

# Responses to the comments of Patricia Kablach Casano on behalf of the General Electric Company

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## **EXECUTIVE SUMMARY**

### **Comment 1.**

*The WHO TEFs are based on feeding studies and, consequently, should be used only to assess risks from dietary intake. Using the WHO TEFs to assess inhalation risks is not appropriate.*

### **Response**

Although human risk assessments ideally use human data or animal data that have the exact same exposure route as humans do, inhalation values can be generated from other routes (e.g., dietary) with proper conversion factors. The TEF methodology is not restricted to any particular exposure route. Its use in this way was endorsed by previous guidance issued under the Toxic Air Contaminants program (AB1807) and Air Toxics Hot Spots program (AB2588), as well as by the WHO and U.S. EPA.

### **Comment 2.**

*Basic assumptions upon which the WHO TEFs are based do not withstand scrutiny.*

- *The WHO TEFs assume that there are no differences in the response of humans and rodents to TCDD and PCBs, including PCB 126.*

### **Response**

The TEF methodology has been developed by a series of respected expert committees, of which the WHO 2005 TEF paper (van den Berg et al. 2006) is the latest report, and has been endorsed by a wide range of authorities including various California programs and the U.S. EPA. We concur with these generally accepted conclusions. The methodology does not claim that there is no difference between humans and animals, but rather uses the assumption that responses in animals are a reasonable analogue of the responses in humans. This is a basic assumption in toxicology.

### **Comment 3.**

- *Repeated investigations have shown that:*
  - (a) humans are an order of magnitude less sensitive to TCDD than responsive rodents;*

*(b) humans are two to three orders of magnitude less sensitive to the most toxic PCB – PCB 126 – than responsive rodents.*

### **Response**

OEHHA does not agree with these sweeping assertions, but in any case they relate to possible values of the TCDD potency rather than to the validity or implementation of the TEF methodology.

### **Comment 4.**

- *The TEF approach assumes that the interactions of dioxinlike chemicals with the AH Receptor are additive (i.e., combining such chemicals increases toxicity).*

*The assumption of additivity ignores competition among molecules to bind with the Ah receptor. Additivity has not been demonstrated across congeners and endpoints in animal studies.*

### **Response**

The additive property of dioxin-like effects was confirmed at low doses (NTP study), which is the important dose range for environmental risk assessment. However, as noted in our document, higher doses may show either competitive or synergetic effects.

### **Comment 5.**

- *The WHO TEFs assume that the dose-response curves for dioxinlike PCBs are parallel to that for TCDD. Studies done by EPA's National Toxicology Project have shown that this assumption is invalid.*

### **Response**

The shape of the dose-response curve for TCDD and the DLCs may not be exactly the same. But the general consensus of the WHO expert committees and other scientifically informed commentators is that the similarities are sufficient to allow use of the TEF methodology in estimating risks from dioxin-like PCBs, at the low levels generally encountered in environmental exposure situations. OEHHA endorsed this approach in adopting the I-TEF methodology in 1999 and the WHO TEF methodology for dioxin-like PCBs in May 2009 after extensive public comment and peer review. The currently proposed action makes no change in this part of the established guidance.

### **Comment 6.**

- *The WHO TEFs assume that there is a reliable estimate of the carcinogenicity of TCDD itself, but there is no scientific consensus on that cancer slope factor.*

## **Response**

OEHHA has a cancer slope factor for TCDD which was adopted after extensive peer review and public comment, and which is similar if not identical to values adopted by other regulatory agencies.

### **Comment 7.**

*The WHO TEFs are not appropriate for body burden assessments.*

## **Response**

It is unclear what evidence the commenter is using as a basis for the assertion, and also what bearing it has on the proposal to update the TEF table to the latest version proposed by the WHO expert committee.

### **Comment 8.**

*Human epidemiological studies do not support the view that there is a causal association between exposure to PCBs and cancer in humans. In fact, the epidemiological studies show that PCBs do not cause cancer in humans at environmental or occupational exposures.*

## **Response**

Although there is evidence for increased cancer risk/mortality from both occupational and environmental PCB exposures (De Roos et al., 2005; Demers et al., 2002; Nelson, 2005; Salehi et al., 2008), PCBs are classified as “probable human carcinogens” by the WHO and “class 2B” by the International Agency for Research on Cancer (IARC), based on insufficient human evidence (Carpenter, 2006), but sufficient evidence of carcinogenicity in animals. Both DL- and non-DL-PCB congeners can promote cancers (Knerr and Schrenk, 2006). It is important to recognize that non-positive results in studies of limited power cannot be used to “show that PCBs do not cause cancer”.

### **Comment 9.**

*There is no validated method for performing the PCB congener analysis required to implement the TEF approach for PCBs. EPA's interlaboratory study demonstrates that Method 1668A, which purports to analyze all 219 PCB congeners, does not produce reliable data, and cannot be used consistently across labs.*

## **Response**

We acknowledge that the reliability and sensitivity of detection methods are a developing area of science and technology. However, there are plenty of examples in the literature, including some that were provided in the current revised draft of Appendix C, which show that useful results can be obtained with currently available methodology. Adoption or recommendation of analytical methods is not OEHHA's responsibility, but rather for the current purpose is undertaken by the California Air Resources Board.

### **Comment 10.**

*The TEFs were not developed in accordance with established principles for ensuring the reliability of science, including the principle that a review of a mass of relevant studies should include an exposition of the reasoning that led the reviewers to (1) include some studies and exclude others; (2) give more weight to some studies than to others; and (3) reach the conclusions that were drawn.*

### **Response**

OEHHA is satisfied that the WHO's expert committee's process and conclusions meet the accepted standards for expert evaluation and reporting, especially when the most recent report is considered in the context of an ongoing process of development and updating of the TEFs which is extensively reported in the scientific literature. However the important point in the present context is that OEHHA has considered use of this type of methodology on a number of previous occasions, starting with the original Toxic Air Contaminants document in 1986. It is on these deliberations, which used the statutorily mandated process of public comment and peer review, which OEHHA relies in applying the TEF methodology. The current proposal merely updates the table of values, without requiring or proposing any change to the underlying method.

### **Comment 11.**

*Appendix C purports to "update[] the background and methodology for use of the TEF method for dioxins and DL [dioxin-like]-compound[s]" as compared to the 2003 version of Appendix C. Appendix C does not, however, cite or discuss numerous relevant scientific papers that have been published since 2003.*

### **Response**

Appendix C does cite some related literature, but it is not intended as a general review paper and does not include papers not directly related to the proposed use of the TEF table. Appendix C describes the background, history, method, and usage of the WHO TEFs. The description of the methodology is the same as before except for including items related to the updating of some TEF values.

### **Comment 12.**

*OEHHA has framed its proposed TEF approach as "guidance," but it will effectively revise the Toxic Air Contaminant (TAC) listing and TAC health effects values for co-planar PCBs. Both the listing of TACs as well as the establishment of TAC health effects values are expressly subject to the California Administrative Procedure Act, Cal. Gov. Code §11340 et seq. ("CAPA"). Appendix C clearly was not adopted in accordance with the requirements of CAPA.*

### **Response**

OEHHA is making the current proposal as a revision to the Air Toxic Hot Spots guidelines required by Health and Safety Code section 44360. OEHHA is following the requirements of the

law concerning preparation of the guidelines (see specifically, Health and Safety Code section 44360 (b)(2), which contains an exemption to the Administrative Procedure Act).

## **DETAILED COMMENTS**

### **Comment 13.**

#### ***The WHO TEFs Are Not Appropriate For Assessing Inhalation Risks***

*The OEHHA notice states that Appendix C is intended to be used to “develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2)).” However, the WHO TEFs have no demonstrated relevance to assessing risks from inhalation. The WHO TEFs generally were developed from rodent feeding studies, and are useful only for analyzing risks from dietary exposure. As the authors of the WHO2005 TEFs observed: “The present TEF scheme and TEQ methodology are primarily meant for estimating exposure via dietary intake situations because present TEFs are based largely on oral uptake studies often through diet.” van den Berg, et al. (2006, p. 237). There is no scientific basis for using TEFs to assess inhalation risks.*

### **Response**

Although health risk assessments ideally use human data or animal data that have the same exposure route as humans do (e.g. inhalation exposure), inhalation values can be generated from other routes (e.g., dietary) by extrapolation if no inhalation data are available. TEFs are used for calculation of TEQ and do not deal explicitly with exposure routes: given that dioxin-like compounds are relatively involatile, slowly metabolized and systemically acting there is no *a priori* reason to expect major route-specific differences. The focus on oral uptake is a reflection of the predominance of this route in most practical exposure scenarios. This fact is reflected in the designation of dioxin-like chemicals as “multimedia” chemicals (i.e. those for which multiple routes of exposure should be considered, not just inhalation) in Hot Spots risk assessments.

### **Comment 14.**

#### ***The WHO TEFs Are Based Upon Assumptions That Are Not Supported by Evidence or Have Questionable Evidentiary Support***

*The accuracy of the WHO TEFs for PCBs, and the reliability of the TEF approach for evaluating any health risks of PCBs, has not been established. The WHO TEFs and TEF approach are based on a number of assumptions that are not well supported by scientific evidence.*

### **Response**

The TEF methodology has been developed by a series of respected expert committees, of which the WHO 2005 TEF paper (van den Berg et al. 2006) is the latest report, and has been endorsed by a wide range of authorities including the U.S. EPA. We concur with these generally accepted conclusions. The TEF methodology has been previously adopted for use in the Air Toxics Hot Spots program following public comment and peer review. The current proposal does not make

any changes to this situation, but merely updates the table of values used to reflect the latest scientific consensus.

### **Comment 15.**

*The TEF approach assumes that:*

- *There is no difference between species in sensitivity to dioxin and the dioxin-like compounds (DLCs);*

### **Response**

The methodology does not claim that there is no difference between humans and animals, but rather uses the assumption that responses in animals (and the results of test systems *in vitro*) are a reasonable analogue of the responses in humans. This is a basic assumption in toxicology.

### **Comment 16.**

- *The toxic effects of all the congeners in a mixture are additive;*

### **Response**

The additive property of dioxin-like effects was confirmed at low doses (NTP study), which is the important dose range for environmental risk assessment. However, as noted in our document, higher doses may show either competitive or synergistic effects.

### **Comment 17.**

- *The shape of the dose-response curve is the same for TCDD and the DLCs;*

### **Response**

The shape of the dose-response curve for TCDD and the DLCs may not be exactly the same. But the general consensus of the WHO expert committees and other scientifically informed commentators is that the similarities are sufficient to allow use of the TEF methodology in estimating risks from dioxin-like PCBs, at the low levels generally encountered in environmental exposure situations.

### **Comment 18.**

- *There is a reliable estimate of the carcinogenic potential of TCDD itself.*

### **Response**

OEHHA has a cancer slope factor for TCDD which was adopted after extensive peer review and public comment, and which is similar if not identical to values adopted by other regulatory agencies.

## **Comment 19.**

*None of these assumptions has merit. We discuss the assumptions and the state of the relevant evidence below.*

### **a. *The WHO TEFs Fail To Recognize Compelling Evidence That Humans Are Less Sensitive To Dioxin And PCBs Than Rodents***

*The TEFs presently in use by California, as well as those which OEHHA is now proposing, are based primarily upon studies performed on rodents, and do not vary between species. For example, the WHO TEF for dioxin (2,3,7,8-TCDD) is 1, and that for PCB 126 is 0.1, whether the TEF is applied to rodents or to humans. The assumption that underlies the WHO TEFs is that TCDD and each of the listed DLCs has the same potency for humans as for rodents, at least within an order of magnitude. However, it has long been recognized that humans are approximately 10 times less sensitive than rodents to TCDD, and recent evidence, discussed below, indicates that humans are about 100 times less sensitive than rodents to PCBs. The WHO TEF for TCDD does not account for the observed difference in species sensitivity to this reference chemical, and the WHO TEFs for PCBs do not account for the even greater differences in species sensitivities to PCBs.*

*Appendix C proposes to continue to rely on the assumption of equal sensitivity, even though Appendix C recognizes that differences in species responsiveness to DLCs "could be important." Appendix C, p. 20. Appendix C then states that "[t]here is a large difference between species in the pharmacokinetics of TCDD and related compounds"; notes that "liver/adipose tissue distribution can vary significantly between species and dose levels used"; and observes that "[d]ifferences in tissue distribution can significantly influence TEFs, when they are based on tissue concentrations."*

## **Response**

OEHHA does not make an assumption of "equal sensitivity", nor is this implicit in the TEF methodology. Interspecies differences in sensitivity to the spectrum of Ah receptor based responses are accommodated by the use of species-specific potencies for TCDD. Thus many of the differences between species noted above relate to possible values of the TCDD potency rather than to the validity or implementation of the TEF methodology. We discussed this variation in the document as did the WHO TEF expert committee. The TEF's are based on the potency of the congeners relative to TCDD in a variety of assays. They are then applied to the cancer potency to address human exposure to a wide range of congeners. We are not addressing the value for the potency of TCDD in this document, but rather the relative potencies of the congeners. The WHO TEF method is the best approach so far available for accommodating these relative potencies in developing standards to protect human health and the environment, although it is recognized that species and tissue differences exist.

## **Comment 20.**

*Since 2003, the issue of whether the evidence supports the assumption that humans and rodents exhibit essentially the same sensitivity to TCDD and DLCs has been addressed in The 2005*

*World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds by Martin van den Berg et al. (2006). The views expressed by the authors of that report – the scientists who derived the WHO2005 TEFs -- carry particular weight in interpreting the TEFs and identifying the critical evidentiary issues they contain. On species differences, the authors state:*

*Literature data also indicate that the PCB 126 REP [relative effect potency] for enzyme induction in human cell systems, including primary hepatocytes, breast cancer cell lines and primary lymphocytes, may be one or two orders of magnitude lower [citations omitted]. In addition, the apparent binding affinity of 2,3,7,8-TCDD to the human AhR is generally 1/10<sup>th</sup> that of the AhR of the more sensitive species, but significant variation among individual humans occurs [citations omitted]. It has been suggested that on average, humans are among the more dioxin-resistant species, but the human data set is too limited to be conclusive. ... Taken together, this information warrants more research into REP values in human systems to establish if the present TEFs based on rodent studies are indeed also valid for humans. [Citations omitted.]*

*van den Berg et al. (2006, pp. 225-226)(emphasis added).*

## **Response**

We are aware of, and concur with, the discussions of Dr. van den Berg and his colleagues on the difficulties and limitations of the method. We also concur with his overall conclusion that despite these limitations the TEF methodology is a useful tool for evaluating the effects of low-dose exposures to dioxin-like compounds, including some PCBs. There are different sensitivities and activities for AhR binding and activating among different species. Since there are no sufficient human data available, risk assessors must use the best available data and assumptions to protect human health, including sensitive subpopulations such as young children.

## **Comment 21.**

*Very similar views on this issue, which is central to the use of TEFs in human risk assessments, were expressed in 2006 by the National Academy of Sciences in its review of EPA's Dioxin Reassessment:*

*Numerous investigators have reported species-specific differences in AHR ligand binding affinity of TCDD, other dioxins, and DLCs. Depending on the system examined, the estimated affinity of binding of TCDD (and related compounds) to the human AHR is about 10-fold lower than that observed to the AHR from "responsive" rodent species and is comparable to that observed to the AHR from "nonresponsive" mouse strains (Roberts et al. 1990; Ema et al. 1994; Poland et al. 1994; Ramadoss and Perdew 2004).*

*NAS (2006, p. 81).*

*As the following review of recent literature will show, enough is known about rodent and human sensitivity to TCDD and dioxin-like compounds that recognition of species differences,*

*particularly those between the rodents on which the studies are typically performed and humans for whom risk assessments are undertaken, needs to be taken into account in calculating TEFs.*

*In the following review of the recent literature, we focus on the differences in the potency of TCDD and PCB 126 for rodents and humans. TCDD is addressed because it is the reference point for the WHO TEFs. PCB 126 receives special emphasis for two related reasons: (1) PCB 126 is regarded as the most potent of the PCBs assigned TEFs. It has a value half an order of magnitude greater than PCB 169, and at least two and a half orders of magnitude greater than any other PCB with an assigned TEF. (2) As a result of its assigned TEF, PCB 126 frequently contributes much of the total dioxin-like toxicity of an environmental mixture. Hence, it is considered to be the PCB congener by which people are exposed to the greatest toxic potency. It dominates the calculation of risk in most human health risk assessments that employ the WHO TEFs. This dominant position is not supported, however, by recent data from studies conducted using human-derived tissue and cell lines.*

*A rudimentary understanding of the biological effects of TCDD and dioxin-like compounds is necessary to understand the advances in scientific knowledge that have been achieved in the last decade. There is agreement that seven dioxins, ten furans, and the twelve coplanar PCBs, collectively referred to as DLCs, bind a receptor protein, the aryl hydrocarbon receptor (AhR); this is followed by the induction, or turning on, of various genes. Many of these genes produce enzymes, particularly those in the cytochrome P450 (CYP) family, which includes CYP1A1, CYP1A2, and CYP1B1. These are considered to be among the early key events in the biological sequence or pathway that leads to tumor development in Sprague-Dawley female rats. The hepatic tumorigenicity of TCDD and DLCs in some strains of rodents has, of course, been established through feeding studies, but such tests cannot be conducted with humans. Consequently, in order to compare the biological action of TCDD and dioxin-like compounds in humans to that in rodents, exposure is conducted in cell cultures in vitro, studying in particular the initial key events that lead to toxicological response in vivo. In these tests, immortal human cell lines, typically from the liver, as well as fresh human donor tissue have been treated with the compound under study, and the amount of EROD activity or CYP gene expression has been measured and compared to that in similarly treated rat cells. Often, the EC50 of the compound – the concentration that is halfway between baseline and maximum response after a given exposure time – is used as a metric for the toxicity or potency of the compound. The lower the EC50, the more sensitive the test species is to the compound. Recently, the use of microarrays in making these measurements has allowed results to be simultaneously achieved for many more genes than in the past.*

*In 1996, Wiebel et al. compared the effect of TCDD on AH hydroxylase induction in rat hepatoma cells, H4IIEC3/T, and human liver-derived HepG2 cells. The human HepG2 cells were found to be twenty times less sensitive than the rat cells.*

*In 2000, Xu et al. compared the effects of TCDD on CYP1A activity measured by EROD activity, protein, and gene expression in primary cultures of rat and human hepatocytes. The authors reported that rat hepatocytes generally responded to dioxin at concentrations ten times lower than required for human cells.*

*In 2001, Zeiger et al. treated human HepG2 cells and rat cells, H4IIE and rat primary hepatocytes, with TCDD as well as an array of dioxin-like compounds. The EC50 values for induction of EROD activity were compared. The human cells were an order of magnitude less sensitive to TCDD than were the rat cells.*

*These studies and others are reported in a 2006 review article by Connor and Aylward that addresses the relative sensitivity of rodent cells and human cells to TCDD. The authors concluded that “human cells have been consistently less sensitive to TCDD for induction of EROD or AHH activity, generally requiring approximately 10-fold higher TCDD concentrations to obtain a half-maximal [EC50] result.” These studies and this conclusion are consistent with that reached by the National Academy of Sciences, relying on other studies, and set out above:*

*[T]he estimated affinity of binding of TCDD (and related compounds) to the human AHR is about 10-fold lower than that observed to the AHR from “responsive” rodent species and is comparable to that observed to the AHR from nonresponsive” mouse strains.*

*NAS (2006, p. 81). Thus, the evidence supports the view that the WHO TEFs should reflect the differential potency of TCDD to rats and humans of an order of magnitude. Given the assumptions that underlie the WHO TEFs, the implication of this correction is that one would anticipate that humans would be at least an order of magnitude less sensitive than rats to the DLCs as well.*

## **Response**

Thank you for your review of recent evaluations of the TEF methodology, and in particular the review by the NAS. We found the content quoted above from NAS (2006) is located at p. 57, rather than p. 81. We cite additional material below which we think provides some clarification:

### **“Rodent-to-Human Prediction**

Assumption: REP of DLCs in rodent models is predictive of REP in humans, given that the rank order potency of the DLC is similar between species. Results from available in vivo, in vitro, and accidental and occupational exposure studies are generally consistent with this assumption. Numerous investigators have reported species-specific differences in AHR ligand binding affinity of TCDD, other dioxins, and DLCs. Depending on the system examined, the estimated affinity of binding of TCDD (and related compounds) to the human AHR is about 10-fold lower than that observed to the AHR from “responsive” rodent species and is comparable to that observed to the AHR from “nonresponsive” mouse strains (Roberts et al. 1990; Ema et al. 1994; Poland et al. 1994; Ramadoss and Perdew 2004). This reduced affinity appears to be at least in part due to a single amino acid substitution within the ligand binding domain of the human and “nonresponsive” mouse AHRs (Ema et al. 1994; Poland et al. 1994; Ramadoss and Perdew 2004). Although the affinity of binding of TCDD and related compounds to the human AHR is reduced compared with rodent AHRs, the qualitative and quantitative rank-order potency of these chemicals is similar.” (NAS, 2006, p. 57).

It is evident that overall the Academy's review (NAS, 2006) supports the use of the WHO TEFs. We are aware of the various considerations raised in your commentary and in the NAS review, and note that these issues have also been discussed extensively by the WHO's expert committees. The WHO reports in particular do discuss the limitations on accuracy and applicability of the method, which we have already noted, including the complications which arise at higher dose levels where interactions between compounds are sometimes observed. The issue of considerable variability in the overall sensitivity of the *Ah* receptor system is important not so much in determining the applicability of the TEF methodology or the specific values used, but rather in the selection of a suitable TCDD potency value for human risk calculations. The observed variability between human individuals seems to be at least as great (proportionally) as that seen in different rodent strains. This makes the often-repeated assertions about the relative sensitivity of rodents and humans, which are frequently based on small and selected groups such as workers, intrinsically questionable, and argues for a cautious choice of TCDD potency to ensure protection of sensitive subpopulations. In any case, we are not revisiting the TCDD potency in our TEF document.

### **Comment 22.**

*Turning to the effects of PCB126, in 1996, Vamvakas et al. compared the level of AH hydroxylase and EROD activity in H4IIE rat hepatoma cells, HepG2 human hepatoma cells and MCF-7 human breast cancer cells that had been treated with TCDD and PCBs 77, 126, and 169 – all coplanar, dioxin-like PCBs. Rat cells were found to be generally more sensitive to all treatments. In particular, the rat cell line demonstrated dose-dependent increases for both AH hydroxylase and EROD activity for all three PCB congeners, while the human cell lines were unresponsive to PCB 77 and PCB 169. For the TCDD treatment, the AH hydroxylase EC50 for the rats was 6 times lower than that for humans, and the EROD EC50 for the rats was 42 times lower than that for humans. For PCB 126 treatment, Vamvakas, et al. (1996) estimated that human cells were 310 and 110 times less sensitive than rat cells for AHH and EROD induction, respectively.*

*In the 2001 study already cited, Zeiger et al, treated rat cells and human HepG2 cells with all twelve dioxin-like PCBs: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. The EROD activity in the cells was compared. The human cells showed lower sensitivity to EROD induction. In fact, for eight of the PCB congeners (PCBs 169, 105, 118, 123, 156, 157, 167, and 189), EROD activity could not be induced. For PCBs 77, 81, and 114, the EC50 values for H4IIE rat cells ranged from 3.8 to 47.4 times lower than in the human HepG2 cells. For PCB 126, which has a WHO2005 TEF more than two orders of magnitude greater than any PCB with a detected EC50 value, the rat EC50 value was more than 1200 times less than that of the human.*

*In 2005, Silkworth et al. compared CYP1A gene expression in fresh hepatocytes from human donors, rats, and rhesus monkeys, and HepG2 human hepatoma cells exposed to TCDD and PCB 126 in culture. EROD activity and CYP1A1 and CYP1A2 mRNA were measured. In the case of PCB 126, the EC50s for both EROD activity and CYP1A1 mRNA in the rat were found to be two to three orders of magnitude lower than in the human cells, indicating that human cells were clearly much less sensitive to this PCB congener than rat cells. More recent studies, using more precise gene array technology, have demonstrated that EC50s in rats for CYP1A1,*

*CYP1A2, and many additional genes are also two to three orders of magnitude lower than in humans. Carlson, et al. (2009) and see below.*

### **Response**

Thank you for your review of these studies. In general we do not see any matters here which conflict with the overall judgments of the several WHO TEF expert committees (which we support) that recommended including dioxin-like PCBs in the TEF table. There are limitations in the applicability of the data described above to human risk assessment. In general, *in vitro* experimental data are less reliable compared to *in vivo* data. Enzyme induction levels in cellular test systems may show considerable variation depending on the source and treatment of the cells, and may or may not be reflective of the situation *in vivo* either in rodents or humans. The WHO expert committees have weighed these factors in their consideration of the overall range of evidence which they included in assigning their recommended TEFs.

### **Comment 23.**

*In 2008, Westerink et al. compared CYP1A activity in rat H4IIE cells and human HepG2 cells for an extensive array of chemicals including TCDD and most dioxin-like PCBs. The PCB 126 EC50 for induction was three orders of magnitude lower in rats than in humans. PCBs 77, 114, 123, and 167 similarly elicited much weaker responses in human cells compared to rat cells. In addition, PCBs 105, 118, 156, 157, 169, and 189 did not induce CYP1A activity in the human cells, in some cases at concentrations three magnitudes greater than those found effective in rat cells.*

### **Response**

Westerink et al. (2008) also reported no differences for cytochrome P450 1A induction measured with CEC between human HepG2 and rat HeIIE cells for some PCB congeners (i.e., PCBs 105, 118, and 123). In addition, CYP1A1 and 1A2 competition for PCB congeners are not consistent between human and rat. For example, the IC<sub>50</sub> for CYP1A1 inhibition by PCB 118 in rat cells ( $2.96 \times 10^{-6}$ ) was higher than in human cells ( $>1.00 \times 10^{-4}$ ), while for CYP1A2 this was reversed (human  $2.54 \times 10^{-5}$  vs rat  $>1.00 \times 10^{-4}$ ) (Westerink et al., 2008). Furthermore, WHO TEFs were weighted more heavily by *in vivo* data than *in vitro* data.

### **Comment 24.**

*In 2009, Carlson et al. investigated whether the difference in relative potency of PCB 126 between rats and humans, as measured by induction of CYP1A1, was also true for other AHR regulated genes that could be important to toxic effects subsequent to AHR binding. Two species-specific gene microarrays that could test more than 4,000 genes shared by both rats and humans were used to generate dose response models for genes responding to both TCDD and PCB 126. The median PCB 126 EC50 for 47 human genes responding in a dose response manner consistent with the TEF concept was more than 100 times greater than the median for 79 similarly responding rat genes. Further, the species-specific relative potency of PCB 126 for these genes was estimated. The median relative potency estimate for the human genes was similar to the human hepatocyte-derived potency estimate of 0.003 based on EROD activity*

*developed by Silkworth et al. (2005), while rat genes were consistent with the 0.1 value. This demonstrates that the lower sensitivity of human cells previously observed for both TCDD and PCB126 and the much lower potency of PCB126 relative to TCDD can be extended to many more genes in addition to the CYP genes.*

## **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 AhR agonists 2,3,7,8-TCDD and PCB 126. Dose response of primary cultures of rat and human hepatocytes were determined using species-specific gene microarrays sharing 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 that 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. For instance, in Figure 2 of the paper, the REP for PCB 126 in humans ranged from about  $10^{-6}$  to 1, whereas for the rat the range is relatively smaller (about 0.1 to less than 1000). This suggests that humans have higher variability compare 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 in the paper for EC<sub>50</sub>. In addition, it is not clear whether the authors confirmed the five responsive orthologous genes by other methods such as Northern Blot or RT-PCR, as microarray data may give false positive or negative results. OEHHA does not agree that this paper impacts our proposal to adopt the WHO 2005 TEFs.

## **Comment 25.**

*In sum, the research conducted by Wiebal et al. (1996), Xu et al. (2000), and Zeiger et al. (2001), as well as earlier studies reported in Connor and Aylward (2006), show that human cells are at least ten times less sensitive to dioxin (TCDD) than are rat cells. Next, the work of Zeiger et al. (2001), Silkworth et al. (2005), and Westerink et al. (2008) demonstrates that humans are two to three orders of magnitude less sensitive to PCB 126 than are rats, as measured by CYP1A induction and associated EROD activity. Finally, Carlson et al. (2009) have presented evidence that the human insensitivity to PCB 126, shown through induction of CYP1A1 and EROD activity, applies to other potential AHR-regulated genes that respond to both TCDD and PCB 126; however, many of these potential AHR-regulated genes are not shared between the species. This last point is important because it indicates that TCDD and the DLCs act on different genes in humans and rodents, and that there are significant differences in the biological systems of the two species. This evidence contradicts the fundamental assumption that the TEFs may properly be applied to these and other mammalian species because the mode or mechanism of biological action is the same regardless of the species under consideration.*

## Response

OEHHA disagrees with the interpretation of these data in this comment, as do the various expert panels referred to in earlier comments, including but not limited to WHO, NAS, U.S. EPA, and previous reviews by California's Scientific Review Panel for Toxic Air Contaminants. Specifically, the various studies *in vitro* represent a selection of a widely variable range of data, most of which were available to the WHO expert committee responsible for the 2005 update to the TEF table. That committee's overall judgment was that the TEF methodology is valid and useful although subject to certain well-known and acknowledged limitations.

## Comment 26.

*The authors of the WHO 2005 TEFs have recognized that our knowledge of TCDD and dioxin-like compounds is rapidly expanding, and agreed that TEFs be reviewed in the light of advancing scientific knowledge every five years.<sup>1</sup> OEHHA is undertaking such a review now. In order to perform that task credibly, OEHHA must review the relevant literature of the past ten years. By doing so, we are confident that OEHHA will conclude that the evidence does not support the assumption that TEFs for rodents and humans should be the same, and that the 0.1 WHO TEF for PCB 126 is not supported by the evidence. The difference in rodent and human sensitivity to TCDD calls into question all the WHO TEFs. In practical terms, addressing the TEF for PCB 126 is of most importance. For use in human risk assessment, the TEF for PCB 126 should be lowered by two to three orders of magnitude to 0.0003 to be consistent with recently collected data. Silkworth (2005, p. 514, Table 2). In addition, the WHO TEFs for the other dioxinlike PCBs should be subject to careful scrutiny.<sup>2</sup>*

## Response

This comment illustrates a basic misapprehension of what we are proposing, which has resulted in a large volume of comment which is not relevant to the actual proposal on which comment was invited. The use and applicability of the TEF methodology was evaluated a number of years ago and has been adopted for risk assessment of dioxin-like compounds: the original table of TEF values (the "ITEF" table) was adopted after review by the Scientific Review Panel for Toxic Air Contaminants in 1999. Subsequent updates have been concerned strictly with the adoption of revised tables of TEF values, and have not found any basis for proposing changes to the basic methodology. OEHHA reviewed the most important recent literature and concluded that the WHO 2005 TEFs are the best currently available values for assessing risks of exposure to low doses of DLCs. OEHHA cannot find sufficient evidence to support the suggestion to lower the TEF for PCB 126 by 2 to 3 orders of magnitude. OEHHA notes, furthermore, that the TEF of 0.1 for PCB 126, originally proposed by Safe *et al.* in 1990, underwent three successive WHO reviews, in 1994, 1997 and 2005, and none of these reviews identified a case for changing the value. Finally, any difference in sensitivity between rodents and humans would be reflected in the potency value for TCDD, rather than the TEFs. As noted in the response to comment 19 above, the relative potencies for the congeners appear to be broadly consistent across species. However, we do intend to update the table of TEFs as necessary when future reviews by the WHO committee are completed.

## **Comment 27.**

### **b. The WHO TEFs Fail to Recognize Substantial Evidence That The Potencies of DLCs Are Not Additive**

*The TEF approach assumes that the potencies of individual DLCs in a mixture are additive. The authors of the WHO TEFs have twice recognized the tenuousness of this assumption. In the initial publication of the WHO TEFs, the authors stated: “The most important limitation is that the combined toxic effects of the components of a given mixture would be additive, neglecting possible synergism or antagonism.” Ahlborg et al. (1994, p. 1050). Similarly, the authors of the WHO2005 TEFs acknowledged that deviations from additivity are not uncommon. van den Berg et al. (2006, p. 224).*

*Knowledge of the mechanisms by which AhR-active chemicals cause effects suggests that the congeners’ toxicities represented by TEFs should not be additive. The AhR binds with a variety of molecules. Whether the AhR binds with a chemical, and the strength of the bond, is a function of the shape of the chemical molecule. A chemical that binds weakly to the AhR may be replaced by a “competitor” chemical that forms a stronger bond with the AhR, so that the binding is competitive rather than additive (Gray et al, 2006; Safe, 1990; Walker et al., 2005).*

*The fact that a chemical binds with the AhR does not indicate that it will cause an adverse effect. In fact, chemicals that bind with the AhR can have a beneficial effect (e.g., triggering a normal physiological response like enzyme induction), an adverse effect (e.g., triggering the events that lead to tumors), or no effect. The adverse effects caused by chemicals that bind with the AhR can range from minor (e.g., inhibiting the production of certain cells useful in fighting infection) to major (e.g., causing reproductive disorders). A chemical that binds to the AhR and causes any effect is called an “agonist.” A chemical that binds but has no effect (or inhibits a “normal” event) is called an “antagonist.” The term “antagonist” results from the fact that chemicals that bind with a receptor with no adverse effect compete with agonists for sites on receptors – while an antagonist occupies the site, an agonist cannot occupy it and cause an effect. Moreover, even agonists can have antagonistic properties. For example, if an agonist that produces either a normal physiological effect or a minor adverse effect competes for a receptor and blocks it from another agonist that causes a more serious adverse effect, substantial harm has been avoided (Newsted et al., 1995; Walker et al., 1996; 2005). Agonists that have antagonistic properties are sometimes called “partial” or “weak” agonists.*

*This understanding of the AhR mechanism substantially weakens the assumption of the TEF approach that the potencies of individual DLCs in a mixture are additive (i.e., combining DLCs increases toxicity). Where antagonists are present in concentrations higher than the concentration of agonists, it is difficult for agonists to bind to receptors. Moreover, partial agonists or incomplete agonists compete with complete agonists for receptor binding sites. Thus, whenever a human body contains a mixture of complete agonists, partial agonists, and antagonists, the total impact on the body cannot be predicted by the sum of the various agonist concentrations.*

*Empirical data indicate that some congeners may have antagonistic properties. For example, Starr et al. (1997) reported that “some PCDFs antagonize AhR-mediated responses including*

*fetal cleft palate, hydronephrosis, immunotoxicity, embryotoxicity and induction of CYP1A1-dependent activities.” The initial results of two-year bioassays recently performed by the National Toxicology Program (“NTP”) also provide evidence of non-additive interactions among DLCs. NTP (2006a; 2006b; 2006c; 2006d).*

## **Response**

The above “definition” of antagonist and agonist for AhR is an oversimplification. DLC-induced AhR activities may include both simple competitive interactions at the receptor site and increases in the numbers of receptors and sizes of organs.

The use and applicability of the TEF methodology, including the necessary assumption of additivity at low doses, was evaluated a number of years ago and has been adopted for risk assessment of dioxin-like compounds. At each stage proposed methodology was adopted after public comment and review by the Scientific Review Panel for Toxic Air Contaminants. The assumption of additivity, and its limitations, has been extensively evaluated by successive WHO committees. OEHHHA has not found any basis for proposing changes to the basic methodology. Other credible authorities agree: the NTP dioxins mixtures research fact sheet (2006) reported that

“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>.

## **Comment 28.**

*This issue of additivity is further complicated when PCBs are considered. At sites where PCBs and PCDD/F are both present, PCBs are often present at substantially higher concentrations than are PCDD/F. In his consideration of the potential additivity of mixtures of these compounds, Safe (1993) concluded that “the TEF approach may significantly overestimate the TEQs for environmental extracts containing PCB, PCDD and PCDF mixtures in which the concentrations of the PCBs were >100-fold higher than the PCDDs and PCDFs.” Other studies have indicated that additivity in PCDD/F and PCB mixtures has not been demonstrated across congeners and endpoints in animal studies (Harper et al., 1995; Safe, 1990; Starr et al., 1997).*

*Thus, additivity does not appear to be demonstrated generally across congeners and endpoints in animal studies, and the applicability of this assumption to humans is even less certain. In these circumstances, it is unwarranted to assume that the toxicity of dioxin and dioxin-like mixtures can be predicted by multiplying the TEFs for the individual congeners by their respective concentrations in the mixture, and summing the results.*

## Response

The WHO TEF method, based on consensus among worldwide experts, is the best available method for assessing risks from exposure to DLCs, although it is acknowledged that there are some limitations. Although the assumption of additivity is both plausible and demonstrable at low doses it is known to be modified by inter-compound interactions at high dose levels. However, this is unlikely to be a substantial problem when assessing low-level, long term environmental exposures. In circumstances where it does affect the predicted risk it may result in either under- or over-prediction of risk. It should also be noted that DLCs including PCBs cause serious adverse health effects by other mechanisms in addition to those mediated by the Ah receptor. A paper published recently used thyroid hormone impacts as a biomarker to generate a new series of TEFs for non-dioxin-like effects that supplement the WHO TEFs for assessing risk of thyroid hormone disruption and neurotoxic effects of DL-PCBs and also some non-DL-PCB congeners (Yang et al., 2010).

### Comment 29.

#### *c. The Dose-Response Curves for TCDD and PCBs Are Not Parallel*

*Because TEFs are used to equate the toxicity of individual congeners to that of TCDD at any dose, the TEF approach assumes that the dose-response curves for PCB congeners are parallel to the carcinogenic dose response curve for TCDD. However, this assumption is overly simplistic. The effect caused by any particular amount of a DLC relative to that of TCDD depends on several dynamic pharmacologic characteristics of each ligand, including, but not limited to, receptor affinity, competition, the duration of receptor occupancy, individual ligand efficacy, and even ligand bioavailability. Because of these features, the validity of the systematic use of a single TEF to predict an outcome at any particular dose by assuming its equivalence to an otherwise proportional amount of TCDD is uncertain. The pharmacologic basis for this uncertainty has been described (Putzrath, 1997). Furthermore, the inability of a single TEF to accurately predict outcomes across various endpoints or animal strains has been demonstrated (Pohjanvirta et al., 1995).*

*As a part of a National Toxicology Project 2-year rodent carcinogenicity study, Toyoshiba et al. (2004) looked at the effects of TCDD, 4-PeCDF, PCB-126, and a mixture of the three on the activity of two liver enzymes induced by TCDD and DLCs. The authors concluded that the dose-response curves for each of the three compounds and for the mixture were significantly different from one another.*

## Response

Toyoshiba et al. (2004) compared 3 DLCs to WHO-98 TEFs and found that the TCDD EROD activity is  $925.19 \text{ pmol min}^{-1} \text{ mg}^{-1}$  compared with  $243.67 \text{ pmol min}^{-1} \text{ mg}^{-1}$  for the equivalent PeCDF dose (a ratio of 0.26 that is more similar to the WHO-05 TEF of 0.3 [van den Berg, 2006] than the WHO-98 TEF of 0.5) after 14 weeks of exposure in the rat study. This demonstrates that the newer WHO TEFs have resolved some inconsistencies. However, the major studies support WHO TEF additivity at low doses of DLCs exposure, although not all

DLCs have perfectly parallel dose-response curves throughout the experimentally accessible dose range.

### **Comment 30.**

*Walker et al. (2005) analyzed cancer incidence data from the same NTP 2-year rodent carcinogenicity study. The dose-response curves were modeled using four different model conditions. When each data set was modeled with parameters that allowed the individual curves to provide an independent optimal fit, none of the resultant dose-response curves were parallel.*

*Walker et al. (2005) also modeled the data sets using parameters that forced the curves to assume the same shape. Walker et al. (2005) then conducted a statistical analysis of the error associated with the fit of each model, and concluded that the hypothesis that the dose-response curves were all the same shape could not be rejected. The researchers did admit, however, that the statistical power of the tests used to determine whether the null hypothesis could be rejected was rather low, ranging from 0.1 to 0.5.*

### **Response**

Walker et al. (2005) evaluated the TEF approach in experimental 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).

### **Comment 31.**

#### ***d. There Is No Consensus on the Cancer Slope Factor for TCDD***

*The TEF approach was developed so that TEQ concentrations of dioxins and furans could be summed and used with the empirical cancer slope factor (CSF) for TCDD to estimate potential cancer risks. However, the cornerstone of the TEF approach – the CSF for TCDD – is missing: there is no agreement within the scientific community as to the appropriate CSF for TCDD. Absent a "dose-response function" for TCDD, the equation for applying TEFs to a mixture of DLCs that Appendix C (p. 2) recommends cannot be solved because an essential factor is unknown.*

*A wide range of CSFs has been proposed for TCDD based on animal studies and using a linear, non-threshold cancer model to extrapolate risks to humans at environmentally relevant doses. The proposed CSFs have ranged from 9,000 to 156,000 (mg/kg-day)<sup>-1</sup>, with differences in values resulting largely from the tumor classification scheme and interspecies scaling factor applied (EPA, 1994, 2000; FDA, 1993, 1994; OEHHA, 2007; Keenan et al., 1991). Recently, in two*

revisions of the draft Dioxin Reassessment, EPA proposed a CSF for TCDD of 1,000,000 (mg/kg-day)<sup>-1</sup> based on EPA's evaluation of human epidemiological data (EPA, 2000; 2003) and use of a linear, non-threshold model, but did not identify and discuss the full range of plausible CSFs for TCDD that could be based on peer-reviewed scientific studies.

## Response

OEHHA risk assessment guidance includes a cancer slope factor for TCDD which was adopted after extensive peer review and public comment, and which is similar if not identical to values adopted by other regulatory agencies. The present proposal does not include any change to this value. Although no change to the value is included in the current proposal OEHHA may in the future decide to update the value if new data or methodologies warrant a change. The cancer slope factor itself does not influence the relative potencies of dioxin and DL-compound congeners, which is the basis of the TEF approach.

## Comment 32.

*The 2000 draft Dioxin Reassessment was reviewed by EPA's Science Advisory Board, which could not "reach consensus on a single value for a dioxin potency factor" (EPA, 2001; p. 6). More recently, the National Academy of Sciences (NAS) completed its review of EPA's 2003 draft Dioxin Reassessment (NAS, 2006). Among its conclusions, NAS determined that the data support a threshold, nonlinear relationship rather than the default, non-threshold, linear model that EPA has used historically. NAS concluded:*

*[A]lthough it is not possible to scientifically prove the absence of linearity at low doses, the scientific evidence, based largely on mode of action, is adequate to favor the use of a nonlinear model that would include a threshold response over the use of the default linear assumption. The committee concludes that four major considerations of the scientific evidence support the use of a nonlinear model for low-dose extrapolation.*

\* \* \* \*

*The committee unanimously agrees that the current weight of evidence for TCDD, other dioxins, and DLCs carcinogenicity favors the use of nonlinear methods for extrapolation below the point of departure (POD) of mathematically modeled human or animal data.*

NAS (2006, pp. 122, 190). NAS further commented that –

*[A] risk assessment can be conducted without resorting to default assumptions.*

- *To the extent that EPA favors using default assumptions for regulating dioxin as though it were a linear carcinogen, such a conclusion should be supported with scientific evidence.*

NAS (2006, pp. 141-142).

*The "four major considerations" listed by NAS as supporting a nonlinear relationship rather than the default linear model were that:*

- *TCDD, other dioxins, and DLCs are not genotoxic;*
- *Receptor-mediated agents have sublinear dose-response relationships;*
- *Dioxin-induced liver tumors are secondary to hepatotoxicity; and*
- *Bioassays provide evidence of nonlinearity.*

*NAS (2006, p. 141).*

*In light of NAS's finding that scientific evidence supports the use of a nonlinear model for low-dose extrapolation of the carcinogenicity of DLCs, NAS recommended that EPA consider the full range of animal bioassay data, "including the new NTP animal bioassay studies on TCDD, for quantitative dose-response assessment." NAS (2006, pp. 190-191). When OEHHA calculated a CSF for TCDD on an administered dose basis using the NTP studies, the result was a CSF of 26,000 (mg/kg-day)<sup>-1</sup>, as compared to the CSF of 1,000,000 in EPA's 2003 draft dioxin reassessment. OEHHA (2007, p. 47).*

### **Response**

OEHHA is aware of the several conflicting opinions expressed by U.S. EPA's Science Advisory Board and the NAS following the several versions of US EPA's dioxin reassessment document. However, we were not part of that process, and are not bound by (nor do we necessarily share) any of the opinions expressed by the various parties to that discussion. On the specific point of using a non-linear dose response model, OEHHA notes that the mechanistic arguments for this are largely theoretical and hard to translate into specific mathematical values for use in risk assessment. Also, analyses by various authors including U.S. EPA and NTP have shown that even if there is a threshold for response to dioxin-like compounds it is likely to be below the background exposure to these compounds experienced by the general human population. Therefore, the response to any incremental exposures above this general background is expected to be continuous rather than showing any threshold, and is likely to approximate linearity at least at lower doses.

### **Comment 33.**

*Appendix C does not mention the CSF of 26,000 (mg/kg-day)<sup>-1</sup>, but does reference a 1986 CSF for TCDD of  $1.3 \times 10^5$  (mg/kg-day)<sup>-1</sup>. Appendix C, p. 3. Appendix C does not, however, propose a specific CSF, or range of CSFs, for use in implementing the TEF approach. Without a reliable estimate of an appropriate CSF, the TEF approach cannot be used to reliably estimate the cancer risk posed by any of the DLCs.<sup>3</sup> Without a CSF, it also is impossible to reach a judgment as to whether the use of the TEFs will result in a realistic or entirely fanciful estimate of the risks posed by TCDD and the DLCs.*

### **Response**

The document undergoing review is a revision to Appendix C of the Air Toxics Hot Spots Technical Support Document dealing with cancer potency factors. This appendix describes how to use TEFs for assessing risk of DLCs in mixtures. Cancer slope factors for individual

chemicals, including that for TCDD -  $1.3 \times 10^5$  (mg/kg-day)<sup>-1</sup>, are listed and clearly described in other parts of the TSD, but are not part of this revision, and not currently undergoing public or peer review. The CSF of  $2.6 \times 10^4$  (mg/kg-day)<sup>-1</sup> appears in a draft document from a different California program and has not been adopted for the Air toxics Hot Spots program, nor so far for any other California program.

#### **Comment 34.**

##### **3. The WHO TEFs Are Not Appropriate For Body Burden Estimates**

*As discussed previously, the WHO TEFs are based on intake or dose and therefore are limited in their application. As the original WHO expert panel observed:*

*It was recognized that the recommended TEFs have been developed for exposure scenarios, i.e., they are intake TEFs. These values may – or may not – be appropriate for body burden assessments. They may also be reexamined for eco-toxicity purposes. Thus, there may be different classes of TEF-values depending upon whether the consideration relate to intake, body burden, or ecological concerns.*

*Ahlborg, et al. (1994, p.1059).*

*In the same vein, the authors of the WHO2005 TEFs stated:*

*Concern is expressed about the application of the TEF/TEQ approach to abiotic environmental matrices such as soil, sediment, etc. The present TEF scheme . . . and TEQ methodology are primarily meant for estimating exposure via dietary intake situations because present TEFs are based largely on oral uptake studies often through diet. Application of these “intake or ingestion” TEFs for calculating the TEQ in abiotic environmental matrices has limited toxicological relevance and use for risk assessment, unless the aspect of reduced bioavailability and environmental fate and transport of the various dioxin-like compounds are taken into account. If human risk assessment is done for abiotic matrices, it is recommended that congener-specific equations be used throughout the whole model, instead of using a total TEQ-basis, because fate and transport properties differ widely between congeners.*

*van den Berg, et al. (2006, p. 237)(emphasis added).*

*Recent evidence bears out these concerns. Gray et al. (2006) conducted dose-response modeling of the results of the recent NTP bioassays. The authors concluded that the WHO98 TEFs, which were derived from data evaluated on an administered dose basis, substantially overpredict the cancer potency of 4-PeCDF and PCB 126 on a body-burden basis.*

*In the NAS (2006) review of EPA’s draft Dioxin Reassessment, NAS reached a similar conclusion to that of Gray et al. (2006) concerning the use of the WHO98 TEFs for evaluating risks based on a body burden metric:*

*It remains to be determined whether the current WHO TEFs, which were developed to assess the relative toxic potency of a mixture to which an organism is directly exposed by*

*dietary intake, are appropriate for body burden toxic equivalent quotient (TEQ) determinations, which are derived from the concentrations of different congeners measured in BF [body fat]. If body burdens are to be used as the dose metric, a separate set of body burden TEFs should be developed and applied for this evaluation. Without these corrected values, the overall TEQs estimated by use of intake TEFs might be substantially in error. [NAS (2006) p. 138.]*

*Appendix C does not mention these recommendations of the authors of the WHO TEFs or the NAS. Instead, Appendix C proposes straightforward application of the uncorrected WHO TEFs.*

## **Response**

With regard to the point about oral exposures as opposed to other routes, we noted in a previous response that route-to-route extrapolation is a reasonable procedure in risk assessment when route-specific data are unavailable. Moreover, since DLCs are assessed as “multimedia” chemicals in Hot Spots site risk assessments the most important part of the overall exposure may actually be by the oral route. The characteristics of DLCs are not such as to suggest large route-specific differences in potency.

In relation to the point about body burden vs. applied dose assessments, the standard methodology for Hot Spots risk assessments uses an applied dose methodology. We interpret this comment as supportive of our proposal.

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 (NAS, 2006; p. 137).

## **Comment 35.**

### ***4. Epidemiological Studies Do Not Support the Carcinogenicity of PCBs at Environmental or Occupational Exposures***

*The TEF approach ignores the vast body of human epidemiological studies, which indicates that PCBs are carcinogenic to humans only at very high doses, if at all. More than 50 peer-reviewed, epidemiological cancer studies specific to PCBs have been published over the past 30 years. Many of those studies involved thousands of workers with occupational exposures far greater than those that would result from environmental exposures. None of those studies support a finding that PCBs are human carcinogens. Two review articles, Golden et al. (2003) and Golden and Kimbrough (2009), are particularly noteworthy.*

*Golden et al. (2003) discusses the findings in Kimbrough et al. (1999) and Kimbrough (2003), which reported on a cohort of over 7,000 occupationally exposed workers in two GE capacitor manufacturing plants. Kimbrough found no statistically significant increase in deaths due to cancer regardless of degree of exposure to PCBs or length of employment in the plants. Golden also reviewed all of the other human evidence relating to the potential carcinogenicity of PCBs. This paper concluded that “[a]pplying a weight-of-evidence evaluation to the PCB epidemiological studies can only lead to the conclusion that there is no causal relationship*

*between PCB exposure and any form of cancer.” A more detailed review of all the relevant human cancer studies involving exposure to PCBs (Golden and Shields (2000)) concluded that the weight of the human evidence does not support an association, much less a causal relation, between PCB exposure and any type of cancer.*

*In the 2009 review article, Golden and Kimbrough reviewed an additional 15 articles that had been published since 2003. The review was done using EPA's 2005 Cancer Risk Assessment Guidelines and a method endorsed by ATSDR. None of the studies changes the conclusion drawn in 2003: “the weight of evidence does not support a causal association for PCBs and human cancer.” The authors found no evidence that PCBs would result in human cancer at the level of environmental or occupational exposures.*

*All of this information indicates that application of the TEF approach to PCBs would lead to human health risk assessments that significantly and improperly exaggerate risk.*

## **Response**

Occupational exposure to PCBs has occurred primarily in workers employed in the manufacture of electrical equipment. Workers had daily skin contact with PCBs for many years, inhaled relatively high levels, and probably ingested some while eating near their workstations (Priha et al. 2005). Scientists in Finland found that workers removing sealants had about 10-fold higher PCB exposure than the reference dose (0.02 µg/kg/day). The estimated excess cancer risk by using the EPA cancer slope factor (2 kg-day/mg) was low ( $4.6 \times 10^{-4}$  cancer cases per lifetime) and no liver cancer cases would probably be noted among 150 – 300 workers (Priha et al. 2005). In an Indiana cohort, overall mortality was reduced relative to workers with lower exposure duration (possibly reflecting the healthy worker effect), but non-Hodgkin's lymphoma, brain cancer, and melanoma mortality were increased, especially for women (Ruder et al. 2006). A retrospective cohort mortality study of 2,567 workers in the U.S. found that all cause and all cancer mortality were lower than expected, while rectal cancer and liver cancer mortality were increased, but not to a statistically significant extent (Brown and Jones 1981). Later an expanded study found increased liver cancer mortality and a strong exposure-response relationship for prostate cancer mortality, but no clear association of rectal, stomach, and intestinal cancers with PCB exposure (Prince et al. 2006). A study of 17,321 U.S. workers in the 1970s reported that their serum PCB levels were approximately 10 times higher than community controls. No overall excess of neurodegenerative diseases was observed, but women had increased incidence of amyotrophic sclerosis, Parkinson's disease, and dementia (Steenland et al. 2006). An international multicenter case-control study from six European countries found an increased odds ratios (OR 2.8, 95% CI 1.3-5.9) for cancer of the extrahepatic biliary tract in men who were occupationally exposed to PCBs (Ahrens et al. 2007). Although there is some evidence for increased cancer risk/mortality from both occupational and environmental PCB exposures, PCBs are classified as “probable human carcinogens” by the WHO and “class 2B” by the International Agency for Research on Cancer (IARC), based on insufficient human evidence, but sufficient evidence of carcinogenicity in animals. Both DL and non-DL-PCB congeners can promote cancers (Yang et al, 2010). OEHHA notes in particular the low power of most occupational epidemiological studies to detect effects of these types, and their inability due to small size and focus on selected adult populations to address concerns about the sensitivity of children and other subpopulations.

PCBs have been identified as a hazardous air pollutant pursuant to subsection (b) of Section 112 of the federal Clean Air Act (42 U.S.C. Section 7412(b)) and have therefore been designated by the California Air Resources Board to be a toxic air contaminant pursuant to Health and Safety Code Section 39657. This determination, in combination with the formal identification by IARC noted above, and various assessments developed for the Air Toxics Hot Spots program, require that these compounds be assessed as probably causing cancer in humans. The current proposal has neither the intent nor the authority to alter these determinations.

### **Comment 36.**

#### ***There is No Validated Method For Performing the PCB Congener Analysis Required to Use the TEF Approach for PCBs***

*The TEF methodology, by definition, requires analysis of individual DLC compounds, including PCB congeners that are DLCs. The only method of which we are aware that purports to analyze the co-planar PCB congeners is EPA's Method 1668B --Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS (Nov. 2008)<sup>4</sup>. That method was developed for "wastewater, surface water, soil, sediment, biosolids and tissue matrices", but states that "[o]ther applications and matrices may be possible, which may or may not require modifications of sample preparation, chromatography, etc." Method 1668B, p. 1. As discussed below, Method 1668B has not been validated as called for by EPA's Agency Policy Directive No. FEM-2005-001, Ensuring the Validity of Agency Methods – Methods Validation and Peer Review Guidelines (2005) (hereinafter "Validation Policy") or FEM Document No. 2005-01, Validation and Peer Review of U.S. [EPA] Chemical Methods of Analysis (hereinafter "Validation Guidance").*

*An interlaboratory variability study of Method 1668A – the immediate predecessor of Method 1668B -- conducted for EPA by qualified labs in 2003-2004 (hereinafter "EPA study") indicates that the Method is highly problematic. Almost half of the labs that submitted data to EPA were not able to produce data that EPA regarded as usable. Method 1668A Interlaboratory Validation Study Report (Nov. 2008) (hereinafter "EPA Report"), pp. 9-10.<sup>5</sup> That fact should have prompted EPA to consider whether this Method can be implemented correctly and consistently by different labs. Instead, EPA simply disregarded the results from the labs whose data EPA regarded as unusable, and proceeded to analyze the data from the remaining six labs that EPA deemed to be usable. Regulated entities, of course, do not know which labs produced usable data, and therefore have at least a 50% chance of hiring a laboratory that will not produce usable data, let alone correct data.*

*To the extent that EPA regarded data obtained in the EPA Study as usable, that data does not demonstrate that Method 1668A attained the goals set forth in EPA's Validation Policy (data must be "suitable for its intended purpose (i.e., yields acceptable accuracy for the analyte, matrix and concentration range of concern))"<sup>6</sup> or has the "method reproducibility" required by EPA's Validation Guidance.<sup>7</sup> Instead, the EPA Report identifies many problems with the "usable" data, including:*

- *Extreme differences in the recoveries obtained by the labs. Figure 4-1 on p. 14 of the EPA Report shows recoveries ranging from almost 0% to more than 100% for*

concentrations of PCB congeners in wastewater in the 0 to 2000 pg/L range, and from 60% to more than 100% for concentrations in the 2000 to 3000 pg/L range – the ranges that are targeted by Method 1668A. If EPA had included data from all 11 labs that submitted data, the differences in recoveries might have been even greater.

- *Unexplained, higher than expected variability at both high and low concentrations of PCBs in wastewater. EPA Report, Fig. 4-3. The higher variability is consistent with the large differences in the minimum and maximum recoveries in Table 4-3. In fact, the EPA Report states, “The variability is somewhat higher than expected at the higher concentrations, with RSDs of approximately 40%. The reason for these higher than expected RSDs is not known.” Section 4.5.1.2. Again, if EPA had included data from all 11 labs that submitted data, the differences in recoveries might have been even greater.*
- *Inability to calculate recoveries of congeners from tissue and biosolids samples, because the true congener concentrations in those samples were unknown. EPA Report at 14. Consequently, EPA cannot determine the accuracy of Method 1668A for those matrices.*
- *Insufficient calibration data “to permit revision of the QC acceptance criterion for calibration linearity” (EPA Report, Sec. 5.1) and to calibrate verification data (EPA Report Sec. 5.2), which limits EPA’s ability to evaluate fully the performance of Method 1668A.*
- *Precision that is “proportional to concentration” (EPA Report, Sec. 6), meaning that Method 1668A is less precise for lower concentrations of PCB congeners – the concentration ranges actually targeted by Method 1668A – than for higher concentrations.*

*Presumably in recognition of these and other problems, the EPA Report does not state or claim that the Method yields acceptable accuracy for PCB congeners in the matrices and concentration ranges of concern, with adequate precision and reproducibility across labs. Indeed, the EPA Report says only that “[t]his study demonstrated that PCB congeners can be measured in water, biosolids, and tissue in multiple laboratories using EPA Method 1668A.” EPA Report, p. 23 (emphasis added). Even if that is true, the fact that PCBs “can be measured” does not mean that Method 1668A measures PCBs with the requisite precision, accuracy and reproducibility. In addition, EPA has not demonstrated that the changes that it made to Method 1668A to produce Method 1668B are sufficient to correct the problems encountered in the EPA study. Consequently, unless and until a validated method for analyzing soil, sediments and other matrices for individual co-planar PCB congeners is developed, the TEF approach can not be implemented reliably for PCBs.*

## **Response**

We acknowledge that the reliability and sensitivity of detection methods are a developing area of science and technology. However, there are plenty of examples in the literature, including some

that were provided in the current draft appendix C, which show that useful results can be obtained with currently available methodology. Adoption or recommendation of analytical methods is not OEHHA's responsibility, but rather for the current purpose is undertaken by the California Air Resources Board. It should be noted that most site assessments for the Air Toxics Hot Spots program use air dispersion modeling and emissions inventories to estimate exposures in the community surrounding a site, rather than direct measurement of environmental media or biological materials. The analytical demands for this modeling approach are generally less severe than those for assessments relying on environmental measurements.

### **Comment 37.**

#### ***The Development of the WHO TEFs Has Not Followed Established Practices For Ensuring Scientific Reliability And Clarity***

*Among the core principles of science is the precept that observations and experiments must be replicable to be reliable and trustworthy. For other scientists to attempt replication, the authors of the original study must describe their method, materials and reasoning in detail. Further, in critical review work, the authors must explain clearly the reasoning that led them to include some studies and exclude others, and to place weight on one experimental result but not another, so that other scientists can make a fair judgment as to whether the evidence has been properly assessed. One of the functions of peer review is to assure that these principles and standards are met.*

*Development of the WHO TEFs has not conformed to these fundamental principles. The scientists who developed and updated the WHO TEFs have provided little rationale for considering some studies and not others. They have not provided sufficient detail on how they evaluated and weighed the studies from which the TEFs were derived. They have provided opinion, with virtually none of the reasoning that would make it replicable.<sup>8</sup>*

*The lack of adherence to these fundamental principles has pervaded the WHO TEF process. The initial set of WHO TEFs was developed during a nonpublic "expert consultation" consisting of 12 experts, two observers, and WHO staff. Ahlborg, et al. (1994, p. 1050). Of 1200 articles on PCBs, 146 were considered useful for developing a database for determining TEFs.<sup>9</sup> These articles were analyzed, and the data included in the final database were selected from 57 articles, manuscripts and personal communications using the following criteria:*

- *At least one PCB congener was studied*
- *TCDD or a PCB-reference (PCB 77, 126, or 169) also was studied in the same experiment or*
- *TCDD or a PCB-reference (PCB 77, 126 or 169) was studied with the same experimental design and by the same authors in another experiment*
- *Endpoints were affected by TCDD or the PCB-reference (PCB 77, 126, 169).*

*Ahlborg et al., 1994 (p. 1051).*

*The requirements that PCBs be studied on a congener basis, and that such studies include or be tied to a study of TCDD, is understandable when the aim is to look at relative potencies, but the reality is that those criteria exclude from consideration a great deal of the established literature on PCBs. Two broad areas of PCB analysis are entirely excluded: studies that examined PCBs on an Aroclor basis rather than a congener basis, and studies that focused on PCBs without reference to TCDD. An excellent example of the effect of these exclusions is to consider the basis on which EPA established the IRIS values for PCBs. All of the animal studies that EPA relied on involved doses of Aroclors – the commercial mixtures of PCBs actually manufactured, marketed and used in the United States -- rather than individual congeners. EPA, IRIS, Polychlorinated biphenyls (PCBs) (CASRN 1336-36-3). The authors of the WHO TEFs did not consider this evidence, thereby ignoring the entire basis on which EPA traditionally has assessed the health risk of PCBs. Similarly, the epidemiological studies obviously do not reflect exposure to a single congener, and those studies also have been ignored by the authors of the WHO TEFs. The authors' consideration only of experimental studies that focused on specific congeners does not represent the best available science on the toxicity of PCBs.*

*These and other deficiencies have persisted through the latest iteration of the WHO TEFs. For the 2005 update, the WHO expert panel used a “combination of . . . unweighted REP distributions, expert judgment and point estimates to re-evaluate TEFs.” van den Berg et al. (2006, p. 227). Certain TEFs were “extensively re-evaluated.” Id. For each TEF, however, only a one-paragraph explanation of the expert panel’s analysis and conclusion is provided.*

*The expert panels that developed and updated the TEFs have consistently failed to provide sufficient information to replicate their analyses. Consequently, the expert panels’ work cannot be peer reviewed in the usual manner. Only the results of the work can be tested against the broader evidentiary material available. As we have shown, such testing demonstrates critical flaws in the assumptions on which the TEFs are premised. Use of the TEF approach for assessing PCB toxicity should be abandoned.*

## **Response**

OEHHA disagrees with the assertions made in this comment. First, in order to compare relative potency, the WHO committee appropriately used studies analyzing specific congeners. Studies that could not provide toxicity information on specific congeners are not useful for determining relative potencies. Second, the WHO committee and others who have contributed to the development of the method have provided an extensive account of the development of the method in the scientific literature extending over the past twenty years (Safe et al., 1990; Van den Berg, 1994; 1997; 2005). Contributors include a wide range of respected scientists.

## **Comment 38.**

### ***Appendix C Does Not Discuss Recent, Important Scientific Publications***

*GE has compared Appendix C with OEHHA’s 2003 “Proposal for the Adoption of the Revised Toxicity Equivalency Factor (TEF<sub>WHO-97</sub>) Scheme – Public Review Draft” (“Draft 2003 Proposal”). Appendix C and the Draft 2003 Proposal are substantially similar except for Appendix C’s discussion of the 2005 WHO TEFs and the addition of a few references to more*

*recent papers. Numerous relevant post-2003 studies and other materials – including the NAS's review of EPA's 2003 draft Dioxin Reassessment – are not discussed in Appendix C. Those studies are identified by the use of boldface text in the attached list of references.*

*Appendix C should be withdrawn pending OEHHA's review of these studies and the NAS report and analysis of their content.*

## **Response**

Appendix C is specifically about how to use WHO TEFs to calculate TEQs. OEHHA does not need or intend to produce an extensive review of the entire literature on DLCs at this point. The methods and procedures addressed in the appendix are the same as before, except for the updated TEF values, so the updates to the document are confined to addressing those changes. However, we do include some related new references including a paper on development of TEF by using other biomarkers (Yang et al, 2010). The comprehensive review of dioxin toxicity and risk assessment by U.S. EPA in 2003, and the various commentaries thereon, address a different and much larger objective, which is not relevant to the current proposal.

## **Comment 39.**

***Appendix C is Not “Guidance,” Has Not Been Promulgated in Accordance with the California Administrative Procedure Act, and Should Be Withdrawn***

*OEHHA has framed its proposed TEF approach as “guidance.” However, if adopted, the approach will effectively revise the Toxic Air Contaminant (TAC) listing and TAC health effects values for co-planar PCBs. Both the listing of TACs as well as the establishment of TAC health effects values are expressly subject to the California Administrative Procedure Act, Cal. Gov. Code §11340 et seq. (the “APA”). (Cal. Health & Safety Code §39650 et seq). The APA mandates various procedural requirements, such as a 45-day comment period; a detailed explanation of the basis for the regulation; and a public hearing (Id.; Cal. Gov. Code §11346.4). The current proposal was not issued in compliance with these APA mandates, and hence is legally insufficient and should be withdrawn.*

## **Response**

As noted in the response to Comment 12 above, there is an express statutory exemption from the APA for development of this guidance (see Health and Safety Code section 44360(b)(2)).

## **Comment 40.**

*Even if the adoption of the TEF approach would not constitute a regulation, OEHHA still must provide the public with sufficient information regarding the nature and effects of the proposed change (see, e.g., Cal. Health Code §44360(b)(2)). Appendix C does not meet this standard because, among other things, it does not explain the effects of the new TEFs on the TAC and Hot Spots programs or how the new TEFs would be used under the Children’s Environmental Health program.*

## **Response**

The effects of the proposed change are demonstrated in the example analysis provided at the end of the document. This Appendix C is accompanied by the other parts of the Hot Spots Technical Support documents, which include a volume describing the application of the health protective levels and assessment methodologies described in the other volumes to site risk assessments for the Air Toxic Hot Spots programs. No specific actions under the TAC program are currently implied by this proposal: any such actions in future would be implemented as Air Toxics Control Measures by the California Air Resources Board. The requirements of the Children's Environmental Health Protection act have been explained in various OEHHA documents addressing this program, and in the revised Hot Spots Technical Support Documents addressing non-cancer and cancer risk assessment: the primary action is to require OEHHA to update health protective values to take account of potentially greater susceptibility of children to toxic effects. Our recent activities for the Air Toxics Hot Spots and TAC programs clearly address this requirement.

## **CONCLUSION**

### **Comment 41.**

*The evidence presented above leads to one conclusion: the WHO TEFs and the TEF approach do not constitute the best available science on the toxicity of PCBs. In particular, key assumptions that underlie the WHO TEFs and the TEF approach are not supported by sufficient evidence or have been demonstrated to be incorrect. Consequently, OEHHA should withdraw Appendix C for further consideration in the light of these comments, and remove all references to PCB congeners and the WHO TEFs for such congeners from Appendix C. If OEHHA proceeds to finalize Appendix C with PCBs included, OEHHA should reduce the TEF for PCB 126 by two to three orders of magnitude, and re-evaluate the TEFs for other PCB congeners, to reflect PCB-specific evidence and the generally accepted consensus that humans are less sensitive than rodents to dioxin.*

### **Response**

The commenter makes clear the objection of the interested parties to the use of the TEF procedure for PCB congeners, but fails to offer any specific proposal as an alternative. OEHHA believes on the contrary that the WHO TEF method is the best available method for assessing the dioxin-like effects from exposure to all DLCs, although obviously it cannot address every issue, especially the complex inter-compound interactions expected at high doses. If OEHHA were to withdraw the current proposal it would have no effect on the use of the TEF methodology or the currently recommended WHO<sub>97</sub> TEF values for DLCs including PCBs. These depend on earlier recommendations which have already been adopted. The principal effect of the current proposal is to actually reduce the TEFs for some PCBs following the recommendation of the WHO 2005 expert committee, to take into account some of the recent literature referenced in our document and in these comments.

## **REFERENCES**

For references see the list provided in OEHHA's proposed revision to Appendix C, and that included in the comments submitted on behalf of General Electric Co.