

Response to Comments in the External Peer Review of *Recommended Toxicity Equivalency Factors (TEFs) for Human Health Risk Assessments of Dioxin and Dioxin-Like Compounds*

On October 22, 2009, an independent scientific peer review panel, organized and implemented by Versar, Inc. under EPA Contract No EP-C-07-025, reviewed and discussed the EPA external review draft document, “*Recommended Toxicity Equivalency Factors (TEFs) for Human Health Risk Assessments of Dioxin and Dioxin-Like Compounds*” in a teleconference open to the public. The EPA document that was under review updates the U.S. EPA’s approach for evaluating the human health risks from exposures to environmental media containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and dioxin-like compounds (DLCs).

The purpose of this summary is to provide a disposition of EPA’s response to major comments raised as part of the External Peer Review of the Agency’s draft. The peer review offered a number of general comments, as well as some more narrowly focused and editorial comments from individual reviewers. Many of the comments from individual reviewers have been incorporated into the document and are not presented here (see the *Peer Review Summary Report* of November 4, 2009 for all of the reviewers’ comments). The peer review was conducted during October 2009, culminating with a conference call meeting open to the public on October 22, 2009 and final report on November 4, 2009. Information concerning the peer review meeting results can be found online at: <http://www.epa.gov/raf/hhtefguidance>. External peer reviewers included:

- Peter L. deFur, Ph.D. [Chair]
- Moiz Mumtaz, Ph.D.
- Thomas B. Starr, Ph.D.
- Martin van den Berg, Ph.D.
- Nigel J. Walker, Ph.D., DABT

EPA appreciates the useful feedback and recommendations of the peer review panel and believes their comments have further improved the overall quality of the document. A summary of the major comments and EPA’s responses are organized as follows:

- A. Summary of peer review teleconference
- B. Overview of general comments and specific observations
- C. Responses to charge questions

A. Summary of Peer Review Teleconference

A.1. The External Peer Review of *Recommended Toxicity Equivalency Factors (TEFs) for Human Health Risk Assessments of Dioxin and Dioxin-Like Compounds* was held as a teleconference on October 22, 2009, from 1:00 to 3:45 PM EDT. The purpose of the

teleconference was to facilitate discussion among five experts to provide feedback on EPA's draft document. Five reviewers were selected by Versar, Inc. for this review based on their experience in areas such as: dioxin toxic equivalency factors (TEFs), toxicology, mixtures component methods, and risk assessment. Each reviewer offered general impressions on the document and contributed to the discussion of ten specific charge questions, taken in order and led by the chair.

In addition to the five reviewers, approximately 25 public observers were present on the call to listen to the reviewers' discussion. Four observers spoke during the public comment period of the teleconference, offering comments on the TEF guidance document. These verbal comments were provided in addition to the written comments submitted to the docket, which the reviewers considered as part of their review.

Each reviewer provided their perspectives on the document, all noting that the authors were to be commended for producing a concise and understandable document. Most reviewers recommended some augmentation of sections of the document to provide additional context or more complete description, with limited additional text. The most commonly occurring comment was the need for more specifics and detail in the section on uncertainty analysis. The uncertainty and variability of the relative potency data (REPs), and the underlying toxicity tests, were the most commonly raised issues during the peer review discussion.

EPA Response: In an ideal situation, a full quantitative uncertainty analysis would be provided, however, at present, the U.S. EPA is aware of limitations in both the underlying ReP database (Hawes et al., 2006) and in the ability to statistically analyze it that preclude a detailed evaluation of the various sources of heterogeneity inherent in a quantitative analysis of uncertainty. Therefore, the document now states this fact and suggests the conduct of a TEF sensitivity analysis that would have at least two purposes: (1) to identify plausible upper and lower estimates of the Toxicity Equivalence (TEQ), and (2) to identify the influence of TEF values for specific compounds on the TEQ. Table 4 of the document summarizes EPA's recommendations for risk characterization.

A.2. Most reviewers agreed that the document is accurate in terms of descriptions of the TEF mechanism and process, although several reviewers requested some additional explanatory text for using the TEFs. The additional text would be intended to provide users at the state or regional level with more "how to" direction. Several reviewers were quite clear that more history and background would improve the guidance and the usability of the document because there is more experimental background on TEFs and this information would be helpful to the reader. Additional references are also needed on specific background topics.

EPA Response: EPA reviewed the document to ensure that references regarding the background of the TEQ were included. Additionally, the document's text was revised to more explicitly say how the WHO developed the 2005 TEFs. However, EPA considers implementation issues to be out of the scope of this document.

A.3. Most reviewers thought that the document needs to provide more description or direction on using or capturing the underlying uncertainty in the information/data used to generate the TEFs. One example of this point is that the van den Berg paper lists the underlying assumptions for the TEF approach and these need to be listed in this EPA guidance.

EPA Response: The underlying assumptions for the TEF methodology are detailed in the document. Equations and TEF range data were added to the text of the document to allow the reader to 1) calculate a basic TEQ and 2) conduct a sensitivity analysis on the TEQ.

A.4. Most of the reviewers felt that additional information needs to be added in describing the Relative Potency (REP) database presented in Haws et al. (2006). Information on REP variability and distribution needs to be added from the Haws publication. One reviewer suggested that an Excel spreadsheet of the data file supplements provided in the publication could be created and made available to all interested parties and placed on EPA's website for downloading.

EPA Response: The publication authored by Haws et al. (2006) includes supplemental materials containing information on the ReP database that are available to the public. Additionally, Tables 2 and 3 were added to the document to give percentiles of the Haws et al. (2006) ReP for use in sensitivity analyses.

A.5. Reviewers recommended alternative ways to approach uncertainty analysis for the TEFs. Alternatives recommended by one reviewer include the use of weighted distributions, classifying congeners based on database strength, assigning different congeners a different level of uncertainty, and classifying TEQs as high, medium, or low potency so the toxicity equivalent (TEQ) contributions from weak congeners can be evaluated.

EPA Response: No uncertainty analysis is recommended based on deficiencies in the underlying ReP data, but a sensitivity analysis is suggested and information is provided in Tables 2 and 3 to assist the reader in undertaking such an analysis.

A.6. All the reviewers were disappointed with the extent and scope of the uncertainty analysis section in the document. At least one reviewer thought the document had no real uncertainty section. The reviewers agreed that the guidance document needs a specific section on uncertainty analysis. This section should provide specific direction on conducting qualitative and quantitative uncertainty analysis for use of TEFs. At present, the guidance offers some useful and valuable direction on sensitivity analysis that the reviewers agreed needs to be included in a thorough and careful application of TEFs.

EPA Response: See the responses provided for A.1., A.3., and A.5.

A.7. Several of the reviewers took issue with the recommendation that the TEFs should be used for all cancer and non-cancer effects that are mediated through aryl hydrocarbon

receptor (AHR) binding by the dioxin-like compounds (DLCs). Several reviewers were confused by the phrase “all cancer” and felt clarification in the document was needed. Most reviewers felt that TEFs are also appropriate for non-cancer effects because these effects are also likely mediated through the AH receptor, though one reviewer cautioned that use of TEFs is as questionable and uncertain for non-cancer as it is for cancer effects. Another reviewer did not agree with a linear approach for carcinogenicity.

EPA Response: In this document EPA assumes that all cancer and noncancer effects of TCDD and DLCs are AhR dependent and recommends TEFs be used for all cancer and noncancer effects that appear to be mediated through AhR binding by the DLCs. EPA also recognizes that this issue will require further evaluation as additional toxicity data become available. Nearly all TCDD and DLC experimental data appear to be consistent with the hypothesis that binding to the AhR is the first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic responses observed in both experimental animals and human epidemiologic cohorts. Binding to the receptor appears to be necessary, but not sufficient, to generate the wide variety of toxic effects caused by dioxin-like halogenated aromatic hydrocarbons.

A.8. The reviewers questioned the use of TEFs if cancer potency factors for specific congeners are available. One reviewer noted that the National Toxicology Program has completed toxicity tests for carcinogenicity of 4 of the 5 DLCs that are the top contributors to TEQ, nationally. EPA should add in the option for using these DLC congener-specific cancer slope factors in those cases where applicable, and provide some guidance on what situations would be most applicable.

EPA Response: The document now states that “the TEF methodology is most commonly applied to situations where exposures are to mixtures of DLCs, and the assessment goals include the analysis of human health risks posed by the mixture. When appropriate dose-response information is available on IRIS, other approaches may be considered for exposures to single compounds or chemical classes. For example, PCB cancer slope factors are available on IRIS based on commercial Aroclor mixtures bioassay data and may be used as appropriate for PCB mixture exposures that do not involve significant amounts of DLCs from the PCDD and PDCF classes.”

A.9. The reviewers had a range of opinions on the recommendation that the TEFs may be applied to other exposure routes (i.e., dermal or inhalation) as an interim estimate. Some felt that application of the current TEFs to non-dietary exposure routes is not scientifically supportable at the present time. Application to the inhalation route may be justified, but again, there is no scientific evidence supporting this application. Others felt that the recommendation was justified based on evidence of systemic responses after dermal exposure and the expected high bioavailability through inhalation.

EPA Response: The EPA agrees with Van den Berg et al. (2006) that the TEFs are most appropriate for dioxin exposures via the oral exposure route and states that the bioavailability of DLCs encountered through various sources of oral exposure need to be evaluated in risk analyses. EPA believes the TEFs may be applied to other exposure

routes (i.e., dermal or inhalation) as an interim estimate. EPA recommends that, if considered in an assessment, the fractional contribution of oral, dermal and inhalation route exposures to the predicted TEQ be identified. Also, within each route of exposure, the fractional contribution of each congener to the predicted TEQ should be identified, as well as the fraction of the TEQ associated with each chemical class.

A.10. The reviewers had various perspectives on alternative approaches to TEFs in dealing with mixtures of DLCs. Some reviewers thought that no other approach is ready for use, some reviewers that that some alternatives could be ready soon or were ready now for application. Several reviewers urged EPA to move forward with implementation of this TEF approach to improve consistency and transparency in risk assessment, management, and communication.

EPA Response: EPA will consider the suggested alternative approaches when the TEF approach is re-evaluated in the future.

B. Overview of General Comments and Specific Observations

B.1. The document is a good and accurate description of the TEF methodology, with background, and explanation of the concept. The technical description is correct, and the development of the TEF is explained well. EPA's conclusions that the TEF method should be used for all Ah mediated responses are sound and supported by a substantial body of evidence. The guidance is concise and to the point, as it should be.

As far as I know, the guidance documents are used by EPA and state agency staff in writing permits, implementing regulations, conducting risk assessments and carrying out cleanup plans. I am not sure that all of the intended users of this document will be sufficiently familiar with all the technicalities of TEFs to be able to pick this document up and use it. I think otherwise, based on my experience with state and local efforts and programs. To correct this problem, I think some text should be added to the current document that gives a bit more context of the TEFs. The reader also needs to be directed to specific references for more information on aspects of the TEF concept and application.

EPA Response: Implementation issues are considered outside of the scope of this document and EPA did not add much text on this topic. However, EPA did ensure that basic references on the subject were cited so that the reader can refer to those for additional information on TEFs.

B.2. The document does discuss uncertainty, but does not really provide much in the way of direction on how to perform an uncertainty analysis for a TEF approach. The issue here is that the writers and reviewers are familiar with both qualitative and quantitative uncertainty analysis. It is not clear to me what the target is for any uncertainty discussion in this guidance.

As far as risk characterization, the guidance could be more clear and direct in expressing the high degree of certainty (low uncertainty) over the TEF approach. In addition,

characterizing risk requires expressing not only the probability, nature and magnitude of harm, but also the quality of information at each step. The TEF approach has high quality information at multiple levels of biological organization.

EPA Response: No uncertainty analysis is recommended based on data deficiencies, but a sensitivity analysis is suggested and information is provided on TEF ranges. A new table illustrating risk characterization recommendations (Table 4) has been added to the document to improve clarity.

B.3. I am a toxicologist interested in mixtures risk assessment methods development. Thus, my comments will bring this perspective that is based on my experience as a mixtures risk assessor. The guidelines for risk assessment of chemical mixtures (EPA, 2000; ATSDR, 2004) recommend three alternative methods viz., whole mixture, similar mixture method, and component based approach. Very few whole mixtures have been tested. For risk assessment of mixtures of environmental chemicals, the most often used method is the component based approach because data are often available on individual chemicals as required by various environmental laws passed by the U.S. congress. Component-based approaches, involving an analysis of the toxicity of components of the mixture, are recommended when appropriate toxicity data on a complex mixture of concern, or on a “sufficiently similar” mixture, are unavailable. The approach proposed in this document focuses on this approach.

TEFs have been proposed and used in risk assessment of chemical mixtures for about two decades. This is a brief document that is technically sound and it very concisely but accurately explains the TEF methodology to derive individual chemical TEF values. The EPA has used similar methodologies for polycyclic aromatic hydrocarbons (PAHs) and other such chemicals that occur in mixtures for a long time. In this document it is formally recommending the adoption of the 2005 consensus TEF values of the World Health Organization (WHO).

There are several areas of the document that are written very well and can be easily followed with people with a good background or experience in this area of toxicology. The EPA had deliberately tried to be concise so that it can be used by field workers however, some additional text might benefit novices.

A quick look at the table of contents shows that all technical aspects of the TEF approach are included in the document even though some are in brief.

EPA Response: Implementation issues are considered outside of the scope of this document and EPA did not add much text on this topic. However, EPA did ensure that basic references on the subject were cited so that the reader can refer to those for additional information on TEFs.

B.4. The draft is generally well-written and straightforward. I enjoyed its brevity. In a few places, the narrative is a bit overreaching in terms of its characterization of the extent to which dose additivity has been, or ever will be, confirmed for DLCs. I called this

“wishful thinking” on EPA’s part. I think it’s also overreaching for EPA to want to use TEFs developed primarily with data from oral feeding studies to assess risks from dermal and inhalation exposures, especially since Van den Berg et al. (2006) have recommended against doing this for a number of very good reasons. Overall, though, the draft is reasonably well-balanced and it does note a number of important limitations of the TEF approach, even when it is implemented with the updated TEFs.

EPA Response: The text of the document was revised to more clearly state that dose additivity is an assumption. EPA added text regarding risk characterization when applying TEFs to multi-route exposures. EPA believes the TEFs may be applied to other exposure routes (i.e., dermal or inhalation) as an interim estimate. EPA recommends that, if considered in an assessment, the fractional contribution of oral, dermal and inhalation route exposures to the predicted TEQ be identified. Also, within each route of exposure, the fractional contribution of each congener to the predicted TEQ should be identified, as well as the fraction of the TEQ associated with each chemical class.

B.5. I was quite disappointed with EPA’s proposed approach to dealing with uncertainty/variability, but, at least in my view, the authoritative bodies that have developed the TEF values have also missed the boat on this. They just don’t seem to understand that the uncertainty/variability of individual RePs needs to be characterized adequately before the uncertainty/variability of any linear combination of RePs, such as a TEF, can be characterized adequately. Sadly, this aspect of the problem has been ignored by just about everyone.

EPA Response: In an ideal situation, a full quantitative uncertainty analysis would be provided, however, at present, the U.S. EPA is aware of limitations in both the underlying ReP database and in the ability to statistically analyze it that preclude a detailed evaluation of the various sources of heterogeneity inherent in a quantitative analysis of uncertainty. Therefore, the document now states this fact and suggests the conduct of a TEF sensitivity analysis.

B.6. TEFs remind me of an amusing story. I trained academically at the University of Wisconsin-Madison as a theoretical nuclear physicist, and we physicists, especially the theoretical ones, had a tendency to look down our erudite noses at engineers, who saw fit to get their hands dirty in the real macroscopic world. We had a saying about them: engineers work really hard at finding the best way to do things that probably shouldn’t be done at all. That’s pretty much the way I feel about TEFs. The feeling originates in my deep conviction that a concept as clean and simple as dose additivity, that ignores the realities of competitive antagonism, partial agonism, and the profound differences in efficacy of the various AhR ligands for inducing such a wide spectrum of toxic endpoints, cannot possibly, by any stretch of the imagination, be true. So why should we base a process as important and critical as risk assessment on a false foundation? I don’t have a good answer to that question.

EPA Response: EPA disagrees with this comment. After evaluating the empirical data on TCDD and some DLCs, WHO reconfirmed that the combined effects of these compounds

generally are consistent with dose additivity (Van den Berg et al., 2006). This is consistent with EPA's understanding of the literature as well. However, EPA also recognizes the use of TEFs as an interim method until improved data and risk assessment methods are available. To this end, in the document, EPA states that "the TEF methodology is most commonly applied to situations where exposures are to mixtures of DLCs, and the assessment goals include the analysis of human health risks posed by the mixture. When appropriate dose-response information is available on IRIS, other approaches may be considered for exposures to single compounds or chemical classes."

B.7. In essence the guidance this document provides is:

- 1. EPA recommends to use the WHO-2005 TEFs**
- 2. Calculate a single value TEQ for your matrix**
- 3. Apply it to oral, pulmonary and dermal routes and note how much comes from each,**
- 4. For abiotic matrices consider bioavailability fate and transport**
- 5. Note how much TEQ is due to TCDD, PCB126, 23478PeCDF , 12378PCDD and 123678HxCDD**
- 6. Consider a sensitivity analysis.**

Given the topic, this is a short document that succinctly describes the derivation of the WHO TEFs and justifies their use for the cumulative risk assessment for dioxin-like compounds. While this reviewer is in support of the EPAs recommendation to use the WHO TEFs and the TEF scheme in general, given that it is meant to be "guidance" and read as a "stand-alone" document, I felt it was too short and did not provide sufficient specific guidance for a lot of the recommendations and especially on issues regarding uncertainties in the TEF scheme and its use, that risk managers may need. I fear that many folks may be left scratching their heads about exactly what to do beyond simply calculating a TEQ, and that subsequent analyses and risk management decisions may vary considerably in how different issue are approached.

EPA Response: This document does not deal with implementation issues. However, information on the conduct of a sensitivity analysis has been added.

B.8. In addition, supporting information is not readily available or presented. It repeatedly references the 2003 draft assessment (which EPA note should not be quoted or cited) and publications that may be inaccessible to many readers.

EPA Response: To aid the reader in finding information on TEFs, EPA has added the link to the 2003 Reassessment in the reference section of this TEF document. In the body of the document there is also a link to the WHO website where TEF can be located. Further, the Van den Berg et al. (1998, 2006) and Haws et al. (2006) papers can be located using PUBMED.

B.9. The description of the Haws and Van den Berg papers I found to be somewhat superficial and did not fully capture much if the nuance of the either the ReP database, or how it was used in the derivation of the TEFs.

EPA Response: The text of the document was revised to more carefully describe the process that the WHO undertook to derive TEF values using the Haws database.

B.10. In addition I found it confusing whether this document was trying to provide guidance on which TEFs to use, how to calculate a TEQ using the TEFs, or application of the TEQ in calculating risks and ranges of risks, interpretation/communication of the TEQ based risks and its uncertainty in the context of an overall site-specific risk assessment.

EPA Response: EPA believes that the document accurately shows the reader which TEFs to use and how to calculate a TEQ. In addition, equations were added regarding the calculation of lower and upper bounds on the TEQ based on the Haws et al. (2006) ReP database. The calculation of risks or interpretation/communication of risks are considered to be outside of the scope of this document.

B.11. This reviewer had to get clarification that this is meant to be a stand alone document and not either as a replacement or addendum to chapter 9 of EPA's draft dioxin health assessment. As such this could lead to confusion.

Also if this is meant to be read as a stand alone document, it requires significantly more detail to provide the scientific basis for many of the assumptions used in TEF scheme (that are already in chapter 9 but need updating in several areas).

EPA Response: This is meant to be a stand alone document. Additional text from chapter 9 of EPA's 2003 Dioxin Reassessment was added to clarify some points related to the assumptions used in the TEF scheme.

B.12. The issue of uncertainty and how it should be handled is generally confusing as it seems to be only addressed by assessing the sensitivity. I.e. how much of the TEQ is due to lung/dermal vs. oral. This is not an uncertainty analyses to my mind. The WHO panel recognized that there is uncertainty around the TEF value but no guidance on this provided in this document in any way. The NAS review also noted that the uncertainty needs to be addressed.

EPA Response: The U.S. EPA is aware of limitations in both the underlying ReP database and in the ability to statistically analyze it that preclude a detailed evaluation of the various sources of heterogeneity inherent in a quantitative analysis of uncertainty. Therefore, the document now states this fact and suggests the conduct of a TEF sensitivity analysis.

B.13. p 1, lines 8-9: The phrase starting with "Because" is too strong. See my comments on Charge Question 1.

EPA Response: This recommendation has been incorporated into the text of the document.

B.14. p 2, lines 7-9: The word "similar" is much too vague. Dose addition implies identical kinetics, dynamics, and dose-response curves up to dose-scaling constants, namely, the

TEFs. See my comments on Charge Question 1.

EPA Response: EPA disagrees with this comment and has not made this change. The current wording using “similar” is consistent with EPA’s Supplementary Guidance on Health Risk Assessment of Chemical Mixtures (U.S. EPA, 2000).

B.15. p 3, lines 8-10: I think I know what this sentence is getting at, but it is a clumsy construction. How does one compare “this sum” to “the dose-response function for TCDD”?

EPA Response: This recommendation has been incorporated into the text of the document.

p 4, lines 7-8: “similarities between interspecies metabolism”? What is interspecies metabolism? Actually, the assumptions included essentially identical metabolism across species, because any material differences would throw off (contradict) the dose addition assumption across species.

EPA Response: This recommendation has been changed to read, “similarities between interspecies kinetics and potency.”

B.16. p 4, lines 14-19: It would be useful to distinguish more clearly between “scientific consensus” and “consensus judgment of expert panels”. I see the former as far more inclusive, important, and difficult to achieve than the latter.

EPA Response: This specific text was not changed. However, additional details were added to the document to improve the description of the WHO consensus process.

B.17. p 5, lines 20-21: I realize this is a quote from the NAS report, but why call it to the reader’s attention. I for one, am embarrassed by it. What the heck does “valid, at least in the context of risk assessment” mean? Is it like “good enough for government work”? You can’t prove additivity no matter how hard you try. See my comments on Charge Question 1.

EPA Response: EPA extended this quote to clarify NAS’s meaning. It now reads, “from an overall perspective, this assumption appears valid, at least in the context of risk assessment. Additivity in biochemical and toxic responses by the indicated DLCs has been supported by numerous controlled mixture studies *in vitro* and *in vivo* and is scientifically justifiable” (NAS, 2006, p. 80).

B.18. p 6, lines 15-17: I find it ironic that on the few occasions where data were available that shed light on the uncertainty/variability of specific RePs, that information was purposefully excluded from the Haws et al. (2006) analysis. This is exactly the wrong thing to do! See my comments on Charge Questions 4 and 5.

EPA Response: The EPA document depicts the Haws et al. (2006) process as it was

published, therefore, no change has been made in response to this comment.

B.19. p 7, lines 1-2, 9-10, 11-12, and 13: Same problem as immediately above. This information is precious, as it informs us about ReP-specific uncertainty/variability. Yet it was purposefully excluded. How tragic!

EPA Response: The EPA document depicts the Haws et al. (2006) process as it was published, therefore, no change has been made in response to this comment.

B.20. p 7, lines 20-21: There is nothing statistical about the distributions of the RePs for each DLC. They are simply cumulative frequency distributions. Replace “statistical” with “cumulative frequency”.

EPA Response: EPA concurs with this comment and has made appropriate wording changes in the document.

B.21. p 7, line 27 - p 8, lines 1-2: I think it would be clearer to say that the expert panel looked at all the included RePs and then decided on a TEF value by “expert judgment”. They just didn’t choose a consistent percentile, say the 50th percentile, of the cumulative frequency distributions of the included RePs. Too bad that the process they did use is not transparent, objective, or reproducible.

EPA Response: Text has been added to the document to clarify this process.

B.22. p 9, lines 14-15: First, the phrase “all cancer” needs clarification. Second, I disagree with this recommendation See my comments on Charge Questions 6 and 10.

EPA Response: EPA has added clarifying text regarding the Ah receptor’s role in producing toxic effects. EPA disagrees with changing this recommendation.

B.23. p 9, lines 15-18: I think EPA should already be working on endpoint-specific TEFs and/or separate TEFs for systemic toxicity and carcinogenicity endpoints. This is easy to get started. A good first step would involve stratifying the existing RePs data base by endpoint and seeing what’s there and what’s not. Then a research plan to get the needed data could be formulated.

EPA Response: The EPA agrees with this comment, but has not made any changes in the document based on this recommendation.

B.24. p 13, lines 16-17: The phrase “ReP variability that appears to be small” is unclear. Is this meant to reflect differences in the ReP values for a single endpoint-single DLC, ReP differences across endpoints for a single DLC, ReP differences for a single endpoint across DLCs, or what? Perhaps the inclusion of a relevant figure from Haws et al. (2006) would be helpful in making this point more readily apparent to the reader.

EPA Response: This discussion was meant to be a risk characterization example and has been moved to a text box. To reflect ReP variability, Tables 2 and 3 were added to the document to give percentiles of the Haws et al. (2006) ReP database for use in sensitivity analyses.

B.25. p 14, lines 3-5: It would be useful to be more specific about what EPA has in mind when it uses the phrase “sensitivity analysis” here.

EPA Response: This recommendation has been incorporated into the document. EPA also added additional equations with supporting tabular information to clarify the phrase sensitivity analysis.

B.26. p 14, lines 9-11: The ReP ranges developed by Haws are deficient as a starting point for a sensitivity analysis. Without information on the uncertainty/variability of individual RePs, we have no natural scale on which to measure interReP differences. Determining standard errors and or 95% confidence intervals for individual RePs is the most appropriate starting point. See my comments on Charge Questions 4 and 5.

EPA Response: To reflect ReP variability, Tables 2 and 3 were added to the document to give percentiles of the Haws et al. (2006) ReP database for use in sensitivity analyses.

B.27. p 14, line 17: I would strike the word “more” from the phrase “more consistent”.

EPA Response: This recommendation has been incorporated into the text of the document.

B.28. p 15, lines 1-2: Replace “Despite these challenges” with “Nevertheless”. Replace “recognizes” with “believes”.

EPA Response: This recommendation has been incorporated into the text of the document.

B.29. p 16, lines 6-7: “new consensus processes” are mentioned. Are there specific plans? If so, how will they differ from previous consensus processes?

EPA Response: EPA is not aware of any current plans to revisit the TEFs. This recommendation has not been incorporated.

B.30. The TEFs that EPA has proposed to utilize for risk assessment purposes are all based on in vivo data collected in mammalian species. I originally thought this was a good idea, because previous versions of the TEFs relied heavily on in vitro data, particularly for enzyme induction, and these data are best viewed only as biomarkers for DLC exposure, and not as biomarkers for toxic responses. They are about as far removed from toxicity and carcinogenicity as it is possible to be.

However, I was very impressed with the very recent work that was presented by Dr. Jay Silkworth in the public comment period, which showed how markedly different human cells are from rodent cells in their in vitro gene array responses to some DLCs. This

information should be included in the section on uncertainty because it suggests strongly that humans are not only much less, but also differently (in a qualitative way) responsive, than are rodents to DLCs. In fact, I would go so far as to say that such data, when available, should be employed in place of the TEFs, because they shed light on the very important and still unanswered question of why humans appear to be so refractory to DLC exposure in comparison to the hypersensitive rodent species.

EPA Response: EPA agrees *in vitro* data provide important information and believes they may be useful in the development of ranges for sensitivity analyses. Both *in vivo* and *in vitro* data are included in the TEF ranges shown in Table 3 of the revised document.

B.31. A general comment is that the document notes TEF scheme should evolve as time goes on to incorporate new data. My question is does EPA envision a point of diminishing return? I.e. is there a point where the impact of new data will be negligible relative to the already known uncertainty, and what new data would be required to reduce uncertainty. It would be good if specific research needs that would address specific deficiencies were articulated. E.g. there is a lot of effort developing human *in vitro* RePs for some of the DLCs using primary human cells. Under the current scheme these *in vitro* RePs would be given less weight than rodent *in vivo* data.

Another concern is how the next “reevaluation” will happen, if at all. My concern is how and when and the process to do this. The desire to “reevaluate periodically” seems to be more of a pragmatic decision that is often driven by specific researchers in the field than it is an agency policy driven to ensure that schemes used are as up to date as can be. There has been much new data since 2005 with no clear idea of how that could impact the EPA TEFs unless WHO decided to do another reevaluation, which based on the 4-yr time cycle is already overdue.

While it is unrealistic that at this time the TEF scheme can be “real-time” an outline for the future refinement of the scheme such that it can be responsive to new data would be a valuable addition.

EPA Response: EPA agrees that the TEFs will likely evolve over time. EPA felt that development of a future refinement scheme was outside the scope of this document.

B.32. Note- throughout you cannot cite the EPA draft assessment (that says do not quote or cite!) - cite primary literature.

EPA Response: EPA agrees and has cited primary literature wherever possible. However, the 2003 Reassessment external review draft contains basic information on the TEFs that is valuable for the reader to know. EPA has continued to cite the 2003 Reassessment in certain parts of the current document.

B.33. Page v- ED50 definition is incorrect- It is the 50% of the maximal response above background.

TEQ is Toxic equivalents not equivalence.

EPA Response: EPA prefers to use a more generic definition for the ED50, but has added the reviewer's definition as an example. EPA prefers to use the term, "toxicity equivalence" when referring to either the TEF or TEQ; EPA has found that this varies significantly across publications on dioxin.

**B.34. Page vii
Define "dioxin"**

EPA Response: "Dioxin" as a stand alone term has been deleted throughout the document and "TCDD" is used in order to eliminate confusion. The title of the document has been changed to now read, *Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds*.

B.35. "Index chemical" needs better clarity- it is the one whose dose response function is used for the estimation of risk. Note PCB 126 was used as an index chemical for derivation of specific RePs

EPA Response: EPA agrees. The text referring to PCB 126 has been revised.

B.36. ReP-better definition needed: It's an estimate of relative potency for a specific endpoint, not study. Multiple RePs can be obtained from a single study. Also what is a general toxic equivalency value? Are you referring to TEFs? TEFs are not an average- In some cases RePs are derived from an average of ratios of dose at different effect levels- see papers by Van Birgelen et al... Note ReP has a different definition in the Van den Berg paper.

EPA Response: Relative Potency (ReP) is now defined as, "The ratio of the potency of a compound to the standard toxicant in that specific study; a concept similar to toxic equivalency but based on a single study, species, or matrix, etc., and not averaged to obtain a general toxic equivalency value."

B.37. TEFs are consensus estimates of potency relative to an index chemical- that are applied to different responses, some of which are "toxicity" endpoints

EPA Response: EPA agrees and has incorporated this recommendation into the text of the document.

B.38. TEQ- Toxic equivalents is the sum of the products ...

EPA Response: This recommendation has been incorporated into the text of the document and is shown in equation 1.

B.39. Page 1 line 9-This is written as a factual statement – it is not – it is a scientific conclusion based on various levels of evidence- provide supporting references

EPA Response: This recommendation has been incorporated into the text of the document.

B.40. Page 2 line 15- note PCB126 is also used as an index chemical for lots of PCB studies.

EPA Response: This recommendation has been incorporated into the text of the document; EPA states that the WHO panel used PCB₁₂₆ as the index chemical for some compounds in some studies used to develop RePs (Haws et al., 2006).

B.41. Page 2 line 21- not all endpoints are “toxicity” endpoints

EPA Response: EPA clarifies in the text that the toxicological data considered for these comparisons of toxic potency are from both *in vitro* and *in vivo* studies as well as structure-activity relationships and are based on the following classes of measure: biochemical changes, toxicity and carcinogenicity.

B.42. Page 3- line 1- delete “toxicological”-

EPA Response: This recommendation has not been incorporated.

B.43. Page 3- by definition PCB₁₂₆ is 0.1 really, since it is used to derive a TCDD ReP under the assumption that its REP is 0.1.

EPA Response: This recommendation has been incorporated into the text of the document.

B.44. Page 3 line 8 and line 16; poorly written. The TEQ is used as the dose metric in the dose response function for TCDD. It is not “compared” to it.

EPA Response: This recommendation has been incorporated and the text of the document.

B.45. Page 4 line 3- TEFs goes back to 1984- need better history here.

EPA Response: This recommendation has not been incorporated. EPA attempted to describe succinctly the history of TEFs, referring to some of the primary texts in the field for readers seeking a more comprehensive discussion of their history.

B.46. Page 4 line 12- replace considered vs. provided

EPA Response: This recommendation has not been incorporated.

B.47. Page 9- line7- not sure what this means?

EPA Response: This recommendation has been incorporated by clarifying the text of the document.

B.48. Page 9 line 14- does this mean that it has to have been shown that the effect is mediated via the AhR?

EPA Response: This recommendation has been incorporated by clarifying the text of the document.

C. Responses to Charge Questions

Charge Question 1: Please comment on whether the TEF methodology is accurately explained and referenced in the document.

C.1.1. The explanation of the TEF approach in this guidance document is explained perfectly and referenced adequately. I do think the referencing would be improved by directing readers who need more information to specific references for specific purposes. To some extent, the guidance is written for readers who are more expert than novice and I think additional information for non-experts in dioxin will enhance the use of the guidance.

EPA Response: EPA has added additional text clarifying the WHO (2005) TEF process, but has not added additional history on TEFs or details for novice users to the document. However, the TEF values and method are appropriately referenced.

C.1.2. Criteria for using TEF approach include a well defined group of chemicals, a good database, and consistency across endpoints, with a well understood common mechanism or mode of action of toxicity. However, all of these criteria cannot be always met. Even if we could collect enough information to derive TEFs for each of these chemicals (or congeners) the final behavior of all congeners in a particular mixture cannot be certainly predicted. Given this general understanding, the approach has been summarized well and previously published studies have been included for further reading.

EPA Response: None.

C.1.3. My understanding is that the recommendations in these documents are used by EPA's technical staff to conduct preliminary risk assessments of mixtures of chemicals and to provide guidance to representatives of risk assessment community who seek EPA's help.

I believe approaches such as TEFs are needed to advance mixtures risk assessment methodologies because whole mixtures data are rarely available. Thus, often the risk assessors have no other option but to use single chemical data and this is done employing potency weighted dose or response addition. The biggest hurdle to this approach is the potential for interaction among chemical components of a mixture. But there is ample documentation in the literature that shows interactions occur at high doses, and do not play a significant role at environmental levels.

EPA Response: The text was not changed in the document since the document clearly states that the TEF approach does not account for interactions among the DLCs, nor between DLCs and other chemicals to which individuals may be exposed.

C.1.4. The description of TEF methodology is generally accurate, well-written, and easy to follow. There are some statements that, at least in my view, are not accurate. On p. 1, lines 8-9, a sentence starts: “Because the combined effects of these compounds have been found to be dose additive, ...” I see this as “wishful thinking” on EPA’s part. Toyoshiba et al. (2004) found numerous statistically significant departures from additivity for CYP1A1 and CYP1A2 activity in rats exposed to a mixture of three DLCs in a very well-designed, well-conducted study. There are numerous other demonstrations of synergy and antagonism of DLCs in combination in the literature. A number of these are cited in Van den Berg et al. (2006). It must also be kept in mind that studies that have failed to find significant departures from additivity may have been very weak, i.e., they may have had little statistical power to detect such departures even if they were present. Remember that an absence of evidence is not evidence of absence.

In 2005, EPA more appropriately characterized additivity as an *assumption*, one of many that underlie the TEF methodology. In truth, how could one ever prove additivity? Any scientific test has limited sensitivity (power), so the possibility always exists that non-additivity occurs but goes undetected. This is exactly same problem that threshold proponents face when they attempt to prove the existence of thresholds.

EPA Response: The document was edited to clarify that dose additivity is an assumption and that there are data in the scientific literature that support such an assumption. Also, the document addresses the point that after evaluating the empirical data on TCDD and some DLCs, WHO reconfirmed that the combined effects of these compounds generally are consistent with dose additivity, a key underlying assumption of the TEF methodology (Van den Berg et al., 2006).

C.1.5. On p. 2, lines 7-9, a sentence states: “Under dose addition, the toxicokinetics and 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.” The word *similar* has been overused in this context (TEFs) and is much too vague. The assumption of dose addition implies *identical* toxicokinetics and toxicodynamics and *identical* dose-response curves, after normalization of the doses by constant, compound-specific dose-scaling factors, i.e., the TEFs.

EPA Response: EPA feels that the term *similar* is a better descriptor when referring to both dose response curve and kinetic/dynamic comparisons across chemicals; this term is used in the EPA’s Supplemental Guidance for Health Risk Assessment of Chemical Mixtures (U.S. EPA, 2000).

C.1.6. In general, the document provides an adequate reflection of the procedures followed by the World Health Organization to derive consensus TEFs for humans and wild life. I appreciate the effort put in by the EPA to describe this process and suggest to accept the

WHO TEF values. Accepting this methodology and associated TEF values is of global importance with respect to the risk assessment of these compounds for humans and wildlife. Most of the remarks made in the document indeed reflect the development and limitations of the TEF/TEQ methodology. However, a number points in the document could be either more clear or provide more background info. In providing this, it will avoid unnecessary criticism from both industry as well as NGOs. A number of points that could be discussed or provided in more detail will be outlined below and in the follow up questions asked to this reviewer. General comments regarding the EPA TEF document:

In the introduction/background a short information could be given regarding the milestones achieved during the different WHO TEF evaluations. Chronologically these are:

- **Ahlborg 1994 – first global consensus TEFs, inclusion of PCBs including di-ortho congeners;**
- **van den Berg 1998 – use of database compiled by the Karolinska Institute, deletion of di-ortho PCBs from the concept, recognition that TEFs for fish and birds need to be differentiated from humans, and importance of in vivo above in vitro results;**
- **van den Berg 2006 –Extensive use of Haws (2006) database, incorporation of NTP results, stakeholders meeting at the beginning of evaluation, identification of significant shortcomings of the present TEF system and potential other compounds for inclusion**

EPA Response: These three references are included in the document.

C.1.7. Page 7 lines 24-2. Note that the 75th percentile was used a general cut off point to decide whether or not a TEF value had to be re-evaluated (See Van den Berg et al 2006). After this, expert judgment was used with priorities as mentioned in the EPA document.

EPA Response: Additional text has been added to the document to clarify this point.

C.1.8. Page 9 line 6 -9. I think that the critical remarks made in the last TEF evaluation in 2005 regarding the use of TEF values for abiotic matrices like sediment and soil are not sufficiently covered in the EPA document. This especially has consequences for contaminated soils and sediments in which no direct ingestion takes place. In reality there is no scientific rationale for using the TEF system for these situations, except for scaling different environment matrices without a toxicological significance but for prioritization for remedial actions. Direct oral ingestion of particles can be included in the TEF system, although bioavailability will play a significant downplaying role in the overall mixture toxicity (TEQs). I agree that for inhalation this system can also be used (e.g. combustion particles) as an interim approach. Dermal uptake from particles under dry conditions is to my opinion a marginal exposure pathway. It should not be considered using e.g. 100% bioavailability for the human skin and can represent a gross overestimation of systemic exposure and associated risk.

EPA Response: This document does not consider the implementation of the TEF methodology; rather it focuses on exposure routes. This discussion of abiotic matrices

was considered to be an implementation issue and, therefore, is outside of the scope of this document.

C.1.9. Page 13 lines 17-19. I think that AT LEAST these five compounds should be known. In addition, such a priority approach might not work from a risk assessment approach, if it concerns an accidental (food) poisoning from a specific source (food process) or for populations living around an environmental hotspot.

EPA Response: The discussion on identifying these five compounds for instances where the dietary pathway is important was meant to serve as an example of this element of risk characterization. To eliminate any confusion over this example, it has been included in a text box to separate it from the rest of the text.

C.1.10. Page 13 lines 21-21 and next page. I sincerely question the validity of the statement about using TCDD only here. Sufficient information regarding dose response relationships of 23478-PeCDF and PCB 126 is also known from many studies. Especially the recent NTP studies provide excellent reference material for this, including both neoplastic as well as non neoplastic effects.

EPA Response: Text was added to the document stating that PCB 126 has been used by WHO as the index chemical in some studies from which RePs have been developed and that, in such cases, it has a TEF of 0.1. To the best of the EPA's understanding, the other compounds identified by the reviewer have not been used as an index chemical in the TEF approach.

C.1.11. The basic TEF methodology is adequately described though wording could be improved.

It should be noted that not all assays for which RePs are developed are "toxic responses." In addition it should be noted that PCB126 was often used as an index chemical, under the assumption that it has a TEF of 0.1.

EPA Response: Text was added to the document stating that PCB 126 has been used as the index chemical in some studies from which RePs have been developed and that, in such cases, it has a TEF of 0.1.

<p>Charge Question 2: Is the history of the mammalian TEFs and the process used to develop them by the World Health Organization accurately described and in sufficient detail? Are the WHO (2005) mammalian TEF values and their derivation accurately reported?</p>
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C.2.1. The information that is in the guidance is perfectly accurate and clear regarding the mammalian TEFs and the WHO process and experience. This topic is one that I would recommend a bit more text, perhaps ½ - 1 page more. The authors and reviewers are more than familiar with all the details of the TCDD TEF concept, but I think more context is needed for non-toxicologists and those who are not steeped in dioxin and the TEF concept. The scientific community began developing the conceptual and practical basis for the TEF concept for some years before the first WHO list of TEFs was developed and published. This history is important and should be included to demonstrate the strong scientific foundation and long history of the TEF approach. WHO did not just create the TEF approach all of a sudden. There are still detractors from the TEF concept, despite the scientific consensus and international support.

The document should point the reader to specific references for more information. These references can include the EPA Dioxin Reassessment, the two volumes edited by Arnold Schecter (*Dioxins and Health*), and several papers in the peer-reviewed literature that include mode and mechanism of action.

EPA Response: EPA disagrees that a long and comprehensive history of the TEF development is appropriate in this document, which has been designed to be concise. The authors believe sufficient supporting information on the history of TEF development has been provided in the form of references, such as those from the WHO efforts (dating back to Ahborg et al., 1994) and from EPA's efforts (dating back to U.S. EPA, 1989).

C.2.2. I also recommend referring the reader to the literature on non-mammalian TEFs for two reasons. First, the application of TEFs to birds and fish provides further evidentiary support for the TEF method. Second, many TEF applications involve non-mammalian vertebrates in addition to mammals.

EPA Response: Reference to these TEFs is made in the introduction section of the document where it is stated that the WHO has used a process based on consensus judgment of scientific expert panels to develop TEFs for mammals, birds, and fish and has re-evaluated them on a schedule of approximately every 5 years (Ahlborg et al., 1994; Van den Berg et al., 1998, 2006).

C.2.3. Yes. Briefly but concisely the history has been captured in essence for mammalian TEFs derivation. Enough details are given for those who are familiar with such approaches. How much more can or should be added depends on its intended use. If a lot more information is added this will not remain a quick reference for teams of toxicologists, risk assessors and managers but will become another federal agency document.

Hopefully, this will be part of the overall dioxin risk assessment document that EPA has undertaken to complete by this year, December 2009 and this will be in perspective. EPA and the risk assessors need this methodology to meet its mandate otherwise by default, lack of data, so many mixtures cannot be evaluated without such methodologies. From a public health perspective, we need a practical method that can be used in the field to perform

mixtures risk assessment and to present the findings in community and public health meetings.

EPA Response: None.

C.2.4. The description of the history and process by which the group of WHO-sponsored experts developed the mammalian TEFs is accurate and about as detailed as it is possible to be. After all, the TEF final values were determined behind closed doors by a select panel of experts, including representatives from EPA. It would be useful and perhaps less self-serving to note in this document that there is not unanimous agreement among all scientists that the TEFs approach (and the process by which TEF values have been established) is an appropriate and scientifically validated way to assess potential health risks from exposures to mixtures. There is a “loyal opposition” who has called for more openness and transparency, objectivity, and sensitivity and attention to limitations, and it is noteworthy that they are not all dependent upon financial support from the various interested parties.

EPA Response: Additional text was added to the document to describe the WHO 2005 process as articulated in Van den Berg et al. (2006).

C.2.5. As a stand alone document this document does not fully describe the history of how we came to this point in the development of TEFs. Chapter 9 of the dioxin health assessment document is much more comprehensive and some of the background from that document ought to be included. Moreover it has a fuller description of the database used for the 1998 TEFs and as such provides better context of why there was a need for Haws et al to generate a new database. The current document focuses mainly on the recent 2005 reevaluation by WHO.

EPA Response: EPA disagrees that a long and comprehensive history of the TEF development is appropriate in this document, which has been designed to be concise. Language from the 2003 Reassessment was not added.

<p>Charge Question 3: Is the development of the Relative Potency (REP) database presented in Haws et al. (2006) accurately described and in sufficient detail? If not, please provide recommendations for enhancing this description.</p>
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C.3.1. The REP database development is well described and covered in the guidance, but somehow the publication by Haws et al. (2006) needs to be more closely associated. I retrieved Haws et al. (2006) when the latest WHO TEFs were published, and find the Haws et al. (2006) publication to be invaluable in effectively dealing with all the issues that arise over TEFs. There is really no way to incorporate Haws et al. (2006) into the Guidance.

EPA Response: Additional text was added to the document to describe how the WHO 2005 process incorporated the Haws et al. (2006) ReP data into its deliberations, as articulated in Van den Berg et al. (2006).

C.3.2. Yes, the relative potency factor (RPF) approach has been well described in this document by technical staff. Also, please see the comments to question 2.

TEF and RPF values have been proposed for a few of the thousands of chemicals in commerce that have been studied fairly well. For a vast majority of chemicals there are very limited data and no time or financial resources available for traditional testing. Hence, recently both alternative in vitro testing methods and computational toxicology methods are being employed. The NAS has recommended more drastic changes to toxicity testing in the 21st century. As risk assessors and toxicologists, we have to move forward with the development of guidance of using these methods (TEF, RPF) that have been proposed and used for specific scenarios for about two decades.

Several uncertainty factors are employed in the risk assessment and risk characterization process that the estimates are kept with a window referred to as “order of magnitude.” Within this document it could be clarified that RPFs can be used for initial screening of mixtures for the purpose of prioritization. Eventually a more thoughtful consensus approach that engages the interested parties (the polluter, the concerned public, the regulators and the public health officials) right from the initial scoping process can help resolve such problems.

EPA Response: This document does not consider the implementation of the TEF methodology. Application of the TEFs as a screening approach is an implementation issue that is outside of the scope of this document.

C.3.3. I have a recommendation relating to gaining access to the underlying data. The Haws et al. (2006) publication has two data file supplements that are available in pdf format, which is an awkward format for most people to work with beyond simple viewing and printing. I think it would be extremely useful to create Excel spreadsheets of the data in these files and make them available to all interested parties via download from the EPA website.

EPA Response: Since the Haws et al. (2006) paper includes supplemental materials containing information on the ReP database that are available to the public, the authors did not find it necessary to recreate these files. However, Tables 2 and 3 were added to the document to give percentiles of the Haws et al. (2006) ReP for use in sensitivity analyses.

C.3.4. Yes. However, I miss a more thoughtful point of view by the EPA about e.g. using the distribution range for setting TEF values. From a risk management and political point of view different arguments could be given for using e.g. a specific percentile. The major drawback of this approach is that different countries maybe going to use different cut off points and the consensus aspect of the present WHO TEF approach is lost. In addition, such an approach might also cause economical problems as the estimated toxicity in TEQs like with food products could then vary between countries with associated import and export problems.

EPA Response: See response to C.3.3 above.

C.3.5. This document provides a relatively short summary of the Haws et al paper but omits lots of detail and nuance and does not bring forward from that paper many of the distribution information that is critical to understanding the ReP variability that underlies the uncertainty in the TEF derivation. Given that some readers may not have ready access to the journal article.

This document consistently refers to “exclusion criteria” which is not quite correct. Criteria on what type of info was needed were established, and then each ReP evaluated vs. those criteria. Also it is important to note that this was for the ReP distributions only. During the WHO deliberations, all ReP data was available (even that that did not meet the criteria) for use by the WHO expert panel.

EPA Response: The EPA authors’ reading of the Haws et al. (2006) paper is that “exclusion criteria” is a correct interpretation.

C.3.6. Description of the process used by WHO panel for the TEFs is also not well described. There is much more detail in the Van den Berg paper that is not fully captured in the document, especially use of the 75%ile as key point on the distributions. This is important since REPs were evaluated relative to the ReP distributions and chosen to be on the conservative side vs. a central tendency. This is noted in the Van den Berg paper. In addition it would not be apparent that prior to the WHO meeting was an open public meeting that had presentations on issues for the expert panel, including new data on human specific in vitro RePs.

EPA Response: The text in the document was revised to clarify this process.

C.3.7. The issue that the TEF is an “order of magnitude” estimate is not fully discussed either. This is a key point that has been lost in the whole TEF discussion and should be part any uncertainty analysis. Essentially it means that if this order of magnitude of uncertainty were applied to each DLC, in a mixture that has no TCDD, the TEQ could vary +/- half log. The uncertainty associated with the output from TCDD dose response functions is well appreciated, it is probably less well appreciated, and not articulated in this document that this means that the input dose (based on the TEQ) should be considered as range and not a single value. Also, it should be noted that while not weighted, in vivo and in vitro RePs were handled differently and distributions were separately presented in the Haws et al paper

EPA Response: Text and Tables 2 and 3 were added on sensitivity analysis which now clearly addresses these points.

C.3.8. A major omission is any guidance about TEFs for mono-ortho PCBs and use of TEQs derived from them. These were a major discussion point in the Van den Berg paper especially concerning possible contaminants, the high uncertainty of the TEFs, the wide

range of the ReP distributions. Moreover since these have “mixed” activity there are possible effects that may not be AhR dependent.

EPA Response: This issue is addressed in Van den Berg et al. (2006), so EPA does not need to repeat this information in this document. The range of the underlying ReP statistics has now been included in Tables 2 and 3 for use in sensitivity analyses.

C.3.9. In addition there is no guidance issue of interactions of non-dioxin like PCBs and dioxin-like PCBs. Chapter 9 from the dioxin reassessment discusses this in detail. While this has no impact on the TEF for a DLC (since a TEF is derived based on studies of individual congeners) some guidance on the interpretation of what the TEQ is for a mixture that contains both DLCs and interacting compounds for which there is known co-exposure, needs to be included.

EPA Response: EPA does not feel a need to address this in detail in the document but does acknowledge in the text that the TEF methodology does not account for the interactions of TCDD and DLCs with each other or with other chemicals to which individuals are exposed.

C.3.10. As an alternative EPA simply needs to state that it is making certain assumptions in the application of the TEF method to mixtures that cannot be fully addressed due to incomplete scientific knowledge. Eg A policy decision that while interactions have been observed, application of the TEF method and interpretation of the TEQ based risk calculation assumes that there are no interactions with non-DLCs, either positive or negative.

EPA Response: See response to C.3.9 above.

Charge Question 4: Is the uncertainty analysis approach described by EPA reasonable?

C.4.1. This question asks about the “uncertainty analysis approach” which would seem to be the paragraphs on page 14. The guidance presents a reasonable perspective on uncertainty analysis, but not ideal. The text does not present a roadmap of how to consider or evaluate uncertainty for the reader who is faced with data from an effluent or a contaminate site. The document would be improved if a subsection were labeled “Uncertainty Analysis” and expand some (1 page) on the present text. The guidance also needs to make a clear written distinction between uncertainty and sensitivity analysis. The uncertainty approach that seems to be presented is to list the individual congeners/compounds (DLCs), identify the major contributors to total toxicity, and repeat much of the same material that is contained in Haws et al. (2006). I do not read a step by step sequence that should/might be conducted in an uncertainty analysis, and the guidance would be greatly improved with such text.

EPA Response: Text has been added to the document on how one might conduct a sensitivity analysis (including some available statistical information on ReP data in

Tables 2 and 3) and that data are not available to conduct a full quantitative uncertainty analysis.

C.4.2. Uncertainty analysis also needs to address the underestimate of toxicity if the possible DLCs listed in the Guidance are not included and these compounds do, in fact, add to the total toxicity.

EPA Response: It is not clear to the EPA at this time how one might accurately quantify this uncertainty in the risk estimate; thus, EPA has not changed the recommendation that risk associated these compounds based on their possible dioxin-like activity be addressed qualitatively at this time.

C.4.3. As a toxicologist I see the need for a practical method for use in the field. The values derived have to be conceptually understood and derived based on good data to be used by risk assessors and practitioners.

The uncertainty analysis and the recommended approach lack clarity for the casual reader. This section should include a good discussion regarding uncertainties related to selection of chemicals in this approach, dose response of individual chemicals, derivation of recommended values, use of animal data to predict human cancer, assumption of common mode of action, and dose additivity for mixtures risk assessment.

EPA Response: EPA has addressed several of these topics in the document, including the assumption of dose additivity, the derivation of the TEFs and the ranges of the TEFs for use in sensitivity analyses. Subjects such as use of animal data to predict human cancer and dose response of individual chemicals are not germane to only TEFs, but are relevant to single chemical risk assessment as well; thus, special treatment of these subjects here is not warranted.

C.4.4. No, it is not. When measurements are compared or contrasted in science, the uncertainty/variability of those measurements is an essential component of the evaluation. A proper uncertainty analysis requires that the uncertainty/variability inherent in the individual RePs which underlie each TEF be identified explicitly. At a minimum, every ReP should have a standard error of estimate or an associated 95% confidence interval. Without such information, it is impossible to test rigorously whether two or more RePs are significantly different from one another.

It is astonishing to me that none of the authoritative bodies who have developed the various sets of TEFs has explicitly considered this uncertainty/variability in their development processes. I can't find a single ReP standard error (or 95% confidence interval) in Haws et al. (2006), van den Berg et al.(1998), or Ahlborg et al. (1993), and I don't understand why this is so. Apparently, these authoritative bodies just don't get it (not that I and others haven't told them!). The individual RePs have instead been treated as if they have no inherent uncertainty/variability at all.

How then can one judge whether a difference between RePs, whether it's $\pm 10\%$ or 100-

fold, is too large to be consistent with the underlying null hypothesis that they come from a common distribution whose mean is the true relative potency that a TEF is supposed to reflect? One cannot, so the experts have simply assumed that they do. Are we to just blindly trust that they have somehow got it right in spite of this complete neglect of a critical component of the scientific method? I think not.

There are many reasons to suspect a priori that RePs would be qualitatively different from one another: different endpoints, different species, different strains, different doses, different investigators, different experimental protocols, and different data analysis methods. These are just some of the sources of potential heterogeneity among RePs that need to be assessed objectively, quantitatively, and reproducibly, before RePs can be combined legitimately somehow into a TEF. The numerous pitfalls of meta-analytic evaluations of multiple data sets are very well-known outside of toxicology. Scientists tread very, very carefully even when conducting meta-analyses of well-controlled, randomized clinical trials. Careful attention must be paid to these pitfalls, and so far, this has not been done. If it isn't done before TEFs are incorporated into regulatory decisions that end up costing interested parties lots of money, I foresee a very profitable open-season on those regulatory decisions for defense litigators.

EPA Response: EPA recognizes that there are many issues with using the Haws et al., (2006) data in quantitative uncertainty analysis. Haws et al. (2006) discuss limitations that are also included in this document. Such limitations include the calculation of the RePs using various approaches, ranging from comparing dose-response curves to developing ratios of ED_{50s} to estimating values from graphs of dose-response data. The RePs also represent a wide variety of study types and endpoints, including biochemical changes, systemic toxicity and carcinogenicity. Some of these data may provide estimates that are more consistent than others with individual PCDD, PCDF, or PCB compound toxicity at higher levels of biological organization and such considerations will need to be included in a risk characterization. Finally, Haws et al. (2006) note a number of issues associated with the dose-response data (e.g., nonparallel dose-response curves, differences in maximal response among PCDD, PCDF, or PCB compounds within a study, incomplete dose-response data due to insufficient dose levels). Thus, in this document, EPA suggests using the ranges of the Haws et al. (2006) data in sensitivity analysis.

C.4.5. There is a need for better clarity and guidance in the document since it is not clear what uncertainty analysis approach is being recommended. A sensitivity analysis is recommended to see how much of the TEQ is driven by route and by the “Big Five” congeners.

Uncertainty could be inferred to refer in some cases to the “variability” in the original ReP calculation, the variance in the distribution of the RePs, uncertainty in the assigned TEF value, the uncertainty in resulting TEQ, uncertainty about relative contribution of route to the TEQ, uncertainty about proportion of some congeners to the TEQ, or uncertainty in the ultimate risk estimates.

As noted above better guidance on the application of the uncertainty is also needed- e.g. if the TEFs have uncertainty of +/- half log then is EPA giving guidance that risk assessors should use the TEF +/- half log in calculating the TEQ?

**E.g. upper bound of [TEQ] = [TCDD] + Sum([DLCi]*TEFi* 3.162)
Lower bound of [TEQ]= [TCDD] + Sum([DLCi]*TEFi/3.162)**

This is implied but not stated.

EPA Response: A quantitative approach for identifying upper and lower TEQ estimates has been added to the document (specifically, equations 2 and 3) in order to specify how to conduct this calculation. Additionally, data on the range of the TEFs is also provided in Tables 2 and 3.

C.4.6. Also the uncertainty associated with different classes of DLCs is not addressed at all yet was of clear concern in the Van den Berg paper. EPA gives guidance to note how much TEQ is from the “top Five” but not how much TEQ is driven by mono-ortho-PCBs which have a very wide ReP range.

EPA Response: EPA has attempted to provide additional clarity to the recommendations. The document was changed to recommend that the reader, 1) identify the fraction of the TEQ attributable to TCDD, each DLC, and to each chemical class, i.e., the PCDDs, PCDFs and dioxin-like PCBs, 2) for multi-route exposures, identify the fractional contributions of oral, dermal and inhalation route exposures to the predicted TEQ. Within each route of exposure, identify the fractional contribution of each congener to the predicted TEQ and identify the fraction of the TEQ associated with each chemical class, and 3) address the implications of the identified fractional contributions to the TEQ for the risk assessment being conducted, in particular, their impacts on the overall confidence in the analytic results. Also-the “top five” are now discussed in a text box to highlight that this is an example.

Charge Question 5: Are there alternative ways to approach uncertainty analysis for the TEFs that you could recommend?

C.5.1. One could calculate a range of values, using TEFs from Haws et al. (2006), in a bounding exercise. That said, uncertainty should not use a range of TEF values in a probabilistic analysis that applies a distribution to the TEFs. Haws et al. (2006) give ranges for the TEFs developed by WHO, but these ranges are not distributions. These ranges should be the basis for any range of TEF values used in a quantitative uncertainty analysis.

Haws et al. (2006), EPA (Dioxin Reassessment) and Schecter (Dioxins and Health) present some of the factors that affect the cascade of events in the Ah receptor mediated mode of action. A comprehensive uncertainty analysis could discuss/present all that is known of

these modifying factors for the specific site or application. This step is merely a more detailed version of what is presented in the guidance document.

EPA Response: EPA recognizes this concern, however, we are not recommending a comprehensive uncertainty analysis be conducted.

C.5.2. To do this right, the absence of significant heterogeneity must first be evaluated and confirmed by objective statistical testing. I suspect that a lot of RePs will fall by the wayside from just this one testing step. Furthermore, the power of the test(s) chosen for this purpose must also be characterized explicitly. Only then is it appropriate to consider combining those RePs that are determined not to be inhomogeneous to generate a TEF estimate. Only then is it possible to quantitatively assess the uncertainty/variability inherent in the resulting TEF estimate.

A TEF is just a weighted average of the associated RePs. Right now, however, the weights that the experts used in constructing the TEFs are unknown, subjective, and irreproducible. More complex probabilistic risk assessment approaches with great promise, such as those alluded to in Haws et al. (2006), are nevertheless hamstrung at the outset by these serious limitations. This will not change until a bottom-up approach is taken to constructing TEFs in which the uncertainty/variability of individual RePs is characterized explicitly and then propagated through whatever explicit and objective weighting schemes (one simple example is inverse variance weighting) are used in constructing TEFs.

EPA Response: EPA concurs with this idea and will consider this bottom-up approach to constructing TEFs once this methodology is available.

C.5.3. With the present state of art, No.

EPA Response: None.

C.5.4. There are several alternatives that have been proposed but these were not discussed. Use of weighted distributions were discussed in the Haws et al and Van den Berg papers but were rejected for the TEF derivation, but nonetheless could be explored. Alternatively classifying based on database strength could be considered. The Haws et al. paper lays out a lot of detail about the distributions, number of endpoints, ranges classified by endpoint etc. These could be used in a non quantitative way as a sensitivity analysis to see how much of the TEQ contributions are from congeners that have a weak dataset. EPA could also choose to give some classes of congeners a different level of uncertainty-e.g. the mono-orthos PCBs could be given a 2 orders of magnitude range of uncertainty- which would be supported by the note in the Van den Berg paper that the ReP range spans 4 orders of magnitude. Alternatively EPA could choose to classify TEQ by high (0.1 and above), med (0.001-<0.01 and low potency (<0.001) TEFs, such that TEQ contributions from weak congeners can be evaluated.

EPA Response: EPA concurs with these concepts and feels that these are valid ideas that need to be explored further. The discussion surrounding a sensitivity analysis has now been expanded and clarified in the document.

Charge Question 6. Please comment on the recommendation that these TEFs should be used for all cancer and non-cancer effects that are mediated through AHR binding by the DLCs.

C.6.1. The EPA is correct that the WHO TEFs should be used in assessing or estimating the effects of DLCs for cancer and non-cancer endpoints. The TEF approach is also valid for non-mammalian vertebrate systems, as described in the WHO recommendations (Van den Berg, 2006). The scientific literature supports the use of TEFs, based on experimental and ecological results at multiple levels of organization.

EPA Response: None.

C.6.2. EPA is correct in making these recommendations for those health effects that are mediated through AhR binding. However, some noncancer effects such as developmental effects are a major public health concern. For developmental toxicity, the window of exposure is small, well defined and there is no latency period associated as is with cancer. EPA should add caveats and provide insights in the guidance for specific conditions.

EPA Response: EPA recognizes the concern for developmental toxicity, however, guidance on specific endpoints is out of the scope of the current effort.

C.6.3. I'm not sure I understand this recommendation. The phrase "all cancer" is confusing. Does it mean any specific cancer, e.g., hepatocellular carcinoma or squamous cell carcinoma of the lung? Or does it mean any and all forms of cancer, as would be included in an estimate of "all cancer" mortality, i.e., death from any of all specific cancers?

EPA Response: The text of the document was modified to clarify this point. Specifically, the document states, "In this document EPA assumes that all cancer and noncancer effects of TCDD and DLCs are AhR dependent. The U.S. EPA recommends these TEFs be used for all cancer and noncancer effects unless they are known to not be mediated through AhR binding by DLCs. U.S. EPA recognizes that this issue will require further evaluation as additional toxicity data become available. Eventually, endpoint-specific TEFs or separate TEFs for systemic toxicity and carcinogenicity endpoints may need to be developed."

C.6.4. In either case, I have difficulty with the recommendation. For example, mammary cancer was significantly reduced by TCDD exposure in the original Kociba et al. (1978) study. Would the TEFs methodology be used to predict corresponding reductions in human or other mammalian mammary cancer from exposure to the other DLCs? Or would the liver cancer excess seen in the recent NTP study be used to predict an increase in human all cancer mortality, including mortality from breast cancer? This makes little sense to me. It

is worth noting that, at least to my knowledge, no specific form of cancer has yet been linked causally to human DLC exposures, and IARC raised this fact as a cautionary point in its 1997 monograph. IARC also characterized the polychlorinated dibenzo-*para*-dioxins other than TCDD as “not classifiable as to their carcinogenicity to humans (Group 3). In any event, I would want the separate risk contributions of TCDD, other dioxins and furans, and the PCBs to estimated overall cancer risks split out and identified explicitly in any risk assessment that made use of TEFs.

EPA Response: EPA concurs and has added recommendations to identify the contributions of these classes of compounds to the TEQ (see Table 4).

C.6.5. I agree with this approach (but not with linear risk assessment approach by itself for carcinogenicity of these compounds)

EPA Response: None.

C.6.6. EPA needs to more explicitly state the assumptions it accepts when using the TEF scheme, which ones are science based and which ones are “best interim judgment and policy” based. A lot of these are in the original chapter 9 but missing here.

EPA Response: EPA concurs and has added text to succinctly summarize these in the document.

Charge Question 7. Please comment on the recommendation that the TEFs are most appropriate for exposures to dioxin-like compounds via the oral exposure route.

C.7.1. I agree that the oral (water and food) exposure is most appropriate because that exposure route is where the most data are. The dermal and inhalation will most likely affect absorption and not the steps subsequent to internalization of a DLC.

EPA Response: None.

C.7.2. Agree with EPA since most often the exposure is by oral routes, at least at hazardous waste sites. The database for this route of exposure is quite extensive. At the present time this is the best available method that can be employed for such a group of chemicals.

EPA Response: None.

C.7.3. I agree with this statement. Van den Berg et al. (2006) provide a number of good reasons for this limitation on the risk extrapolations that should be made with the current TEFs.

EPA Response: None.

C.7.4. Justified given that most of the studies used for deriving RePs are based on in vivo studies oral routes

EPA Response: None.

Charge Question 8: Please comment on the recommendation that the TEFs may be applied to other exposure routes (i.e., dermal or inhalation) as an interim estimate.

C.8.1. This recommendation is warranted, based on the available data for experimental and ecological results for mammals. The dermal and inhalation routes of exposure do pose somewhat different conditions and some of these differences can be taken into account in a site specific assessment. The principle issue would be differences in absorption between oral, dermal and inhalation. But the basic toxicity level of a specific DLC should be unaffected by route of exposure once the DLC is in the body.

EPA Response: None.

C.8.2. Bioavailability and absorption are key issues that should be mentioned in the guidance if TEFs are being recommended for other routes of exposure. Aging in the environment changes the bioavailability of some of these chemicals and could be a source of uncertainty in their absorption.

EPA Response: EPA agrees that kinetics may vary for different exposure routes but has not added a detailed discussion of this to the document, but indicated that it should be considered.

C.8.3. Van den Berg et al. (2006) cite concerns regarding differential bioavailability and the very limited (“almost nonexistent”) data that is available from studies using environmental matrices contaminated with DLCs. They cautioned against the extrapolation of risk estimates, obtained using the current TEFs which are largely based on results from dietary intake studies, to non-dietary exposure routes. I agree wholeheartedly with their concerns. In my opinion, application of the current TEFs to non-dietary exposure routes is not scientifically supportable at the present time.

EPA Response: EPA agrees that dietary exposures are the most appropriate application of TEFs and therefore, recommends that TEFs be applied to other routes as an interim estimate.

C.8.4. Justified based on clear evidence of systemic responses after dermal exposure. Justified for pulmonary given expected high bioavailability from this route. Need to note very limited (if any?) data on clear pulmonary routes studies.

EPA Response: No changes were made in the document based on this comment,

Charge Question 9: Please comment on the recommendation that, if considered in an assessment, the fractional contribution of dermal and inhalation route exposures to the

predicted toxicity equivalence (TEQ) should be identified as part of the risk characterization.

C.9.1. The risk characterization should specifically address the oral and inhalation route of exposure contributions, as well as a range of other factors. EPA and NRC have an abundance of guidance on risk characterization, and there is no doubt that a complete risk characterization would include specific discussion of the dermal and inhalation route contributions.

EPA Response: EPA agrees with the need to add additional text regarding risk characterization. As a result, a summary of risk characterization recommendations has been added to the text of the document (Table 4, copied below).

TABLE 1

Summary of Risk Characterization Recommendations

- 1) Apply the TEF methodology to situations where exposures are to mixtures of DLCs, using TCDD as the index chemical, and the assessment goals include the analysis of human health risks posed by the mixture.
- 2) Identify the fraction of the TEQ attributable to TCDD, each DLC, and to each chemical class, i.e., the PCDDs, PCDFs and dioxin-like PCBs.
- 3) For multi-route exposures, identify the fractional contributions of oral, dermal and inhalation route exposures to the predicted TEQ. Within each route of exposure, identify the fractional contribution of each congener to the predicted TEQ and identify the fraction of the TEQ associated with each chemical class.
- 4) Address the implications of the identified fractional contributions to the TEQ for the risk assessment being conducted, in particular, their impacts on the overall confidence in the analytic results.
- 5) Include occurrence or exposure data, if available, for the following compounds:
 - PCB 37
 - Polybrominated dibenzo-*p*-dioxins and polybrominated dibenzofurans
 - Mixed halogenated dibenzo-*p*-dioxins and mixed halogenated dibenzofurans
 - Hexachlorobenzene
 - Polychlorinated naphthalenes and polybrominated naphthalenes
 - Polybrominated biphenyls
- 6) Consider conducting a sensitivity analysis to characterize the impact of TEF variability on the TEQ.
 - For the TEQ_U and TEQ_L estimates that are generated, identify the fraction of the TEQ attributable to TCDD, each DLC and each chemical class.
 - Identify the TEF_{*i*} values that are most influential to changing the TEQ estimate.

C.9.2. The more explicit the risk characterization the more confidence in the overall risk assessment, so it is a good idea where possible to apportion route specific contributions.

EPA Response: See response to C.9.1.

C.9.3. If this is to be done, and I recommend strongly against doing it, I would want to see the separate contributions from the different exposure routes split out explicitly. Also, as I mentioned in my response to question 6, the separate contributions to risk from TCDD, other dioxins and furans, and the dioxin-like PCBs should be split out and provided for each exposure route. Furthermore, the limitations and cautions noted by Van den Berg et al. (2006) against using what is essentially dietary exposure TEFs to do this should be stated explicitly.

EPA Response: See response to C.9.1.

C.9.4. This approach I can agree with, although the quantitative relevance is most likely very limited. From what we know from exposure analyses (where most of the dioxin money went to during the last decades) oral exposure via food is by far the most important source. In specific situations exposure other than via food might play some role. This could for example occur in children playing on contaminated soil (oral and dermal) and inhalation (malfunctioning municipal/chemical incinerator) by the neighboring population.

EPA Response: None.

C.9.5. Good idea -but also need to better to characterize contributions by classes of materials. This does not substitute for uncertainty analyses.

EPA Response: EPA agrees and has added a recommendation and text of the document to address this concern. See response to C.9.1.

Charge Question 10: Is there a currently available approach for calculating the cumulative exposures to DLCs that is more appropriate than the WHO TEF methodology being proposed by EPA?

C.10.1. No, not that has been scientifically supported and documented.

EPA Response: None.

C.10.2. The use of the TEF approach using additivity as default is the most practical approach available for risk assessment of mixtures of these chemicals. Physiologically based pharmacokinetic (PBPK) modeling is a possibility but development of such models is data dependent and technically challenging to apply consistently in the field.

EPA Response: None.

C.10.3. I suspect that estimated cancer risks will be the primary drivers in many site-specific risk assessments that involve DLCs. Usually, exposures at such sites are dominated by just a few DLCs, and these may turn out to be DLCs for which valid cancer bioassays have been conducted. 2,3,4,7,8-PeCDF is one specific example. In such cases, it makes little sense to me to “degrade” a DLC-specific cancer potency estimate that is derived from

directly relevant carcinogenicity data by substituting the corresponding TEF for that cancer potency. I recommend instead that EPA consider allowing (in fact, encouraging) the use of RePs obtained from more recent, high quality endpoint-specific data in place of TEFs in risk computations on a case by case basis.

EPA Response: It has been stated in the document that the Agency will update this guidance when new consensus TEFs are developed.

C.10.4. No. Theoretically, a methodology that includes a combination of congener specific exposure information, toxicokinetic tissue specific modeling and tissue specific biological/toxicological response analysis could do the job more accurately. In this case the additivity prerequisite has to be applied, but I think there is enough scientific evidence available for this as a default approach. However, in view of the relative large number of congeners involved, I question if there is sufficient scientific information regarding these aspects available.

EPA Response: None.

C.10.5. Other possible approaches are almost totally ignored: They are in chapter 9 but not here. Simply in terms of clarity those that are “inappropriate” should still be stated, e.g. inappropriate methods: TCDD only, sum all without addressing different potencies.

Other methods that may have equivalent levels of uncertainty and have not been discussed or explored:

Use of DLC specific dose-response functions where available and use of TEF for those where a specific function is unavailable. E.g. for cancer risk, in vivo rodent cancer bioassay data now exist for 4 of the top 5 TEQ contributors (TCDD, PeCDF, PCB126, HxCDD and also PCB118) and slope factors could be developed for each of these and cumulative risk for these calculated. For the remaining congeners, the TEF/TEQ scheme and TCDD dose response could be used.

This would reduce uncertainty for those congeners in a matrix. The TEQ based risk could also be calculated and applied to the dose response functions for TCDD and this would provide a measure of uncertainty.

One could group chemicals in classes and in some cases apply class specific TEF/TEQ- e.g. all mono ortho PCBs with a TEF of 0.00003 could be simply summed and then applied to use a PCB118 slope factor.

Group DLCs by their TEF potency and use dose response functions by “potency class” where available or use TCDD - express risk estimates by this type of class and uncertainty in the TEQ estimate relative to the classes.

Classify DLCs by database uncertainty relative to the number of endpoints- TEQ uncertainty

Use dose response functions where possible? Noted in chapter 9, but not addressed

EPA Response: EPA appreciates these ideas and agrees that such ideas need to be explored further. At this time the authors have not incorporated these concepts in the current document in order to keep the document focused and concise. However, EPA has added text to indicate that the TEF methodology is most applicable to situations where exposures are predominantly to mixtures of dioxins, furans and PCBs, and the goal of the assessment is to analyze the health risks posed by the mixture. Thus, other approaches may be considered when exposures are to single compounds or chemical classes.