Responses to Major Comments on Technical Support Document

Public Health Goal
For
Styrene
In Drinking Water

Prepared by

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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TABLE OF CONTENTS

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 styrene. The first group of comments, from University of California peer reviewers, is based on the pre-release review draft. Changes were made in response to these comments, and were incorporated into the draft posted on the OEHHA website on May 30, 2008. Further comments are based on that first posted draft and on the second draft posted February 5, 2010. For the sake of brevity, we have selected the more important or representative comments for responses.

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 on first review draft, March 2006

Comments from University of Southern California (Dr. Landolph)

Comment 1: "In general, this document represents a very large amount of effort on the part of six authors with support and review from another six scientists. The literature review on the toxicity and carcinogenicity of styrene is very comprehensive, if not exhaustive, and should be commended. The writing of the document is very clear in most places. The calculations of the PHGs for the toxicity and the carcinogencity of styrene are appropriately transparent and use conventional methodologies. The calculated PHG for non-cancer toxicity of 4 ppb should be fully protective against the non-cancer toxic effects of styrene. The calculated PHG of 0.5 ppb deriving from the carcinogenicity of styrene is appropriate and should be health-protective for the public at the risk of 10^{-6} (one in a million). Overall, this is an excellent document. The Summary and the section on calculation of the PHG values for toxicity and cancer are commendably clear and concise. The scientific literature review is very comprehensive, particularly the sections on toxicity, carcinogenicity in animals and humans, and especially the sections on metabolism and pharmacokinetics."

Response 1: Comment noted and appreciated.

Comment 2: "In a few places, as I have outlined in the attached review containing specific comments, the authors should consider some slight modifications to the document to improve its quality from very strong to excellent. In a number of places, simply moving sections of the document to different places would make the document flow more logically and thereby make it easier to read and comprehend quickly. In a few places, some sections can be condensed to reduce the wordiness of the document, which would make it more concise and more interesting for the reader."

Response 2: Many of the suggested changes were made, including reorganizing the Summary by rearranging the paragraph sequence, moving text in other places, adding short text in places suggested by the reviewer for clarity, and condensing the text in various places.

Comments from University of California, Davis (Dr. Witschi)

Comment 1: "This is a very and thorough review of the toxicity of styrene. The voluminous literature is well represented and well covered. The document conveys a large and important body of information.

I want to make a special comment: the chapter on Genetic Toxicology (pages 37 through 71) is truly outstanding and was a great pleasure to read. I really appreciated the thoughtful introduction to genetic toxicology, the discussion of its purpose and limitations and, above all, the short introductory pieces to the different assays, such as for

example “Cytogenetic Alterations” on page 38 or “DNA Strand Breaks” on page 50. They admirably review the pertinent information and manage to refresh the rusty memory or to add some new insights for someone who is not specialized in genetic toxicology. This chapter is an outstanding work of thoughtful and well informed scholarship and the authors have to be congratulated.”

Response 1: Comment noted and appreciated.

Comment 2: “I wish that the chapter dealing with cancer potency, particularly the cancer potency estimates from human data, would make for similar easy reading. There is no doubt that the models that were used are well outlined and discussed. However, I found it difficult how to deal efficiently with the wealth of data presented in the tables. For example, in table 35 (page 203), unit risk (or potency) for pancreatic cancer or malignant lymphoma and derived from human data is presented in a familiar format. Later however, several tables which report human potency numbers from animal data do not seem to provide the same information, unless I miss something (see, for example, table 49, page 219).”

Response 2: The lack of potency units in Tables 48 to 51 and supporting text has been corrected in the revised technical support document.

Comment 3: “A summary table of ‘selected’ cancer potency estimates (page 221, Table 52) gives data in a form similar to table 35 (mg/kg-d), but with scaling factors that make direct comparison not exactly easy. And why present only selected data –if so, what were the criteria for selection?”

Response 3: The scaling factors are used to adjust animal values to a human equivalent so values in the same units are by definition comparable at some level. The “selected” refers to the estimates, not the data, and refers to cancer potency factors which are most relevant for the risk assessment. All relevant data sets analyzed are included in Table 52.

Comment 4: “It would be of great interest, given the wealth of both human and animal data, to compare estimates derived from human data with those derived from animal data.”

Response 4: Table 60 directly compares the animal based potency values and derived health protective drinking water concentrations for styrene with the best human data set, that of Kogevinas *et al.* (1994) for malignant lymphoma by inhalation. The best animal-based cancer potency values are quite similar to the best human value, i.e., 0.028 and 0.017 $(mg/kg\text{-}d)^{-1}$, respectively.

Comment 5: “P.146/147: why different mortality rates (PMR, Obs/Exp, and SMR). Could they be “normalized”, i.e. given always in the same unit, such as SMR, or is the information provided in the individual papers not sufficient to allow doing this?”

Response 5: PMR (proportional mortality ratio) and SMR (standardized mortality ratio) are different measures of effect. SMR is actually calculated as observed/expected and is calculated by the "indirect" method of standardization. The results of SMR and PMR cannot be "normalized" because they are based on different kinds of information. PMR is proportionate mortality and is calculated as the proportion of deaths in a population due to a specific disease in the exposed, which is compared with the proportion of deaths due to that disease in the unexposed population. Usually the authors present results based on what kind of information they have, which can differ among studies. No change to the document was made in response to this question.

Comments from University of California, Riverside (Dr. Eastmond)

Comment 1: "The information presented appeared to be accurate in the majority of the areas that I checked. I did notice a number of mostly minor errors which are listed in the technical comments section below."

Response 1: The various minor errors were corrected as needed.

Comment 2: "[T]here are several recent reports and review articles that, probably due to their relatively recent publication, are not cited in the document. However, these appear to come to very different conclusions in evaluating the experimental data and the significance of the results seen. This should be investigated in more detail as it may reflect an underlying difference in the evaluation of the data or a bias in its interpretation either by OEHHA or the other groups. Perhaps of most concern are what appear to be major differences in the conclusions on developmental and reproductive effects of styrene presented in this document and those recently published by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction."

Response 2: In the revised PHG document, we have acknowledged the differences of opinion about the reproductive and developmental toxicity studies expressed in the recent NTP report (NTP, 2006). However, because no reproductive or developmental studies or endpoints were used in calculation of non-cancer (or cancer) health-protective levels, these differences in conclusions have no influence on the PHG value.

Comment 3: "Recent reviews on genotoxic effects in humans and animals (Henderson and Speit, 2005; Nestmann *et al*., 2005) also seem to have reached substantially different conclusions. In addition, at least one important article that could significantly influence the interpretation of the cancer bioassay data seems to have been missed (Cruzan *et al*., 2002)."

"While there have been multiple reports that styrene can exhibit genotoxic effects, I do not believe that the evidence that this occurs *in vivo* is as strong or consistent as portrayed in the conclusions of the document. The largely negative results seen at high styrene doses in the majority of the chromosome aberration and micronucleus animal bioassays are a serious concern. The positive results seen in the SCE assays *in vivo* may be more

relevant as a biomarker of exposure or a measure of an efficient repair of adducts than as a biomarker of effect."

Response 3: The two reviews of genotoxicity sponsored by the Styrene Steering Committee (SSC) of the European Chemical Industry Council (CEFIC) (Henderson and Speit, 2005; Speit and Henderson, 2005) were reviewed as well as that by Nestmann *et al.* (2005), members of a Canadian consulting firm. Henderson and Speit (2005) reviewed the genotoxicity of styrene in humans, downgrading nine studies (of a total of 15), considered by the original authors (and accepted by the peer reviewers of the studies and the editor of the peer-reviewed journal) to be positive, from positive to inconclusive or negative. As a result of their reanalysis Henderson and Speit (2005) decided that the genotoxicity evidence was weak. We do not agree with the downgrading of these studies, nor with their conclusion. Nestmann *et al*. concluded that styrene, through metabolism to styrene oxide, could be clastogenic in humans at workplace levels greater than 125 mg/m^3 (29 ppm). However, they also note that animals exposed only to styrene do not show clastogenic effects up to 1500 mg/m^3 (350 ppm). The greater genetic heterogeneity in the human population than in inbred laboratory animal strains could explain the finding of an apparent higher human sensitivity to clastogenesis. The study by Cruzan *et al.* (2002) is now discussed in the PHG document.

Comment 4: "For deriving a PHG from an animal study, the Cruzan mouse study would seem to be the best study for reasons outlined in the document. However, additional information should be provided such as a justification for using the combined mouse benign and malignant lung tumor data, historical control frequencies for the combined tumors, etc. In the IARC monograph, it states that the historical control range for the female mouse bronchiolo-alveolar carcinomas was 0-13.5% for the breeder's database based on nine oral studies. This should be confirmed. If correct, the 14% incidence reported for the styrene-treated female mice is just outside of the historical range and lessens the biological significance of the observed increases."

Response 4: Notwithstanding the importance of historical control rates for specific tumors, the concurrent controls are the most important for dose-response assessment. In the Cruzan *et al.* (2001) inhalation study the concurrent control rates for bronchioloalveolar carcinoma were 8 percent in males and 0 percent in females. In our view this study is adequate and appropriate to serve a key role in the risk assessment of styrene. No change was made in response to this comment.

Comment 5: "The described risk assessment used a variety of current approaches including PBPK modeling, the benchmark dose methods, and the most recent EPA low dose extrapolation method. However, it does not appear that the metabolic differences between mice and humans described in the Cruzan *et al.* (2002) paper have been considered. These should be evaluated as they may significantly alter the PBPK modeling and the low dose extrapolation. Similarly, based on the material in the document, it would appear that styrene requires metabolic activation to exert its toxic and carcinogenic effects and that the bioactivation in humans would appear to occur at a significantly lower rate than that in mice or rats. Yet, this information does not appear to

have been considered in the modeling. Indeed, the standard defaults that were used indicate that humans are much more sensitive than rodents."

Response 5: The Cruzan *et al*. (2002) report hypothesizes that the R-styrene oxide metabolite of styrene produced in the mouse lung's Clara cells by CYP 2F2 is primarily responsible for the species differences observed, i.e., the lack of lung cancer in styrene-exposed rats and potentially in humans. However, subsequent findings by Hoffmann *et al*. (2006) show that the metabolism of styrene to styrene oxide is only about 2-fold higher in mice than in rats and that the interspecies difference in lung burden of styrene oxide is insufficient to conclude that styrene oxide generation is the likely cause of the observed lung tumors. These authors postulate an indirect mode of action wherein styrene metabolism to styrene oxide and 4-vinylphenol causes disruption in cellular glutathione homeostasis, apoptosis and cell proliferation in the bronchi and terminal bronchioles. Our assessment uses styrene oxide as a surrogate for styrene metabolites generally and does not identify a specific mode of action for styrene-induced carcinogenicity.

Comment 6: "It is not clear why scaling was based on a 0.025 kg mouse as the mice in the Cruzan study weighed significantly more (Page 219)."

Response 6: Error noted. The potencies have been recalculated and inserted in the relevant tables.

Comment 7: "While commonly presented, some aspects of the metabolic schemes shown in Figure 1 seem unlikely to me. For example, the direct conversion from mandelic acid to benzoic acid would be more likely to proceed through multiple reactions or through a different pathway. The same would seem to be the case for the conversion from phenylaceturic acid to hippuric acid. I believe the schemes presented by Johanson *et al*. (2000) to be more likely. Since there are multiple steps between the glutathione conjugates and the mercapturic acid conjugates, I would represent these reactions with broken arrows."

Response 7: Figure 1 is a general metabolic scheme showing the relations between a number of styrene metabolites and is not meant to catalog all metabolic intermediates or the number of steps between them. This has been clarified in the text.

Comments on first posted draft, May 2008.

Comments from Styrene Information and Research Center (SIRC) Sept 13, 2008

From the SIRC cover letter:

Comment 1: "[O]ur review of the document indicates that it excludes much of the newest styrene science, and we therefore are pleased to provide OEHHA with our detailed scientific assessment of the document, including recommended additional

references that SIRC believes significantly contribute to the scientific database for styrene."

Response 1: Yes, our review of the mechanistic literature needed updating. We have evaluated the additional references cited by SIRC, and have included the most relevant ones.

Comment 2: "The draft styrene PHG is not based on sound principles of science or toxicology."

Response 2: A Public Health Goal (PHG) is the level in drinking water at which a contaminant will (a) cause no known or anticipated adverse effect on human health, plus a margin of safety, if the contaminant is acutely toxic; or (b) pose no significant risk to health, if it is a carcinogen or otherwise causes chronic disease (California Health and Safety Code 116365(c)). The draft PHG document discusses and evaluates a large number of toxic effects associated with styrene, favoring public health where there is uncertainty. Differences of opinion as to interpretation of the toxic effects reported for styrene are substantial, as represented by the similarly unfavorable SIRC comments (SIRC, 2008) on the National Toxicology Program (NTP) Report on Carcinogens Background Document for Styrene (NTP, 2008a). NTP's evaluation is consistent with OEHHA in that there is significant concern for potential carcinogenicity of styrene. The NTP's Styrene Expert Panel in its meeting on July 21-22, 2008 recommended (NTP, 2008b; see http://ntp.niehs.nih.gov/go/29682) that styrene be listed in the next NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen."

Comment 3: "Real effects are repeatable by different investigators; effects found in isolated studies, but not in others, are probably artificial."

Response 3: The meager animal cancer database for styrene was one reason for the inhalation studies in CD-1 mice and SD rats. The studies found no lung tumors in rats (Cruzan *et al.*, 1998) but found increased lung adenomas and adenocarcinomas in male and female mice (Cruzan *et al.*, 2001). The results corroborated earlier results of alveolar/bronchiolar adenomas and adenocarcinomas in male B6C3F1 mice by NCI (1979) and with male and female O20 mice by Ponomarkov and Tomatis (1978). These latter investigators found that strain C57Bl mice did not develop lung tumors in response to styrene, but the other three studies showed positive results with a dose-response. Thus we have three positive studies, by two routes in one sex in one study and both sexes in the other two. OEHHA concludes that, overall, there is sufficient evidence that styrene causes cancer in animals and limited evidence in humans. For these reasons, it is prudent to assume carcinogenicity for the purposes of risk assessment.

Comment 4: "The dose makes the poison. Especially for mammary tumors in female rats, the PHG document cites increased incidences in one study at relatively low doses as evidence of a styrene effect and seeks to invent reasons why decreased mammary tumors at higher doses should be ignored."

Response 4: Non-linear dose-response curves are not unusual, in the context of specific toxic effects predominating at particular dose ranges. The reasons for such effects are frequently uncertain. If one study shows a significant increase in mammary tumors (especially at low doses) and a second study does not, or reveals a decrease at very high levels, the increase should not necessarily be ignored. In this case increased tumor rates have been confirmed in other studies, in other tissues. OEHHA concludes that the weight of evidence supports an increased carcinogenic risk from exposure to styrene. The PHG must favor public health where there is uncertainty.

Comment 5: "The epidemiology data are evaluated to make a case for cancer. Data from human epidemiology studies are selected to support a conclusion of carcinogenicity without examining consistency or evidence against an effect. As pointed out in the comments by independent epidemiologists, there is no consistency of response."

Response 5: OEHHA has found suggestive but not definitive evidence of cancer in the human cancer data. The excess malignancies observed are most frequently of the lymphatic and hematopoietic systems. OEHHA concurs with the IARC (2002) finding of "limited" evidence of carcinogenicity for styrene in humans and the recent recommendation of an NTP expert panel (NTP, 2008a,b) that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals." We believe these statements accurately represent the scope of the epidemiology evidence.

Comment 6: "The evidence in the animal studies is overstated. Data from selected rat studies are given extra weight and countervailing studies are dismissed by speculation to assert increased mammary tumors in rats. Overall, the IARC, EU, Harvard, and ATSDR concluded there was no evidence of cancer in rats exposed to styrene."

Response 6: Our analysis found a significant, dose-related increase in mammary tumors in female Sprague-Dawley rats in the study of Beliles *et al.* (1985) by pairwise comparison and by trend test after exposure to styrene in drinking water. We also found a significant increase in malignant mammary tumors and in combined malignant and benign mammary tumors in female Sprague-Dawley rats by both pairwise comparisons and trend test in the study of Conti *et al.* (1988), after chronic inhalation exposure. There was also a statistically significant increase in mammary tumors in the lower of two exposure groups in female Sprague-Dawley rats in the study of Jersey *et al*. (1978), in which interpretation of results is weakened by concurrent infections, low survival in the high-dose group, and an unusually low incidence in the controls. In the study of Cruzan *et al.* (1998), there was a statistically significant *decrease* in mammary adenocarcinomas in female Sprague-Dawley rats over a range of inhalation exposure concentrations (50, 200, 500 and 1000 ppm), complicated by an unusually high incidence of tumors in the controls. These observations of significant changes in incidences of mammary tumors in all four studies are described very similarly by NTP (2008a).

We concluded that female rats have developed increased mammary tumors after both drinking water and inhalation exposures. However, these results in female rats were not used in the calculation of a health-protective level for styrene in drinking water. We do not agree that reporting that increases in mammary tumors have been observed is an overstatement of the results.

Comment 7: "Data from two oral mouse studies that provide limited or equivocal evidence of lung tumors are given the status of clear evidence. The Panomarkov and Tomatis study so exceeded the MTD that dosing was stopped after 16 weeks; this does not provide a meaningful assessment of carcinogenicity at non-MTD doses. NCI asserted that there was "suggestive evidence" of increased lung tumors in male mice; the PHG document treats this as definitive evidence. IARC, Harvard and the EU concluded there was limited evidence of carcinogenicity in mice."

Response 7: The multiple studies reporting increased lung tumors in male and female mice, by both gavage (Ponomarkov and Tomatis, 1978; NCI, 1979) and inhalation (Cruzan *et al*., 2001), do provide definitive evidence, in our opinion, that styrene causes increased lung tumors in mice. This conclusion is supported by ample evidence of genotoxicity and formation of DNA adducts *in vivo* and *in vitro*, in both humans and experimental animals. IARC (2002) rates styrene as group 2B, possibly carcinogenic to humans, based on the limited mouse data plus several lines of supporting evidence, including the carcinogenicity of styrene oxide, a styrene metabolite. An NTP expert panel (NTP, 2008b) recently recommended that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals."

Comment 8: "The document asserts that styrene lung toxicity occurs in terminal bronchioles and tumors originate in alveolar cells, based on staining of normal cells. Recent evidence indicates that bronchiolar Clara cells lose their ability to secrete the normal CC10 early in the carcinogenic process and one cannot determine cell of origin by secretion of CC10 or SPC. … Mode of action data are evaluated based on the hypothesis that all effects of styrene are related to styrene oxide, despite evidence to the contrary. A data supported mode of action for mouse lung tumors is not even discussed. The last 4 years of research on mouse lung tumor MOA are not included in the document. Metabolism of styrene (and related compounds) occurs by cyp2f2 in mouse lung and nasal tissue. This produces novel cytotoxic metabolites (not styrene oxide). Humans are not able to carry out this metabolism to any biologically significant extent. Therefore, mouse lung tumors produced by this MOA are not relevant to human risk assessment."

Response 8: The PHG document has been updated to include descriptions of the more recent mouse studies. However, these interesting studies related to molecular mechanisms of tumor development in mouse bronchiolar Clara cells do not provide definitive information on the possible toxic mechanisms in humans. The PHG document discusses interactions of styrene with various tissues, and several hypotheses for mode(s) of action. Tissue concordance for tumor development is not assumed across species, and

the evidence of genotoxic interactions of styrene with various cell types in humans is not based on interactions with lung Clara cells.

Comment 9: "SIRC believes that the PHG should not be based on cancer endpoint in animals or humans. Further, the PHG for non-cancer endpoints should not be based on nasal or lung toxicity in mice because the MOA is not-relevant for humans. The PHG should be based on avoidance of neurobehavioral effects from styrene and, as stated on p. 224, this would be 100 ppb, the same as the US EPA MCL."

Response 9: OEHHA believes that the evidence of carcinogenicity of styrene in animals, the suggestive evidence of increased tumor rates in humans from multiple epidemiology studies, and the ample evidence of genotoxicity of styrene in experimental animals and humans, *in vivo* and *in vitro*, justify the health-protective assumption that styrene may have carcinogenic effects in humans. We also do not agree with the commenter that the mouse toxic effects, which provide the lowest NOAELs for styrene in animals, should be considered to be irrelevant for non-cancer risk assessment. Using the Benchmark approach, these data yield a health-protective concentration of 4 ppb.

From the detailed comments provided by SIRC:

Comment 10: "[T]he document is not consistent in its evaluation of data. The document often cites slight, non-significant numerical increases over control values as evidence of effect, but dismisses statistically significant decreases below control as irrelevant or develops hypotheses to dismiss them without evidence to support the hypothesis. The document needs to state up front the basis for data evaluation. For example, how much greater than control should a non-significant difference be to constitute evidence of effect? Is it 50%, 10%, 1% or 0.1%? The criteria need to be thought about and stated, not just applied on the spot and inconsistently."

Response 10: The risk assessment calculations are based exclusively on significant increases in toxic effects ($p < 0.05$). Fluctuations in response rates above and below controls are expected because of the stochastic nature of the process, but trends in one direction or another may be worth mentioning. Decreases in tumor rates can occur for a variety of reasons, but are not the basis of the risk assessment.

Comment 11: "The styrene PHG document starts from the premise that styrene is metabolized to styrene-7,8-oxide, which is a genotoxic carcinogen; therefore, styrene is carcinogenic. All data are weighed against this mantra and any data that disagree are discounted."

Response 11: Styrene has been well documented to be metabolized to styrene-7,8-oxide, which has been judged independently to be a genotoxic carcinogen, and these basic points do not seem to be disputed. The PHG document provides over 200 pages of detailed discussion of styrene itself, and the many lines of evidence concerning its toxicity. The document estimates a health-protective level for styrene based on the styrene toxicity studies, not from studies on styrene-7,8-oxide.

Comment 12: "The animal carcinogenicity section asserts that styrene causes increased mammary tumors in female rats by inhalation, but not by gavage. The main basis of this conclusion is a study reported by Conti *et al*., 1988 (p. 121). First, based on data quality, this study should not be included in the PHG because it is not published in the peer-reviewed literature. This study is not peer-reviewed (published in non-peer-reviewed journal) and does not meet data quality guidelines."

Response 12: The study was published in the Annals of the New York Academy of Sciences. This journal publishes presentations made to the Academy by speakers invited to address the academy on specific areas of interest. The peer review occurs up front and an editor assembles the presentations in a volume. This study appeared in Volume 534, Living in a Chemical World: Occupational and Environmental Significance of Industrial Carcinogens, edited by Maltoni and Selikoff. We consider this to be a credible source.

Comment 13: "The PHG notes Maltoni's initial report of this [Conti *et al*.] study (1978) as indicating that all animals were alive when the first mammary adenocarcinoma was observed. In that initial report, Dr. Maltoni indicated that the mammary tumors were not likely related to styrene exposure because the incidence of mammary tumors in Sprague-Dawley rats was high and variable."

Response 13: Comment noted. However, the final data show the lowest incidence of tumors in the control group. Several of the styrene-exposed groups show significantly elevated mammary tumor incidence relative to the control group by the Fisher Exact Test.

Comment 14: "[A]s stated by the NTP guidelines (McConnell *et al*., 1986) benign and malignant mammary tumors should not be combined. This comparison should be deleted from the PHG document."

Response 14: OEHHA practice follows the U.S. EPA (2005a) cancer guidelines, which state (p. 2-19) "The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately but may be combined when scientifically defensible (McConnell *et al*., 1986)." Our own peer-reviewed guidelines (OEHHA, 2009) state essentially the same. Combining benign and malignant tumors at the same site is judged to be scientifically defensible when the benign tumors are considered to have the potential to progress to the associated malignancies of the same histogenic origin. The Preamble to IARC monographs states, "When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation" (IARC, 2006). However, this seems somewhat a moot point since there were significant increases by

both pairwise and trend tests for malignant tumors alone as well as combined with benign tumors.

Comment 15: "The only study (Cruzan *et al*., 1998) that was performed using current study guidelines and good laboratory practices found no increase at similar doses to the Conti study and decreased malignant mammary tumors at higher doses. From a scientific standpoint these data should not be ignored."

Response 16: OEHHA has not ignored these data from a scientific standpoint. Both studies are described and the results contrasted. The existence of a negative study does not automatically invalidate a positive study, or vice-versa.

Comment 16: "The PHG (p. 120) reports increased % of female rats at 500 ppm with mammary fibroadenomas [in the study of Cruzan *et al*., 1998], based on those examined histopathologically. The appropriate denominator is the number of animals/group. Mammary tumors are almost never found microscopically if not seen at necropsy. The incidences should be 21/60, 16/60, 13/60, 18/60, 17/60. Although there is not a decreased trend with increasing dose, all treated groups had a lower incidence than the control group. The PHG asserts that this does not indicate a decreased response. If this approach is taken in the PHG for reduced values compared to control (i.e., dismiss if not statistically significant), then in other instances when there are higher values in treated (or exposed) groups that are not statistically significant, they should also be dismissed."

Response 16: OEHHA estimated the mammary fibroadenoma incidence on the number of animals at risk, based on the animals surviving until the occurrence of the first tumor. This is standard practice. Thus the incidences are 21/60 (35%), 16/44 (36%), 13/43 (30%), 18/38 (47%), and 17/59 (29%). The PHG document accurately describes the fibroadenomas as having no significant change at any dose, without ascribing any particular importance to the excursions in either direction. We agree that statistical significance is an appropriate criterion for evaluating the potential biological significance of effects.

Comment 17: "The PHG (p. 112, 113) reports increased mammary tumors (benign and malignant combined) in the drinking water study of Beliles *et al*., 1985. The authors of the drinking-water study reported no increase in mammary (or any other) tumors. In an analysis of this and other studies, Huff (1984) asserted that there was a statistically significant trend for increased mammary tumors if one combined the malignant and benign tumors (fibroadenoma, adenoma, adenocarcinoma) in females. According to the NTP publication on combining benign and malignant tumors for statistical analysis, McConnell *et al*. (1986) indicate that mammary fibroadenomas should not be combined with malignant mammary tumors unless a continuum has been demonstrated within a given study. No such continuum was demonstrated in the Beliles drinking water study. Therefore, combining them, as Huff did (1984) and as was done in the PHG draft is not appropriate and should be removed from the report."

Response 17: As stated in the response to Comment 14, combining tumors of similar derivation is recommended in several cancer guidelines, including our own. We agree that the level of detail provided by Beliles *et al.* is inadequate to ascertain what degree of continuum may have been exhibited in the mammary histopathology. In our view it makes more sense to combine these tumors rather than assume a lack of a continuum.

Comment 18: "The PHG document (p. 125) concludes that inhalation exposure to styrene resulted in increased mammary tumors. This is NOT supported by a weight of the evidence evaluation. Table 22 (p. 131) reports the female dose groups of females exposed to styrene by cumulative exposure. These values are similar to Table 8 of Cruzan *et al*., 1998. As shown in Table 8 of Cruzan *et al*. the only groups with increased mammary tumors were at the lowest end of the exposures."

Response 18: As discussed in the above comments and responses, there were statistically significant increases in mammary tumors in female rats after inhalation exposures. In OEHHA's view, as summarized on p. 125 of the draft, clear or suggestive evidence was seen in three of the four studies reviewed. We agree that observing increased tumors only at the lower end of the dose-response is relevant to interpretation of their toxicological significance, although in view of the differences in study methodology and dosimetry it is not surprising to see a lack of tumor site concordance in different studies. We see no need to discount these tumors as irrelevant, but agree that they should not be used in derivation of a health-protective value because there are better, clearer data for that purpose.

Comment 19: "The PHG cites individual studies reporting respiratory toxicity, nephrotoxicity, or hepatotoxicity in short term studies as interfering with styrene's ability to induce mammary tumors at higher doses. It MUST be recognized that none of these organs were adversely affected in any of the chronic studies, by gavage or inhalation. Thus this is not a likely explanation of increased mammary tumors in the Conti *et al*. study and not in the other studies."

Response 19: Various organ changes do occur in the studies cited by OEHHA, including significant induction of liver enzymes and depletion of lung glutathione. We agree that such effects do not provide a ready explanation for any dose-dependent variations in incidence of mammary tumors.

Comment 20: "The PHG further indicates that when styrene is administered by gavage most of it is metabolized in the liver and only low levels of styrene and styrene oxide reach the mammary tissue, as an explanation for increased mammary tumors by inhalation and not by gavage. The blood level of styrene and styrene oxide were measured at the end of a 6-hour exposure in the Cruzan *et al*. 1998 inhalation study. Similar measurement were not taken in any of the oral studies, but using the Sarangapani *et al*., 2002 PBPK model, the peak blood level of styrene and styrene oxide can be calculated. In the inhalation study, the blood levels of styrene were 9460 and 29,680 ng/ml in females exposed at 500 and 1000 ppm, respectively. The PBPK model predicts

peak styrene blood levels of 149,448, 321,048, and 707,616 ng/ml in females receiving gavage doses 5 days/week after 10 weeks of dosing at 500, 1000, and 2000 mg/kg/day, respectively. Clearly, gavage dosing in the NCI, 1979 study of styrene in rats resulted in much higher blood levels of styrene available to the mammary tissue than inhalation of styrene in any of the inhalation studies. Furthermore, the blood levels of styrene oxide (194, 200, and 202 ng/ml from 500, 1000, and 2000 mg/kg/day, respectively) exceeded the level from inhalation (92 and 152 ng/ml at 500 and 1000 ppm, respectively). Thus one cannot dismiss the negative tumor findings in the gavage studies on the basis that styrene and styrene oxide are not available to the mammary tissue from oral exposure."

Response 20: Styrene metabolism is saturable, and it induces its own metabolism at higher repeated doses as well as the activity of multiple hepatic P450s (Filser *et al*., 1993; Hirasawa *et al*., 2005). These effects complicate the PBPK modeling of effects of gavage (bolus) doses versus the continuous dosing in an inhalation exposure. Styrene levels will swing wildly in bolus dosing, but the variations in blood levels of the reactive metabolites are much more restricted. Nevertheless it is clear that styrene oxide is formed in significant amounts after gavage administration of styrene, and the reports of increased tumors after gavage administration are consistent with this. We agree that the negative results for mammary tumors in the study of Cruzan *et al*., 1998, cannot be ascribed to inadequate availability of styrene or styrene oxide on the basis of the available data.

Comment 21: "The findings of these studies need to be evaluated in the context of dose-response. Table 8 from Cruzan *et al*., 1998 evaluated all the individual dose groups from all the rat studies of styrene. It is clear from this table that the only dose groups suggesting increased mammary tumors were at the low end of the dose range and other studies did not find increased mammary tumors at similar doses. In the cited animal cancer bioassays, recent and comprehensive reviews by the International Agency for Research on Cancer (IARC), the European Union (EU), the Harvard Center of Risk Analysis, and the Agency for Toxic Substances and Disease Registry (ATSDR) have all concluded styrene does not increase cancer in rats. The PHG document should do the same."

Response 21: The PHG document accurately reports the results of studies which show statistically significant increases in mammary tumor rates in female rats, as well as the studies which show no increase or a decrease in mammary tumors. OEHHA's judgment that the increased mammary tumors are likely to represent a carcinogenic response in female rats (rather than a statistical artifact) rests on the weight of evidence, including multiple studies *in vitro* and *in vivo* showing indicators of carcinogenicity (genotoxicity, production of reactive metabolites, increased tumors in multiple organs).

Comment 22: "The PHG document describes the Ponomarkov and Tomatis, 1978 studies of styrene in O20 and C57Bl mice very thoroughly. However, it does not point out the limitations of these studies to evaluate carcinogenic effects of styrene in humans. In O20 mice, the MTD was severely exceeded. One can conclude that doses of styrene that result in death might increase cancer risk, but no conclusions about lower doses can be drawn. The C57 study used much lower doses and produced no increased cancers, but

used relative few animals. p. 108 Table 14 – should be O20 not C57Bl mice. Neither of these studies should be used in assessing the carcinogenic potential of styrene."

Response 22: The high-dose toxicity is noted in the study description. The caption of Table 14 has been corrected. We believe the limitations of these studies for risk assessment have been stated, and that the results of Ponomarkov and Tomatis are relevant to the styrene assessment although not appropriate for a cancer potency calculation.

Comment 23: "p. 124. The PHG cites the short duration of the Brunnemann *et al*, 1992 study in A/J mice as a reason to discount its lack of increased lung tumors. This is a screening assay for tumor initiation by genotoxic chemicals. The importance of the study is not as a complete carcinogenicity study, but as a genotoxicity study, especially in mouse lung."

Response 23: We agree.

Comment 24: "The PHG indicates (p. 117, 118) that staining of the bronchiolar regions for CC10, a product of normal Clara cells, indicates that the toxicity of styrene occurs in Clara cells, but that the lack of staining for CC10 in the lung tumors indicates they are of alveolar type II cell origin. Thus the document proposes that toxicity and tumorigenicity in mouse lung can be separated." *Various lines of evidence are presented that* "indicate that one cannot determine the cell of origin of mouse lung tumors based on immunohistochemical staining."

Comment 24: There is considerable uncertainty in the literature as to the cell type origin of mouse alveolar-bronchiolar tumors (see Hicks *et al*., 2003). We agree that CC10 may not be a suitable marker of tumor cell origin. We have added some text and references addressing this point to the discussion on pp. 118-119 and have edited the discussion on pp. 193-194 of the final PHG document.

Comment 25: "Beginning on p.184, the PHG document discusses direct action of styrene-7,8-oxide on DNA as the mode of action for mouse lung tumors. There is much evidence that argues against styrene oxide having role in these tumors that is not included in the PHG document. … An alternative MOA hypothesis has been proposed and should be included and discussed in the PHG document. Through this alternative MOA, CYP2F2 metabolizes styrene, and several structurally related compounds, to form unique metabolites that cause cytotoxicity in the terminal bronchioles of mice but not of humans. The resulting ongoing regenerative cell proliferation in mice leads to prolonged hyperplasia and eventually lung tumors that are specific to mice (Cruzan *et al*., 2002)."

Response 25: The discussion in the PHG document has been updated to cite the recent literature which provides more perspective on the mouse lung tumors, including papers by Carlson (2004a), Cruzan *et al*. (2005), Vogie *et al*. (2004), and Hoffmann *et al*. (2006). Considering the clear genotoxic effects of styrene metabolites and the multiple possible MOAs, differences in a specific isozyme in specific tissues between mice and humans provide an insufficient rationale to discount potential human carcinogenicity of

styrene. This risk assessment does not assume tumor site concordance between mice and humans.

Comment 26: "SIRC believes the Draft Document should be revised to consider fully the hypothesized alternative MOA for mouse lung tumors, and to review and evaluate the significant evidence supporting the alternative MOA for mice lung tumors, and to revise the Draft Document to conclude that these mouse-specific tumors provide insufficient evidence to support a conclusion that the Public Health Goal for styrene should be based on carcinogenicity."

Response 26: The revised PHG document provides an expanded discussion of the alternative hypothesized modes of action of styrene in producing lung tumors. OEHHA continues to believe that the weight of evidence supports a presumption of relevance of styrene carcinogenicity to humans. We also note that an NTP expert panel (NTP, 2008b) recently recommended that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals." The vote for this identification was eight to two, and the two members of the panel who voted against the motion are reported to have done so because, in their opinion, styrene should be listed as "known to be a human carcinogen" (NTP, 2008b).

Comment 27: "Given that the qualitative impacts of the proposed [mouse] MOA on tumor outcomes are not fully defined, quantitative differences between mice and humans must also be considered.… Given these species differences, the MOA is assumed to be plausible in humans, but humans are expected to be much less sensitive than mice to the pulmonary effects of these chemicals. Because rat lungs contain more cyp2f4 than human lungs contain CYP2F1 and rats do not develop cytotoxicity or lung tumors from these chemicals, it is very unlikely that any chemical that causes mouse lung tumors by this MOA and does not cause rat lung tumors will cause human lung tumors."

Response 27: OEHHA does not agree that the lung tumors demonstrated in mice are irrelevant to humans, considering that genotoxic metabolites can be formed in human as well as mouse lung. Multiple cytochrome P450s capable of metabolizing styrene are found in both human and mouse lung and other tissues, providing the potential for several specific pathways to reactive metabolites as well as considerable genetic variability among humans. In any regard, classification of chemicals as potential human carcinogens does not assume concordance of tumors in specific organs or tissues between species. Benzene, for example, causes leukemia in humans but tumors at multiple sites in rodents. The animal data, based on the most potent carcinogenic responses, were considered to provide the most appropriate quantitative extrapolation to negligible risk levels, as per standard cancer risk assessment practice (U.S. EPA, 2005a; OEHHA, 2009).

Comment 28: "Another mode of action that must be considered is direct genotoxicity. For styrene and divinylbenzene, mouse lung tumors are the only tumorigenic response in

rats or mice. For naphthalene, only lung tumors in mice and nasal tumors in rats (also high in CYP2F) were found. For the remaining chemicals of this group, other tumors were increased in mice and/or rats. Finding increased tumors in multiple organs and/or species leads to a suspicion of a genotoxic MOA."

Response 28: We agree with the basic point that a genotoxic mechanism of action should be considered as the cause of styrene carcinogenicity.

Comment 29: "Quantitiative Relevance of this Proposed Animal MOA for Humans. Whether the lung tumors are caused by genotoxic reactions or cytotoxic reactions of metabolites, neither will happen in humans unless there is metabolism by CYP2F1. Since human CYP2F1 is barely detectable and for the chemicals studied to date has very little metabolic activity, lung tumors are extremely unlikely in humans."

Response 29: OEHHA rejects the basic premise that this proposed mechanism of action of styrene is the only one worth considering. Since several cytochromes in lung, liver and other tissues can be involved with styrene metabolism, and exhibit genetic variability in humans (Gadberry *et al*., 1996; Bartsch *et al*., 1998; Pavanello and Clonfero, 2000; Carlson, 2003, 2004a; Norppa, 2004; Wenzlaff *et al*., 2005; Chung *et al*., 2006), this assumption is far too restrictive.

Comment 30: "The PHG erroneously cites the Kolstad and Kogevinas studies as independent. One third of the Kogevinas cohort comes from the Kolstad study (those companies where resin suppliers thought more than half of the employees were involved in RPC manufacture). Furthermore, the owners of the companies disagreed with the resin suppliers in over one-third of the cases on being involved in RPC manufacture. Kolstad indicated he discarded the company owners' evaluation because there were no significant increases in cancer risk if he used their assessment. In addition, no attempt was made to determine if any of the leukemia cases was actually exposed to styrene."

Response 30: It is true that the reports of Kolstad *et al.* (1994) and Kogevinas *et al.* (1994) include partially overlapping cohorts. Kolstad (but not the other authors) was a coauthor of the Kogevinas study, but not vice-versa. However, we think that the separate data-gathering and statistical analysis makes these two publications distinct enough to cite and describe separately. As to the remainder of this comment, we accept the judgment of Kolstad and his coauthors from the Danish Cancer Society for purposes of this study review.

Comment 31: "SIRC believes there is insufficient scientific justification to conclude that styrene is a carcinogenic risk to humans. Therefore, the Public Health Goal should not be based on cancer potency, but on non-cancer effects. The PHG indicates that lung pathology in mice is the appropriate endpoint for calculating a non-cancer PHG. As previously noted in these comments, the pathology in mouse lung following styrene exposure is not related to metabolism to styrene oxide, but is driven by cyp2f2 metabolism. This metabolism does not occur in human lung and is not relevant for human risk for the PHG. The PHG should be based on the avoidance of neurobehavioral

effects in humans. Based on the Mutti *et al*. 1984 study (p. 224), the PHG for styrene should be 100 ppb."

Response 31: Discounting all the evidence for a carcinogenic risk of styrene would be a violation of OEHHA (2009) guidelines on cancer risk assessment. The suggestion completely ignores the principal directive in California Health and Safety Code 116365 that PHGs should be set at a level which does not pose any significant risk to human health, because there is in fact ample evidence of reasons for concern. Styrene-derived DNA adducts have been found -in humans occupationally exposed or exposed under controlled laboratory conditions to styrene. The reactive metabolite styrene-7,8-oxide, which is known to be formed in humans, is classified by IARC (1994) as a probable human carcinogen and by the National Toxicology Program Board of Scientific Counselors (NTP, 2001) as "reasonably anticipated to be a human carcinogen." An NTP expert panel (NTP, 2008b) recently recommended that styrene should be listed in the NTP Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals." There is suggestive evidence that styrene exposure increases the risk of cancer, particularly at lymphohematopoietic sites, in exposed workers. Several animal studies have reported increased tumors in multiple tissues. Use of the most sensitive tumor site for cancer risk extrapolation is fully justified by the available data.

Comments on second posted draft, February 2010.

Comments from Styrene Information and Research Center (SIRC) May 14, 2010

From the SIRC cover letter:

Comment 1: "Because most of SIRC's comments have not been addressed or reflected in the revised Technical Document, we again are submitting substantive comments on what we find to be profound deficiencies in OEHHA's assessment of the collective body of health effects data on styrene."

Response 1: OEHHA believes it has adequately addressed the earlier comments. One principal conclusion is that we fundamentally disagree with SIRC's interpretation of much of the data. For this reason, the comments in this 44-page submission that are duplicative and redundant will be addressed relatively briefly. We acknowledge the many alternative reviews of studies provided by SIRC, but specific responses to or discussions of most of those are not included here, since these are not comments on the PHG document.

Comment 2, p. 2: "SIRC's overarching frustration with the styrene PHG document is that the use of toxicology data in risk assessment rests on an evaluation of all the available data to develop a weight of the evidence conclusion. The approach taken in the styrene PHG goal document is, however, merely a speculative search for theoretical risks. Such an approach may even fall short of a *strength* of the evidence determination, which

may be warranted in instances when little data exists on a substance. Typically, little data results in a high degree of uncertainty as to both potential health effects and the exposure levels at which potential health effects might be anticipated. In styrene's case, however, an extensive body of high-quality scientific data is available on the chemical; a body of data that should be *viewed as a whole* and can readily serve to support a scientifically supportable *weight* of the evidence conclusion."

Response 2: We sympathize with the frustration expressed by SIRC, but disagree as to their interpretation of much of the data. The large body of toxicity information available on styrene makes clear that there are significant reasons for concern over potential toxic effects of styrene, including a risk of cancer. OEHHA follows the mandate of Health and Safety Code Section 116365 to place a primary emphasis on the protection of public health, and, with respect to carcinogens, to avoid any significant risk to human health.

Comment 3, p. 2: "The draft document, among its many deficiencies, does not apply tests of significance to distinguish statistically significant data from variations in data that may have occurred by chance, invents a mode of action that conflicts with existing data, and bases a characterization of probable carcinogen status on data OEHHA characterizes as inconclusive."

Response 3: The PHG document carefully discusses the statistical significance of every relevant indication of a toxic effect of styrene. Our discussions of mode of action are apparently notable to SIRC mainly because they fail to agree with SIRC's conclusions. We conclude that styrene toxicity can be exerted through multiple modes of action – a very common conclusion about chemical toxicity - and that the data are consistent with possible toxicity mediated through formation of styrene oxide, a well-recognized carcinogen. The opinion of SIRC that the documented formation of this genotoxic, carcinogenic metabolite *in vivo* should be considered inconsequential and irrelevant is, in our opinion, scientifically insupportable.

Comment 4, p. 2: "OEHHA's draft abandons both evidence-based science and accepted risk assessment principles. In doing so, it violates the California statute that sets the standards for conducting a risk assessment. Health & Safety Code section 116365 states that OEHHA shall prepare an assessment of the risks to public health, and the risk assessment "shall be prepared using the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology.""

Response 4: The styrene risk assessment has been written by a large team of OEHHA scientists experienced in toxicology, epidemiology, and risk assessments. They have prepared in-depth evaluations of the available data over a number of years. The conclusions reached by our public health professionals are consistent with our obligation under California law (Health and Safety Code 116365 *et seq*.) to ensure protection of public health.

Comment 5, p. 2: "OEHHA primarily has based the proposed Public Health Goal number (0.5 ppb) on an assumption that styrene is carcinogenic in animals (i.e. – mouse lung tumors). However, looking at the continually expanding *full* body of data, we feel strongly that it increasingly supports a conclusion that styrene simply is *not a significant concern as a human carcinogen*. This conclusion has been supported by regulatory determinations in both Europe and Japan that styrene *does not warrant classification as a human carcinogen*." [Emphasis in original.]

Response 5: OEHHA does not consider it an "assumption" that styrene is carcinogenic in animals. For instance, in the chronic inhalation study of Cruzan *et al*. (2001) in mice, there were in fact significant dose-related increases in bronchioloalveolar adenomas and combined adenomas and carcinomas. Other animal bioassays are consistent with this response (i.e., NCI, 1979). Genotoxic damage has been reported in lymphocytes of styrene-exposed reinforced plastics workers, including chromosomal aberrations, sister chromatid exchanges (SCE), single-strand breaks (SSBs)/DNA damage, mutations in the hprt gene or glycophorin A locus, and formation of styrene-derived DNA adducts (Koskinen *et al*., 2001; Vodicka *et al*., 2003). Considering the tumors in animals and the genotoxic effects in humans, we must agree with the conclusion of the NTP expert panel (NTP, 2008b) that styrene should be considered as "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence in animals."

Comment 6, p. 2: "OEHHA has concluded using its selective justification approach that styrene should be considered a *probable human carcinogen*."

Response 6: The PHG document does not make any such conclusion. As stated in the summary, "Overall OEHHA concludes that there is sufficient evidence that styrene causes cancer in animals and limited evidence in humans. For these reasons, it is prudent to assume carcinogenicity for the purposes of risk assessment."

Comment 7, p. 3: "In SIRC's view, OEHHA's continued application of a strength of the evidence approach to hazard assessment results in a profound disconnect relative to the larger regulatory community, given the vast leap from the European and Japanese conclusions of *no* concern to OEHHA's *probable* concern. With the remarkable and continually evolving research advances that aid in understanding the cause of cancer – such as tumor mode of action data – OEHHA's adherence to such simplistic default assessment approaches seems regressive at best. **Fundamentally, California's conclusion that styrene exposure will *probably* cause cancer in its citizens – when other countries and continents have deemed it of little concern – does not serve to accurately inform its citizens; most especially given that this conclusion was based on a failure to embrace current principles, practices, and methods used by experienced practitioners in hazard assessment techniques.**" [Emphasis in original.]

Response 7: OEHHA's weight of evidence approach for risk assessment of carcinogens is detailed in our most recent cancer guidelines (OEHHA, 2009), which are generally consistent with the most recent cancer guidelines of U.S. EPA (2005a,b). These

guidelines recommend use of the default linear extrapolation for genotoxic carcinogens, unless it can be established that the tumors in question are not the result of a mutagenic/genotoxic mechanism of action (MOA). OEHHA finds that styrene toxic effects can be mediated through both genotoxic and non-genotoxic MOAs, and that it has not been adequately established that the lung and mammary tumors are produced solely by a non-genotoxic mechanism for which a non-linear (threshold) MOA should be assumed.

Our conclusions on carcinogenicity are consistent with the most recent evaluation by a U.S. agency (NTP, 2008a,b). U.S. EPA has no recent styrene carcinogenicity review (IRIS, 2010; last drinking water review 1985). The draft ATSDR (2007) styrene review concluded, "Overall, human and animal studies suggest that styrene may be a weak human carcinogen." Furthermore, OEHHA notes the conclusion of the European Commission's Scientific Committee on Health and Environmental Risks (SCHER, 2008) review of styrene that, "based on the observations in human workers regarding blood styrene 7,8-oxide, DNA adducts and chromosomal damage, it cannot be excluded that this [formation of carcinogenic styrene oxide] and other mechanisms are important for other organs [than lung]." The European Commission styrene evaluation also states, "SCHER disagrees with the conclusion, that there is no concern for human carcinogenicity." OEHHA has found no recent evaluation from Japan to substantiate the above statement about lack of concerns in Japan. OEHHA believes that a prudent public health position on styrene includes an evaluation of its potential risk to humans based on its demonstrated carcinogenic activity.

"Summary Technical Concerns"

Comment 9, p. 3: "The styrene draft PHG document ignores the principle that true effects are repeatable by others or in other studies, and differences that are not repeatable are probably not true effects. For example, it reports liver toxicity in one study, but does not report that several other studies of equal or greater dose for equal or greater duration did not find any liver toxicity."

Response 9: OEHHA reported the major toxicological observations made in each of the studies.

Comment 10, p. 3: "The PHG document assumes that mean values of groups of individuals are precise representatives of the true means for an endpoint and any numerical difference between groups represents a toxicologic effect. This approach ignores sampling theory, confidence intervals and statistical analysis. In the styrene document, the result is that a number of cancer types are represented as "increased, but not statistically significant." This is a term without definition. Instances of "increased" incidence or relative risk are cited where, based on the 95% confidence intervals, the "true" incidence may be decreased by as much as 50% less than expected."

Response 10: We agree that effects are less likely to be meaningful when statistical significance is not achieved. However, patterns of effect which do not reach statistical significance in a given study or at a given dose may nevertheless be relevant when

repeatedly observed in different studies or at other doses. Thus we report the incidences of effects when they appear potentially relevant to a toxicity evaluation.

Comment 11, p. 3: "The PHG document sets no criteria for considering numerical differences to be treatment effects. In several instances in the styrene document, SMRs are called "increased, but not statistically significant" when the observed number of cases is only a fraction of 1 case more than expected. Obviously actual cases come in whole numbers of people, not in fractions. No such consideration is presented when the number of cases is less than expected."

Response 11: OEHHA follows the standard practice of reporting SMRs and other estimates of relative incidence rates in fractions, with confidence intervals. For completeness in discussing a study, observed rates which do not reach statistical significance may be tabulated and mentioned. These ratios are derived by counting actual deaths. Our conclusions are based on the study observations which are judged to reflect toxicological effects, based on statistical significance.

Comment 12, p. 4: "The results of the epidemiology studies are described toward the conclusion of the document as supportive of findings of an increased cancer risk from styrene. The overall conclusion of the epidemiology studies is stated on page 188 as "In summary, we acknowledge that the epidemiological data are inconclusive," however, the executive summary (page 1) says, contrary to current principles, practices, and methods employed by experienced practitioners, the data provide "suggestive, but not definitive direct evidence of cancer in the human cancer data." … Inconclusive and Suggestive are not the same! Neither constitutes a finding of Limited Evidence."

Response 12: Based on the data available as discussed in our document, we disagree with SIRC's conclusion that the human data do not suggest a potential human risk. The comment is incorrect in its understanding of Limited Evidence as described by IARC: "A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC, 2006). In this case, there is suggestive evidence that occupational exposures to styrene have increased the risk of cancer but data are limited for making a definitive evaluation, which fits the IARC definition of Limited Evidence.

Comment 13, p. 4: "The document concludes that small numerical or inconsistent differences represent styrene-related risks and ignores large and statistically significant decreases in risk. Examples include:

a. The citing of cancer incidences as "increased" when the number of cases is less than 1 greater than the expected number and 95% confidence intervals indicating there may actually be a decreased incidence;

b. The citing of small differences in specific studies as evidence of increased risk, when the other studies do not show such an increase;

c. The citing of increased risk from average exposure in the Kogevinas study and dismissal of the lack of increase from cumulative exposure due to imprecision in the duration of exposure estimates. However, the imprecise estimates affect average exposure more than cumulative exposure because average exposure was calculated by dividing the cumulative by the duration."

Response 13: Odds ratios or relative risks greater than 1.0 by definition represent an increased risk; the statistical significance is an estimate of the probability that the numerical increase reflects a true population increase rather than a sampling event. All of our epidemiological reports and observations attempt to evaluate and interpret the strength of the data as to potential toxic effects. We agree that the exposure estimates are imprecise in the available studies. Statistically significant decreased risks may or may not represent physiologically relevant effects, but in these studies such results were not judged adequately informative to support any inferences.

Comment 14, p. 4: "When the incidences of specific cancer types are evaluated using the whole reinforced plastics cohort (Ruder, Wong and Kogevinas), there is no significant increase in any cancer type (Boffetta *et al*. 2009)."

Response 14: The review of styrene epidemiology studies conducted by Boffetta *et al*. (2009) for SIRC stated: "A study of reinforced plastic workers reported an association between average estimated styrene exposure and non-Hodgkin lymphoma (NHL, $P < 0.05$) but no trend with increasing duration of exposure." Considering that $P < 0.05$ is generally considered to be a finding of statistical significance, it is not clear why SIRC claims the study found no significant effect. We acknowledge that Boffetta *et al*. downplayed the positive finding(s) as being "inconsistent" but, as stated in the PHG document, we came to a different conclusion on the positive findings than did these reviewers.

Comment 15, p. 4: "Based on the PHG document, there is no evidence of a dose-related increase in mammary tumors from styrene exposure by oral or inhalation routes of exposure. The NTP pathology guidance for combining tumors (McConnell *et al*., 1986) indicates that rat mammary fibroadenomas are species specific, are benign, do not become malignant, and have no human counterpart. Therefore they recommend that fibroadenomas, *not* be combined with other mammary benign or malignant tumors. The PHG document, contrary to current principles, practices, and methods employed by experienced practitioners, combines fibroadenomas with adenomas and adenocarcinomas in several instances to provide evidence that styrene causes increased mammary tumors in rats."

Response 15: We believe this comment is incorrect, and that we have classified tumors in accordance with the NTP pathology guidance. Table 1 in McConnell *et al.* (1986) clearly shows that mammary adenomas, adenofibromas and fibroadenomas may be combined. Also the table indicates that "benign epithelial neoplasms and malignant epithelial neoplasms" may be combined "if a continuum is observed in a given study." We think a continuum is evident, and OEHHA routinely combines such tumors in cancer

risk assessment. For example OEHHA (2005) combined mammary adenocarcinomas and fibroadenomas in female F344 rats exposed to acrylamide (Friedman *et al*., 1995).

Comment 16, p. 5: "In the Beliles drinking water study, there was an increase in fibroadenomas, but not adenomas or adenocarcinomas; yet the PHG document cites this study as demonstrating increased mammary tumors."

Response 16. Yes, that is correct. In view of the observations referred to in the response to the previous comment, fibroadenomas were considered tumors.

Comment 17, p. 5: "The document cites a statistically significant increased incidence of mammary adenocarcinomas in the low dose animals of the Jersey study as evidence of styrene-induced mammary tumors, despite the fact that there was no increase in the high dose and the low dose incidence was within the control range for the laboratory."

Response 17: Some of the wording in the PHG document has been revised; we think it accurately describes the data. See p. 128: "There was a suggestion of mammary tumors and leukemia in female rats in another inhalation study (Jersey *et al.*, 1978), although this study had methodologic issues." And on p. 130: "Jersey *et al.* (1978) suggested that the increased incidence of mammary adenocarcinoma in female rats exposed by inhalation (600 ppm styrene, 8.2 percent) could be an artifact of an unusually low concurrent control rate (1.2 percent); both the control rate and the rate at 600 ppm were within the historical range of rates from the same laboratory (0 to 9 percent)."

Comment 18, p. 5: "The PHG document cites increased malignant mammary tumors at low doses in the Conti inhalation study as evidence of carcinogenicity of styrene. The document acknowledges that Dr. Maltoni had previously stated there was a high and variable rate of formation of mammary tumors within the colony. The PHG document *did not add* that the same report stated because of this high and variable rate, the differences in tumor rates were *not* considered evidence of a styrene-related increase in mammary tumors."

Response 18: That is correct. OEHHA staff do not always reach the same conclusions as study authors in reviewing animal bioassay data. We concur with the thinking expressed in a recent article by Melnick *et al.* (2008), which discusses some of the issues involved in the assessment of animal bioassay data for public health decisions, that conflicting views on the results of these studies are frequent and expected.

Comment 19, p. 5: "The document dismisses a dose-related significant *decrease* in malignant mammary tumors in the Cruzan study because the doses are within a historical control range developed for the document. Note that this is in contrast to the treatment of an *increase* within the historical control range in the Jersey study. Furthermore, the studies considered by OEHHA occurred several years earlier than the Cruzan study, and the incidence reported in the two highest doses in the Cruzan study are lower than incidences contained in Charles River's historical database of 20-plus studies."

Response 19: OEHHA places more reliance on concurrent control data than historical control data. When the latter are considered they must be recent historical data more likely to reflect current conditions. In addition, in establishing a PHG, data which indicate that a chemical is carcinogenic are of more concern than data indicating no carcinogenic activity and even data indicating possible anti-carcinogenic activity under certain conditions.

Comment 20, p. 5: "The document acknowledges that there were no increases in malignant mammary tumors at higher doses in 5 other studies and, therefore, it is questionable if this is a styrene effect. The document then launches on a justification for calling it a styrene-related effect. Two arguments were used:

- Toxicity at higher do[s]es prevented the formation of mammary tumors and;
- Because of saturation of metabolism, less styrene and styrene oxide would be present in the mammary tissue of rats at high doses of styrene than at low doses of styrene."

Response 20: As stated above, data which indicate that a chemical is carcinogenic are of more concern than data which do not reveal carcinogenic activity. Thus the positive data of Beliles, Jersey, and Conti on mammary tumors are more important than the negative data of Cruzan.

It is true that toxicity at high doses can prevent tumor formation. Thus in a lifetime study the body weights in the highest dose group should be at least 90% of the body weights in the control group. (A Maximum Tolerated Dose should cause no more than a 10% reduction in body weight.) Figure 1 of Cruzan *et al*. (1998) indicates that the final body weights in the control group females were 480-500 g, while in the highest dose groups (500 and 1,000 ppm) the final body weights were 400 g, i.e., 80-83% of the controls.

We did not find a statement in the draft PHG document that saturation causes decreased levels of styrene oxide, and agree that that would be incorrect. If styrene metabolism approaches saturation with increasing doses, disproportionally higher levels of styrene would be expected, with styrene oxide falling proportionally behind, but still increasing. Thus it could be said that lower levels of styrene oxide would be present in the mammary tissue of rats at high doses of styrene than would be expected if metabolism were not saturated (or approaching saturation).

Comment 21, p. 6: "The PHG document asserts that a bolus dose by gavage would somehow result in a lower blood (and mammary tissue) level of styrene and styrene oxide (SO) than either water administration or inhalation. The argument makes two contrary statements. First, it says that styrene by inhalation bypasses the liver and more passes directly to mammary tissue, but then it indicates that styrene from drinking water also somehow bypasses the liver and more escapes the liver than does from gavage. The primary criticism of gavage studies, in general, has always been that the large bolus dose allows much of the dosed chemical to escape the liver into the blood, overestimating the hazard of a chemical. The PHG draft, however, indicates that bolus gavage dosing reduces the amount of styrene in circulation. As shown later in detail, PBPK modeling of

styrene and styrene oxide as peak level or area under the curve (by either Sarangapani *et al.*, 2002, or Csanady *et al*., 2003) show that the arguments regarding the level of toxicant to mammary tissue are speculation and FALSE."

Response 21: No such interpretation was intended. Discussions of absorption and distribution have been modified for clarity.

Comment 22, p. 6: "[T]he PHG document states that styrene metabolism to SO begins to be saturated at around 200 ppm. This is a true statement for styrene metabolism in humans, but not in rodents. In rats, saturation of styrene metabolism occurs at around 600 ppm. Furthermore, NO principle of toxicology or pharmacokinetics would say that the level of styrene oxide is lower at doses above saturation than from doses below saturation. *Saturation means the level of metabolite ceases to increase with increasing dose; it does not decrease with increasing dose!*" [Emphasis in original.]

Response 22: It is not clear what passage in the draft document this was referring to. We revised a description of the blood levels of styrene and styrene oxide in rats and mice after inhalation exposures, in the studies of Cruzan *et al*. (1998, 2001), to more carefully describe the reported results (found on page 12 in the draft PHG). We note, for example, that Cruzan *et al*. (2001) reported increasing styrene ng/mL in blood of mice, per ppm in air, over air concentrations from 20 to 160 ppm in air. This supralinear increase could be interpreted as evidence of approaching saturation of styrene metabolism in this exposure range. However, the ratio of styrene oxide to styrene in blood did not decrease, indicating no detectable effect on this specific metabolic pathway.

We also revised the discussion of metabolism on pp. 128 and 129 to correct any possible implication that the levels of styrene oxide would decrease in blood at doses above saturation, and agree with SIRC that this would be a misstatement.

Comment 23, p. 6: "Mammary tumors: The PHG document asserts that styrene exposure causes increased prolactin resulting in increased mammary tumors. The document cites Mutti *et al*., (1984a) and Luderer *et al*., (2004) showing increased prolactin levels in styrene exposed women. It must be pointed out that there is a range of normal values for women (and men), and that increases above normal result in oligomenorrhea, amenorrhea, and infertility. First of all, the levels of prolactin reported are *within the normal range* for women. Secondly, evidence of clinically significantly increases is lacking; i.e., there is *no* evidence of oligomenorrhea, amenorrhea, and infertility in styrene-exposed women, or in rats exposed to styrene. Furthermore, rats exposed by oral or inhalation routes did not have increased prolactin (Jarry *et al*.). Thus there is *no* evidence of a styrene-related increase in mammary tumors in rats and no evidence of increased prolactin in rats exposed to styrene."

Response 23: The draft PHG on pp. 99 and 137 referring to Mutti *et al*., 1984a on prolactin in women should have cited Mutti *et al*., 1984c. This has been corrected. The sections in question were merely reporting on the results of these studies, and did not mention mammary tumors. If the reference above is to the discussions of potential mechanisms of carcinogenicity on pp. 189 and 193 of the draft, referring to a possible

indirect mode of action of styrene on mammary tumors mediated through an increase in the CNS pathway that controls prolactin, we think that this is the appropriate place and appropriate wording to use in describing hypothetical MOAs.

The commenter may wish to examine a recent paper by Harvey *et al*. (2008), Adverse effects of prolactin in rodents and humans: breast and prostate cancer. J Psychopharm 22(2) Suppl. 20-27. The authors conclude that "hyperprolactinaemia is associated with an increase in breast cancer risk in both post and premenopausal women, that rat carcinogenicity studies are predictive of the human response, and that in a regulatory toxicology context prolactin-induced mammary tumors from non-genotoxic drugs and chemicals are an adverse effect." OEHHA believes that the subject statements regarding a potential mode of action are adequately supported by the available research.

Comment 24, p. 7: "The PHG document asserts that styrene-induced mouse lung tumors are caused by genotoxicity from SO and cite evaluations by Cohen *et al*. (2002), IARC (2002), and Hofmann *et al*. (2005) as supporting this conclusion. In fact, both Cohen and Hofmann state that differences in SO levels do not explain why mice develop lung tumors and rats do not. Further, IARC concluded "the lung tumors were caused by mouse lung metabolism of styrene and the process does not occur to a meaningful extent in humans."

Response 24. The IARC (2002) conclusions are at odds with the earlier IARC (1994a) and more recent NTP (2008a,b) evaluations. The expert panel reviewing the NTP evaluation concluded that "Sufficient evidence of carcinogenic activity comes from multiple studies in mice exposed to styrene by multiple routes. Styrene induced benign and malignant lung tumors in male and female mice by inhalation (Cruzan *et al.*, 2001) and in male mice by intubation (NCI, 1979). This is supported by findings of lung tumors in both sexes of mice in studies of more limited design (Ponomarkov and Tomatis, 1978). There is also the finding in rats of malignant mammary tumors by inhalation (Conti *et al*., 1988) and a small increase in mammary fibroadenomas in a relatively low dose drinking water study (Beliles *et al*., 1985). Mammary tumors were not increased in an adequate inhalation study in the same rat strain exposed for two years (Cruzan *et al*., 1998), limiting the weight given to the other mammary tumor findings. In earlier reviews (IARC 1994b, NTP, 2002), sufficient evidence in animals was found for the carcinogenicity of styrene-7,8-oxide" (NTP, 2008b). We concur.

Comment 25, p. 7: "In the detailed description (p. 119), the PHG document includes a paragraph of more recent research showing that Clara cells lose the ability to produce (or stain for) CC-10 as they progress toward tumors and that they may produce SP-C. These more recent research data negate the conclusion that staining for SP-c and not for CC10 means the tumors are of alveolar origin. The PHG document acknowledges the recent research, but continues to assert the staining proves the tumors to be of alveolar origin. *This needs to be corrected.*" [Emphasis in original.]

Response 25: The discussion in the PHG document has been updated and clarified. The lung has at least 40 types of cells. The tumors may be derived from Clara cells that have

dedifferentiated and lost their CC10 marker. Alveolar macrophages may be involved. Type II cells may be involved. The most important conclusion is that inhalation of styrene leads to lung tumors in mice.

Comment 26, p. 7: "Conclusion: In addition to the summary comments outlined above, attached with this letter are detailed, specific comments on the many inaccuracies, conflicting statements, and questionable conclusions identified by SIRC – most of which, SIRC points out, *have not been adequately addressed from our earlier comments*. SIRC strongly urges to OEHHA to fully address the concerns expressed in these comments, to ensure that the styrene draft Technical Support Document reflects a *through, balanced and accurate* assessment of the full body of data on styrene. As written, this document does not provide a scientifically credible assessment of styrene that applies hazard identification techniques (i.e. – weight of the evidence) that are viewed as the appropriate standard by the larger scientific community."

Response 26: OEHHA has reviewed the SIRC comments and continues to disagree with many of the SIRC interpretations of the data. OEHHA has applied a weight of evidence approach to the assessment of styrene as a drinking water contaminant and derived a public health protective value based on the results of our extensive review. Our conclusions with respect to styrene carcinogenicity are wholly in line with IARC (1994a) and NTP (2002) and the Styrene Expert Panel's review of NTP's current assessment of styrene (NTP, 2008a,b). OEHHA believes that our draft PHG document is a through, balanced and accurate assessment of the *relevant* data on styrene. Many of the voluminous comments supplied by SIRC are inconsequential and, if incorporated, would do little to improve the scientific credibility of this document, which has been favorably reviewed by independent experts and other interested members of the public. OEHHA has responded to SIRC's comments, extended the comment deadline and made risk assessment staff available for discussions with SIRC representatives. We believe that SIRC's concerns and opinions have been heard and adequately addressed in the document in the context of public health protection.

Specific comments

Comment 27, p. 9: "p. 5. This section on exposure implies that the general public's only oral exposure to styrene is from leaching of styrene from styrene-based packaging. Styrene is found in many raw foods. The average intake from styrene in foods is about equal to the styrene that migrates from styrene-based packaging. Both are quite small (Cohen *et al.* 2002; IARC 2002)."

Response 27: The exposures are poorly documented in the references cited. IARC (2002) states that styrene occurs at very low levels in raw foods, whereas estimates of styrene intake from packaging may be significant, e.g., 9 µg/d or 0.13 µg/kg-d compared to overall intakes of 0.19 to 0.85 µg/kg-d from all sources. Additionally, IARC (2002) states: "The styrene in food occurs mainly by migration from polymer packaging materials." However, OEHHA has revised the document to indicate the potential presence of styrene in raw foods.

Comment 28, p. 9: "p. 13 and p. 17. The same data from CD-1 mice in the Cruzan *et al*., 2001 study are used in on page 13 to argue that saturation of metabolism of styrene-7,8-oxide in mice does not occur. On page 17, the document states "The dose-response suggested saturation of styrene-7,8-oxide metabolism (Cruzan *et al.*, 2001)." It is not clear that there was saturation of styrene metabolism at 160 ppm in mice."

Response 28: We agree; the discussions have been revised.

Comment 29, p 9: "p. 20. "In a study on the histochemical properties of mouse lung tumors induced by styrene, Brown (1999) reported evidence for Type II cells in the tumors. These results suggest an inconsistency between the mouse lung tumors that are associated with alveolar Type II cells (Brown, 1999) and the higher level of styrene metabolic activation associated with Clara cells (Hynes *et al*., 1999)." Statements are repeated and expanded on pages 118, 119. These are not true statements. Brown did not find evidence for type II cells in the tumors.

"Similar statements are repeated on page 119 in an apparent attempt to negate mouse lung-specific metabolism of styrene as an explanation of MOA. On page 119, the PHG document acknowledges (in the next paragraph) that CC10 is reduced or lost during tumor development and may not be a suitable marker for lung tumor cell of origin. Furthermore, both Hicks *et al*., 2003 and Yao *et al*., 2003 report increased surfactant protein in bronchiolar-derived tumors. CC10 is a marker of normal Clara cell and SP-C is a marker of normal Alveolar cells. Neither is a marker for the origin of lung tumor cells.

On page 20 and 119, the document must delete the statements that the mouse lung tumors are derived from Type II alveolar cells and that the tumors are not related to the cytotoxicity in terminal bronchioles."

Response 29: The discussions on page 20 and 119 of the draft were revised extensively in updating the document, and these statements are no longer included.

Comment 30, p. 10: "p. 21, par. 2. The document states "The apparent lack of involvement of CYP2E in Clara cell styrene bioactivation may reflect an intrinsic property of Clara cell CYP2E or it may be due to a lack of uptake of the CYP2E inhibitor diethyldithiocarbamate by the Clara cell preparation." A third alternative is more likely; CYP2E1 does not produce the metabolites that are toxic to mouse Clara cells. The PHG document needs to include several articles submitted last summer by SIRC showing that, in addition to lack of inhibition of styrene lung toxicity by administration of diethyldithiocarbamate, styrene lung toxicity is not reduced in CYP2E1-knockout mice (Carlson G. 2004b. Influence of selected inhibitors on the metabolism of the styrene metabolite 4-vinylphenol in wild-type and CYP2E1 knockout mice. *J Toxicol Environ Health A* 67(12): 905-9; Carlson GP. 2004a. Comparison of the susceptibility of wild-type and CYP2E1 knockout mice to the hepatotoxic and pneumotoxic effects of styrene and styrene oxide. *Toxicol Lett* 150(3): 335-339; and Vogie K, Mantick N, Carlson G. 2004. Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice. *J Toxicol Environ Health A* 67(2):145-52.)."

Response 30: The alternative explanation has been added to the text on p. 21.

Comment 31, p. 10: "The draft document starts from the premise that a positive genotoxicity result is indicative of cancer, but a negative result is meaningless. (page 37). The citations for this indicates that one cannot dismiss a positive in an endpoint (e.g., Ames assay) because several other endpoints did not give positive results. It appears that OEHHA has reinterpreted this caution to mean that one positive in a particular endpoint makes the endpoint positive regardless of how many negative studies have been reported for that endpoint; i.e, for endpoints with positive and negative reported findings there is no analysis of which studies are more informative, or why 1 positive should outweigh 7 negatives."

Response 31. Positive results always carry more weight than negative results since they are more difficult to dismiss as a result of inadequate dosing or other methodological failures. Study design and general quality also figure into the weight of evidence assessment, as discussed by Melnick *et al*. (2008). In this case the weight of evidence strongly suggests that styrene is genotoxic in humans, rodents, and non-mammalian species.

Comment 32, p. 11: "IARC showed mainly negative results for induction of chromosomal aberrations, micronuclei or sister chromatid exchange and concluded that workers having no significant increase in chromosomal aberrations were exposed to styrene at concentrations ranging from 2 to 70 ppm (8.5-298 mg/m^3). In contrast, the PHG report interpreted essentially the same studies as a consistent proof of genetic damage in styrene exposed workers. However the data presented in Table 8 of the PHG document demonstrates that out of 34 referenced studies 15 were positive for CA, two slightly positive and 17 – negative; more so, Oberheitmenn *et al*, 2001 (authors of the study) claimed slight but not significant (0.22% exchange type aberrations vs. 0.14% in controls) increase in CA that was within historical control and in Andersson *et al*, 1980 and Lazutka *et al*, 1999 studies workers were exposed to genotoxins such as methylethylketone peroxide, phenol and formaldehyde that could confound response. Furthermore there are a predominant lack of induction of micronuclei (four out of 17 studies were positive) and SCE seven of 20 studies were positive, three –slightly positive and ten negative."

Response 32: Nearly half of the studies OEHHA reviewed reported positive findings for chromosomal aberrations (CA). In view of the fact that positive findings have more weight than negative findings, OEHHA concludes that the results indicate genotoxicity in exposed workers.

In addition, a recent study of chromosome aberration frequency in lymphocytes was found to predict risk of cancer in a pool of 22,358 subjects from 11 countries (Bonassi *et al*., 2008). In this study the relative risk (RR) of cancer for subjects in the medium tertile was 1.31 (95% CI = 1.07-1.60) and in the high tertile was 1.41 (95% CI = 1.16-1.72) when compared to the low tertile. The presence of ring chromosomes increased RR to 2.22 (95% CI = 1.34-3.68). We believe that this study further supports a link between

CAs and cancer and explains why human CA data needs to be seriously considered in human risk assessment.

Comment 33, p. 11: "Therefore, it would be helpful to define how OEHHA interprets "consistently" and "clearly defined". In the document OEHHA stated that a dose relationship between increasing styrene exposure and genotoxicity has been clearly defined. In fact, only Yager *et al* (1993) showed linear regression of SCE frequency on styrene concentration in exhaled air and workplace air. Artuso *et al*. 1995 showed increase in CA in high exposure group only. The response in the low-exposure group, however, was weak (2.13% in control, 2.75% in low and 4% in high exposure group). Somorovska *et al*. (1999) reported elevated CA frequencies compared to the control but no dose-response (frequencies of 1.37, 3.27, 2.50 and 3.75% in control, low, medium and high group, respectively). The PHG document's analysis of individual studies together with a consideration of dose-response relationships and lack of the common profile of positive responses for the various endpoints in different studies, provide no clear evidence that styrene exposure in workers results in detectable level of genetic damage."

Response 33: The cited studies repeatedly reported genotoxic responses. Different magnitudes of effect in different studies are expected due to the varied exposure conditions. Given the limitations of group sizes and statistical power, smooth dose-response curves are not expected.

Comment 34, p. 12: "IARC (2002) concluded that variable results have been reported with regard to association between exposure to styrene and chromosomal damage. Increased frequencies of chromosomal aberrations in vivo were not reported in most studies and only weak evidence was reported for induction of sister chromatid exchange. It is strange that the OEHHA document cites the 1994 IARC statement on genotoxicity and not the more recent 2002 conclusions. SIRC concurs with the IARC 2002 conclusion: 'Inconsistent results have been reported for chromosomal aberrations, micronuclei and sister chromatid exchange in approximately 30 studies of workers exposed to styrene in various industries. These studies were predominately from the reinforced-plastics industry where styrene exposure is high, but there was no indication of a dose-response relationship in any of the studies reporting positive results. Induction of chromosomal aberrations was reported in 12 of 25 studies, sister chromatid exchange in six of 16 studies and micronuclei in three of 14 studies.'"

Response 34: Yes, OEHHA agrees with the conclusions that were reached by the authors of the 1994 IARC styrene document, and disagrees with the conclusions reached by the panel members who produced the 2002 review.

Comment 35, p. 13. "The PHG document ignores overwhelmingly negative results for CA and MN in rodent studies with controlled exposures as being contradictory to their conclusion that styrene induces MN and CA in humans with unknown, estimated and variable exposures to styrene and potentially other substances."

Response 35. Rodent studies do not supersede human studies. OEHHA uses human data when possible. The data may simply indicate that humans are more sensitive to the genotoxicity of styrene and its metabolites than are rodents.

Comment 36, p. 13: "Overall, the *in vitro* and submammalian data are of limited use in the evaluation of the genotoxicity of styrene since the test systems utilized do not reflect *in vivo* mammalian conditions, particularly in humans, where there is limited metabolism of styrene to SO, and rapid detoxification by EH of any SO that is formed (Scott & Preston, 1994)."

Response 36: As stated above in response to comment 35, OEHHA is more concerned about the genotoxic effects observed in humans.

Comment 37, p. 15: "Several studies indicate low levels of DNA adducts in laboratory animals and humans following exposure to styrene. The presence of DNA adducts shows exposure to agents capable of binding to DNA, but is not necessarily correlated with mutagenic endpoints or with tumor formation. The lack of a clear connection between DNA adduct formation and tumor induction is exemplified by the study of Boogaard *et al*. (2000). Boogaard *et al*. (2000) examined DNA adduct formation in the liver, lungs, and isolated lung cells of rats and mice exposed to styrene by inhalation at concentrations of 500 ppm and 160 ppm respectively for 6 hours. DNA adduct levels in the lung tissue of mice, the target tissue in the 2-yr carcinogenicity study (Cruzan *et al*., 2001) were found to be lower than in liver, a nontarget tissue. In addition, DNA adduct levels in the lung, liver and lymphocytes of mice were about the same as in rat, yet there was no indication of an oncogenic effect in the latter species."

Response 37: These results are not unique. La and Froines (1992a) report a similar difference between adduct formation and carcinogenicity for 2,4 and 2,6-dinitrotoluene. The compounds gave qualitatively and quantitatively different adducts. The 2,6-DNT gave a higher adduct yield at low concentrations but following a high concentration the adduct yield for 2,4-DNT predominated. The maximum adduct yields measured were 3.0 and 1.8 per million nucleotides for the 2,4-DNT and 2,6-DNT, respectively. Adduct persistence was similar at two weeks post-exposure but the 2,6-DNT was much more cytotoxic than the 2,4-DNT and a more potent carcinogen.

The same authors studied the adduct formation of the carcinogen 2,4- diaminotoluene in liver, mammary gland, kidney and lung (La and Froines, 1992b). Qualitatively identical adducts were detected in all the tissues with the target tissues of liver and mammary giving the highest yields (up to 30 times that found in non-target tissues). Comparison with the weakly carcinogenic analog 2,4-DNT showed that identical adducts were formed but the yield with the latter was lower. Similarly, the noncarcinogenic 2,6-diaminotoluene analog gives a much lower adduct yield and cytotoxicity than 2,6-DNT (La and Froines, 1993). These studies indicate that adduct formation is complex and several factors may influence their role in carcinogenesis. DNA adduct formation by styrene and its metabolites is one factor in a weight of evidence approach in assessing the potential risk of lifetime exposure to styrene via drinking water.

Comment 38, p. 16: "Koshinen *et al.* (2001) analyzed and quantified the presence of β1-adenine adducts of styrene-7,8-oxide in the white blood cells of eight men and 10 women employed as hand laminators in reinforced plastics industry. The styrene exposure concentrations were calculated to average 76.2 mg/m^3 (17.9 ppm). Three of the nine exposed workers had β1-adenine DNA adducts present at the levels of 0.67 to 1.03 per 10^9 nucleotides. There were no correlations between β1-adenine adduct level and measured styrene concentrations."

Response 38. This study (actually Koskinen *et al*., 2001) is too small in numbers of subjects to be used to establish a dose response; adducts were only detected in three of the workers. However, various adducts are consistently measured in blood of styrene-exposed workers by this Czech Republic group, and are not detectable in controls (Vodicka *et al*., 2003; Kuricova *et al*., 2005).

Comment 39, p. 17: "The evidence to date suggest that low levels of DNA adducts are formed in rats, mice and humans following exposure to styrene. Mouse lung does not form unique DNA adducts nor does it form DNA adducts in greater amount than in liver. The level of adducts (<10 per 10^8 nucleotides) is at least 100 fold lower than the level necessary for genotoxic carcinogens to induce a 50% tumor incidence in mouse liver (Otteneder & Lutz, 1999). No comparison is available for genotoxic lung carcinogens. Such a low level of DNA binding, especially in the target organ, does not suggest a genotoxic mode of action for styrene."

Response 39: Otteneder *et al*. (2002) continued the line of research referred to above, examining levels of 2'-deoxyguanosyl-O6-adducts at the alpha(7)- and beta(8)-positions in lungs of rats and mice after 2 weeks of inhalation of styrene. They found no adducts above their limit of detection of 1 adduct per 10^7 DNA nucleotides in either rat or mouse samples. They concluded that "species- and organ-specific tumor induction by styrene is not reflected by DNA adduct levels determined in tissue homogenate. The particular susceptibility of the mouse lung might have to be based on other reactive metabolites and DNA adducts, indirect DNA damage and/or cell-type specific toxicity and tumor promotion." Although the exposure time was relatively short, we favor their conclusion, that other genotoxic mechanisms may be involved in the effects of styrene in mouse lung.

Comment 40, p. 20: "p. 91, last paragraph states that swimming ability was increased on PND 24. The time for swimming the straight channel was increased, but this is not an increase in swimming ability."

Response 40: Agreed; the sentence has been corrected.

Comment 41, p. 20: "p. 93 paragraph 1 lists qualitative effects of styrene exposure. These are meaningless without dose information. Furthermore, the text says that styrene exposure causes "decreases in testicular function in male fetuses." The Cruzan *et al*., 2005 2-generation reproduction study found no effect on testicular function after exposure of dams

to 500 ppm. Furthermore, as noted in the next section, styrene exposure in rats had no effect on the ability of males to reproduce or on sperm parameters."

Response 41: The sentence has been revised to indicate the effect on neonates as reported by Srivastava *et al*., 1992.

Comment 42, p. 20: "p. 103. Should add that no liver pathology was found in F344 rats exposed to 500 mg/kg/day styrene by gavage for 13 weeks (NCI, 1978) or in Sprague-Dawley rats exposed to 1500 ppm for 13 weeks (Cruzan *et al*., 1997)."

Response 42: Text has been added to address this issue.

Comment 43, p. 21: "p. 103. No kidney toxicity was found in F344 rats exposed to 200 mg/kg/day styrene for 13 weeks by gavage (NCI, 1978) or in Sprague-Dawley rats exposed to 1500 ppm for 13 weeks by inhalation (Cruzan *et al*., 1997)."

Response 43: Text has been added to address this issue.

Comment 44, p. 21: "p. 106. Need to add that liver toxicity was not seen in Sprague-Dawley rats exposed to 1000 ppm styrene for 2 years by inhalation or at 500 mg/kg/day for two years by gavage in F344 rats."

Response 44: Text has been added to address this issue.

Comment 45, p. 21: "p. 106. Need to add that no kidney toxicity was found at 2000 mg/kg/day by gavage in F344 rats for 2 years (NCI, 1978) or from 1000 ppm by inhalation for 2years (Cruzan *et al*., 1998)."

Response 45: Text has been added to address this issue.

Comment 46, p. 21: "p. 106. Need to add that no effects on spleen, thymus indicative of immunotoxicity were found at 2000 mg/kg/day by gavage in F344 rats for 2 years (NCI, 1978) or from 1000 ppm by inhalation for 2 years (Cruzan *et al*., 1998)."

Response 46: Text has been added to address this issue.

Comment 47, p. 21: "p. 114. Beliles study: Beliles *et al.* 1985 reported total mammary tumors; as indicated by NTP, this is a meaningless comparison as fibroadenomas should not be combined with adenomas/carcinomas. In 1984, Huff reported the incidences of specific mammary tumors (Table 4-4 from NTP RoC Background document for styrene). The only increase in this study was for fibroadenoma. There was no increase in adenoma, adenocarcinoma, or adenomas/adenocarcinoma combined. The Beleiles *et al*., 1985 study does not show an increase in mammary tumors that are relevant for human risk."

Response 47: OEHHA disagrees with this comment and the supporting opinion. Fibroadenomas of the mammary gland are benign epithelial cell tumors comprised of a mixture of glandular tissue, connective tissue and ducts. On occasion, fibroadenomas can give rise to adenocarcinomas (Boorman and Everitt, 2006). We think that the mammary tumors could be relevant to human risk and are not willing to assume that they are not, in the context of potential exposure of millions of Californians. Since OEHHA uses the most sensitive site approach, the PHG is based on lung rather than mammary tumors.

Comment 48, p. 23: "p. 122. The only study (Cruzan *et al*., 1998) that was performed using current study guidelines and good laboratory practices found no increase at similar doses to the Conti study and decreased malignant mammary tumors at higher doses. From a scientific standpoint these data should not be ignored. The PHG (p. 120, 121) dismisses the decreased mammary adenocarcoinomas in the Cruzan *et al*. study by asserting that the control incidence was extremely high and the incidences in the treated animals were normal by citing incidences of adenocarcinomas from several studies at earlier time points than the Cruzan *et al*. 1998 study. The Charles River database reports control incidences over 26 studies for mammary adenocarcinomas averaging 17% with a range from 7 to 43%. The control incidence of 33% in the Cruzan *et al*., 1998 study is well within the CRL historical control range. The incidence at the highest dose in the Cruzan *et al*., 1998 study (3%) was below Charles River historical control range. Furthermore, as noted in the PHG document, there was an earlier communication of this study in which Dr. Maltoni said the incidence of mammary tumors at his laboratory was high and variable and there was no styrene-related increase in mammary tumors in this study."

Response 48: Good Laboratory Practice (GLP) is the formal name of a set of procedures for toxicity studies, established for submission of studies for regulatory purposes. Studies which do not follow these procedures are not necessarily bad science. NTP studies, for example, are not carried out according to these guidelines. OEHHA does not accept the inference that non-GLP studies are necessarily inferior to GLP studies.

We have not ignored the Cruzan *et al*. (1998) data on mammary tumors in rats. However, in establishing a PHG, data which indicate that a chemical is carcinogenic are of more concern than negative data or data indicating a possible anti-carcinogenic activity. Several known human carcinogens (e.g., azathioprine, busulphan, tamoxifen, bis(chloromethyl)ether) are used in the chemotherapy of cancer or other diseases, but if they become environmental contaminants, it is the carcinogenic effects that we would focus on. Thus the positive rat mammary tumor data of Beliles and Conti, and suggestive data of Jersey are of more concern than the negative data of Cruzan.

We do not now know what data the earlier communication by Maltoni was based on. OEHHA's analysis of the Conti *et al*. (1988) data (Table 20) indicate a dose-related increase in mammary tumors.

Comment 49, p. 27: "From IARC (2002): "Styrene was tested for carcinogenicity in rats in four gavage studies, one drinking water study and two inhalation studies. Overall, there was no reliable evidence for an increase in tumour incidence in rats."

Response 49. OEHHA disagrees with this conclusion. Our findings are more in line with those of IARC (1994a) and the more recent review by NTP (2008a,b).

Comment 50, p. 27: "p. 134. Ototoxicity. This section is grossly negligent of the studies assessing hearing loss from styrene exposure. The most recent study (Triebig *et al*., 2009) indicates hearing loss only for those exposed to high levels for long periods, and only at low frequency sounds."

Response 50: We have added more of the available information on ototoxic effects of styrene in occupational exposures to the document, including the review of Triebig *et al.* (2009). Documentation of auditory effects of long-term exposures at levels in air of 50 ppm or less makes this endpoint potentially relevant for development of health-protective levels.

Comment 51, p. 27: "p. 135. Neurotoxicity. The section describes the Mutti *et al*., 1988 as the most comprehensive study. A more recent study evaluated more endpoints, contained a much larger cohort and examined effects during periods of work and vacation (Seeber *et al*., 2009). It did not find the neurotoxic effects reported by Mutti and was not included in the Benignus *et al*., 2005 meta analysis. Furthermore, the Benignus analysis concluded there was a linear relationship between duration of exposure and reaction time because their model assumed linearity and could not determine if there was nonlinearity. Those studies included in Benignus that examined linearity, as well as the Seeber study, found no effect of duration of exposure on neurobehavioral responses."

Response 51: A description of Seeber *et al*. (2009) has been added to the document. Actually Seeber did report two exceptions to a general conclusion of no effects, namely significant dose responses for the Benton test of visual perception and memory of figures with lifetime weighted average exposure (LWAE) of styrene ($P < 0.05$), and the peg board test of finger dexterity with LWAE ($P < 0.01$) and chronic exposure index (CEI) ($P < 0.05$). These results are presented in the revised document.

Comment 52, p. 27: "p. 142. Heart Disease. The PHG document cites the 2002 Matanoski and Tao analysis of ischemic heart disease in a cohort of SBR workers. The document also needs to include the follow-up study of this cohort adding more than 10 years of data. Delzell *et al.* (2005) found no increased risk of death from ischemic heart disease in the SBR cohort when followed through 1989.

"p. 142. The U.S. RPC cohort had no such increases (Wong and Trent, 1999)."

Response 52: A description of Delzell *et al.* (2005) has been added to the PHG document. Wong and Trent (1999) are now cited in the introduction to the cardiovascular toxicity subsection.

Comment 53, p. 29: "p. 157, the document states "Wong *et al.* (1994) considered styrene exposure based not on styrene level as done earlier, but on exposure duration (the number of years from 1948-1977 during which exposure occurred to each worker), and on cumulative exposure (calculated by multiplying duration of exposure by the estimated TWA exposure level)." The statement regarding cumulative exposure is not correct. Wong *et al.* state (p. 387) "A cumulative exposure in ppm-years, **calculated as the sum of products of TWA and duration of exposure in each job, developed for each cohort member.**" [Emphasis in original.] That is, for each member they multipled the TWA for the first job the person held by the length of time he held that job, did the same for the second job he held, etc. and summed those values for a cumulative exposure.

"p. 157, the document states "As noted by IARC (2002), Wong *et al.*'s use of both cumulative exposure and exposure duration, two correlated exposure indices, in the regression models may have artificially reduced the coefficients of both." It must be noted that Wong *et al*., 1994 also conducted regression analyses using only cumulative exposure, so the above statement is wrong."

Response 53: We believe that our description of these studies is accurate.

Comment 54, p. 29: "p. 157. The document states "Rate ratios of specific LHCs were elevated in different ways in relation to latency, with elevations at the longest latency for lymphosarcoma (≥20 years, SMR = 162.6) and "cancer of all other lymphopoietic tissues" (≥20 years, SMR = 150.4), and elevations at the shortest latency for Hodgkin's disease (<10 years, SMR = 128.5) and leukemia (<10 years, SMR = 110.9). All were based on small numbers, and none was statistically significant." These data are not taken from the Wong *et al.*, 1994 publication, but from Table 13 of the more complete final report submitted to SIRC. It is extremely misleading to call these increases."

Response 54: The statement (now on p. 158) has been changed to read "Rate ratios of specific LHCs showed possible elevations at the longest latency for lymphosarcoma (≥20 years, SMR = 162.6) and "cancer of all other lymphopoietic tissues" (≥20 years, SMR = 150.4), and at the shortest latency for Hodgkin's disease (<10 years, SMR = 128.5) and leukemia (<10 years, SMR = 110.9). All were based on small numbers, and none was statistically significant." The rate ratios reported in the OEHHA document are drawn from Table 3, "Observed deaths and SMRs by cause and latency for all cohort members" in Wong *et al.*, 1994, page 389.

Comment 55, p. 33: "Overall, the document works very hard to discredit any studies that did not report increased cancer, and accepts without question any finding reporting increased cancer. Why are the studies not treated the same?"

Response 55: We believe the most relevant studies have been presented, whether or not an increased cancer risk was found. However, attention has necessarily focused on the studies that appear to show potential toxic effects, discussing the pluses and minuses of each, because consideration of potential toxicity is the point of the evaluation.

Comment 56, p. 33: "p. 189. The PHG document identifies styrene-7,8-oxide (SO) as "a reactive chemical classified by IARC (1994) as a probable human carcinogen and by NTP (2001) as "Reasonably anticipated to be a human carcinogen." These are true statements, but is the carcinogenicity of SO relevant to the "carcinogenicity" of styrene? In the preceding sections the PHG document asserts that styrene causes increased mammary tumors in female rats, increased lung tumors in male and female mice, and increased leukemia and lymphoma in humans. SO caused increased forestomach tumors in rats and mice. Increased forestomach tumors were not reported in any of the 13 carcinogenicity studies of styrene. Administration of SO did not cause increased mammary tumors in female rats, did not cause increased lung tumors in male or female mice. No increases in leukemia or lymphoma have been reported from administration of SO. **Thus, on the first analysis, the tumor spectrum of styrene and SO do not support a conclusion that formation of SO is the MOA for styrene carcinogenicity.**" [Emphasis in original.]

Response 56: The SIRC commenters earlier made the point that mouse lung tumors caused by styrene are the result of local (lung) metabolism of styrene to SO. That is, the most potent tumor site is where the highest concentration of the chemical is. In the comment above, they point out that SO causes forestomach tumors, i.e., at the site of highest concentration when SO is given orally, which would be consistent with the first observation. If one were to continue this line of thought for styrene, one would have to determine which tissues received the highest concentration of styrene oxide, the presumed proximate carcinogen, to predict where tumors are most likely. Claiming that styrene-induced tumors should occur at the same (route-specific) locations as those from styrene oxide is therefore illogical; it does not pass the "on first analysis" caveat stated above.

The spectrum of tumors observed depends on the dose, mode of administration, species, and a host of other factors.

Comment 57, p. 34: "p. 193. The document states "Biochemical studies in experimental animals suggest that exposure to styrene may lead to decreased levels of dopamine in the hypothalamus (reviewed by Mutti, 1993). Prolactin secretion is under the control of the hypothalamus through negative feedback, and increased serum levels of this hormone have been observed in styrene exposed workers (Bergamaschi *et al*., 1997). In rats, increased prolactin levels have been associated with increased spontaneous mammary tumors and an enhanced growth of preformed mammary tumors (spontaneous or carcinogen induced) (Meites, 1980)." The CERHR report on styrene indicates that the levels of prolactin reported in the human studies are within the normal range for humans. Furthermore, the Mutti study did not measure prolactin, only dopamine and speculated that prolactin would be increased. In the only rodent studies of styrene and prolactin, five day exposures to styrene at 150, 500, or 1500 ppm did not cause increased prolactin or dopamine (Jarry *et al*., 2002, 2004).

Response 57: Comment noted; the Jarry *et al.* studies (2002, 2004) are now cited in the PHG document.

Comment 58, p. 34: "p. 196, 197. Respiratory Effects. Respiratory tract toxicity has been demonstrated to occur in rodents in tissue that are high in CYP2F; i.e., rat nasal epithelium and mouse nasal epithelium and lung Clara cells. Toxicity is inhibited by inhibition of CYP2F. Since metabolism of styrene is very limited in both nasal and lung tissue, styrene is Not likely to cause toxicity in either the nose or lung of humans. These endpoints are not appropriate for developing human RfC or RfD."

Response 58: OEHHA estimated a non-cancer health-protective level from effects of styrene in deep lung (bronchiolar epithelia, alveolar ducts, and terminal bronchioles) of male mice. We agree that the same effect (decreased eosinophilic staining) has not been observed in human tissues – biopsies would be out of the question - but we are not comfortable with the assumption of the commenters that comparable effects would not occur in humans. It should be noted that increased lung tumors were found in mice after styrene by gavage (NCI, 1979) as well as by inhalation.

Comment 59, p. 35: "Interspecies Extrapolation - OEHHA appears to rely upon a crude allometric scaling approach for interspecies extrapolation rather than use the PBPK model for this purpose. The assumption that the allometric scaling factor of ¼ is equal parts toxicokinetics (1/8) and toxicodynamics (1/8) is a policy decision by OEHHA, and may not be valid. It does not appear that OEHHA relied upon the human PBPK styrene model at all in this assessment (e.g., no table of human model parameter values are provided as they were for rodents). The PBPK model should be used to extrapolate across species to estimate human equivalent doses for the LED10 values."

Response 59: Yes, the standard allometric scaling estimates were used in this risk assessment. We felt that these were more appropriate than use of the limited PBPK models for the scaling of the responses to humans.

Comment 60, p. 36: "Low-Dose Extrapolation – The mode of action for styrene carcinogenesis in rodents may include a cytotoxic component (see mode of action summary below). For this reason, the dose-response relationship may well be nonlinear and consistent with a threshold. Nonlinear estimates of cancer potency for styrene should be discussed and presented as alternatives to linear estimates."

Response 60: Following OEHHA (and U.S. EPA) cancer guidelines, cancer risk for genotoxic chemicals is estimated by linear extrapolation to low doses. OEHHA sees no purpose in estimating cancer potency using the method proposed by the commenters.

Comment 61, p. 36: "Exposure Assessment – OEHHA relied upon CalTOX predictions, which generally produces conservative exposure estimates. By including inhalation and dermal pathways OEHHA assumes an effective drinking water exposure of 5.25 L/day. Because of this conservative exposure assumption, OEHHA's proposed PHG for styrene is approximately 2.6-fold lower than would be calculated for ingestion alone. Alternatives to CalTOX (e.g., addressing all three routes of exposure within the PBPK model) should be explored and discussed."

Response 62: CalTOX is a relatively crude model, but few alternatives are available, and none are demonstrably more accurate. PBPK analyses do not address exposures to volatile organic chemicals from other household uses of drinking water.

Comment 62, page 36: "**Dose-Response Assessment.** The proposed mode of action summarized above was used to guide key decisions in the dose-response assessment to derive an alternative PHG value for styrene using the best available science. Each step of the dose-response assessment is summarized in Table 2-2 … and described below."

Response 62: This comment is actually the summary of an alternate analysis and derived PHG value of 42 ppb (by our calculation 46.8 ppb). While some of the approaches are interesting, in our view the overall analysis falls short of an adequate assessment, as follows:

Endpoint: The most serious problem is the combination of the oral and inhalation data. This has the effect of diluting the response, which can be readily seen by the loss of adequate dose-response model fit to the data sets. In the male mouse lung single study and combined data sets in their Table 4-1, none of the combined sets give adequate fits by any model (X^2 fit criterion, $P \geq 0.1$). In their Table 4-2 the female mouse data give no adequate fits either singly or combined. If there is a rationale for combining data (not clear) it must be supported by improved dose-response.

Dose Measures: The area under the curve for "SO Clara Cell" as defined by the Sarangapani *et al*. (2002) PBPK model might be a reasonable metric.

Dose Measure Adjustments: These are essentially the same adjustments that were used by OEHHA in our PHG document.

Response Measure: Essentially the same as used by OEHHA.

Dose Response Model: The multistage cancer model is preferred with a benchmark response. The use of more complex models (logistic, probit and their log forms) is not recommended for cancer data sets.

Point of Departure: Same LED_{10} or $BMDL_{10}$.

Route to Route Extrapolation: The PBPK model looks reasonable.

Interspecies Extrapolation: Assumes that AUC/effect in mouse = AUC/effect in human. OEHHA thinks this is an optimistic assumption which makes no allowance for different responses in humans vs. mice, i.e., pharmacodynamic differences.

Low Dose Extrapolation: Must be linear, unless there is strong evidence for a nonlinear approach; OEHHA does not agree that the required strong level of evidence has been reached for this genotoxic carcinogen.

Cancer Potency: Geometric mean of values for each sex. OEHHA uses either the most sensitive single sex/site or, when it seems appropriate, combined sites.

Exposure Assessment Pathways: No suitable Dermal/Oral/Inhalation human model for styrene is available. OEHHA used CalTOX to estimate the relative contributions by each route from low level contaminated drinking water in a household setting. Any human

PBPK model would need to develop and incorporate adequate in-home exposure scenarios.

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