Responses to Major Comments on Technical Support Document

Public Health Goal
For
TCDD
In Drinking Water

Prepared by

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INTRODUCTION

The following are the combined responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for 2,3,7,8-tetrachlordibenzodioxin, commonly known as TCDD. The comments from the University of California reviewers and the Chlorine Council are based on the pre-release review draft, completed in 2005, while the last two reviewers were commenting on the second posted version (June 2007). Changes in response to these comments have been incorporated into the final version posted on the OEHHA website; no comments were received on the third posting. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA web site at www.oehha.ca.gov. OEHHA may also be contacted at:

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RESPONSES TO MAJOR COMMENTS RECEIVED

Comments from Christopher Vogel, University of California, Davis

Comment 1: "The authors might be not correct finding that there is no report of human exposure only to TCDD. The accident of Seveso in 1976, which is cited later in the PHG document, describes a scenario where humans were exposed to high levels of almost pure 2,3,7,8-TCDD. Besides the non-cancer effects listed in the document, the authors should consider the results of the Seveso accident cohort showing evidence of excesses of several specific types of cancers (Bertazzi *et al*., 2001) which is in line with findings of epidemiologic studies from the U.S., the Netherlands, or Russia."

Response 1: Indeed, the 1976 Seveso, Italy accident differs in several important ways from other epidemiological studies involving exposure to dioxin. The Seveso accident exposed potentially large numbers of individuals in the local population to almost pure TCDD (the contents of a TCP reactor in a chemical plant were vented directly into the atmosphere). This is in contrast to most of the occupational studies, which entailed concomitant exposure to other chemicals in addition to dioxin, and were comprised mostly of male workers. A summary of the Bertazzi *et al.* (2001) study, including both cancer and nonmalignant results, has been included in the PHG document.

Comment 2: "With respect to developmental effects, the authors might consider a recent report showing that the AhR is not only important to mediate the toxicological response of TCDD but is also required for the developmental closure of a hepatic vascular shunt known as the ductus venosus (Walisser *et al.*, 2004)."

Response 2: This study has been reviewed and added to the PHG document.

Comment 3: "Regarding the dose-response assessment of noncarcinogenic effects another subchronic study could give some more support to the existing data: Vogel *et al.,* 1997."

Response 3: This study has been reviewed and added to the PHG document.

Comment 4: "The proposed PHG value for non-carcinogenic effects at 7 pg/L is based on a study with sensitive endpoints in mice finding a relatively low LOAEL of 1 ng/kg-day (Toth *et al*., 1979). This value might be too high and reconsidered for sensitive individuals and children since it is calculated for adults with a bodyweight of 70 kg."

Response 4: Studies in animals do suggest that females and children may be more or especially susceptible to the toxic effects of TCDD. Also, since some segments of the population consume many times the average level of fat per day, the principal exposure pathway for dioxins in the general population, they may be at higher risk. These factors should be accounted for with the revised calculation, with a health-protective concentration for non-carcinogenic effects of 2 pg/L.

U.S. EPA has not seen fit to determine an RfD (a non-cancer health protective value) to which our non-cancer value might be compared. Their stated reason is that human body burdens are already "at or near levels associated with adverse health effects" for both cancer and non-cancer (with the customary large uncertainty factors used for non-cancer risk assessment). U.S. EPA has indicated that the estimated safe level would likely be far below background environmental exposure levels (U.S. EPA, 2003). We agree, but think it is useful to provide a value.

As for health-protectiveness, our PHG is based on cancer findings, which results in a still lower level. We believe that this PHG provides an adequate margin of safety to protect potential sensitive subpopulations against all of the noncarcinogenic effects of TCDD, including adverse effects on the immune system, cardiovascular system, liver, and reproductive/developmental effects, as well as the carcinogenic effects.

Comments from Daniel Chang, University of California, Davis

Comment 1: "In the section dealing with "Environmental Occurrence and Human Exposure" it might be useful to provide a context for the public regarding exposure to 2,3,7,8-TCDD and biologically related compounds. It could be pointed out that environmental releases of "dioxins" are dominated by releases to air from combustion sources, and that ambient air levels of "dioxins" have declined since about the mid-1970's."

Response 1: Additional information on temporal trends in release of dioxins, expressed as TCDD/TEQs, has been added under the Environmental Occurrence and Human Exposure section.

Comment 2: "In the last sentence of the second paragraph the statement is made, "The U.S. EPA (2000) has estimated that the general human population is exposed to daily doses of ~0.3 pg/kg-day". Because of the location of that statement in the context of the discussion on "air", confusion may result over whether this is the "airborne" dose".

Response 2: This sentence, which included, "from all sources," has been moved to avoid any potential confusion.

Comment 3: *The developmental studies listed in Appendix A should be added to the PHG document.*

Response 3: The following *in vivo* and *in vitro* developmental studies, in primates and human trophoblasts, respectively, were added to the PHG document: Moran *et al.* (2001, 2004), Scott *et al.* (2001), Chen *et al.* (2003).

Comment 4: "The document states that differences as large as a factor of 1000 are observed for the same biological endpoint between the most sensitive and least sensitive species. A statement of where humans lie on that spectrum, in the case of TCDD might assist the public in assessing the conservatism that is built into the proposed PHG. These data might also be added to Table 5 so that the dose at which the observed effect occurred would be more readily seen"

Response 4: The discussion has been expanded, which should clarify that humans are one of the less sensitive species to dioxins with regard to acute effects. Table 5 would not, in our opinion, be useful for providing much perspective on this point because the observations on toxic effects in humans involve far different conditions as well as endpoints. With respect to cancer, comparisons of human and animal ED_{01}s for increased tumors, on a body burden basis, show approximately equal potency for TCDD. This also should be more clear in the present form of the discussion – although the data from the animal studies are quantitatively more precise.

Comment 5: "In the summary it is stated that the PHG is based on TCDD alone rather than all its congeners, and a reason is provided in the first complete paragraph on page 2…little sense is provided as to the relative proportion of TCDD's contribution to the TEQ compared with congeners typically present. Thus the public may have difficulty gauging the level selected for TCDD provides an adequate margin of safety…."

Response 5: The PHG document is based exclusively on the 2,3,7,8-isomer because this compound is specified for the California MCL in California regulations (Title 22, Div. 4, Chap. 15,Art. 5.5, Sec 64444, Table 64444A). The relative proportion of TCDD to other congeners depends on many factors, including the source of environmental contamination and the physical environment, However, TCDD is the major contributor to dioxin toxicity equivalent (TEQ) in most environmental media, and many researchers have chosen to measure only TCDD. For drinking water, relatively little data on levels of TCDD or any of the PCDDs are available; that is, none of the PCDDs are detectable in finished water. Therefore we think that, for the intended purposes of this risk assessment, there should be no particular reason for confusion.

Comment 6: "State clearly whether the USEPA max allowable concentration for dioxin in drinking water is isomer specific or TEQ. The public may also be confused then when they see the USEPA MCL of 0.03 ng/L on pg. 1. Did the

authors of the draft document mean to clarify “surface water guideline” rather than drinking water on pg. 7?”

Response 6: The U.S. EPA Maximum Contaminant Level (MCL) of 0.03 ng/L refers specifically to TCDD. Page one of the PHG document has been changed to reflect this. The reference to “surface water guideline,” now on page 5, specifically refers to ITEQ, or International Toxicity Equivalent Quotient, which refers to a mixture of isomers.

Comment 7: “The relationship of the calculation of daily intake, D, in the equation provided and Table 15 is not clear for the lay reader. The assumed value of the absorbed fraction, A(0.5), of the dose, D, is not provided and it is not explained that D should be divided by bodyweight in order to compute the human equivalent doses in Table 15 from the rat adipose tissue dose”

Response 7: Additional information on calculation of the Human Equivalent Doses has been added to the document.

Comments from Clifford Howlett, Executive Director, Chlorine Chemistry Council

Comment 1: “OEHHA’s reliance on the USEPA Draft Reassessment document is inappropriate. The analyses should not be relied upon until they have undergone a rigorous, thorough and final review. OEHHA should await the release of the NAS report before finalizing the draft PHG for TCDD.”

Response 1: The PHGs are developed from scientific studies in the peer-reviewed literature. U.S. EPA documents serve as one important source of information, and reports by NAS are another. However, as a practical matter, we do not wait for promised updated evaluations, because updates are sometimes delayed for years. The Calderon-Sher Safe Drinking Water Act of 1996 requires OEHHA to review and update the risk assessments of water contaminants at least every five years. Although with the present staffing level this is not generally possible, our timely production of chemical reviews would be made even more difficult if we waited for other agencies to finalize each of their evaluations.

However, it should be noted that the NAS committee’s report has now been released (NAS, 2006), and its conclusions are now cited in the risk assessment. Both the U.S. EPA’s Dioxin Reassessment Review Subcommittee (DRSS) of the Science Advisory Board (SAB) (U.S. EPA, 2001) and the NAS committee basically concurred with the major observations and conclusions in the U.S. EPA’s draft dioxin risk assessment.

The major concerns were on cancer risk modeling methods, quantitation and acknowledgement of uncertainty, and points related to risk communication, i.e., better acknowledgment of the uncertainty. The U.S. EPA SAB DRRS panel acknowledged that the various issues are not resolvable with current data, and

recommended that U.S. EPA complete the risk assessment with available data. The NAS (2006) recommended that the approach be justified better and the uncertainty and variability be more explicitly stated, in the final draft.

OEHHA concurs with the SAB recommendation. Despite the uncertainty associated with risk extrapolation to low environmental levels for this (or any other) chemical, public health protection requires prudent assumptions such as the use of the linearized multistage method for cancer risk assessment in this case. We see nothing in the recommendations of either the SAB or the NAS which would justify further delay in publication of a drinking water standard.

Comment 2: "The use of a benchmark dose of a 1 percent response as the basis of non-cancer risk assessment results in additional, unstated conservatism in the risk assessment process"

Response 2: A benchmark dose approach was not used in the calculation of the non-cancer public-health protective level for TCDD. For the final version of the document, a lowest-observed-adverse-effect-level (LOAEL) of 3 ng/kg-day has been selected for calculation of a public health-protective concentration for noncarcinogenic effects of TCDD in drinking water, based on the NTP (2004) toxicology/carcinogenesis gavage studies of TCDD in female Sprague-Dawley rats. At the LOAEL, there were significant increased incidences of cell proliferation, gingival squamous hyperplasia, cytochrome P450 induction, as well as significant increases in lung and liver weights. The health-protective level was calculated from the estimated human body burden comparable to the LOAEL in rats.

Comment 3: "The info on general population exposure levels is out of date. See pg. 8 of PHG. Look at Lorber 2002 and Aylward and Hays, 2002 studies. Patterson et al. 2004…The exp characterization should be updated to include the most current information…"

Response 3: A section on temporal trends in TCDD/TEQs has been added to the document and updated information on general TCDD exposure levels is included in it. However, present estimates of national background levels of dioxins in tissues are uncertain because current data cannot be considered statistically representative of the general U.S. population, as discussed by Lorber (2002), Aylward and Hays (2002), and Patterson *et al*. (2004).

Comment 4: "OEHHA's cancer potency calc is incompletely documented…"

Response 4: The calculation has been revised and additional information has been added to the PHG document to clarify calculation of the TCDD cancer potency.

Comment 5: "In the NTP (2004) carcinogenesis study, the predominant responding tumors are hepatic tumors, responding both at the lowest doses and to the greatest degree. Bioassay reports provide both adipose and liver tissue concentration data. Given the availability of hepatic tissue concentration data, use of adipose tissue concentrations as the dose metric for assessing dose-response in the NTP (2004) bioassay for hepatic tumors may be problematic."

Response 5: The choice of dose metric is dependent upon the data available. Liver and adipose tissues showed the highest levels of TCDD. No measurable concentrations of TCDD were observed in blood from treated rats at any of the study time points; thus metabolic rates for TCDD could not be calculated. Because of the liver toxicity, changes in physiological parameters (e.g., tissue volumes, organ perfusion rates) due to growth and toxicity (cell death) would have to be accounted for if one were attempting to use the liver concentration data in estimating steady-state tissue concentrations. Also, the liver/fat concentration ratio changes with TCDD dose because of an increase in the amount of microsomal TCDD-binding protein, CYP1A2, in the liver (Anderson *et al.,* 1993; Diliberto *et al.*, 2001). For high doses in chronic exposure studies, this leads to nonlinearity in the concentration of TCDD in the liver whereas, at low doses, TCDD concentration of liver as a function of dose is more or less linear. Therefore, we judged that applying estimated body burden (from adipose tissue concentrations) to cancer response data would provide the best approach.

Comment 6: "The data supporting a threshold should be acknowledged and discussed – even if OEHHA chooses to use a non-threshold approach to derive the PHG."

Response 6: We acknowledge in the Dose Response and Risk Characterization sections of the PHG document the varied opinions on the cancer dose-response extrapolation, as well as the quantitative uncertainty with regard to extrapolation to low doses and cancer risk levels for TCDD. OEHHA has utilized the approach used by the U.S. EPA (2003) and recommended in the current U.S. EPA cancer risk guidelines (U.S. EPA, 2005).

Comment 7: "[The PHG document]…does not contain key recent studies by Cole *et al.* (2003) and Bodner *et al.* (2003)…. [and] Aylward *et al*. 2005"

Response 7: The Cole *et al.* (2003) review, sponsored by the Chlorine Chemistry Council, concludes that, "The long-term accumulation of negative, weak, and inconsistent findings suggests that TCDD eventually will be recognized as not carcinogenic for humans." This is simply not supported by the weight of the scientific evidence, either in humans or experimental animals. The 1976 Seveso, Italy industrial accident was one in which several thousand people were potentially exposed to relatively pure TCDD. Bertazzi *et al.* (2001) conducted an extended follow-up of this population 20 years later. An excess of lymphohemopoietic neoplasms was found in both genders. In previous

experimental studies, a dose-related increase of lymphoma was found in both male and female mice (NTP, 1982, 2004; Della Porta *et al.*, 1987). In the Bertazzi *et al.* (2001) study, all-cancer deaths were *significantly* in excess after 15 years amongst males living in the high-exposure zones. The magnitude of the excess was similar to that estimated in previous long-term studies of high-exposure, male occupational cohorts (Saracci *et al.,* 1991; Flesch-Janys *et al.*, 1995; Kogevinas *et al.,* 1997). Mortality from rectal cancer and lung cancer was also elevated among males. The lung is one of the organs targeted by the carcinogenic action of TCDD in rats and mice (Kociba *et al.*, 1978; NTP, 1982, 2004). Also, at least one other occupational cohort study found an increase in rectal cancer (Flesch-Janys *et al.,* 1998).

Cole *et al.* (2003) state that, "The epidemiologic studies of occupational exposures, pesticide applicators, and community exposures following industrial accidents, notably Seveso, have generated overall risks of all cancer of about 1.0." In fact, in the Seveso population, the relative risks of Hodgkin's disease, non-Hodgkin's lymphoma, myeloid leukemia, and rectal cancer were 4.9, 2.8, 3.8, and 2.4, respectively (Bertazzi *et al.,* 2001). Although we reject the conclusions of Cole *et al.* (2003), it is now cited in the PHG document.

The epidemiological investigation of Bodner *et al*. (2003), which reports no significant increase in cancer mortality in a cohort of chemical workers, has been added to the cancer section.

Aylward *et al.* (2005) argue that current PBPK models need to be modified to account for elimination of unchanged TCDD via lipid partitioning from the circulation into the large intestine. Their study is based on published human data from 39 persons, in which the hepatic elimination rate parameter for each person was varied to optimize model fit to the data. According to the authors, the data and model results indicate that, for males, the mean apparent half-life of TCDD ranges from less than 3 years at serum lipid levels above 10,000 ppt to over 10 years at serum lipid levels below 50 ppt. Aylward *et al.* (2005) state that "specific values of the individual parameters used in this modeling should be interpreted with caution." We agree; this is not a model that has been rigorously tested or scientifically validated.

A number of other investigators have proposed that the elimination kinetics for TCDD are concentration-dependent, which is at least partly related to AhR-mediated induction of cytochrome P450 1A2 (CYP1A2). In both the human and animal data, as the dose increases the apparent half-life decreases, indicating an inducible elimination of TCDD. These studies are discussed in the PHG document. At present, human data are insufficient to determine the shape and parameters of the dose-response curve for the liver fraction due to induction of CYP1A2 in the liver. Increased elimination rates have typically been observed in instances where body burdens are substantially elevated, compared to exposures at environmental levels, although the data are too limited to validate a PBPK model that incorporates an inducible elimination of TCDD. Therefore the decision has been made to use the human half-life for TCDD of 7.1 years, which has been accepted by U.S. EPA (2003), for the PHG cancer calculation. (A

number of studies entailing TCDD exposure in both occupationally and non-occupationally-exposed cohorts have reported that the half-life for TCDD ranges from about 7 to 9 years (Flesch-Janys *et al.*, 1996, Michalek and Tripathi 1999; Needham *et al.,* 1994, 1997; Rohde *et al.*, 1999)).

Comment 8: "Finally, the benchmark dose modeling methodology used by the USEPA results in comparison of 1% responses across a wide range of biochemical, tissue, and adverse response endpoints, all with differing biological significance and control animal variability. A 1% percent change in enzyme activity is biologically trivial (and undectable); a 1% incidence of cleft palate is not trivial but is still undetectable in most experimental protocols. The USEPA (2000) analysis incorporates factors of unstated additional conservatism (several to more than 10-fold) compared to traditional risk assessments."

Response 8: We agree with the U.S. EPA that changes in biochemical indices can be linked to toxic responses, and that applicable data are certainly available for this purpose for TCDD. However, our analysis is not based on the benchmark approach used by U.S. EPA.

Comments from Minnesota Department of Health

Comment 1: "Why is the Goodman and Sauer (1982) re-evaluation of the Kociba data not discussed or included in Tables 10-12?"

Response 1: A discussion of the Goodman and Sauer (1982) paper was inadvertently omitted from the PHG document. This has now been corrected. The Goodman and Sauer (1982) tumor incidence data have also been added to Table 11 of the PHG document. Table 10 presents only male rat tumor incidence data, and Goodman and Sauer (1982) only re-evaluated liver sections; liver tumors were not found in male animals in this study. Similarly, Table 12 compares tumor incidences between the Kociba *et al*. (1978) and Squire (1980) reports, which include more than just liver tumor incidence data.

Comment 2: "Table 14 should include NTP 2004 data/calculations for comparison."

Response 2: We agree. These are now included.

Comment 3: "Did you attempt to account for the stop-dosage group in your analyses of the NTP 2004 data? The data seem to suggest that timing (of dosing and evaluation) may be very important – and may be more important than some of the human equivalent dose (HED) adjustments."

Response 3: For development of the PHG cancer-based number, the issue of concern is chronic exposure, so the cancer analysis focused on the most

relevant data for that endpoint. The NTP (2004) stop exposure data comprised an exposure duration of only 30 weeks (and at only a single dose level), so we decided not to attempt to incorporate these data into the analyses.

Comment 4: "The document specifies an absorbed dose of 0.5 – NTP cites 66-93% (84%). Typically, GI absorption is not corrected in the calculation of risk, especially when the difference from 100% is minimal. Adjustments are often incorporated into exposure equations."

Response 4: For this calculation, we are assuming that 100 percent of the TCDD present in drinking water would be absorbed, but that a lesser fraction would be absorbed under the conditions of the NTP study. The 0.5 estimate is more health-protective (in effect, doubling the potency per mg dioxin administered), but not excessive, in our opinion.

Comment 5: "How was the Monte Carlo used? It is not clear what the independent and dependent variables were in the Monte Carlo, nor is it clear what the uncertainty is for the data used. Why weren't deterministic calculations used?"

Response 5: The linear term (q_1) of the multistage model is first estimated based on dose-response data for each of the treatment-related tumor sites (tumor incidence data taken from Table 9). Statistical distributions, rather than point estimates, are generated at each site by tracing the profile likelihood of the linear term (q_1). The distributions of q_1 for each of the treatment-related sites are then statistically summed using a Monte Carlo approach and assuming independence. The sum is created by adding the linear term for each tumor site, according to its distribution, through random sampling with 100,000 trials. The upper 95 percent confidence bound on the summed distribution is taken as the multisite cancer potency estimate (q_1*). Deterministic calculations are less useful when summing results from multiple sites.

Comment 6: "HED conversions are not shown or explained. You appear to be normalizing to "rat adipose tissue concentrations" but your units in Table 15 are pg/g-day. Should this be pg/g? It appears that you are using the mean of the adipose tissue concentrations (pg/g) from the NTP study (Table 13). What is the adipose tissue equivalence q_1* in Table 16? Why is it calculated and how is it used in your risk calculations?"

Response 6: Yes, the correct units for Rat Adipose Tissue Concentrations in Table 15 are pg/g. This has now been corrected. For calculating body burden, we used each of the adipose tissue levels at four different time points. The trapezoid rule was then used to estimate the overall average. Then, using this data and U.S. EPA's steady-state, Human Equivalent Doses (HEDs) were calculated for the various dose levels. In the previous draft of the PHG, the applied dose q_1* combined site estimate was mistakenly used in the final cancer

calculation in place of the adipose equivalence q_1*, which no doubt caused considerable confusion. This has been corrected.

Comment 7: "How can a combined site estimate for q_1* be less than the q_1* at one site (see Table 16, third column, Lung and combined site estimates)?"

Response 7: The value for lung (applied dose q_1*) in Table 16 should have been entered as 2.66×10^3. This has been corrected in the final document.

Comment 8: "The potency estimates in Table 16, second column, appear to over-represent tumors that were not observed very often in the study (pancreas acinar adenomas, lung epitheliomas, liver hepatocholangiomas). Further – the estimates for the three tumors are identical. Again, more transparency in how these calculations were made is needed."

Response 8: The human cancer potency estimates for pancreas acinar adenomas, lung epitheliomas, and liver hepatocholangiomas (Table 16) are 0.268×10^4, 2.66×10^3 (corrected), and 0.265×10^4, respectively. The same equation is used to fit all three sites. Those are the upper confidence bound on the lowest estimates.

Comment 9: "You do not appear to have used the actual dose to the animals anywhere in your calculations. Instead you use the adipose tissue concentration as your metric. This should be stated in your document."

Response 9: Our use of body burden (i.e., adipose tissue concentration) as a dose metric for the PHG cancer calculation is stated in several key sections in the PHG document (in the introduction, in the dose response assessment section, etc.). This follows the approach of U.S. EPA for TCDD (U.S. EPA, 2003). According to U.S. EPA, body burden (estimated at steady-state conditions) provides for a more reasonable description of dose.

Comment 10: "'Because this study and cancer potency derivation appears to be superior to earlier approaches, OEHHA has chosen these for development of the proposed PHG for TCDD'. This is not a reason to use this method – but a summary evaluation. Why is this method superior?"

Response 10: Our cancer potency derivation utilizes body burden as a dose metric, as opposed to the more traditional method of using daily intake, for species extrapolation. Body burden takes into account the considerable difference in half-life of TCDD in rats vs. humans. Although the assumption of a single TCDD half-life is uncertain, because limited data exist to validate a PBPK model that incorporates an inducible elimination of TCDD, the decision was made to use the human half-life of 7.1 years recommended by the U.S. EPA (2000, 2003) for the PHG cancer calculation because it accounts for more

uncertain variables. The cancer PHG is derived from the NTP (2004) gavage study because this provides a superior data set compared to the study of Kociba *et al*. (1979). In the latter study, which U.S. EPA used to estimate human cancer risk, survival was poor in all groups of control and exposed rats; at 2 years, only 8-22 percent of males, and 8-32 percent of females were still alive. The early mortality reduced the sensitivity of this study for determining the actual number of neoplasms induced by two years of exposure to TCDD. We believe that the NTP (2004) study, given its careful design and conduct, as well as improved survival rate, provides a superior basis for risk assessment.

Comment 11: "Can you justify the use of new female rat data and the methods used to calculate the CSF rather than other CSFs that could be calculated using other species/sex and/or other methods? (e.g., CSF calculations from male mice data (NTP 1982) would likely be different."

Response 11: All long-term carcinogenicity studies on TCDD have produced positive results. TCDD is a carcinogen at multiple sites in both sexes of rats and mice (U.S. EPA, 1985; IARC, 1997; NTP, 2004). Several studies in animals have demonstrated that female rats are more susceptible to TCDD-induced liver neoplasms than males. Sex hormones appear to exert a profound influence on the carcinogenic action of TCDD. Higher tissue concentrations and longer half-lives have been reported in females vs. males (Li *et al.,* 1995). The study design, species, and dose range used in the NTP (2004) study was based on earlier animal carcinogenicity studies. That is, female Sprague-Dawley rats were chosen because of the high incidence of hepatocarcinogenicity in females in this species and strain compared to males of this strain, as well as other species of test animals. Use of the most sensitive species, strain, and sex is standard procedure for health-protective risk estimates. The combined-site CSF calculation is now our default cancer potency calculation method, where data allow.

Comment 12: "Page 48 – carcinogenic effects – the implication is that the EPA human-data derived CSF is not conservative enough. You never say why this EPA CSF shouldn't be used or why your CSF (which is consistently less than the EPA Human CSF) is better."

Response 12: We believe that use of the NTP (2004) animal study from which our CSF was derived (and including U.S. EPA's recommended use of body burden as a dose metric for species extrapolation) constitutes a superior approach to U.S. EPA's derivation of a human CSF using epidemiological data. On pages 44-45 of the PHG document, we discuss the considerable limitations and uncertainties associated with the TCDD epidemiological literature, and in particular the lack of good exposure information. In general, potency estimates from animal studies have been found to be similar to those derived from human data (U.S. EPA, 2000).

Comment 13: *Non-cancer Hazard Calculation:* "If amyloidosis and dermatitis are effects that would be expected after a short-term exposure (prior to reaching steady state), why are they used as the principal/most sensitive chronic non-cancer endpoints? … Assuming that the dose metric used for amyloidosis and dermatitis is correct (dose and not body burden), using other studies that rely on body burden in a HED calculation would undoubtedly result in a lower RfD (e.g. NTP 2004). Why wasn't this discussed?"

Response 13: The critical study for the non-cancer PHG value has been changed to the NTP (2004) chronic exposure of female rats. The health-protective value is based on the LOAEL for significantly increased incidences of cell proliferation, gingival squamous hyperplasia, and cytochrome P450 induction, as well as significant increases in lung and liver weights.

Comments from Cambridge Environmental, Inc., Edmund Crouch

Comment 1: "The first and second entries, for liver, hepatocellular carcinoma, and liver cholangiocarcinomas are inconsistent with the remaining entries. The remaining entries have been calculated using MSTAGE (or a similar program) using a total of 6 parameters. To obtain the values in the "Applied Dose", the confidence limit has been calculated using 5 parameters for liver, hepatocellular carcinoma and 4 parameters for liver cholangiomas. The values using 6 parameters are 5,345 kg-d/mg and 14,134 kg-d/mg, respectively."

Response 1: When calculating values for the applied dose column, OEHHA constrained the MSTAGE model to four parameters (for liver cholangiomas) because of instability in fitting. Use of the later version of the MSTAGE model results in a small percent change in the combined site estimate for TCDD (2.7 vs. 2.6), a change of ~ 4 $x10^{-2}$ (0.1/2.6).

Comment 2: "The entry for "lung" is a factor of 10 too high. The correct value is 2,661 kg-d/mg. This looks like a typo."

Response 2: Agreed; the value for lung has been corrected in the PHG document (stated in the equivalent form of 0.266 x 10^{4} $(mg/kg\text{-}day)^{-1}$).

Comment 3: "A substantial part of pages 42-47 is spent detailing the purported advantages of using body burden to extrapolate to humans. At page 48, we are told "OEHHA agrees with the U.S. EPA's use of body burden as dose metric.…" Despite this, at the top of page 49, we have, "The combined cancer potency for the seven tumor sites identified in the NTP (2004) study is 2.6 x 10^{-2} $(ng/kg\text{-}day)^{-1}$." But this is potency calculated using intake doses and extrapolating to humans in the OEHHA standard way (assuming 70 kg human, 0.35 kg rat, and an interspecies factor proportional to the 1/3 power of the body

weight ratio). See Table 16 where this value is quite clearly derived for the "applied Dose q1*" for the "Combined site estimate for TCDD". This value does NOT correspond to using a body burden metric for extrapolation".

Response 3: The combined site estimate used in the initial PHG draft for the cancer calculation was incorrect. Instead, the adipose tissue equivalence combined site estimate q_1* should have been used. This has been corrected, and the cancer PHG value has been re-calculated.

Comment 4: *Inadequate description of methodology:* "The methodology described at page 46 for "Multi-Site Analysis" is too abbreviated to be adequate, although I believe I have reproduced what was done (see above). I believe that the 0.1 to 99.9 percentile points by steps of 0.1% were calculated (a total of 999 points), and these were sampled with equal frequency (but see item 7 below). The precise methodology should be specified. MSTAGE produces the percentiles one value at a time (there is a tabular facility, but it does not produce that particular table), and I understand some automated procedure was used to run MSTAGE. That procedure should be made publicly available (or I will modify MSTAGE if it is felt desirable to produce such tables; however, it is really unnecessary, see below). It is not clear whether 6, 5, or 4 parameters were used for some of the analyses (see items 1 and 3 above), and the basis for any such selection is not given. The spreadsheet that was used for the Monte Carlo procedure, and the data input to that spreadsheet, should also be provided, in order to allow an adequate technical evaluation. The approach of generating individual percentage points to approximate a distribution is cumbersome. A more elegant approach is to use the tables produced by MSTAGE that provide the change in log likelihood and various gradients as the parameter values are stepped. These tables can be used to fit the log likelihood very accurately with cubic splines, and these splines can then used for the distributions. However, this approach is unnecessary in this case (see item 5)."

Response 4: A combined response to comments 4 and 5 is provided at the end of comment 5, below.

Comment 5: "The Monte Carlo procedure described at page 46 ("Multi-Site Analysis") is unnecessary to sum across multiple end-points. An approach that simply extends the standard EPA style likelihood-based approach (as carried out for single end points in MSTAGE) is much easier, more in the spirit of the original (single-end-point) approach, and is readily implemented in a spreadsheet [indeed, all the calculations performed by MSTAGE are easy to carry out in a spreadsheet]. The standard approach to analysis of these bioassays simply calculates the loglikelihood for the observations, assuming binomial results and a linearized multistage dose-response. See Anderson *et al.* (1983), Quantitative Approaches in use to assess Cancer Risk, Risk Analysis 3(4)277–295. The upper confidence limit on the linear term is found by maximizing that linear term (treating all the parameters of the dose-response model, including the linear

term, as free to vary) subject to twice the decrease in loglikelihood from its maximum value being less than or equal to a critical value (approximately 2.70554). Extension to the sum of multiple end points is straightforward. The log likelihood in this case is formed as the sum of the log likelihoods for all the end points treated in exactly the same way as for evaluation of each end point individually (with individual dose-response curves for each). Then the sum of the linear terms is obtained, and its upper confidence limit is found in exactly the same way (maximizing this sum, treating all the parameters of all the dose-response models for the individual end points as free to vary, subject to twice the decrease in log likelihood being less than or equal to the critical value)."

"If my hypothesis as to the procedure adopted is correct [see item 4 above], the Monte Carlo procedure adopted is slightly incorrect. Sampling the 0.1% step 0.1% to 99.9% points effectively omits the two 0.05% regions at the top and bottom ends. A better approximation would be to generate the 0.05% step 0.1% to 99.95% points, and sample those (1000) points with equal probability. [It is possible that the 0.1% step 0.2% to 99.9% points were sampled with equal probability, which would be correct, but I cannot tell from the material presented]. The effect of this correction would be small (I have not bothered to evaluate it) compared with other approximations involved."

Response to comments 4 and 5: While using MSTAGE tables may be a more elegant methodological approach, it does not alter the overall result (i.e., accuracy). The Monte Carlo procedure has been peer-reviewed through the regulatory setting processes, and provides an acceptable degree of transparency. We believe that the Monte Carlo approach to approximating a distribution is more easily understood by the public than discussing alterations and likelihood functions. OEHHA has adopted several standards based on this approach.

Comment 6: *The introduction of LED_{01} on page 49 is misleading.* "On page 49, following the first equation, the definition of CSF is given as $0.01/LED_{01}$. This is incorrect, however, since the CSF in this case is not so derived".

Response 6: Agreed. This mistake has been corrected.

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