

# Responses to the comments of Todd Abel on behalf of the Chlorine Chemistry Division of the American Chemistry Council

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## EXECUTIVE SUMMARY

### **Comment 1.**

*OEHHA is proposing to replace the Toxicity Equivalency Factors (TEF), which were developed by the World Health Organization (WHO) in 1997, with the more recent TEF values developed by the WHO in 2005. We agree with and support this revision. As noted correctly by OEHHA, the TEF methodology has evolved and improved over the years as additional toxicological data became available. The adoption of the WHO 2005 TEF values is a logical and laudable step in the direction of improving the TEF methodology. Although it is widely acknowledged that there are a number of limitations of the TEF methodology, it provides a useful tool for the health risk assessment of dioxins and dioxin-like compounds. The TEF approach is particularly valuable for screening risk assessment and as an interim approach to risk assessment until more appropriate data become available.*

*According to the OEHHA Notice, the TEF Document is also designed “to review recent scientific literature on this [TEF] methodology.”<sup>1</sup> However, the TEF Document does not include a complete review of the most current scientific literature. We would like to bring to OEHHA’s attention several important scientific publications on this topic. Specifically, the TEF Document does not include:*

- *OEHHA needs to include the most recent technical literature in its review of TEFs and TEF methodology (from cover letter)*
- *the recent review of the TEF methodology by the National Academy of Sciences (NAS, 2006)*
- *the recent 2-year cancer bioassays conducted by the National Toxicology Program (NTP) to evaluate the TEF methodology by assessing mixtures of dioxin-like compounds (NTP, 2006a-g; 2009)*
- *the paper by Haws et al. (2006), which presented the refined database that served as the basis for the 2005 WHO review*
- *the USEPA (2008) document concerning the applications of TEFs in the assessment of ecological risk (which includes concepts that are directly applicable to both human health and ecological risk assessment)*

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<sup>1</sup> [http://www.oehha.ca.gov/air/hot\\_spots/crn080709.html](http://www.oehha.ca.gov/air/hot_spots/crn080709.html)

- *additional publications that provide important new information concerning the mode of action, toxicity, and relative potency for various dioxin-like compounds, as well as the applicability of TEF methodology (e.g., Carlson et al., 2009; Connor et al., 2008; Simon et al., 2008; Zhang et al., 2008; Budinsky et al., 2006)*

*OEHHA should include these references, and others identified in the more complete comments and information that follow, in its review of recent scientific literature on this methodology.*

## **Response**

- OEHHA has added/cited some new literatures in the document following public comments, which include references by Amakura Y et al. 2003; Connor KT et al. 2008; de Waard WJ et al. 2008; Degner SC et al. 2009; Giesy JP et al. 1998; Haws LC et al. 2006; Hong B et al. 2009; Huwe J et al. 2009; Jeuken A et al. 2003; NTP, 2006; Seegal RF et al. 2010; Simon T et al. 2008; Zhang S et al. 2003; and Zhang S et al. 2008. However, none of these references would change the TEF method or values used in risk assessment for dioxin and dioxin-like chemicals (DLCs) based on the WHO TEF criteria.
- OEHHA agrees with the statement in the National Academy of Sciences (NAS, 2006) report that “overall, even given the inherent uncertainties, the toxic equivalency factor (TEF) method provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative toxic potency of DLCs on human and animal health.” (<http://www.ejnet.org/dioxin/nas2006.pdf>).
- OEHHA has cited the National Toxicology Program (NTP, 2006) report. This report provides results of a series of studies in which rodents were exposed to either a single dioxin-like compound or mixtures of them for up to two years, and then evaluated for toxicity and carcinogenicity relative to TCDD. The NTP notes “Analysis of data from one group of completed studies confirms the assumption that the effects of the dioxin-like compounds in mixtures are additive. The number of cancer cases in the rats exposed to the mixture could be predicted accurately by adding the concentration of each compound, adjusted for its potency relative to TCDD using TEFs.” (<http://ntp.niehs.nih.gov/ntp/Factsheets/DioxFacts061.pdf>).
- OEHHA recognizes that the WHO TEF 2005 reevaluation process had used the refined TEF database published by Haws et al. (2006) as a starting point, which facilitated better characterization of the variability and uncertainty inherent in the data (Haws, et al. 2006). Decisions about a TEF value were made based on a combination of unweighted relative effect potency (REP) distributions from Haws’ database, expert judgment, and point estimates (Van den Berg et al. 2006).
- OEHHA cites the U.S. EPA’s new draft (2009) for adopting the WHO 2005 TEF values at ([http://www.epa.gov/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/raf/files/hhtef_draft_090109.pdf)).
- OEHHA has cited some references and rejected others; please refer to our responses below and to comment 24 for further details.

These points are addressed in the responses provided to the detailed comments below. OEHHA has added some necessary new literature citations to the document. It should be noted that the intention of this proposal is merely to update the TEF table to the latest version. The accompanying document titled “Use of the Toxicity Equivalency Factor (TEF WHO<sub>97</sub> and TEF WHO<sub>05</sub>) Scheme for Estimating Toxicity of Mixtures of Dioxin-Like Chemicals” is not a review article regarding TEF methodology, but is intended to identify the key issues and sources used by WHO in deriving the latest TEF table. However, we do update some necessary new references to address the differences between the new WHO (2005) TEFs and the old ones. The underlying TEF/TEQ methodology is unchanged.

US EPA released a new draft TEF document on September 1, 2009. The U.S. EPA recommends the use of the consensus TEF values for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and DLCs published in 2005 by the World Health Organization (WHO). The U.S. EPA recommends these TEFs be used for all effects mediated through aryl hydrocarbon receptor (AhR) binding by the DLCs including cancer and non-cancer effects. Using information that summarizes the range of relative toxicities of the DLCs, the U.S. EPA suggests that “conduct of a sensitivity analysis be considered to illustrate the impact the TEFs have on the predicted risk.” However, in U.S. EPA’s new draft, they propose to adopt the WHO 2005 TEF values ([http://www.epa.gov/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/raf/files/hhtef_draft_090109.pdf)).

OEHHA is also adopting the WHO 2005 TEFs and will update these recommendations in the future based on the evaluation of new toxicity data for DLCs and the results of new consensus processes undertaken to update the TEF approach. A main goal of this document is to assist risk assessors in applying the toxicity equivalence methodology correctly.

For PCB 126, NTP (2006) concluded that “Under the conditions of this 2-year gavage study there was *clear evidence of carcinogenic activity*\* of PCB 126 in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma of the liver, squamous neoplasms of the lung (cystic keratinizing epithelioma and squamous cell carcinoma), and gingival squamous cell carcinoma of the oral mucosa. Hepatocellular adenoma and hepatocholangioma of the liver were also considered to be related to the administration of PCB 126. Neoplasms of the adrenal cortex and cholangioma of the liver may have been related to administration of PCB 126. PCB 126 administration caused increased incidences of nonneoplastic lesions of the liver, lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, thymus, spleen, clitoral gland, and mesenteric artery in female rats.” ([http://ntp.niehs.nih.gov/files/TR\\_520\\_Web.pdf](http://ntp.niehs.nih.gov/files/TR_520_Web.pdf)).

For TCDD, NTP (2006) concluded that “Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity*\* of TCDD in female Harlan Sprague-Dawley rats based on increased incidences of cholangiocarcinoma and hepatocellular adenoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. The increased incidence of squamous cell carcinoma of the uterus was also considered to be related to TCDD administration. The marginally increased incidences of pancreatic acinar neoplasms and occurrences of hepatocholangioma and cholangioma of the liver may have been related to TCDD administration. TCDD administration caused increased incidences of nonneoplastic lesions of the liver, lung, oral mucosa, pancreas, thymus, adrenal cortex, heart, clitoral gland, kidney, forestomach, mesentery, and thyroid gland in female rats.” ([http://ntp.niehs.nih.gov/files/521\\_Web.pdf](http://ntp.niehs.nih.gov/files/521_Web.pdf)).

For PeCDF, NTP (2006) concluded that “Under the conditions of this 2-year gavage study, there was *some evidence of carcinogenic activity\** of PeCDF in female Harlan Sprague-Dawley rats, based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa. Occurrences of cystic keratinizing epithelioma of the lung, neoplasms of the pancreatic acinus, and carcinoma of the uterus may have been related to administration of PeCDF. PeCDF administration caused increased incidences of nonneoplastic lesions of the liver, oral mucosa, uterus, lung, pancreas, thyroid gland, thymus, adrenal cortex, kidney, heart, and forestomach in female rats.” ([http://ntp.niehs.nih.gov/files/525\\_Web\\_Final.pdf](http://ntp.niehs.nih.gov/files/525_Web_Final.pdf)).

For a mixture of TCDD, PeCDF, and PCB 126, NTP (2006) concluded that “Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity\** of the mixture of TCDD, PeCDF, and PCB 126 in female Harlan Sprague-Dawley rats based on increased incidences of hepatocellular adenoma and cholangiocarcinoma of the liver and cystic keratinizing epithelioma of the lung. Neoplasms of the pancreatic acinus may have been related to administration of the mixture of TCDD, PeCDF, and PCB 126.

Administration of the mixture of TCDD, PeCDF, and PCB 126 caused increased incidences of nonneoplastic lesions of the liver, lung, pancreas, adrenal cortex, oral mucosa, uterus, thymus, ovary, kidney, heart, bone marrow, urinary bladder, mesenteric artery, and thyroid gland in female rats.” ([http://ntp.niehs.nih.gov/files/526\\_Web\\_Final.pdf](http://ntp.niehs.nih.gov/files/526_Web_Final.pdf)).

A dioxins mixtures research fact sheet by NTP (2006) reported “the NTP carried out a series of studies in which rodents were exposed to either a single dioxin-like compound or mixtures of them for up to two years and then evaluated for toxicity and carcinogenicity relative to TCDD. Analysis of data from one group of completed studies confirms the assumption that the effects of the dioxin-like compounds in mixtures are additive. The number of cancer cases in the rats exposed to the mixture could be predicted accurately by adding the concentration of each compound, adjusted for its potency relative to TCDD using TEFs.” (<http://ntp.niehs.nih.gov/ntp/Factsheets/DioxFacts061.pdf>).

For a mixture of PCB 126 and PCB 118, NTP (2006) concluded that “Under the conditions of this 2-year gavage study there was *clear evidence of carcinogenic activity\** of the mixture of PCB 126 and PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of holangiocarcinoma and hepatocellular neoplasms (predominantly hepatocellular adenomas) of the liver and cystic keratinizing epithelioma of the lung. The marginally increased incidences of gingival squamous cell carcinoma of the oral mucosa were also considered to be related to administration of the mixture of PCB 126 and PCB 118. Occurrences of cholangioma and hepatocholangioma of the liver may have been related to administration of the mixture of PCB 126 and PCB 118. Administration of the mixture of PCB 126 and PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, thymus, thyroid gland, adrenal cortex, pancreas, kidney, heart, lymph nodes, mesenteric artery, brain, forestomach, spleen, and nose.” ([http://ntp.niehs.nih.gov/files/531\\_Web.pdf](http://ntp.niehs.nih.gov/files/531_Web.pdf)).

For a mixture of PCB 126 and PCB 153, NTP (2006) concluded that “Under the conditions of this 2-year gavage study there was *clear evidence of carcinogenic activity\** of a constant ratio binary mixture of PCB 126 and PCB 153 in female Harlan Sprague-Dawley rats based on

increased incidences of cholangiocarcinoma, hepatocholangioma, and hepatocellular neoplasms (predominantly adenomas) of the liver, squamous neoplasms of the lung (predominantly cystic keratinizing epithelioma), and gingival squamous cell carcinoma of the oral mucosa. Increased incidences of pancreatic acinar neoplasms were also considered to be related to administration of the binary mixture of PCB 126 and PCB 153. The increased incidences of uterine squamous cell carcinoma may have been related to administration of the binary mixture of PCB 126 and PCB 153.

Administration of the binary mixture of PCB 126 and PCB 153 caused increased incidences of nonneoplastic lesions in the liver, lung, oral mucosa, pancreas, adrenal cortex, thyroid gland, thymus, kidney, nose, and forestomach.” ([http://ntp.niehs.nih.gov/files/TR530\\_Web1.pdf](http://ntp.niehs.nih.gov/files/TR530_Web1.pdf)). For PCB 118, NTP (2009) draft concluded that “Under the conditions of this 2-year gavage study, there was *clear evidence of carcinogenic activity* of PCB 118 in female Harlan Sprague-Dawley rats based on increased incidences of neoplasms of the liver (cholangiocarcinoma, hepatocholangioma, and hepatocellular adenoma) and cystic keratinizing epithelioma of the lung. Occurrences of carcinoma in the uterus were considered to be related to the administration of PCB 118. Occurrences of squamous cell carcinoma of the uterus and acinar neoplasms of the pancreas may have been related to administration of PCB 118. Administration of PCB 118 caused increased incidences of nonneoplastic lesions in the liver, lung, adrenal cortex, pancreas, thyroid gland, nose, and kidney.” ([http://ntp.niehs.nih.gov/files/559\\_Board\\_Web.pdf](http://ntp.niehs.nih.gov/files/559_Board_Web.pdf)).

Walker et al. (2005) evaluated the TEF approach in 2-year rodent cancer bioassays with female Harlan SD rats receiving 2,3,7,8-TCDD, PCB-126, PeCDF, or a mixture of the three compounds. By using a statistically based dose–response model, they found that the shape of the dose–response curves for hepatic, lung, and oral mucosal neoplasms was the same in studies of the three individual chemicals and the mixture. In addition, the dose response for the mixture could be predicted from a combination of the potency-adjusted doses of the individual compounds. Finally, they showed that use of the current WHO TEF values adequately predicted the increased incidence of liver tumors (hepatocellular adenoma and cholangiocarcinoma) induced by exposure to the mixture. Their data support the use of the TEF approach for dioxin cancer risk assessments (Walker et al., 2005).

We cited the Haws et al. (2006) paper in the document. WHO TEF experts (2005) also “emphasized that for this 2005 TEF reevaluation, the expert panel used all available REPs, either included or excluded in this database (Haws et al., 2006), and made their own assessment. Studies published since the 1997 reevaluation were also fully evaluated” (Van den Berg, et al., 2006).

US EPA (2009) recommended Toxicity Equivalency Factors (TEFs) for Human Health Risk Assessments of Dioxin and Dioxin-Like Compounds: EXTERNAL REVIEW DRAFT ([http://www.epa.gov/OSA/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/OSA/raf/files/hhtef_draft_090109.pdf)). U.S. EPA proposes adopting WHO 2005 TEFs.

Carlson et al. (2009) employed microarray technology to reveal species differences in response to two prototypical AhR agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin and the PCB 126. Dose responses of primary cultures of rat and human hepatocytes were determined using (Species-specific) over 4000 gene orthologs. Forty-seven human and 79 rat genes satisfied dose-response

criteria for both chemicals and were subjected to further analysis including the calculation of the 50% effective concentration and the relative potency (REP) of PCB 126 for each gene. They reported only five responsive orthologous genes were shared between the two species. The geometric mean of the REPs for all rat and human modeled responsive genes were 0.06 (95% confidence interval [CI]; 0.03–0.1) and 0.002 (95% CI; 0.001–0.005), respectively, suggesting broad species differences in the initial events that follow AhR activation but precede toxicity. As shown in Figure 2, the REP for PCB 126 in humans ranged from about  $10^{-6}$  to 1, whereas for rat the range is relatively smaller (about 0.1 to less than 1000), which suggests that humans have higher variability compared to rats and this species-specific sensitivity must be considered in the risk assessment. Simple comparison of two geometric means may not be enough in the risk assessment to protect sensitive human populations. Similar phenomena were described in figure 5 for EC<sub>50</sub>. In addition, they did not report whether they confirmed that there were five responsive orthologous genes by other methods such as Northern Blot or RT-PCR as microarray data may give false positive or negative results. Furthermore, this paper does not affect the proposal to adopt WHO 2005 TEFs, and thus is not cited in Appendix C.

Connor et al. (2008) measured the activity of AhR-driven reporter gene as an induction equivalent (IEQ) compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or IEQ concentration in human blood samples from 10 volunteers under different dietary regimens. Blood concentrations of polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), as determined by analytical chemistry (HR-GC/MS) and expressed as toxic equivalents (TEQs) with the use of TCDD equivalency factors (TEFs), were within a range that has been reported in the general US population [0.022 to 0.119 ppt (whole blood basis)]. However, the human blood IEQ measured directly via bioassay ranged from 13.4 to 218 ppt (whole blood basis). These order of magnitude greater IEQs compared to the TEQs for dioxins, furans, and certain PCBs suggests that human blood contains a relatively high level of AhR agonists able to activate the CYP1A1 dioxin response element (DRE)-linked reporter gene bioassay, and that this AhR activity is not accounted for by PCDDs/Fs and dioxin-like PCBs based on standard HR-GC/MS and TEF analysis. When study participants switched from a "baseline" to a high-vegetable diet, increases in bioassay IEQ were observed that were statistically significant ( $P < 0.05$ ). In addition, IEQ activity was elevated above levels observed following dietary intervention in two subjects given indole-3-carbinol (I3C) supplements. They concluded that a substantial portion of the IEQ activity occurred as a result of the increased intake of natural AhR agonists (NAHRAs) present in many fruits, vegetables, and herbs. They also suggested that dietary NAHRAs constitute a substantial daily dietary intake of AhR-active compounds, and these NAHRAs could influence AhR status in humans and play a role in a basal level of AhR activation. However, as their study was carried out in human blood samples from 10 volunteers under different dietary regimens, it is not representative of the general population. Intraspecies difference plus some potential confounding factors may overwhelm their findings. Further, dietary AhR agonists have not been shown to induce toxicity similar to dioxin.

Zhang et al. (2008) investigated structure-dependent differences in activation of the AhR by a series of halogenated aromatic hydrocarbons. TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 3,3',4,4',5-pentachlorobiphenyl (PCB126) induced CYP1A1-dependent activities in HEK293 human embryonic kidney, Panc1 pancreatic cancer, and Hepa1c1c7 mouse hepatoma cell lines. They found a structure-dependent difference in the efficacy of TCDF and PCB126 in HEK293

and Panc1 cells since induced CYP1A1mRNA levels were lower than observed for the other congeners. Their results of the mammalian two-hybrid studies demonstrated that activation of pGAL4-luc in cells transfected with VP-AhR and GAL4-coactivator chimeras is dependent on the structure of the HAH congener, cell context, and coactivator, suggesting that the prototypical HAH congeners used in their study exhibit selective AhR modulator activity. We added this paper (Zhang et al, 2008) to the discussion in Appendix C.

Budinsky et al. (2006) analyzed NTP cancer bioassays for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) by chi-squared tests. They indicated that “the current TEF value of 0.5 value is too high” for 4-PeCDF (Budinsky et al., 2006). Actually, the values for 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF are lower in the WHO 2005 TEF compared to the 1998 TEF values. The WHO TEF expert group considered a much wider set of data in arriving at their TEF values. We don’t think it is necessary to cite this paper. The fact is the current TEF value for 4-PeCDF is lower than 0.5 (van den Beg, et al 2006).

## **Comment 2.**

*One of the most significant improvements in utilizing TEFs in dioxin exposure and risk assessment involves the characterization of the variability and uncertainty in TEFs (examined by Haws et al., 2006, 2008 and 2009). The TEF Document should include a statement about the potential benefits of probabilistic treatment of TEF values in risk assessment. Both NTP and WHO, as well as a recent National Academy of Science panel (NAS, 2006), acknowledged the importance of better characterizing the variability and uncertainty inherent in TEFs. Further, the USEPA has indicated its support of the use of probabilistic treatment of TEF values to improve risk assessment (USEPA, 2008). It is well recognized that TEFs represent a point estimate for a range of underlying relative potencies for a specific dioxin, furan or PCB congener compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Moreover, the range of underlying relative potencies from which the TEF estimate is selected, represents a disparate collection of biological effects and studies that vary as to quality and the quantitative dose-response information. Many times, a dioxin, furan, or PCB’s data set is limited to simple liver enzyme induction which is an adaptive rather than a true toxicological effect. Rarely does a dioxin, furan, or PCB congener possess higher tiered toxicological studies (e.g., cancer bioassays or reproductive/developmental studies) for developing relative potencies and TEFs for the most significant and relevant risk assessment endpoints. Because of these limitations and recognized variability and uncertainty in TEFs, Haws and investigators recommend using probabilistic risk assessment methods based on the range of relative potencies that the TEF value falls within. This should be endorsed by OEHHA as a new scientific and risk assessment advancement leading to improvements in how TEFs are applied.*

## **Response**

While we are interested in the probabilistic risk assessment method, there are some problematic issues in the probabilistic analysis of TEF data which have not yet been resolved. The paper by Van den Berg et al. (2006) discusses these issues in detail. They note that “A disadvantage could be that such an approach lumps all data together and gives similar weight to all types of studies. In part, this problem could be avoided by separating in vitro from in vivo REPs (Haws et al., 2006). However, if probabilistic approaches for setting a future TEF are used, it is essential that

weighting factors be applied to REPs that are determined from different types of studies. These weighted REP values could then be used to determine weighted REP distributions in the risk assessment process. Clearly, unweighted REP distribution ranges that bracket the TEFs incorporate biological and toxicological uncertainty. For this reason, in the WHO 2005 TEF reevaluation, unweighted REP distribution ranges, expert judgment, and point estimates were used in combination to assign TEFs. The sole use of a probabilistic approach to determine TEF values also includes other decision points, such as establishing a range instead of a point estimate for the TEF value. However, the use of a TEF range might cause problems for regulatory authorities and international harmonization of TEF values because one or more TEF values could then be selected for risk assessment calculations. This might easily lead to different TEFs being used by different countries depending on the level of conservatism used in the risk management process by national authorities. In this respect, the choice, e.g., of a 50th, 75th, or 95th percentile of the REP distribution range to assign a TEF is a risk management decision.” (van den Berg et al., 2006). Although the USEPA has indicated its support of the use of probabilistic treatment of TEF values to improve risk assessment (USEPA, 2008), the newest TEF draft document (September 1<sup>st</sup>, 2009) by USEPA is still proposing to adopt the WHO TEF 2005 values ([http://www.epa.gov/OSA/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/OSA/raf/files/hhtef_draft_090109.pdf)).

### **Comment 3.**

*The TEF Document should indicate that, in certain circumstances, alternative methodologies for risk assessment may be more appropriate than the current TEF approach. It is important to recognize that the TEF methodology was first developed as an interim approach to assess the potential health risks associated with exposures to complex mixtures of dioxin-like compounds. The TEF Document should state, “When reliable data become available that provide better estimates of risk (either as part of the TEF approach or as an alternative to the TEF approach), such data should be used.”*

- *The TEF methodology should be flexible to allow congener-specific or site-specific toxicity values in risk assessment rather than insist on utilizing TEFs with all their uncertainty and issues. (From cover letter)*

*OEHHA should consider developing cancer potency values for certain dioxin-like compounds in lieu of TEFs, when adequate data are available. For example, NTP has conducted 2-year cancer bioassays for 2,3,4,7,8-penachlorodibenzofuran, PCB 118, PCB 126, and PCB 153. As such, cancer slope factors can now be calculated for these specific dioxin-like compounds rather than having to rely on TEFs. The TEF Document should acknowledge that, under certain circumstances, it may be better to develop cancer potency values than to rely on TEFs.*

### **Response**

The WHO TEF method is a congener-specific risk assessment for DLC mixtures and is the best available method identified by worldwide experts, as we addressed in the document. This appendix is about how to use TEFs to assess the risk of DLC mixtures, rather than individual cancer potencies. We are not against using site-specific toxicity risk assessment when it is appropriate and the supporting data are available.

## Comment 4.

*Additional recommendations for OEHHA's consideration include:*

- *The TEF Document should address the significance of recent data that demonstrate that 2,3,7,8-TCDF does not bioaccumulate, as well as the implications of this finding on the applicability of the TEF approach for this specific compound.*
- *OEHHA should include a discussion of recent studies indicating broad species differences in the initial events that follow Aryl hydrocarbon receptor (AhR) activation, as well as congener-specific differences in genetic responses.*
- *The TEF Document should include the study by Connor et al. (2008) which demonstrated that a dietary exposure regimen in which individuals ingesting food containing naturally-occurring AhR ligands over a period of days is sufficient to alter the amount of AhR activating substances in human blood. This finding conflicts with statements in the TEF Document that naturally-occurring AhR ligands have short half-lives in humans, do not bioaccumulate, and are not relevant to the TEF framework.*
- *Consider the impact of naturally-occurring AhR ligands in the context of TEFs and update the discussion on naturally-occurring AhR ligands to reflect the current state of the science. (From cover letter)*

## Response

OEHHA intends to adopt the WHO TEF values, instead of generating new values at present. Recently, Milbrath et al. (2009) reported that 2,3,7,8-TCDF has a median half-life of 0.9 year, which means 2,3,7,8-TCDF is persistent and bioaccumulative. The current data are not sufficient to support a re-evaluation of WHO 2005 TEFs, although OEHHA will continue to monitor the data available and the status of the ongoing WHO review program for the TEFs.

Simon et al., (2008) calculated cancer slope factor estimates for 2,3,4,7,8-PeCDF that ranged from  $6 \times 10^{-2}$  to  $6 \times 10^{-3}$  (ng/kg/day)<sup>-1</sup>, based on the lifetime average liver and adipose tissue concentration data from the two-year NTP study in female Sprague-Dawley rats. We added this paper to the document.

The WHO 2005 TEF values for PeCDF are lower than in the previous version of TEFs (reduced from 0.05 to 0.03 for 1,2,3,7,8-PeCDF and from 0.5 to 0.3 for 2,3,4,7,8-PeCDF, respectively), which is consistent with the results of the NTP study. WHO (2005) keeps the same value (0.1) for 2,3,7,8-TCDF. OEHHA agrees with WHO and USEPA that overall the TEF values for dioxin and dioxin-like compounds are valid (van den Berg et al., 2006) ([http://www.epa.gov/OSA/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/OSA/raf/files/hhtef_draft_090109.pdf)). OEHHA does not agree with the statement “2,3,7,8-TCDF does not bioaccumulate”.

We discussed the variation issues in the document and added some additional citations that showed AhR activities are tissue and enzyme-specific with wide variability (page 12). However, the WHO TEF method is still the best available approach for assessing DLC mixtures.

As noted in response to comment 1, we updated the citations regarding naturally occurring AhR ligands. Connor et al., (2008) reported that they measured an induction equivalent (IEQ) as compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or IEQ concentration in human blood samples. They studied 10 volunteers under different dietary regimens, which seems insufficient to represent the variability within the human population. Also, some potential confounding factors may exist. However, we agree with van den Berg, et al., (2006) who reported “the majority of toxicity studies demonstrated that these naturally occurring AhR agonists fail to produce AhR-dependent toxicity (Leibelt et al., 2003; Pohjanvirta et al., 2002), although some developmental dioxin-like effects have been reported for indole-3-carbinol (I3C) (Wilker et al., 1996). In addition, naturally occurring AhR ligands, such as I3C and diindolymethane, have been reported to inhibit 2,3,7,8-TCDD-dependent in vivo induction of CYP1A1 and immunotoxicity (Chen et al., 1995, 1996).”

## 2. INTRODUCTION

*The following comments address OEHHA’s intention of adopting the 2005 WHO TEF values and OEHHA’s objective of updating the scientific information behind the WHO TEF methodology. We recognize the utility of modernizing the TEF values to reflect the most recent thinking of the WHO panel (van den Berg et al., 2006). However, our recognition extends only to the application of TEFs as a screening level methodology for assessing exposure and risk to dioxins, furans, and dioxin-like PCBs. As exposure and risk assessments grow in complexity and significance, with respect to remediation efforts or risk characterization of dioxins, it is necessary to thoroughly explore the uncertainty and variability in utilizing TEFs in exposure and risk assessment. According to the OEHHA Notice, the TEF Document is also designed “to review recent scientific literature on this [TEF] methodology.”<sup>2</sup> However, the TEF Document does not include several important scientific publications on this topic. Within these comments we provide more recent scientific data by which OEHHA can meet their objective of updating the TEF methodology. Specific examples of scientific advancements in the use of TEFs are discussed and this information should be included in any updates on the TEF methodology. In addition, any update in TEF methodology must address the significant variability and uncertainty in the use of TEFs and how this variability and uncertainty can be qualitatively and quantitatively addressed in exposure and risk characterization for dioxin.*

### **Comment 5.**

***OEHHA Proposes to adopt the 2005 WHO TEF values as well as update the TEF data and methodology: “This document updates the background and methodology for the use of TEF methods for dioxins and DL-compounds....” (Executive Summary). However, as explained in this general comment and developed in greater detail within the specific scientific examples, the OEHHA Technical Support Document (TSD) does not include and discuss more recent scientific publications and advancements in understanding and applying the***

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<sup>2</sup> [http://www.oehha.ca.gov/air/hot\\_spots/crn080709.html](http://www.oehha.ca.gov/air/hot_spots/crn080709.html)

***TEF methodology. For example, a modern examination of the TEF methodology must include information on the use of probabilistic methodologies around the underlying datasets supporting the TEF value, so that the uncertainty in the use of TEFs can be more fully elucidated. A number of specific scientific advancements in the use of TEFs are described more fully below for OEHHA's consideration.***

*This draft document intended to update the TEFs recommended by the most recent WHO expert panel, as well as to review recent scientific literature on concerning the TEF methodology. The most substantive update in the TSD TEF document is the replacement of the 1997 WHO TEF values with those recommended by the 2005 WHO expert panel as outlined by van den Berg et al. (2006). The update does not include the most important documents released over the last several years concerning the TEF methodology, including the paper by Haws et al. (2006) which presented the refined database that served as the basis for the 2005 WHO review, the recent 2-year cancer bioassays conducted by the NTP to evaluate the TEF methodology by assessing mixtures of dioxin-like compounds (NTP, 2006a-g and 2009; Toyoshiba et al., 2004; Walker et al., 2005 and 2006), the USEPA document concerning the applications of TEFs in the assessment of ecological risk (which includes concepts that are directly applicable to both human health and ecological risk assessment) (USEPA, 2008), and the recent review of the TEF methodology by the National Academy of Sciences (NAS, 2006). In addition to these seminal documents, there have been a host of experimental studies that have been published and provide important new information concerning our understanding of the mode of action, toxicity, and relative potency for the various dioxin-like compounds, as well as the applicability of the TEF methodology (Carlson et al., 2009; Connor et al., 2008; Simon et al., 2008; Zhang et al., 2008). A search of PubMed on September 8, 2009 indicated that 72 published papers explicitly mention the acronym "TEF" and these papers should be screened for relevant information in order to update TEF guidance. Further, the update should acknowledge many of the key issues identified by the 2005 WHO expert panel (van den Berg et al., 2006). In these comments we have addressed a number of these more recent TEF-update studies.*

## **Response**

Our responses to comments 1 and 2 address this point. This document examines the differences between the latest WHO TEF (2005) and the earlier versions, in addition to the history, mechanism, rationale, application, and uncertainty of TEF in risk assessment. There is no proposal to update the TEQ methodology other than the new TEF values, nor to generate new TEFs based on AhR at this point. We cited a limited number of papers for this appendix as it only addresses the latest values adopted by the WHO.

### **Comment 6.**

***TEFs are an interim methodology indicating the need for continual improvement and refinement of the TEF methodology as new science becomes available. These limitations must be acknowledged in order that the uncertainty in characterizing exposures and risk for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans is clearly established.***

*We support OEHHA's proposal to adopt the 2005 WHO TEFs in place of the 1997 WHO TEFs, especially as a screening level methodology. The TEF methodology endorsed in the OEHHA update is applicable to the subset of structurally-related polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) that have been observed to induce a similar spectrum of biochemical and toxic responses in experimental animals that is characterized by severe weight loss, thymic atrophy, hepatotoxicity, edema, fetotoxicity, teratogenicity, reproductive toxicity, immunotoxicity, and enzyme induction (Birnbaum, 1994; Birnbaum and Tuomisto, 2000; DeVito and Birnbaum, 1994; McConnell et al., 1978; Safe, 1990; Schwetz et al., 1973). The TEF methodology is predicated on the assumption that these effects are mediated through a common mechanism of action initiated by binding to and activation of the AhR (Birnbaum, 1994; Hankinson, 1995; Martinez et al., 2003; Okey et al., 1994; Safe, 1990; Sewall and Lucier, 1995). This subset of PCDDs, PCDFs, and PCBs is commonly referred to as the "dioxin-like" compounds (DLCs) and consists of the 17 laterally-substituted (2,3,7,8-substituted) PCDD and PCDF congeners, and 12 non-ortho and mono-ortho chlorine-substituted PCBs.*

*Because the DLCs are typically detected in the environment as part of complex mixtures of structurally-related polyhalogenated aromatic hydrocarbons (Birnbaum, 1999; Safe, 1994) and assumed to act through a common mechanism of action, a toxic equivalency factor (TEF) methodology was developed as an "interim approach" to assess the potential health risks associated with exposures to mixtures of DLCs (Ontario Ministry of the Environment, 1984; USEPA 1987 and 1989; van den Berg et al., 1998 and 2006). The TEF methodology is based on the concept of dose addition, whereby the toxicokinetics and the toxicodynamics of all components are assumed to be similar and the dose-response curves of the components of a mixture are assumed to be similarly shaped, i.e., parallel curves with similar maximal responses. In accordance with these assumptions, the combined toxicity of the individual components is estimated based on the sum of their doses, which are scaled for potency relative to 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD), the most toxic and well-studied member of the class of DLCs (USEPA 1987, 1989, and 2000; van den Berg et al., 1998 and 2006).*

*The additive TEF model has been accepted by numerous regulatory agencies worldwide as the most reasonable method currently available for evaluating potential health risks associated with exposures to DLC mixtures (Birnbaum, 1999; Birnbaum and DeVito, 1995; NATO/CCMS, 1988b; Olson et al., 1989; USEPA 1987 and 1989; van den Berg et al., 1998 and 2006; Yrjanheikki, 1992). However, uncertainties associated with use of the TEF methodology [e.g., non-additive interactions, natural ligands for the Ah receptor, differences in species responsiveness, differences in the shape of dose-response curves (Birnbaum and DeVito, 1995; Haws et al., 2006; van den Berg et al., 1998 and 2006)] continue to challenge their utilization, especially regarding their inherent uncertainty in accurately depicting the true exposure and risk potential. Only a few studies involving mixtures of DLCs have shown approximate mixture additivity as predicted by TEFs (DeCarprio et al., 1986; Silkworth et al., 1989; Suter-Hoffman and Schlatter, 1989). More recently, as part of the NTP's comprehensive evaluation of the ability of the TEF approach to predict cancer potency for dioxin-like compounds, Walker et al. (2005 and 2006) concluded that the 1998 WHO TEFs adequately predicted the increased incidence of liver tumors induced by exposure to a mixture of TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and PCB 126. This position, however, is controversial. Some investigators have concluded that the NTP bioassays demonstrate that the TEFs may not*

*be adequate. As an example, Budinsky et al. (2006) assessed the validity of the 2005 WHO TEF for 2,3,4,7,8-PeCDF by evaluating the liver and adipose tissue concentration data in addition to the administered dose data reported in the 2-year NTP study of 2,3,4,7,8-PeCDF (NTP TR 525). Goodness-of-fit statistical analyses of the NTP bioassay liver tumor response data for TCDD and 4-PeCDF (dose-response curves based on independent Weibull models) failed to support the null hypothesis that 4-PeCDF has a relative potency factor of 0.5 (the current 2005 WHO TEF). In fact, Budinsky et al. (2006) calculated relative potency factors of 0.26, 0.014, 0.021, and 0.036 for administered dose, liver concentration at terminal sacrifice, liver concentration area under the curve, and lifetime average body burden, respectively. Because the cumulative dose parameters take into account the pharmacokinetics of 2,3,4,7,8-PeCDF, using these internal dose metrics in the derivation of relative potency factors or toxicity factors is consistent with the USEPA dose-response modeling for cancer and non-cancer effects.*

*While there are innate limitations and untested assumptions in the TEF methodology, it is more appropriate than other potential alternatives, such as basing the risk on TCDD alone or assuming that all chemicals are equipotent to TCDD. Nonetheless, because of the uncertainties and limitations that are inherent in the TEF methodology, WHO and others have clearly indicated that the approach should be thought of as an interim methodology that should be subject to periodic review as new scientific information becomes available (Birnbaum, 1999; Birnbaum and DeVito, 1995; USEPA, 1987, 1989; van den Berg et al., 1998 and 2006). The need to explore alternative approaches for assessing the potential health risk associated with this class of compounds has been acknowledged by USEPA and others (NAS, 2006; USEPA, 2003; van den Berg et al., 2006). As scientists have gained a better understanding of the modes of action underlying this class of compounds, as more data have become available concerning the relative potencies of these compounds, and as more sophisticated quantitative tools have been developed, it is possible to further improve the TEF methodology. Examples of some of the potential improvements are outlined in the comments that follow.*

## **Response**

As noted in our earlier responses, Budinsky et al. (2006) analyzed NTP cancer bioassays for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF). They indicated that “the current TEF value” of 0.5 for 4-PeCDF is too high (Budinsky et al., 2006). However, in the Budinsky paper, the “current TEF value” refers to the 1998 WHO TEFs, since the paper by Budinsky et al., (2006) was published before the paper by van den Berg et al (2006) describing the 2005 WHO reevaluation of TEFs. The current WHO 2005 TEF values for 2,3,4,7,8-PeCDF is 0.3, lowered from 0.5, and for 1,2,3,7,8-PeCDF is 0.03, lowered from 0.05 (van den Berg et al., 2006). The WHO TEF expert group considered a much wider set of data in arriving at their TEF values. We don’t think it is necessary to cite this paper. In fact, the current WHO 2005 TEF value for 4-PeCDF is lower than 0.5 (van den Beg, et al 2006).

## **Comment 7.**

***Congener-specific toxicity values should be utilized over TEFs when a congener possesses sufficiently robust, relevant toxicity data such as a well-conducted cancer bioassay or a reproductive-developmental study.***

*When adequate data are available, OEHHA should consider developing toxicity criteria for specific DLCs in lieu of using the interim TEF approach. For example, in addition to having adequate cancer bioassay data for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the NTP has conducted 2-year cancer bioassays for 2,3,4,7,8-pentachlorodibenzofuran, PCB 118, PCB 126 and PCB 153. As such, cancer slope factors can now be calculated for these congeners rather than having to rely on TEFs. As an example, Simon et al. (2008) recently calculated cancer slope factors and reference doses for 2,3,4,7,8-PeCDF based on the lifetime average liver and adipose tissue concentration data from the two-year NTP study. They applied linear and nonlinear dose-response methods using these two internal dose metrics and the combined liver tumor incidence (hepatocellular adenomas and cholangiocarcinomas). From this quantitative dose-response assessment, they were able to extrapolate the internal doses calculated for the rats to humans using the Carrier toxicokinetic model. Using both lifetime average tissue concentrations and 1% and 10% points of departure, Simon et al. (2008) calculated cancer slope factor estimates for 2,3,4,7,8-PeCDF that ranged from  $6 \times 10^{-2}$  to  $6 \times 10^{-3}$  (ng/kg/day)<sup>-1</sup>. This effort clearly demonstrates the utility of applying data from two-year cancer bioassays to derive more credible toxicity criteria. It was the recommendations of the 2006 National Academy of Science panel that the USEPA utilize the results of this 2006 NTP cancer bioassay series. Thus, in the context of a tiered approach for assessing dioxin-like compound toxicity (i.e., the use of the interim TEFs in the absence of adequate two-year cancer bioassay data and development of congener-specific cancer slope factors when adequate 2-year cancer bioassay data does in fact exist), the work by Simon et al. (2008) concerning 2,3,4,7,8-PeCDF illustrates that (when available) data from 2-year cancer bioassays should be incorporated into toxicity assessments of these chemicals in lieu of TEFs.*

## **Response**

Environmental exposure is usually to the mixtures and rarely to one or two congeners, but we agree that qualified congener-specific data may be used. Developing slope factors for congeners based on the NPT (2006) data is a possible next step. We are currently proposing to adopt the new WHO TEF (2005) values, which is by far the best method generated by worldwide experts for assessing risks from mixtures of DLCs. To our knowledge, no single method can solve all the issues for DLCs. However, as noted above, the results of Walker et al (2005) support the TEF method for rodent cancer risk assessment by assuming dose additivity for administered doses of dioxin and dioxin-like compounds (Walker et al., 2005).

### **Comment 8.**

#### **3. Examples of Scientific Improvements to the Utilization of TEF Methodology in Dioxin Risk Assessment.**

##### **Example #1: Addressing Relative Potency (REP) Variability Underlying the TEF Point Estimate of Relative Potency**

*A number of scientific publications by members of the WHO expert panel who proposed the 2005 WHO TEFs, have noted the variability and uncertainty with the TEF methodology. Methods*

*have been developed to account for this variability and uncertainty. Therefore, the following comment stated in the OEHHA Document on Page 18 is incorrect and should be: “Variability in estimated REPs for individual congeners may not significantly impact risk estimates.”*

## **Response**

We expanded the statement on page 20 to read: “Variability in estimated REPs for individual congeners may or may not significantly impact risk estimates, which depends on its TEF value and amount inside the body or exposure environment among other factors. More importantly, the most sensitive animal species should be used in risk assessment to protect susceptible human populations”. As noted on page 20, the TEQ in the typical American diet is dominated by 4 congeners, and the variability in REP for those congeners is relatively small.

### **Comment 9.**

*Variability in REPs can significantly impact exposure and risk estimates. The current TEFs represent values recommended by the WHO (van den Berg et al. 2006). In assigning TEFs to each congener, the WHO panel employed scientific judgment and a qualitative weighting scheme to develop single point estimate TEFs based on relative estimates of potency (REPs) that represented a host of different studies, test conditions, species, strains, genders, endpoints, and derivation methods. Because the underlying REPs for each congener represent a heterogeneous data set, it is not surprising that the REP values have been shown to span several orders of magnitude for the different congeners (Birnbaum et al., 2004; Finley et al., 2003; Haws et al., 2006; van den Berg et al., 2006). The range of REP values for different congeners (both vivo and vitro combined) is illustrated in Figure 1-1 (reproduced from Figure A-2, Haws et al., 2006). However, because the TEFs were established using a qualitative approach and are presented as a single point estimates, the variability in the underlying REP distributions is not captured and, as a result, it is not possible to characterize the uncertainty inherent in the risk estimates that are developed based on the TEFs. For example, a rigorous statistical assessment of relative potency estimates and variability via the utilization of quantitative dose-response modeling was not undertaken by the WHO TEF panel. The NAS recently reviewed the TEF methodology and also acknowledged the importance of better characterizing variability and uncertainty inherent in the TEFs (NAS, 2006).*

## **Response**

We are aware of the variability of congener REPs. We also agree with the WHO TEF expert panel. To harmonize risk assessment of DLCs and use consistent TEF for a single congener around the world, we support the point estimate approach for practical purposes that were generated by the WHO TEF expert panel, particularly as the degree of variability of individual REPs is substantial and uncertain. A distributional analysis could be more conservative if the 95<sup>th</sup> percentile were chosen. We prefer the point estimate approach to DLCs risk assessment for the sake of simplicity and consistency.

## Comment 10.

*To address this limitation of the TEF methodology, some investigators have proposed developing distributions of REP values that could in turn be used in probabilistic risk assessments (Finley et al., 2003; Haws et al., 2006). Both the WHO and the NAS have stated that use of distributions of REPs would provide a means of characterizing the variability and uncertainty inherent in the TEFs (van den Berg et al., 2006; NAS, 2006). During their most recent reevaluation of the TEF methodology, the WHO Expert Panel indicated that weighted distributions of REP values could be used to establish TEFs for each DLC. However, the WHO Expert Panel concluded that a consensus-based weighting framework would need to be developed and applied to the REP database prior to using the distributions of REP values in such a manner (van den Berg et al., 2006). Based on this recommendation, Haws and coworkers (2009) have since developed an objective, transparent, reproducible, consensus-based weighting framework that could be used to identify and place greater emphasis on REPs that are believed to be most relevant for purposes of estimating human health risks.*

*In instances where sufficient data are available for the probabilistic evaluation of a chemical's toxicity, there is clearly a benefit to probabilistic treatment of the toxicity values. Quantifying uncertainties associated with the dose-response relationship, such as extrapolation uncertainties (e.g., inter-species extrapolation, low-dose extrapolation), study design uncertainties (e.g., exposure regimens, endpoint selection), calculation techniques (e.g., ED50/LD50, NOEL/LOEL, NOEC/LOEC, benchmark dose), and other factors (purity of reagents, measurement errors) are essential to evaluating how alternative decision choices impact a target population and the consequences of making a decision under a given level of certainty (USEPA, 2008; USEPA, 2009). This is especially true for more complex assessments involving mixtures of compounds. If there is sufficient data to allow evaluation of the toxicity value in a probabilistic framework, inclusion of these data with the use of probabilistic risk assessment (PRA) methods are essential to a comprehensive evaluation of uncertainty. DLCs represent a class of compounds that is well suited for the probabilistic treatment of toxicity.*

## Response

We discuss this issue in our response to comment 2. We agree with the conclusions in van den Berg, et al. (2006):

“Recently, several authors have published papers in which they advocated the use of a probabilistic approach to determine TEFs (Finley et al., 2003; Haws et al., 2006). In using such an approach, there is a clear advantage because it will better describe the level of uncertainty present in a TEF value. The distribution of REPs can be expressed in terms of minimum and maximum values combined with percentiles at different levels (e.g., 25th and 75th percentiles). A disadvantage could be that such an approach lumps all data together and gives similar weight to all types of studies. In part, this problem could be avoided by separating in vitro from in vivo REPs (Haws et al., 2006). However, if probabilistic approaches for setting a future TEF are used, it is essential that weighting factors be applied to REPs that are determined from different types of studies. These weighted REP values could then be used to determine weighted REP distributions in the risk assessment process. Clearly, unweighted REP distribution ranges that bracket the

TEFs incorporate biological and toxicological uncertainty. For this reason, in the WHO 2005 TEF reevaluation, unweighted REP distribution ranges, expert judgment, and point estimates were used in combination to assign TEFs. The sole use of a probabilistic approach to determine TEF values also includes other decision points, such as establishing a range instead of a point estimate for the TEF value. However, the use of a TEF range might cause problems for regulatory authorities and international harmonization of TEF values because one or more TEF values could then be selected for risk assessment calculations. This might easily lead to different TEFs being used by different countries depending on the level of conservatism used in the risk management process by national authorities. In this respect, the choice, e.g., of a 50th, 75th, or 95th percentile of the REP distribution range to assign a TEF is a risk management decision.”

### **Comment 11.**

*USEPA has recognized the potential for probabilistic treatment of DLC TEF values in risk assessment. In their Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk assessment, USEPA identified sources of variability among REP values (e.g., precision of dose and effects measurements, calculation techniques, natural variability among organisms of the same species in their response to DLCs), as well as sources of uncertainty (e.g., purity of chemicals, study design, measurement errors). To address these sources of variability and uncertainty, USEPA proposed that “more sophisticated models may be used to combine the exposure and toxicity information into distributions that may allow for the development of probability density functions, if data are adequate” (USEPA, 2008). In so doing, the USEPA has clearly indicated their support of the use of probabilistic methods for the quantification of variability and uncertainty regarding DLC toxicity.*

*Additionally, the 2000 USEPA Science Advisory Board (SAB) panel charged with reviewing the draft Dioxin Reassessment “questioned whether the uncertainty in the TEFs and the application of this approach to predicting risks due to current levels of exposure was adequately presented” (USEPA SAB 2001, p. 29). The SAB concluded that the USEPA should acknowledge the need for better uncertainty analysis of the TEF values, and although no current method for doing so has been endorsed by the scientific community, several approaches were suggested, such as the use of probabilistic distributions of TEF values in TEQ evaluation (Finley et al., 2003). Further, the SAB concluded that available information indicates a considerable amount of variability in the REP data that was used to derive the WHO TEF values. In addition, they concluded that although the WHO TEFs were derived based on a scientific consensus evaluation of the available REP values using defined criteria to qualitatively weight individual studies, details of the quantitative basis of this weighting scheme were not clearly presented in the description publication (van den Berg et al., 1998). These issues clearly contribute to variability and uncertainty in the application of the WHO TEF values to health risk assessment. Application of a mathematical value or percentage of the overall range of REP values, such as those described by Finley et al. (2003), would be one way to make the process of determining the specific TEFs more transparent and to provide a standard method for developing TEFs for other DLCs that may be added at a later date. Some members of the 2000 USEPA SAB Panel also recommended that, as a follow up to the Reassessment, EPA should establish a task force to build ‘consensus*

*probability density functions' for the thirty chemicals for which TEFs have been established, or to examine related approaches such as those based on fuzzy logic (EPA SAB 2001, p. 29).*

## **Response**

In addition to the responses to comment 2 and the previous comment, please note that US EPA (2009) did not derive new TEFs by a probabilistic method, but instead are adopting the WHO 2005 TEF values. [U.S. EPA, 2009: "Recommended Toxicity Equivalency Factors (TEFs) for Human Health Risk Assessments of Dioxin and Dioxin-Like Compounds: External Review Draft" at [http://www.epa.gov/OSA/raf/files/hhtef\\_draft\\_090109.pdf](http://www.epa.gov/OSA/raf/files/hhtef_draft_090109.pdf)]

### **Comment 12.**

*Subsequent to the review by the 2000 USEPA SAB, the USEPA Dioxin Reassessment was updated and then reviewed by a NAS panel that recognized the need for characterization of variability and uncertainty with regard to the toxicity of DLCs (NAS, 2006). Specifically, the NAS panel concluded that there was a significant degree of uncertainty in the current consensus-based TEFs, and that the weighting considerations that went into their establishment were not clear. As such, the NAS panel strongly recommended that the USEPA consider inclusion of uncertainty analysis of the TEF values and endorsed the recommendation of the 2000 USEPA SAB Panel regarding probability density functions for TEFs*

*The TEF methodology was also recently reviewed by a WHO expert panel (van den Berg et al., 2006). Although this panel once again relied upon qualitative scientific judgment as the basis for establishing the TEFs, the panel acknowledged that distributions could be used in the future once a consensus-based weighting framework had been developed (van den Berg et al., 2006). Further, the panel stated that recent papers advocating the use of a probabilistic approach for determining TEFs (Finley et al., 2003; Haws et al., 2006) provided a clear advantage because such approaches allow for better description of the level of uncertainty present in a TEF value.*

*Several studies have demonstrated the impact of using distributions for TEF values. Finley et al. (2003) suggested that the WHO TEFs are likely to be a significant source of uncertainty and variability in health risk assessments involving complex mixtures of PCDD/Fs and PCBs. To examine this issue more closely, Finley and coworkers obtained the original 1997 WHO REP database that the 1997 WHO panel relied upon to establish the initial WHO TEFs (van den Berg et al., 1998). This database contained 936 REP values, of which 759 were determined to be useable. The number of REPs ranged from 117 (PCB 126) to 1 (1,2,3,7,8,9-HxCDF). Distributions were fit for congeners (where possible), and a simple weighting scheme was developed which gave higher weights based on endpoint (tumor production > P-450 induction > other), and cell lines tested (human > non-human > unknown). Weighted and un-weighted distributions were tested using concentrations of striped bass filet and blue crab muscle in a Monte Carlo probabilistic risk assessment (PRA). It was found that upper bound PCDD/F risk was consistent with point estimates, while upper bound PCB risk increased by approximately 10-fold (weighted and un-weighted results were similar). It was hypothesized that this result reflected the location of the WHO TEF in the distribution of REP values. For PCDD/F the WHO TEF reflects an upper percentile of the REP distribution (75<sup>th</sup>-99<sup>th</sup> percentile), while for PCBs the WHO TEF generally reflects a central percentile (40<sup>th</sup>-57<sup>th</sup> percentile).*

Haws et al. (2006) briefly reviewed the evolution of the TEF methodology and development of the 1997 REP database, and presented definitive criteria for evaluating REPs from different studies. The result of this evaluation was the development of a refined REP database, as well as summary statistics for congeners having more than 10 REPs (min, max, and percentiles: 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, 90<sup>th</sup>) and congeners having less than 10 REPs (min, max, and 50<sup>th</sup> percentile). Summary statistics are presented in Tables 1 and 2 (reproduced from Tables 6 and 7 of Haws et al., 2006). As a note, this refined REP database was relied upon by the 2005 WHO expert panel during their most recent review of the TEF methodology (van den Berg et al., 2006). This refined database provides the structure to assess variability in the underlying data, as well as the uncertainty inherent in the TEF values assigned to individual congeners. Building upon this work, Haws et al. (2009) have proposed a consensus-based weighting framework that incorporates consideration of multiple criteria: study type (in vivo, in vitro), pharmacokinetics, REP derivation quality (maximum response achieved, sufficient number of replicates, at least 3 doses plus control), REP derivation method, and endpoint (toxic, biochemical). This framework is illustrated in the flowchart in Figure 1 (reproduced from Figure 2 of Haws et al., 2009).

**Table 1:** Summary statistics for in vivo + in vitro REPs in the REP2004 database

Congener	N	Min	25th Percentile	50th Percentile	75th Percentile	90th Percentile	Max	WHO <sub>98</sub> TEF	Rank <sup>a</sup>
<b>PCDDs</b>									
12378PeCDD	45	0.044	0.18	0.4	0.6	0.8	1.5	1	94%ile
123478HxCDD	21	0.0076	0.049	0.075	0.12	0.35	0.61	0.1	72%ile
1234678HpCDD	18	0.001	0.0073	0.01	0.028	0.042	0.1	0.01	35%ile
<b>PCDFs</b>									
TCDF	30	0.006	0.026	0.08	0.19	0.3	0.63	0.1	62%ile
12378PeCDF	28	0.0027	0.015	0.048	0.12	0.15	0.95	0.05	52%ile
23478PeCDF	99	0.0065	0.12	0.22	0.46	1.1	3.7	0.5	78%ile
123478HxCDF	13	0.014	0.043	0.07	0.3	0.49	4	0.1	54%ile
123678HxCDF	18	0.0031	0.03	0.072	0.098	0.15	0.16	0.1	76%ile
234678HxCDF	10	0.0085	0.034	0.21	0.31	0.32	0.32	0.1	33%ile
<b>Non-ortho PCBs</b>									
PCB77	49	2.0E-06	0.0001	0.00079	0.018	0.1	0.48	0.0001	25%ile
PCB81	12	0.000042	0.0041	0.0065	0.01	0.023	0.05	0.0001	2%ile
PCB126	115	0.000067	0.05	0.1	0.18	0.42	0.86	0.1	47%ile
PCB169	30	1.8E-06	0.0016	0.0055	0.063	0.53	0.77	0.01	58%ile
<b>Mono-ortho PCBs</b>									
PCB105	26	4.7E-07	1.1E-05	0.000081	0.0003	0.0019	0.074	0.0001	60%ile
PCB118	25	4.2E-07	7.1E-06	0.00002	0.00045	0.0021	0.075	0.0001	68%ile
PCB156	30	2.1E-06	0.000036	0.000095	0.00052	0.19	0.51	0.0005	72%ile

Note. Summary statistics reflect retained in vivo + in vitro REPs combined. Those REP values meeting the following criteria were excluded when developing the summary statistics based on the REP<sub>2004</sub> Database: (1) non-numeric REPs; (2) REPs based on repetitive endpoints; (3) REPs based on repetitive studies; (4) REPs based on a single dose level of the test or reference compound; and (5) REPs identified as invalid for other reasons (e.g., no response; REP based on mixtures study; data omitted from final peer-reviewed publication).

<sup>a</sup>Percentile rank of WHO<sub>98</sub> TEF relative to the distribution of REP values in the REP<sub>2004</sub> Database.

**Table 2:** Summary statistics for congeners in the REP 2004 database having less than 10 in vivo + in vitro REPs

Congener	N	Min	Max	50th Percentile	WHO <sub>98</sub> TEF
PCDDs					
123789HxCDD	6	0.0054	0.07	0.052	0.1
123678HxCDD	5	0.031	0.2	0.043	0.1
OCDD	6	0.00025	0.0032	0.00035	0.0001
PCDFs					
123789HxCDF	2	0.11	0.2	0.15	0.1
1234678HpCDF	2	0.024	0.32	0.17	0.01
1234789HpCDF	2	0.018	0.044	0.031	0.01
OCDF	9	4.0E-06	0.0028	0.0007	0.0001
Mono-ortho PCBs					
PCB114	8	0.000072	0.0024	0.00054	0.0005
PCB123	6	3.0E-06	0.00071	0.000044	0.0001
PCB157	9	0.00004	0.002	0.00042	0.0005
PCB167	5	2.0E-06	0.00063	0.00001	0.00001
PCB189	5	2.0E-06	0.00018	0.000037	0.0001

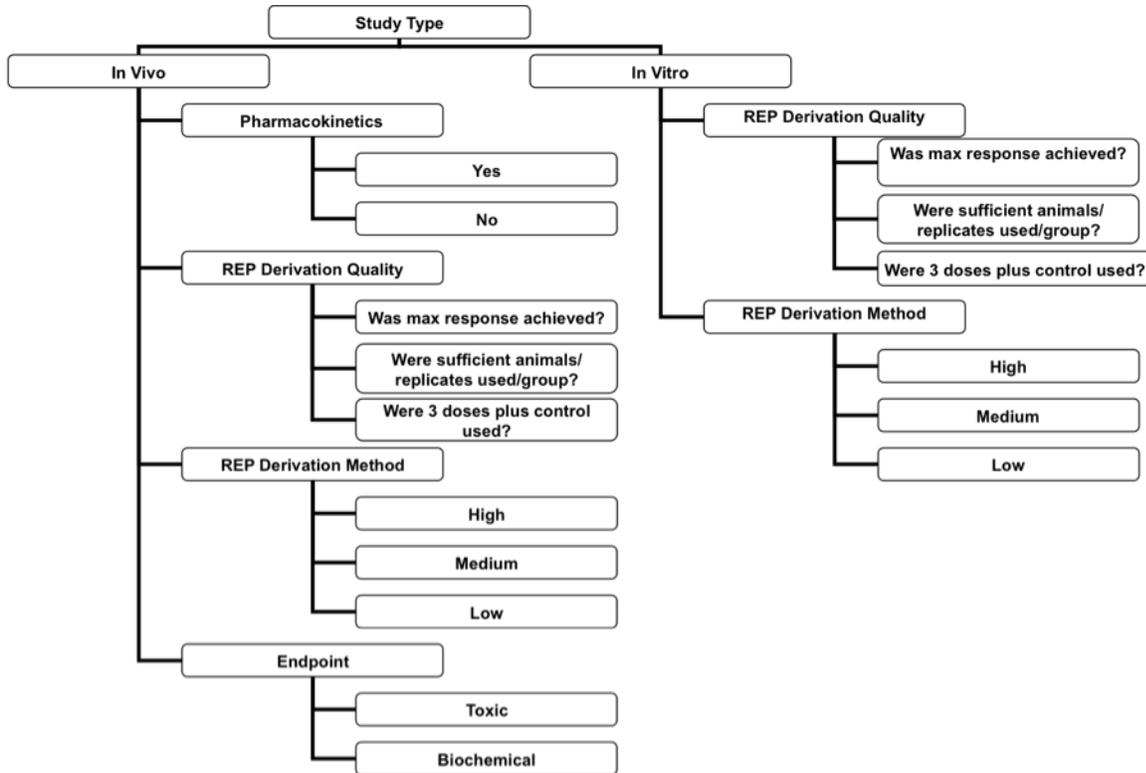
*Note.* Summary statistics reflect retained *in vivo* + *in vitro* REPs combined. Those REP values meeting the following criteria were excluded when developing the summary statistics for the REP<sub>2004</sub> Database: (1) non-numeric REPs; (2) REPs based on repetitive endpoints; (3) REPs based on repetitive studies; (4) REPs based on a single dose level of the test or reference compound; and (5) REPs identified as invalid for other reasons (e.g., no response; REP based on mixtures study; data omitted from final peer-reviewed publication).

## Response

OEHHA does not agree that the analysis method for combining *in vivo* and *in vitro* data together is sufficient to address this issue, as effects studied *in vivo* and *in vitro* may have different mechanisms of action. This may involve confounding and biases that could result in a wrong conclusion. The reviewers should analyze data *in vivo* and *in vitro* separately. One approach to a combined analysis of *in vivo* and *in vitro* studies could be to put all 3 analyses (i.e., *in vivo*, *in vitro*, and *in vivo* + *in vitro*) together for comparison. One example that supports our argument is that DL-PCBs have neurotoxicity *in vivo*, but tests *in vitro* are predominantly negative.

## Comment 13.

Figure 1: Weighting Framework



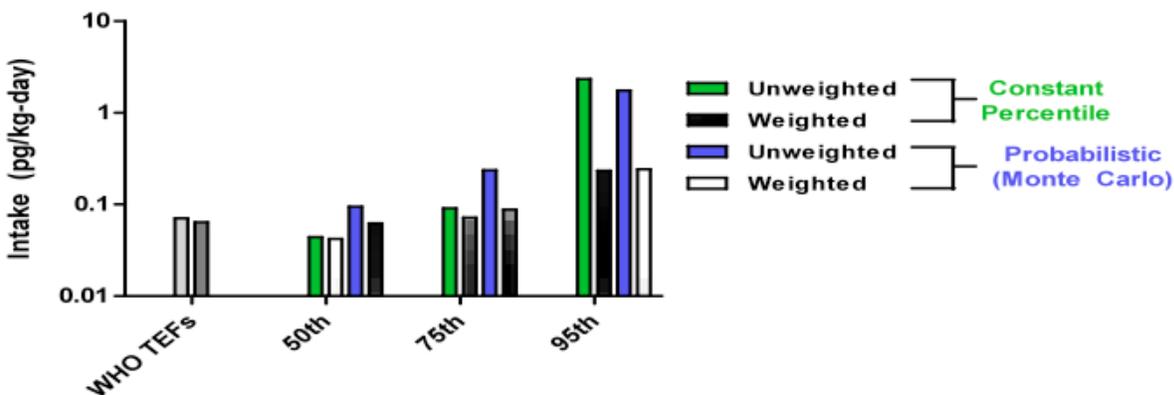
Utilizing this weighting framework, Haws et al. (2008) evaluated the impact of using weighted distributions of REPs by estimating the intake associated with consumption of catfish containing DLCs. This study estimated intake of DLCs using three methods: (1) WHO TEFs, (2) point estimate TEFs based on a series of selected percentiles from the weighted and un-weighted REP distributions, and (3) the full weighted and un-weighted REP distributions in a Monte Carlo PRA. Intake estimates varied by approximately two orders of magnitude across the various approaches, as illustrated in Figure 2 below (reproduced from Figure 2 of Haws et al., 2008). In addition, the intake estimates calculated with the WHO TEFs were consistent with the estimates based on the 50th percentile of the weighted and un-weighted distributions. Intake estimates based on un-weighted distributions were generally higher than those based on weighted distributions, particularly when the upper percentiles were selected. Weighting had a greater impact when percentiles > 75<sup>th</sup> were selected. The ratio of PCB risk to PCDD/F risk increased when PRA was applied with TEF distributions, consistent with the results of Finley et al. (2003). The use of distributions had a greater impact on intake calculations than did the weighting process alone. These results are shown below in Table 3 (Reproduced from Table 1 of Haws et al., 2008). It should be pointed out that PCBs are regulated as PCB mixtures since there is substantial toxicological data on PCB mixtures that show they are not dioxin-like in their activity. Hence, the TEF values for PCBs are largely irrelevant for assessing exposure, toxicity and risk from PCBs.

## Response

The comment that “PCBs are regulated as PCB mixtures since there is substantial toxicological data on PCB mixtures that show they are not dioxin-like in their activity” is only partially true. We agree that there are non-dioxin like toxicities associated with PCB mixtures, including neurological and endocrine toxicity, and are therefore potentially developing an alternative TEF method that will cover both DL- and non-DL-PCB congeners (Yang et al., 2010). We do not agree with the statement that TEFs are largely irrelevant for assessing risks from PCB mixtures. As noted in response to comment 1, there are now studies demonstrating the utility of TEFs for estimating risk from PCBs. For example, showed that use of the current WHO TEF values adequately predicted the increased incidence of liver tumors (hepatocellular adenoma and cholangiocarcinoma) induced by exposure to the mixture. Their data support the use of the TEF approach for dioxin cancer risk assessments (Walker et al., 2005). Further, the dioxins mixtures research fact sheet by NTP (2006) reported “the NTP carried out a series of studies in which rodents were exposed to either a single dioxin-like compound or mixtures of them for up to two years and then evaluated for toxicity and carcinogenicity relative to TCDD. Analysis of data from one group of completed studies confirms the assumption that the effects of the dioxin-like compounds in mixtures are additive. The number of cancer cases in the rats exposed to the mixture could be predicted accurately by adding the concentration of each compound, adjusted for its potency relative to TCDD using TEFs.”

### Comment 14.

**Figure 2:** Comparison of intake estimates (TEQ pg/kg-day) using three different approaches to derive TEF values for each DLC congener. Both the “constant percentile” and “probabilistic” methods utilize the full REP distributions.



**Table 3: Apportionment of intake (TEQ pg/kg-day) by chemical group**

Approach	PCB Intake	PCDD/F Intake	Ratio of PCB Intake to PCDD/F Intake
<b>Deterministic</b>			
1998 TEFs	2.56E-04	1.28E-03	0.2
2006 TEFs	3.21E-04	1.28E-03	0.3
<b>Probabilistic</b>			
<b>50th Percentile</b>			
Unweighted Probabilistic	3.85E-02	1.92E-03	20
Weighted Probabilistic	6.41E-03	1.28E-03	5
<b>75th Percentile</b>			
Unweighted Probabilistic	6.41E-03	1.28E-03	5
Weighted Probabilistic	1.28E-03	1.28E-03	1
<b>95th Percentile</b>			
Unweighted Probabilistic	3.85E-03	1.28E-03	3
Weighted Probabilistic	5.13E-04	6.41E-04	0.8

## Response

TEFs for 8 *ortho*-PCBs in WHO-2005 are significantly smaller than those in WHO-1998; two exceptions are PCB 81 (from 0.0001 to 0.0003) and PCB 169 (from 0.01 to 0.03). The TEQ value of a biological sample depends on what kind of sample, sample size, representativeness, and composition of individual PCB congeners in the mixtures. Thus, variation in TEQ, including the ratio of total TEQ from PCBs and PCDD/PCDF, exists based on varying composition of different samples.

However, we agree with both WHO 2005 and US EPA (2009) new recommendations. A TEF method that is based on AhR mechanism may not cover non-DL-effects, and may under-estimate the risk of PCB mixtures. We are therefore considering evaluating an alternative method, at least for non-cancer risk assessment of PCBs, to cover both DL- and non-DL-PCB congeners. Van Den Berg, et al. (2006) also reported “When reviewing the database of mammalian REPs for dioxin-like compounds, it was observed that even for the most thoroughly studied congeners like 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and PCB 126, significant gaps in knowledge exist (Haws et al., 2006). Reasons for significant differences in REPs for the same congener can be caused by the use of different dosing regimens (acute vs. subchronic), different endpoints, species, and mechanisms (e.g., tumor promotion caused by at least two different mechanisms as for mono *ortho*-substituted PCBs), as well as different methods used for calculating REPs. Thus, different methodological approaches used in different studies clearly provide uncertainties when deriving and comparing REPs. If future study designs to derive REPs were more standardized and similar, the variation in REPs when using the same congener, endpoint, and species might be expected to be smaller.”

Hong et al. (2009) used a major exposure study which examined blood, household dust, and soil levels of dioxin-like compounds in several regions of Michigan to compare TEQ based on the WHO 2005 and 1998 TEFs. They found the mean total TEQ was significantly reduced by 26%, 12% and 14% for serum, household dust, and soil, respectively, when the TEQ was based on the

2005 TEFs compared to the 1998 TEFs. They addressed that decrease in the serum total TEQ was largely due to the down-weighting of the TEFs for the majority of mono-ortho PCBs. In contrast, the decrease in the soil total TEQ was mostly due to the down-weighting of the TEF for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (1998 TEF=0.5, 2005 TEF=0.3). For household dust, the decrease in total TEQ was not due to any single TEF but was due to small changes in a number of compounds. There was a dramatic decrease (-88%) in the mean and 95th percentile for mono-ortho PCB TEQ due to the 2005 TEFs. They suggested that comparisons between studies based on the TEQ-WHO(98) and TEQ-WHO(05) may need to consider an appropriate conversion factor to assure comparability (Hong et al. 2009).

In addition, a statistically based survey of dioxins and dioxin-like compounds in domestic meat and poultry was conducted by the U.S. Department of Agriculture (USDA) from September 2007 to September 2008. Seventeen toxic polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and four non-ortho-polychlorinated biphenyls (no-PCBs) were measured in 510 beef (steer/heifer), market hog, young turkey, and young chicken samples. The results of the survey showed the sum of PCDD/F and non-ortho-PCB toxic equivalencies (sum-TEQs) ranged from not detected to 4.5 pg/g of lipid. Mean sum-TEQ levels for beef, turkey, chicken, and pork were 0.66, 0.61, 0.17, and 0.16 pg/g of lipid, respectively. To compare the new survey data with data from previous USDA surveys in the mid-1990s and 2002-2003, TEQs from all data sets were calculated using the most recent 2005 toxic equivalency factors (TEFs). The results of the recalculation on the older survey data was a small increase (4-13%) in mean TEQs for the mid-1990s data, which initially used pre-1994 TEFs, and a small decrease (2-4%) for the 2002-2003 data, which initially used 1998 TEFs (Huwe *et al.* 2009).

We already discussed the issues in Appendix C. We cited Bhavsar *et al.* (2008) who compared TEFs that have been developed by various agencies over 25 years. Their results from consumption of fish showed that the mammalian PCDD/F-TEQ based on WHO-05 TEF is about 7.5% lower than that based on WHO-97 TEF. The mammalian WHO-05 DL-PCB TEQ is on average 25-26% lower than WHO-97 DL-PCB TEQ. Total WHO-05 TEQ is on average 22% lower than WHO-97 total TEQ. According to the WHO-05 toxicological standards for dioxins/furans, all previous major TEF schemes except Germany-85 TEF and US EPA-87 TEF were conservative (i.e., higher) in estimating TEQs. The major (>75%) contribution to WHO-05 PCDD/F-TEQ is from 2,3,7,8-TCDD (33%), 1,2,3,7,8-PCDD (26%), 2,3,7,8-TCDF (10%), and 2,3,4,7,8-PCDF (9%). The WHO-05 DL-PCB TEQ is dominated by PCB 126 which on average contributes about 88%. The DL-PCB TEQ generally contribute >70% of total TEQ. The author recommends that the congener-specific concentrations, TEF scheme, and names of compounds be presented whenever reporting TEQs (Bhavsar *et al.* 2008).

### **Comment 15.**

*Urban et al. (2009) performed a risk assessment using fish tissue data for the Lower Passaic River. In Phase 1 of this assessment, multiple estimates of risk were generated: 1) a deterministic point estimate; 2) PRA using distributions for exposure parameters and the WHO 2006 TEFs; and 3) a PRA using distributions for exposure parameters and DLC TEFs (including the weighting framework proposed in Haws et al., 2009). These data are illustrated in Figure 3 (reproduced from Figure 2 of Urban et al., 2009). From this figure, it is clear for these specific fish residue data, that while the use of weighted REP distributions has little impact on the*

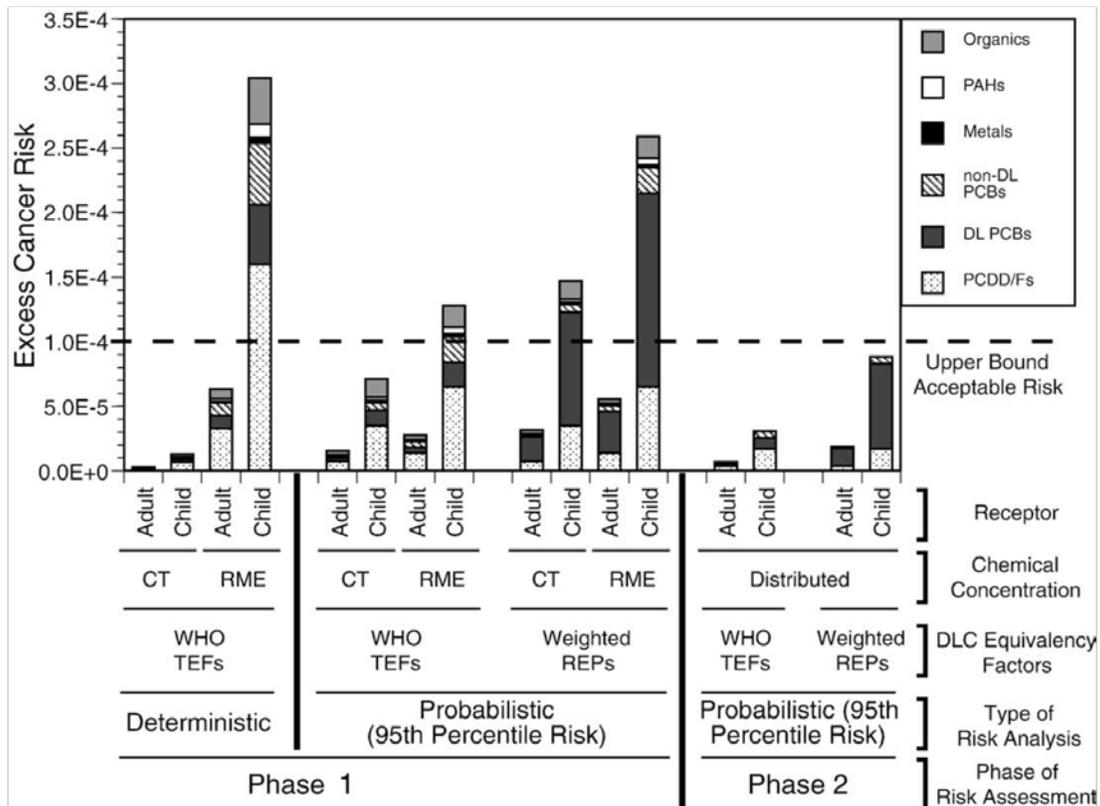
PCDD/F risk, there is a substantial impact on the PCB risks; this is consistent with the findings of Finley et al. (2003) and Haws et al. (2008). Phase 2 included a more refined probabilistic analysis that incorporated distributions for the concentration associated with each congener in fish tissue. These results (also shown in Figure 3) are similar to Phase 1 results with regard to proportion of PCDD/F and PCB contribution to risk. However, it should be noted that total risk is now below the upper bound acceptable risk benchmark of  $1E-4$ . Again, we do not concur with regulating PCB mixtures as dioxin-like since it is the PCB mixture exposure, toxicity and risk that are at issue and the TEF values for PCBs do not reflect the risk from PCB mixtures.

## Response

Again, we agree with the comment that WHO TEF do not address PCB mixtures with non-DL-effects. We are therefore developing an alternative TEF method that will cover both DL- and non-DL-PCB congeners (Yang et al., 2010). However, WHO TEF is still the best method by far for addressing dioxin and DL-effects from the related mixtures.

### Comment 16.

**Figure 3:** Excess cancer risk summary for ingestion of fish from the Lower Passaic River. The central tendency (CT) and reasonable maximum exposure (RME) estimates represent the 50<sup>th</sup> and 95<sup>th</sup> percentiles, respectively, of the sampling distribution of the composite concentration means.



*The impact of using distributions for toxicity criteria in PRA is an essential element of quantifying variability and uncertainty in a PRA. In particular, the use of distribution values for REP values used to derive TEFs for the evaluation of DLCs is established in the literature. There are three criteria specified by USEPA that indicate when a PRA is typically not necessary: when a screening level deterministic PRA indicates that risks are negligible, when the cost of averting the exposure is small, and when there is little uncertainty or variability in the analysis (USEPA, 2009). These three criteria are infrequently met for DLCs. First, the slope factor for 2,3,7,8-TCDD is sufficiently large that the evaluation of DLCs using the TEF methodology can often lead to estimates of unacceptable risk. Second, given that a number of DLC contamination scenarios involve the ingestion of fish associated with a particular waterway, the potential cost of averting exposure is rarely small. Third, the establishment of TEF values is certainly a process in which there is documented uncertainty and variability. For these reasons, the incorporation of variability and uncertainty estimates in risk assessment involving exposure to DLCs is essential.*

## **Response**

The WHO, U.S. EPA, reviewers, and OEHHA all discussed the uncertainty and variability of TEF method. However, there is no better method that can be substituted for the WHO TEF in risk assessment for DLCs. Current attempts to address uncertainty and variability are themselves uncertain and incomplete.

### **Comment 17.**

#### ***Example #2: Toxicokinetics and TEF Uncertainty***

*Although the OEHHA Document addresses several sources of uncertainty (Pages 15-22, Uncertainties Associated with the Use of the TEF Methodology), the review does not mention a key uncertainty with TEFs. This key uncertainty reflects the fact that TEFs are derived on an administered dose basis whereas the administered dose basis does not reflect internal measures of dose (tissue concentrations) that are more accurate for comparing the toxicity of a particular dioxin congener to 2,3,7,8-TCDD.*

## **Response**

Van den Berg et al., (2006) reported that “Some experimental evidence shows that non-dioxin-like aryl hydrocarbon receptor agonists/antagonists are able to impact the overall toxic potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds, and this needs to be investigated further. Certain individual and groups of compounds were identified for possible future inclusion in the TEF concept, including: 3,4,4-TCB (PCB 37), polybrominated dibenzo-p-dioxins and dibenzofurans, mixed polyhalogenated dibenzo-p-dioxins and dibenzofurans, polyhalogenated naphthalenes, and polybrominated biphenyls. Concern was expressed about direct application of the TEF/total toxic equivalency (TEQ) approach to abiotic matrices, such as soil, sediment, etc., for direct application in human risk assessment. This is problematic as the present TEF scheme and TEQ methodology are primarily intended for estimating exposure and risks via oral ingestion (e.g., by dietary intake). A number of future approaches to determine

alternative or additional TEFs were also identified. These included the use of a probabilistic methodology to determine TEFs that better describe the associated levels of uncertainty and “systemic” TEFs for blood and adipose tissue and TEQ for body burden.” (van den Berg et al., 2006). We agree with this analysis that alternative or additional TEFs may be needed. Further studies are warranted.

### **Comment 18.**

*Although OEHHA briefly discusses some of the limitations inherent in the TEF methodology, the document fails to mention a key shortcoming identified by the 2005 WHO Expert Panel – namely, the issue of dose-metrics in deriving and applying TEF values. A single comment in this section on species responsiveness, “Differences in tissue distribution can significantly influence TEF values when they are based on tissue concentrations (DeVito and Birnbaum, 1995),” does not fully characterize the issues associated with applying TEFs based on administered dose to human biomonitoring or risk assessment data. Because current WHO 2005 TEF values are based on administered dose, the WHO International Programme on Chemical Safety expert panel specifically stated that applications of WHO 2005 TEFs to human tissues must be carried out with caution (van den Berg et al., 2006). The panel further noted:*

*“There is emerging evidence suggesting that the relative potency of certain dioxin-like compounds may differ when the REP is determined based on administered dose versus tissue concentration (DeVito et al., 2000; Chen et al., 2001; Hamm et al., 2003). As a result the use of systemic TEFs and TEQ has been suggested as an additional approach to the present WHO TEFs. **From a biological and toxicological point of view the development and use of systemic TEFs is recommended**, but the expert panel was of the opinion that at present there is insufficient data to allow the development of systemic TEFs. If systemic TEFs would be developed in the future, TEF values based on blood lipid concentration might be the preferred choice.”*

*“In view of their direct biological relevance to humans, the expert panel proposed that systemic or body burden TEFs for humans be developed in the near future. These body burden/systemic TEFs would allow a more accurate quantitative human dose-response assessment.”*

*“In addition, body burden TEFs can also be used as the dose metric for interspecies extrapolation. At present, the WHO 2005 TEFs that are based on intake can be applied for characterization of exposure to dioxin-like chemicals in human blood or tissues and comparisons across populations, **but these derived TEQ values have certain caveats from a risk assessment point of view.**”*

*DeVito et al. (2000) evaluated the dose-response relationships for enzyme induction following subchronic exposure to PCBs and reported that some estimates of relative potency were dose-dependent, and some estimates varied by more than an order of magnitude. In this study, REPs for enzyme induction were also tissue- and response-specific, thus warranting a cautious view of some of the assumptions in the TEF methodology, including the assumption that REPs are equivalent across endpoints, tissues, and dose metrics. These authors further evaluated REPs based on administered dose with those estimated based on tissue dose and discussed the*

*importance of pharmacokinetic considerations in estimates of relative potency. Specifically, it was noted that the wide range in REP values was likely due to differences in pharmacokinetic properties, including absorption, distribution, and elimination of the PCB congeners evaluated relative to TCDD. Several other studies have reported similar findings regarding variations in relative potency due to differences in pharmacokinetics (Hamm et al., 2003; Hemming et al., 1993).*

*Gray et al. (2006) specifically evaluated the consequences of applying various dose-metrics for TEFs in risk assessment. This group published findings of an evaluation of NTP bioassay data for three dioxin-like compounds in which relative potencies were compared based on administered dose versus tissue concentration. The authors reported that when compared on a TEQ body-burden basis, at least two of the compounds were substantially less potent than predicted by TEFs based on administered dose. And, thus, for the scenario evaluated, the cancer potency of the DLCs was substantially over predicted. The authors provided several examples of similar scenarios based on other studies in the REP database in which REPs based on administered dose were different (and often higher) than tissue concentration-based REPs.*

*As a result of the emerging evidence suggesting that the REP of certain dioxin-like compounds may differ when the REP is determined based on administered dose versus tissue concentration, the 2005 WHO Expert Panel concluded that the application of the current TEF values, which are by default primarily designed for intake situations, to human tissue samples must be carried out with caution. The WHO Expert Panel therefore recommended that consideration be given to developing systemic TEFs. Given the potential broad application of the OEHHA TEF document, further discussion should be given to this issue and limitations associated with the use of TEFs when evaluating tissue concentration data noted.*

## **Response**

We agree with the WHO expert panel that at present there are insufficient data to develop systemic TEFs. Both WHO and OEHHA discuss that REPs based on enzyme inductions were tissue- and species-specific with large variation. The WHO expert panel also addressed why they used point estimation for generation of TEFs (Van den Berg, et al. 2006). It is not clear what the commenter means by referring to the “potential broad application of the OEHHA TEF document”. The document under discussion is an appendix to a Technical Support Document for risk assessments conducted in connection with the Air Toxics Hot Spots program. As is evident from a reading of the other Technical Support Documents, and in particular that dealing with exposure assessment, an applied dose methodology is specified as the normal approach, which is consistent with the TEF methodology as currently presented.

## **Comment 19.**

### ***Example #3: 2,3,7,8-Tetrachlorodibenzofuran’s Metabolic Clearance and Non-Persistence***

*OEHHA Document Text, Page 12, Ligands for the Ah Receptor, 2<sup>nd</sup> paragraph: “One of the criteria for the inclusion of anthropogenic chemicals in the TEF methodology is their persistence*

and bioaccumulation in wildlife and humans.” At least for one furan congener, 2,3,7,8-TCDF, there are recent scientific data that show this congener is not persistent and does not bioaccumulate in wildlife or experimental animals, especially in the presence of CYP1A1 enzymatic activity induced by other dioxin-like compounds in the mixture.

OEHHA has indicated that elimination half-life and bioaccumulation are important factors in determining if a TEF should be assigned to a given congener/chemical. This is consistent with criteria established by the 1997 WHO Expert Panel for inclusion of specific DLCs in the TEF framework – specifically, that the DLC must be persistent and bioaccumulate in the food chain (van den Berg et al., 1998). Indeed, OEHHA utilized these criteria as a basis for justifying their decision that TEFs were not necessary for naturally occurring AhR ligands. Given this logic, OEHHA should consider recent data demonstrating that 2,3,7,8-TCDF does not bioaccumulate and, as such, assess the applicability of the TEF framework to this congener.

For instance, Zwiernik et al. (2008) recently evaluated the toxicokinetics of 2,3,7,8-TCDF in Mink by conducting a 180-day spiked feeding study. The authors reported that the elimination half-life for 2,3,7,8-TCDF was less than 15 hours and was inversely proportional to dose. These results are consistent with other reports in the literature. For example, the elimination half-life for 2,3,7,8-TCDF has been reported to range from 0.3 to 1.8 days and 1.8 to 2.8 days, in Fischer 344 rats and C57BL6J mice, respectively (Birnbaum et al., 1980; Decad et al., 1981). In contrast, the elimination half-life for 2,3,7,8-TCDD in Sprague Dawley rats ranged from 12 to 31 days (Piper et al., 1973; Fries et al., 1975; Rose et al., 1976) and for C57BL mice it ranged from 9 to 11 days (Gasiewicz et al., 1983; Birnbaum, 1986). Thus, there is a significant disparity between elimination half-life of 2,3,7,8-TCDF and 2,3,7,8-TCDD.

Zwiernik et al. (2008) also reported that the liver:diet bioaccumulation factor (BAF) for 2,3,7,8-TCDF in Mink was very low ranging 0.041 to 0.14 depending on the exposure (see Table 4). In contrast, BAFs ranged from 9.5 to 17 for 2,3,4,7,8-PeCDF in the same study – a very large difference.

**Table 4:** Bioaccumulation Factors for Liver:Diet at 180 days for Mink for 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF (Table taken from Zwiernik et al., 2008)

Treatment	4-PeCDF liver:diet BAF	TCDF liver:diet BAF
Low	9.5	0.14
Mid	12	0.060
High	17	0.041
Mixture	12	0.032
Saginaw River feeding study 10% carp (Tillitt et al., 1996)	43	1
Saginaw River feeding study 20% carp (Tillitt et al., 1996)	53	0.5
Saginaw River feeding study 40% carp (Tillitt et al., 1996)	35	0.25
Tittabawassee River field study (Zwiernik, 2008)	11	0.14

*The results in Table 4 are not unexpected as 2,3,7,8-TCDF is rarely identified in biota or humans. For example, in its Third National Report on Human Exposure to Environmental Chemicals, the National Center for Health Statistics reported that 2,3,7,8-TCDF was not identified in either its 1999-2000 or 2001-2002 survey of chemicals in the blood of people living in the United States (NCHS, 2005). As a part of this survey (the National Health and Nutrition Examination Survey (NHANES) blood was collected from 1,243 people in 1999-2000 and from 1,229 people in 2000-2001 and 2,3,7,8-TCDF was not identified in any individual. In contrast to the results for 2,3,7,8-TCDF, numerous other dioxin-like chemicals were identified in these two surveys. The results suggest that 2,3,7,8-TCDF does not bioaccumulate in humans to any great extent. This suggests that the low constitutive activity of CYP1A1 metabolism is sufficient, along with the small dietary contribution of TCDF, to keep TCDF from bioaccumulating.*

*Additionally, a recent study evaluated the uptake of PCDDs and PCDFs in Sprague Dawley rats and juvenile swine following ingestion of these compounds in an urban soil and a floodplain soil (Budinsky et al, 2008). The floodplain soil was reported to have 2,150 pg/g soil of 2,3,7,8-TCDF (215 pg TEQ/g soil of 2,3,7,8-TCDF). The results from the juvenile swine are most informative as they have an anatomy, physiology, and a small intestine absorption mechanism that is similar to humans (Weis and LaVelle, 1991; Casteel et al., 1998). This similarity has led to increasing use of swine as models of human oral bioavailability (Casteel et al., 2006; USEPA, 2006). In this study, swine were fed floodplain soil on a daily basis for 30 days, receiving an average daily-administered dose of 1,120 pg/kg bw/day of 2,3,7,8-TCDF (or 112 pg TEQ/kg bw/day of 2,3,7,8-TCDF). At the end of the exposure period, liver and adipose tissues were analyzed to determine the concentration of 2,3,7,8-TCDF in these tissues. The average concentration of 2,3,7,8-TCDF in swine liver and adipose tissue was 0.2 and 0.9 pg/g, respectively. These tissue concentrations represented only 0.01% (liver) and 0.3% (adipose tissue) of the administered dose of 2,3,7,8-TCDF. This study provides further evidence that 2,3,7,8-TCDF does not bioaccumulate to any significant extent in tissues.*

## **Response**

In response to “Example #3: 2,3,7,8-Tetrachlorodibenzofuran’s Metabolic Clearance and Non-Persistence”, Milbrath et al. (2009) published a paper titled “Apparent Half-Lives of Dioxins, Furans, and Polychlorinated Biphenyls as a Function of Age, Body Fat, Smoking Status, and Breast-Feeding”. They compared data from more than 30 studies that contained congener-specific elimination rates, and found that half-lives of dioxin and dioxin-like congeners in humans vary widely between and within different dioxin, furan, and PCB congeners. Age, a measure of body fat, smoking habits, and breast-feeding status are strong determinants of the elimination rates observed in humans. Their study integrates these critical factors into an empirical model that predicts the half-lives of the 29 WHO TEF scheme congeners over a human life span. We found that 2,3,7,8-TCDF with a median half-life of 0.9 years, which is shorter than other dioxins and furans, is still long enough (about 11 months) to consider it bioaccumulative in humans (refer to their table 5 following):

**Table 5.** Reference half-lives (in years) and model parameters for Equations 2 and 3 for dioxins and furans.

	Infant half-life	Adult half-life	Median half-life	Reference adult age (years)	Source (adult values)	SF	$K_{BM}$	Intercept ( $\beta_0$ )	Slope ( $\beta_{age}$ )
TCDD	0.4 <sup>a</sup>	7.2	6.3	48.7	<i>b</i>	0.739	0.92	0.26	0.15
1,2,3,7,8-PeCDD	0.3 <sup>a</sup>	11.2	8.5	48.7	<i>c</i>	0.683	1.21	0.09	0.23
1,2,3,4,7,8-HxCDD	0.5	9.8	10.90	48.7	<i>c</i>	0.509	1.44	0.35	0.20
1,2,3,6,7,8-HxCDD	0.4 <sup>a</sup>	13.1	12	48.7	<i>c</i>	0.635	1.32	0.12	0.27
1,2,3,7,8,9-HxCDD	0.3 <sup>a</sup>	5.10	6.8	48.7	<i>c</i>	0.665	1.51	0.18	0.10
1,2,3,4,6,7,8-HpCDD	0.3 <sup>a</sup>	4.9	3.7	48.7	<i>c</i>	0.525	1.87	0.22	0.10
OctaCDD	0.5 <sup>a</sup>	6.7	5.7	48.7	<i>c</i>	0.551	3.3	0.33	0.14
2,3,7,8-TCDF	0.1	2.1	0.9	48.7	<i>d</i>	0.648	1.1	0.08	0.04
1,2,3,7,8-PeCDF	0.2	3.50	1.9	48.7	<i>d</i>	0.648	1.6	0.13	0.07
2,3,4,7,8-PeCDF	0.3 <sup>a</sup>	7.0	4.9	48.7	<i>d</i>	0.648	1.15	0.13	0.14
1,2,3,4,7,8-HxCDF	0.4	6.4	4.8	48.7	<i>c</i>	0.692	1.79	0.23	0.13
1,2,3,6,7,8-HxCDF	0.4	7.2	6	48.7	<i>c</i>	0.695	1.91	0.26	0.15
1,2,3,7,8,9-HxCDF	0.4	7.2	—	40.0	<i>e</i>	0.648	1.39 <sup>f</sup>	0.19	0.15
2,3,4,6,7,8-HxCDF	0.2	2.8	3.4	48.7	<i>d</i>	0.648	1.38	0.10	0.06
1,2,3,4,6,7,8-HpCDF	0.2	3.1	3	48.7	<i>c</i>	0.832	2.59	0.11	0.06
1,2,3,4,7,8,9-HpCDF	0.3	4.6	5.2	48.7	<i>d</i>	0.648	4.28	0.17	0.09
OctaCDF	0.1	1.4	1.6	48.7	<i>d</i>	0.648	3.4	0.05	0.03

—, not available.  $K_{BM}$ , blood lipid to milk fat ratio; SF, smoking factor.

<sup>a</sup>Infant reference values taken from Leung et al. (2006). <sup>b</sup>Flesch-Janys et al. (1996), median value. <sup>c</sup>Flesch-Janys et al. (1996), regression values. <sup>d</sup>Van der Molen et al. (2000). <sup>e</sup>No data for this congener (the half-life values were taken to be the same as 1,2,3,6,7,8-HxCDF). <sup>f</sup>Geometric mean of all  $K_{BM}$  values.

## Comment 20.

### **Example #4: Dioxin Congeners Recruit Different Co-Activators That are Tissue Specific**

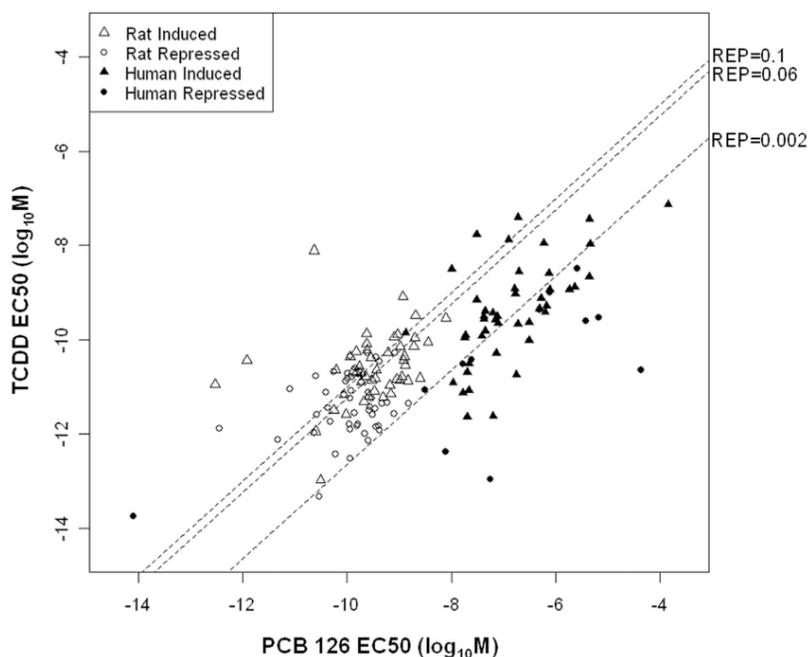
*OEHHA Document Text, Page 14, Basis of TEF and TEQ Calculation: The Assumption of Additivity, 1st paragraph: “The TEF/TEQ methodology is based on the scientific assumption that the AhR mediates the biochemical and toxicological actions of DLCs. Another essential assumption in the development of the TEF methodology is the assumption of additive interactions. Although there are numerous scientific reports on the synergistic or antagonistic interaction of mixture of DL- and/or non-DLCs with TCDD, reports on the additive effects of DLCs predominate. Several published studies aimed to validate the concept of the TEF methodology as a tool to predict the risk of exposure to DLC mixture.” However, recent scientific data shows that AhR-mediated gene expression and the mechanism (i.e., recruitment of coactivators at the level of the interaction between the dioxin-response element and the ligand-bound AhR/ARNT complex) whereby dioxins cause biological effects (activation of the AhR) is not comparable from congener to congener or tissue to tissue.*

*The OEHHA document should include a discussion of recent studies indicating broad species differences in the initial events that follow AhR activation, as well as congener-specific differences in genetic responses.*

*Ongoing research continues to evaluate the mechanism(s) of action associated with the varied toxicities observed following exposure to TCDD and other DLCs. Much of the research has focused on variations in response at the molecular level for a number of components in AhR pathway. Findings generally indicate that clear differences exist between species and among DL congeners with respect to AhR structure and function, genetic responses associated with activated AhR, and dose-response relationships for a variety of endpoints. In a recent publication, Carlson et al. (2009) reported the findings of a toxicogenomic study assessing species differences in response to two prototypical AhR agonists (TCDD and PCB 126) in rat and human hepatocytes. Dose-response data were evaluated for over 4,000 orthologous genes; however, only five responsive orthologous genes were shared between the two species,*

suggesting broad species differences in the initial events that follow AhR activation. These data clearly demonstrate that there are species differences in both the specific genes that responded and the agonist potency and relative potency (Figure 4). The study authors further discussed the rationale for observed species differences in REP estimates for PCB 126 and suggested that although the two species share approximately the same relative affinity, there could be differences in initial receptor binding and occupancy related to species differences that previous studies have failed to quantify. The authors also suggest that species differences in AhR structure affect some other aspect of receptor function downstream of initial ligand binding such as transactivation or interaction with other signal transduction pathways.

**Figure 4.** Relationship between estimated EC50s for TCDD and PCB 126 of successfully modeled genetic probe sets within each species evaluated (Figure 4, Carlson et al., 2009).



Data demonstrate that *in vitro*-derived potencies and PCB 126 REPs vary across individual genes both within and between species.

## Response

Carlson, et al., (2009) published “Divergent Transcriptomic Responses to Aryl Hydrocarbon Receptor Agonists between Rat and Human Primary Hepatocytes” in *Toxicological Sciences*, 2009, 112: 257-272. They employed microarray technology to reveal species differences in response to two prototypical aryl hydrocarbon receptor (AhR) agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin and the PCB 126. Dose response of primary cultures of rat and human hepatocytes were determined using species-specific gene microarrays with over 4000 gene orthologs. Forty-seven human and 79 rat genes satisfied dose-response criteria for both chemicals and were subjected to further analysis including the calculation of the 50% effective concentration and the relative potency (REP) of PCB 126 for each gene. They reported only five

responsive orthologous genes were shared between the two species. The geometric mean of the REPs for all rat and human modeled responsive genes were 0.06 (95% confidence interval [CI]; 0.03–0.1) and 0.002 (95% CI; 0.001–0.005), respectively, suggesting broad species differences in the initial events that follow AhR activation but precede toxicity. As seen in their Figure 2, the REP for PCB 126 in humans ranged about  $10^{-6}$  to 1, whereas for rat the range is relatively smaller (about 0.1 to less than 1000). This suggests that humans have higher variability compared to rats, and this species-specific sensitivity must be considered in the risk assessment. Simple comparison of two geometric means may not be enough in the risk assessment to protect sensitive human populations, especially only based on in vitro data. Similar phenomena were described in figure 5 for EC<sub>50</sub>. In addition, we did not see whether they confirmed that there were five responsive orthologous genes by other methods such as Northern Blot or RT-PCR; microarray data may give false positive or negative results. Furthermore, this paper does not change our proposal to adopt WHO 2005 TEFs, and it is not necessary to cite.

### Comment 21.

*Species- and congener-specific differences in gene expression were also reported by Rowlands et al. (2007). In this study, gene expression profiles were compared following treatment of human and rat hepatocytes with TCDD, 2,3,4,7,8-PeCDF, or TCDF. A comparison of gene expression profiles for the three congeners evaluated is shown in Table 5. These data indicate that the three DLCs did not result in the same genetic response following exposure. The authors report that this trend was observed in both species, and that when the number of genes with altered expression was compared, significantly more genes were altered in rats as compared to humans.*

**Table 5.** Comparison of the number of differentially expressed genes following treatment with three DLCs in human and rat hepatocytes (Table 2, Rowlands et al., 2007).

Species <sup>a</sup>	Filter <sup>b</sup>	TCDD <sup>a</sup>	TCDF <sup>a</sup>	4-PeCDF <sup>a</sup>	Common
Human	0	132	147	181	27
	1.5	92	91	126	17
	2.0	62	55	86	13
Rat	0	320	479	466	77
	1.5	251	331	308	51
	2.0	154	192	178	26

<sup>a</sup>EC<sub>50</sub> based on CYP1A1 mRNA induction in a separate study (data not shown). EC<sub>50</sub>s for humans, 0.3 nM TCDD, 0.3 nM 4-PeCDF, 3.0 nM TCDF; EC<sub>50</sub>s for rats, 0.01 nM TCDD, 0.3 nM 4-PeCDF, 1.0 nM TCDF.

<sup>b</sup>the number of significantly altered genes in each treatment were determined induced/repressed at and beyond the indicated fold level.

*Additionally, data demonstrated that there were only a small number of genes that were altered in both species when gene expression was specifically compared across species (Table 6). In fact, only five common genes were differentially expressed following treatment with TCDD, six with TCDF, and five with 2,3,4,7,8-PeCDF. However, with the exception of one gene, the induced levels were different for the remaining genes. The authors concluded that data suggest that there are important differences in gene expression responses to DLCs that may be explained*

*by differences in congener activation of the AHR. The authors further noted that because of the small number of common genes affected by treatment with the various DLCs, the AhR pathway may not be well conserved between rats and humans beyond the regulation of the AhR core gene battery. These data are supported by other findings indicating that prototypical AhR regulated genes do not appear to play a role in some TCDD toxicities (Nukaya et al., 2009; Pohjanvirta, 2009; Uno et al., 2004).*

## **Response**

We agree that “prototypical AhR regulated genes do not appear to play a role in some TCDD toxicities”, as TCDD not only has DL-toxicities but also has other toxicities that may be mediated by other genes/pathways. However, to our knowledge, no other genes at present can substitute for the AhR regulated genes as a basis for assessing DL toxicity.

In addition, Rowlands et al. (2007) may have introduced large bias in their use of primary hepatocytes from rats and human donors. The biases in term of comparability between two primary cell cultures include age of donors, i.e., 10 weeks of age, about 1/10 of life in rats verses 44 to 77 years old women, about ½ of life in humans; sample source issues that affect cell viability; culture condition issues affecting viability; and DNA microarray data issues that usually need to be confirmed at least for some important genes via other tools to exclude false positive and/or false negative, which are especially important under sub-optimal conditions. This paper has many unanswered questions/problems, and a small sample size (3 human donors). The paper did not address why rat primary hepatocytes seem to be more sensitive compared to human hepatocytes, and whether all common genes they identified are AhR-gene-related and/or all other non-common genes are non-AhR-gene-related. We decided not to cite this paper in our Appendix C document, as we felt it could be confusing or even misleading.

## **Comment 22.**

*Data obtained from gene array assessments and subsequent indications of mechanistic pathways indicate that different DLCs activate the AhR in different ways. Specifically, data have shown ligand-dependent differences in AhR coregulator recruitment as demonstrated by differential coregulator interactions induced by the AhR following exposure to TCDD, PeCDF, and TCDF (Zhang et al., 2008). Further, non-halogenated aromatic hydrocarbon ligands induce different coregulator interactions by the AhR (Boronat et al., 2007; Hestermann and Brown, 2003). Data have also shown that AhR conformation depends on binding of the specific ligand in consideration (Henry and Gasiewicz, 2003).*

**Table 6.** *Comparison of the number of common, differentially expressed genes following treatment with three DLCs in human and rat hepatocytes (Table 3, Rowlands et al., 2007).*

Treatment <sup>a</sup>	Filter <sup>b</sup>	Rat	Human	Common
TCDD	0	147	71	5
	1.5	114	45	4
	2.0	69	27	3
TCDF	0	238	87	6
	1.5	158	53	4
	2.0	89	29	3
4-PeCDF	0	262	75	5
	1.5	170	45	5
	2.0	97	29	4

<sup>a</sup>EC<sub>50</sub> based on CYP1A1 mRNA induction in a separate study (data not shown). EC<sub>50</sub>s for humans, 0.3 nM TCDD, 0.3 nM 4-PeCDF, 3.0 nM TCDF; EC<sub>50</sub>s for rats, 0.01 nM TCDD, 0.3 nM 4-PeCDF, 1.0 nM TCDF.

<sup>b</sup>the number of significantly altered genes in each treatment were determined induced/repressed at and beyond the indicated fold level.

*Collectively, these data suggest that mechanism of action associated with TCDD and other DLCs varies among species, and varies by congener. Thus, these data suggest that not all of the same biological processes are affected similarly following exposure to TCDD and other DLCs. These differences among congeners in regulating genes and recruitment of coactivators raise concerns about a fundamental tenant of the TEF approach - that all DLCs activate the AhR in the same way. Although OEHHA briefly addresses events associated with AhR-mediated mechanisms of action, the issue, as well as the implications on the TEF methodology, is not adequately discussed.*

## Response

To our knowledge, at present there are no other genes more important than AhR genes in mediating DL-toxicities, although obviously AhR regulated genes do not cover all TCDD toxicities. In other words, no other genes can substitute for AhR genes as a basis for the TEFs, and the WHO TEF method is still the best available method to address DL-toxicities. We do consider species differences, in that human cells may have a big range of variation compared to rat cells, indicating a need to have more health-conservative TEF values to protect sensitive subpopulations.

### Comment 23.

#### *Example #5: Natural AHR Ligands*

*OEHHA Document Text, Page 14, Ligands for the Ah Receptor, Top of Page: "Although some chemicals, including chemicals that occur naturally, bind to the AhR and some may elicit DL-activity, it is clearly not sufficient to be considered in the TEQ calculation. Other toxicological factors, such as biological half-life, exposure and toxicity data in vivo should be considered." An ongoing concern in the application of TEFs is the failure to note the occurrence of naturally occurring dioxin activity in human blood that is orders of magnitude higher than the dioxin activity attributable to dioxins, furans and PCBs. This activity is ignored on the premise that*

*these naturally occurring AhR ligands are short acting. However, this may not be a valid dismissal since this activity, measured in human blood, is continually present and is believed to be a function of diet and natural biological processes.*

*OEHHA states that naturally occurring AhR ligands have short half-lives in humans, do not bioaccumulate, and thus are not relevant to the TEF framework. However, Connor et al. (2008) demonstrated that a dietary exposure regimen in which individuals ingested food known to contain naturally-occurring AhR ligands over a period of days is sufficient to alter the amount of AhR activating substances in human blood. The elevated AhR induction activity was observed to occur over a period of days, which negates the argument by OEHHA that a short elimination half-life precludes dioxin-like activity. This is believed to be due to the fact that humans are continuously exposed to naturally occurring AhR ligands in their diets. Jueken et al. (2003) also notes that humans are chronically exposed to these substances. Additionally, de Waard et al. (2008) demonstrated that naturally occurring AHR ligands are capable of inducing gene expression in a number of the same genes as does TCDD and BaP. Citrus pulp extract and grapefruit extract induced gene activity similar to that of TCDD across a wide variety of genes in Caco-2 cells.*

## **Response**

We are aware that DLCs have half- lives of months to decades, which is generally much longer than the natural ligands, although different natural ligands may have different half-lives. At this point, we do not think the data will allow estimation of an exact half-life for natural ligands.

Van den Berg, et al. (2006) reported that the AhR can bind and be activated by a structurally diverse range of synthetic and naturally occurring chemicals that are widely distributed in dietary vegetables, fruits, teas, and dietary herbal supplements sometimes at relatively high concentrations. The ability of metabolically labile phytochemicals to induce or inhibit induction of CYP1A1-dependent activities by 2,3,7,8-TCDD in cell culture model systems has been reported by numerous laboratories. However, the majority of toxicity studies demonstrated that these naturally occurring AhR agonists fail to produce the typical dioxin-like AhR-dependent toxicity, although some developmental dioxin-like effects have been reported for indole-3-carbinol (I3C). In addition, naturally occurring AhR ligands, such as I3C and diindolylmethane, have been reported to inhibit 2,3,7,8-TCDD–dependent in vivo induction of CYP1A1 and immunotoxicity. Other studies have shown synergistic effects on dioxin toxicity of non-dioxin–like compounds, e.g., thyroid hormones, porphyrins, reproductive toxicity, and immunotoxicity. The above studies provide evidence that non-dioxin–like compounds that are weak AhR agonists can modulate the overall toxic potency of 2,3,7,8-TCDD and related compounds.

If occurring under natural background situations, these interactions might impact the magnitude and overall toxic effects produced by a defined amount of TEQ (i.e., from intake or present in the body) but would not impact the determination of individual REP or TEF values for dioxin-like chemicals. The WHO expert panel recognized that there are studies providing evidence that non-dioxin–like AhR agonists and antagonists are able to increase or decrease the toxicity of 2,3,7,8-TCDD and related compounds. Accordingly, their possible effect on the overall accuracy of the estimated magnitude of the TEQ needs to be investigated further, but it does not impact the experimental determination of individual REPs or TEFs.

## Comment 24.

The discussion by OEHHA regarding naturally-occurring AHR ligands should be revised to reflect current data published since 2000. Listed below are relevant publications on this topic and key text from the publication that were apparently not considered by OEHHA:

**1. Degner et al. (2009). Targeting of aryl hydrocarbon receptor-mediated activation of cyclooxygenase-2 expression by the indole-3-carbinol metabolite 3,3'-diindolylmethane in breast cancer cells. *J Nutr.* 139(1): 26-32.**

*Key Text:* “Conversely, the cotreatment of MCF-7 cells with DIM (10 micromol/L) abrogated the TCDD-induced recruitment of the AhR and ACh4 to the COX-2 promoter and the induction of COX-2 mRNA and protein levels. Taken together, these data suggest that naturally-occurring modulators of the AhR such as DIM may be effective agents for dietary strategies against epigenetic activation of COX-2 expression by AhR agonists.”

**2. Amakura et al. (2008). Influence of food polyphenols on aryl hydrocarbon receptor-signaling pathway estimated by in vitro bioassay. *Phytochemistry.* 69(18): 3117-30.**

*Key Text:* “Some vegetable polyphenolics with low molecular weights and planar structures exhibited properties of agonistic and/or antagonistic effects of AhR in the in vitro bioassays. However, in light of the bioavailability of such polyphenols, it can be inferred that they may have an antagonistic function in our usual dietary intake. The AhR for polyphenols in usual intake might function biodefensively to protect from the incorporation of foreign chemical compounds such as dioxin. On the other hand, the large excessive intake of foods that contain AhR-activators may be conducive to dioxin-like toxicity, therefore it may be necessary to pay attention to how much of these foods people eat. The results suggest that a well-balanced meal is also important in preventing dioxin-like toxicity.”

**3. de Waard et al. (2008). Gene expression profiling in Caco-2 human colon cells exposed to TCDD, benzo[a]pyrene, and natural Ah receptor agonists from cruciferous vegetables and citrus fruits. *Toxicol In Vitro.* 22(2): 396-410.**

*Key Text:* “To establish whether or not activation of the AhR pathway by NAhRAs and dioxin-like substances results in similar cellular responses, gene expression profiles induced in Caco-2 cells were studied using microarray analysis. Cells were exposed to indolo[3,2-b]carbazole (ICZ), an acid reaction product from cruciferous vegetables, and to extracts of citrus pulp and grapefruit juice. Gene expression profiles induced by these NAhRAs were compared to those of the xenobiotic AhR agonists TCDD and benzo[a]pyrene (B[a]P). Over 20 genes were found more than 1.5 times up- or down-regulated by TCDD, and the expression of most of these genes was modulated in the same direction and to a similar extent by B[a]P and the NAhRAs. Results were confirmed by RT-PCR, and many of these genes may be involved in dioxin-related toxic effects. In conclusion, this in vitro study showed similar effects induced by NAhRAs, TCDD and B[a]P at the transcriptome level in a human intestinal cell line.”

**4. Connor et al. (2008). AH receptor agonist activity in human blood measured with a cell-based bioassay: Evidence for naturally occurring AH receptor ligands in vivo. *J Exp Sci Env Epi.* 1-12.**

*Key Text: “In the present study, an aryl hydrocarbon receptor (AhR)-driven reporter gene bioassay was used to measure the activity, measured as an induction equivalent (IEQ) as compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or IEQ concentration in human blood samples from 10 volunteers under different dietary regimens. Blood concentrations of polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs), as determined by analytical chemistry (HR-GC/MS), and expressed as toxic equivalents (TEQs) with the use of TCDD equivalency factors (TEFs), were within a range that has been reported in the general U.S. population, ranging from 0.022 to 0.119 ppt (whole blood basis). However, the human blood IEQ measured directly via bioassay ranged from 13.4 to 218 ppt (whole blood basis)... Our findings also suggest that dietary NAhRAs constitute a substantial daily dietary intake of AhR-active compounds, and these NAhRAs could influence AhR status in humans and play a role in a basal level of AHR activation.” Note that this study illustrates that even though naturally occurring AhR ligands may have a short-life, that chronic exposure to these compounds is sufficient to impact AhR induction.*

**5. Zhang et al. (2003). Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. *Environ Health Perspect.* 111(16): 1877-82.**

*Key Text: “These data suggest that dietary phytochemicals exhibit substantial cell context-dependent AhR agonist as well as antagonist activities. Moreover, because phytochemicals and other AhR-active compounds in food are present in the diet at relatively high concentrations, risk assessment of dietary toxic equivalents of TCDD and related compounds should also take into account AhR agonist/antagonist activities of phytochemicals.”*

**6. Amakura et al. (2003). Activation of the aryl hydrocarbon receptor by some vegetable constituents determined using in vitro reporter gene assay. *Biol Pharm Bull.* 26(4): 532-9.**

*Key Text: “Ninety-five vegetable constituents, including flavonoids, tannins, saponins, and terpenes, were tested in vitro. Among them, isoflavones such as daidzein, resveratrol having a stilbene structure, and some flavonoids such as naringenin, hesperetin, and baicalein showed AhR activation.”*

**7. Jeuken et al. (2003). Activation of the Ah receptor by extracts of dietary herbal supplements, vegetables, and fruits. *J Agric Food Chem.* 51(18): 5478-87.**

*Key Text: “Although some food extracts (corn, jalapeno pepper, green bell pepper, apple, Brussels sprout, and potato) were relatively potent activators of AhR DNA binding (30-50% of TCDD), only corn and jalapeno pepper extracts induced AhR-dependent luciferase reporter gene expression. However, dilution of corn, jalapeno pepper, bell*

pepper, and potato extracts dramatically increased their ability to induce luciferase activity, suggesting that these extracts contained AhR antagonists whose effectiveness was overcome by dilution. Overall, these results demonstrate that dietary products can be a major source of naturally occurring AhR ligands to which animals and humans are chronically exposed.”

**8. Connor and Finley (2003). Naturally Occurring Ah-Receptor Agonists in Foods: Implications Regarding Dietary Dioxin Exposure and Health Risk. Hum Ecol Risk Assess. 9: 17.**

*Key Text: “Relative estimate of potency (REP) values were developed for I3C ( $8.7 \times 10^{-7}$ ) and ICZ (0.5). The TEQ doses of I3C and ICZ together comprised >99% of the total daily TEQ dose; the daily ICZ TEQ dose ( $1.4 \times 10^6$  pg TEQ/day) was approximately 45,000-fold greater than the current dietary PCDD/F TEQ dose (32 pg TEQ/day). When 30-year accumulated body burden and area-under-the curve doses were calculated, I3C/ICZ still comprised a significant fraction (up to 95 and 96%, respectively) of the total TEQ dose. Further, reduction or elimination of meat and dairy products yielded a minimal (less than 4%) decrease in total TEQ dose. These findings indicate that reducing the intake of animal products (the primary source of dietary PCDD/Fs) might not achieve a significant reduction in total “dietary dioxin TEQ” dose; the comparisons also suggest that trace levels of PCDD/Fs in the human diet are unlikely to pose a significant health risk.”*

## **Response**

We added citations for number 1 to 5 and 7 above.

For number 6 above, Amakura et al. (2003) also reported that ninety vegetable constituents including flavonoids, tannins, saponins, terpenes, *etc.*, were assayed *in vitro*. Among them, flavones, flavonols, anthraquinones, piperine, coumestrol, brevifolincarboxylic acid, and resveratrol showed marked inhibitory effects on AhR-based bioassay activation by TCDD, and their effects were dose dependent. Curcumin, carnosol, and capsaicin also inhibited the activation of AhR in this assay, although to a lesser degree. These results suggest that several vegetable constituents might play a role in protection against dioxin toxicity (Amakura et al. 2003. Screening of the Inhibitory Effect of Vegetable Constituents on the Aryl Hydrocarbon Receptor-Mediated Activity Induced by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. Biol. Pharm. Bull. **26**(12) 1754-1760).

With regard to number 8 above, in the *in vivo* experiments which the WHO TEFs are based on, the animals in the exposed groups and the controls were fed the same diet. Therefore, dietary AhR agonist and/or antagonist would not affect experimentally determined TEFs. In addition, naturally occurring AhR ligands, such as I3C and diindolymethane, have been reported to inhibit 2,3,7,8-TCDD-dependent *in vivo* induction of CYP1A1 and immunotoxicity (Chen et al., 1995, 1996), although some reported that I3C can significantly induce CYP1A1 activities *in vitro* (Connor and Finley, 2003). Furthermore, based on the WHO criteria for inclusion or exclusion

of an REP, Connor and Finley's (2003) paper was not included in their reevaluation of TEF values (Van den Berg et al., 2006). We support WHO TEFs for assessing the risks of DLCs based on AhR mechanism. This approach is not suitable for assessing natural foods containing AhR ligands which have much shorter half-lives and different toxicities compared to DLCs.

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