate of cancer risk, when exposure occurs *in utero* or in childhood when organ systems and tissues are reproducing most rapidly. Methods were suggested by Murdoch, Krewski and Wargo in 1992 to account for early in life exposures in cancer risk assessments.9"

Response 1: OEHHA is engaged in addressing early childhood exposure to carcinogens and will soon begin to address this issue in the development of PHGs and other health-based criteria.

Comment 2: "The OEHHA used appropriate methods to estimate human exposure to the compound. I agree with the Office's estimate that the majority of exposure is derived

from ingestion, and this is of special concern due to tumor development within the gastrointestinal tracks and nasal cavities of test animals."

Response 2: We agree; no change needed.

Comment 3: "I agree with OEHHA's opinion that the evidence of forestomach tumors is relevant for human risk assessment, given the similarity of cells found in the human stomach and esophagus. The International Agency for Research on Cancer evaluated and supported the relevance of rodent forestomach tumors to human cancer risks. Huff also noted the relevance of forestomach tumors in animal studies to estimate human cancer risk, given the similarity of animal and human cells. Huff also noted that among nearly 400 animal carcinogenicity studies performed by NCI/NTP by 1992, only 7 caused a neoplastic response in the forestomach in 4 experiments, including TCP, DBCP, and 1,3-D (positive in three of three experiments). The National Toxicology Program in its Eleventh Annual Report on Carcinogens relied in part upon forestomach tumor induction (found in both sexes of rats and mice following a 2 year feeding studies) to reach its conclusion that TCP is "reasonably anticipated to be a human carcinogen.""

Response 3: OEHHA concurs.

Comment 4: *For the non-cancer calculation,* "OEHHA's calculation of a health protective concentration for TCP is reasonable, and appropriately relied on a 1,000 fold safety factor to account for the use of subchronic toxicity data to predict chronic effects (10x), interspecies differences (10x), and human variability (10x). The estimated Acceptable Daily Dose of 5.7 mg/kg-day is also reasonable, based upon available data."

Response 4: OEHHA concurs, but wishes to note the typo. Acceptable Daily Dose is stated as 5.7 microgram/kg-day, not mg/kg-day.

Comment 5: "The OEHHA calculated a health-protective concentration for TCP resulting in a proposed limit of 0.0007 ug/L or 0.0007 ppb based upon cancer risk derived from a linear cancer extrapolation model. Given currently available evidence this limit based upon carcinogenic outcomes would be protective against other adverse health effects."

Response 5: OEHHA concurs.

Comment 6: The commenter pointed out that low-dose cancer risks may be higher than estimated, quoting a 2003 WHO report which stated: "Considering the very high incidences of forestomach neoplasms in low-dose groups of rats (33–66%) and mice (nearly 100%), this carcinogenic activity might have been detected even at lower doses, and the LOAELs for significantly increased tumour incidences will be well below 3 mg/kg body weight per day in rats and 6 mg/kg body weight in mice."

Response 6: We appreciate the point, and accept this as a possibility. However, no change was made in the document in response to this comment.

Comment 7: "The State of California's Safe Drinking Water Act of 1996 (Health and Safety Code 116365) requires the establishment of a Public Health Goal that meets the following criteria specified in your Draft PHG released on February 6, 2009:

a. 'PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.'

Comment: Given the uncertainty that arises from the inability to identify the LOAEL with confidence described … above, as well as the absence of testing for numerous endpoints including developmental neurotoxicity and immunotoxicity, it is entirely possible that the margin of safety suggested by OEHHA for non-cancer health effects is insufficient to protect health."

Response 7: We agree that the paucity of non-cancer toxicity data makes the establishment of a non-cancer health-protective level quite uncertain. The 1,000-fold uncertainty factor based on the available data addresses this lack of information. However, for regulatory purposes, attention should be focused on the PHG, based on the carcinogenic effects of TCP, at a much lower level.

Comment 8: '"To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.'

Comment: OEHHA should quantitatively consider the potential for additive and synergistic effects associated with chemicals that are structurally similar, that produce similar types of tumors at similar sites, and that tend to co-occur in water supplies."

Response 8: The PHG for TCP is based on exposure to TCP in water contributing an extra cancer risk of 10^{-6} above and beyond the risk of cancer from other sources and other chemicals. Dibromochloropropane is a similar compound that could potentially interact with TCP in some way. However, we have no specific information on this which could be used to help inform the risk assessment, nor any data on their co-occurrence. There is also no information that other substances potentiate or diminish the cancer risk associated with exposure to TCP.

Comment 9: '"OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.'

Comment: I find the consideration of the potential for heightened susceptibility, exposure, and subsequent cancer risk to fetuses, infants, and children to be limited in the Draft PHG. Given the plausibility of these conditions, and the attention given to many currently registered pesticides by EPA pursuant to the Food Quality Protection Act of 1996, OEHHA might consider a more direct discussion of data adequacy, and its implications for meeting the "ample margin of safety" requirement of the California Health and Safety Code (HSC 116350 et seq.)."

Response 9: Detailed discussions of the potential susceptibility of children, infants and other potentially sensitive subpopulation can be found elsewhere in recent OEHHA and U.S. EPA documents. We note this factor in the risk characterization section of the PHG document, but could find no information related to any subpopulation that would likely be more sensitive to TCP exposure.

Comment 10: "While California OEHHA did not include data on reproductive toxicity, a National Toxicology study that found "clear evidence that TCP at 120 mg/kg is a reproductive toxicant in Swiss mice in the presence of mild systemic toxicity". The study noted "significant" reproductive toxicity in Swiss CD-1 mice exposed to TCP expressed in fewer litters and fewer pups per litter in first generation mice."

Response 10: The OEHHA review of reproductive toxicity in this PHG document includes a discussion of this NTP study (Chapin *et al*., 1997).

REFERENCES

Albrecht (1987). Central nervous system toxicity of some common environmental residues in the mouse. J Toxicol Environ Health 21:405-21.

ATSDR (1992). Toxicological profile for 1,2,3-Trichloropropane. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Atlanta, GA, Sept 1992.

CDHS (1985). Guidelines for Chemical Carcinogen Risk Assessments and Their Scientific Rationale, Sacramento, CA**.**

Chapin R, Gulati D, Mounce R, Russell S, Poonacha K (1997). Trichloropropane. Environ Health Perspect 105(S1)1-2. NTIS #PB91129676. Accessed at: http://ehp.niehs.nih.gov/members/1997/Suppl-1/dfa184.html.

IARC (2003). Predictive Value of Rodent Forestomach and Gastric Neuroendocrine Tumours in Evaluating Carcinogenic Risk to Humans. IARC Technical Publication No 39, International Agency for Research on Cancer, Lyon, France.

IARC (2006). Preamble to the IARC Monographs, accessed 9/19/2008 at http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf. International Agency for Research on Cancer, Lyon, France.

Ito N, La D, Holt S, Craft TR, Sills RC, Swenberg JA (1996). Analysis of ras mutations in forestomach tumors from B6C3F1 mice exposed to 1,2,3-trichloropropane. 87th Annual Meeting of the American Assoc. for Cancer Research, Washington, DC, April 20-24, 1996. Proc Am Assoc Canc Res 37:137.

Jo WK, Kwon KD, Dong JI, Chung Y (2005). Multi-route trihalomethane exposure in households using municipal tap water treated with chlorine or ozone-chlorine. Sci Total Environ 339(1-3):143-52.

La DK, Lilly PD, Anderegg RJ, Swenberg JA (1995). DNA adduct formation in B6C3F1 mice and Fischer-344 rats exposed to 1,2,3-trichloropropane. Carcinogenesis 16(6):1419-24.

La DK, Schoonhoven R, Ito N, Swenberg JA (1996). The effects of exposure route on DNA adduct formation and cellular proliferation by 1,2,3-trichloropropane. Toxicol Appl Pharmacol 140(1):108-14.

La DK, Swenberg JA (1997). The induction of endogenous DNA adducts in tissues of Fischer 344 rats following gavage administration of 1,2,3-trichloropropane. Proc Amer Assoc Canc Res 38:131.

Liu C, Russell RM (2008). Nutrition and gastric cancer risk: an update. Nutr Rev 66(5):237-49.

NTP (1993). Toxicology and carcinogenesis of 1,2,3-trichloropropane (CAS No. 96-18-4) in F344/N Rats and B6C3F1 Mice (gavage studies). Technical Report Series No 384. NIH publication No. 94-2839. National Toxicology Program. Research Triangle Park, NC. 348 pp.

OEHHA (1999). Public Health Goal for 1,2-Dibromo-3-chloropropane (DBCP) in Drinking Water. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

OEHHA (2002). Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II Technical Support Document for Describing Available Cancer Potency Factors. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

Tancrede M, Yanagisawa Y, Wilson R (1992). Volatilization of volatile organic compounds from showers – I. Analytical method and quantitative assessment. Atmospheric Environ 26A:1103-11.

U.S. EPA (1997). Exposure Factors Handbook. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC. EPA/600/P-95/002Fc.

U.S. EPA (2004). An Examination of EPA Risk Assessment Principles and Practices. U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

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

U.S. EPA (2005b). Benchmark dose Technical Guidance Document. External Review Draft, Risk Assessment Forum. U.S. Environmental Protection Agency, Washington, DC. http://www.epa.gov/ncea/pdfs/bmds/BMD-External_10_13_2000.pdf.

U.S. EPA (2007). Toxicological Review of 1,2,3-Trichloropropane (CAS No. 96-18-4). In Support of Summary Information on the Integrated Risk Information System (IRIS), October 2007. U.S. Environmental Protection Agency, Washington DC. NCEA-S-1669.

U.S. Inter-Agency Regulatory Liaison Group (IRLG) (1979). Scientific basis for identification of potential carcinogens and estimation of risk. J Nat Canc Inst 63:241-68.

Tsang AM, Klepeis NE (1996). Results tables from a detailed analysis of the National Human Activity Pattern Survey (NHAPS) response. Draft Report prepared for the US EPA by Lockheed Martin, Contract No. 68-W6-001, delivery order No. 13.

Vainio H, Wilbourn JD, Sasco AJ, Partensky C, Gaudin N, Heseltine E, Eragne I (1995). [Identification of human carcinogenic risk in IARC Monographs.] Bull Canc 82:339-48 (in French).

Weber GL, Sipes IG (1990). Covalent interactions of 1,2,3-trichloropropane with hepatic macromolecules: studies in the male F-344 rat. Toxicol Appl Pharmacol 104(3):395-402.

Weber GL, Sipes IG (1992). In vitro metabolism and bioactivation of 1,2,3-trichloropropane. Toxicol Appl Pharmacol 113(1):152-8.

Wilbourn J, Haroun L, Heseltine E, Kaldor J, Partensky C, Vainio H (1986). Response of experimental animals to human carcinogens: an analysis based upon the IARC Monographs Programme. Carcinogenesis 7:1853-63.