Response to Comments in the External Peer Review Report on the *Draft Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment*

The purpose of this summary is to provide a disposition on EPA's response to major comments raised as part of the External Peer Review of the Agency's draft "Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment" (the Framework).

The peer review offered a number of general comments, as well as more narrowly focused and editorial comments from individual reviewers. Many of the comments from individual reviewers have been incorporated into the Framework and are not presented here. The peer review was conducted over several months from October 2003 to February 2004, culminating with a conference call meeting on December 5, 2004 and final report on February 9, 2004. External peer reviewers included:

- Dr. William J. Adams, Rio Tinto, Magna, UT
- Dr. Scott B. Brown, Environment Canada, Burlington, Ontario
- Dr. Peter L. deFur, Environmental Stewardship Concepts, Richmond, VA
- Dr. John P. Giesy, Michigan State University, East Lansing, MI
- Dr. Mark E. Hahn, Woods Hole Oceanographic Institute, Woods Hole, MA
- Dr. Barbara L. Harper, AESE Inc., West Richland, WV
- Dr. Bruce K. Hope, Oregon Department of Environmental Quality, Portland, OR
- Dr. Sean W. Kennedy, Environment Canada, Ottawa, Ontario
- Dr. Charles A. Menzie [Chair], Menzie-Cura & Associates, Inc., Winchester, MA
- Dr. Christopher D. Metcalfe, Trent University, Peterborough, Ontario
- Dr. Richard E. Peterson, University of Wisconsin, Madison, WI
- Dr. Martin Van den Berg, Utrecht University, Utrecht, Netherlands

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

A. Key issues identified in the reviews
B. Overview of general comments
C. Responses to charge questions

Overall Summary:

<u>Comment</u>: There was broad agreement that the document met its major goals and objectives. Therefore, our comments are intended to provide helpful feedback on how the Framework can be made more useful to the intended audiences. Often, this involves clarification. In some cases, our comments reflect areas where one or more of us disagree with technical statements made in the Framework document. These technical issues were discussed during our December 5th conference call. For the most part, the tenor of our comments is captured by a response (caveat) from John Giesy:

> ***In general, this document is very useful and a much needed improvement on previously available documents and guidance… there are many very positive aspects to the document, but to be concise, I will limit my comments to those where I think that the document can be improved. If I am silent on an issue or section of the document it indicates my concurrence with those conclusions or guidance.***

A. Key Issues Identified in the Reviews

A.1. The following issues were identified prior to our December 5th conference call and considered during the call. There was general agreement that these were the major issues with respect to our review.

- **Management-related considerations including how to judge the strengths and limitations (and costs) of the Toxicity Equivalence Methodology relative to other approaches**
- **Clarification of text and consistent use of terminology**
- **Approaches to estimating the bioaccumulation of chlorinated compounds in animal tissues**
- **More detailed information on dose response**
- **Quantification of uncertainties and possible use of probabilistic methods**

EPA Response: The key issues identified in section A are a subset of the more detailed comments in Sections B and C, and are therefore addressed in the response to the more specific comments.

B. Overview of General Comments

B.1. Provide a short section or perhaps only a few paragraphs in either an Executive Summary or in the Introduction that gives the reader a more complete view of the pluses and minuses of the method. This was considered particularly important for risk assessors and managers who are making decisions on how to proceed for particular sites. It would be useful to a greater range of practitioners, and in a more equitable fashion, if the document directly addressed both the benefits <u>and the costs (burdens)</u> of its key technical recommendations. No matter how "technical" a guidance document tries to be, its recommendations will have non-technical implications, such as driving increased costs onto regulated parties or greater

review burdens onto regulators. Hope, Harper, and Menzie provide further comments concerning this.

EPA Response: Discussion of the prerequisites, strengths, and limitations to be considered in applying the toxicity equivalence factor (TEF) methodology is provided in Sections 1, 2, 3.1, 3.1.1, and 3.2.2 of the Framework. Methodological considerations associated with using the TEF methodology are presented in Sections 3.1 and 3.2 to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations associated with using the TEF methodology against other methods they may be considering. The type and number of other approaches that could be considered will be specific to each ecological risk assessment, such that comparisons of costs and benefits is best conducted during the planning and problem formulation phases of the specific ecological risk assessment. Additional discussion in this regard has been added to the Framework. Specifically, Section 3.1 (*Considerations in Planning*) raises the issue that costs and benefits need to be considered during the planning phase of an ecological risk assessment. However, since costs and benefits will vary depending on the scope and objectives of a specific ecological risk assessment and will also vary over time, EPA does not believe it is appropriate to provide a specific comparative analysis within the Framework. As appropriate, EPA Offices and Regions may consider costs (monetary as well as other resource requirements) as they implement the Framework for their individual programs.

B.2. Provide an illustrative example(s) for the application of the method. This example(s) could be placed in an Appendix. Most reviewers felt that this would help people less familiar with the process to follow the methodology. The Group debated whether this example(s) should use real numbers or simply illustrate the process. There was a concern that the use of numbers would lead readers to view the numbers (e.g., for BSAFs) as the ones that would be used for other sites. The reviewers felt that any use of examples should be caveated to make sure the readers were aware that these were intended only for illustration. There was some discussion on using sensitivity analysis to show how alternative decisions can influence the outcomes of the assessment. Sensitivity analysis could also be used to help judge which parts of the assessment contribute the most uncertainty. This discussion led to either including an example or including some discussion of the value of sensitivity analysis (perhaps in the Uncertainty section).

EPA Response: EPA has integrated illustrative examples in the Framework within the sections discussing the individual steps in applying the TEF methodology. Application of the TEF methodology is illustrated through the examples provided in Tables 4, 5, and 6, which show how to calculate toxicity equivalence concentrations (TECs) in fish tissues, bird tissues, and a mammalian (otter) diet, respectively. A note has been added to Tables 4, 5, and 6 to make clear that the data are for illustrative purposes only and are not recommended default values. Application of the selection logic laid out in the relative potency matrix (Figure 11) is illustrated through the examples provided in Tables 7 and 8, which show how to array and select relative potency data for birds and mammals, respectively.

In lieu of providing another case study, the Framework references the Workshop Report on the Application of 2,3,7,8-TCDD Toxicity Equivalence Factors to Fish and Wildlife (U.S. EPA, 2001a) that includes case studies wherein the TEF methodology is applied to two different ecosystems. The workshop report is available on-line (http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=23763) and will be linked to from the same web site on which the final Framework is posted. In addition, references to a recent peer-reviewed publication (Burkhard et al., 2006) that describes the basis for and examples of extrapolating bioaccumulation factors (BAFs) and/or biota-sediment accumulation factors (BSAFs) across ecosystems have been added in appropriate places in the Framework.

B.3. The document makes various references to other methods for doing PCB risk assessment (specifically aroclors and homologues [totals]). In general, the document points out the advantages of the congener approach relative to these other approaches. However, the document also notes that there are ecological receptors and toxicological endpoints that cannot be addressed with the TEF/TEQ approach (e.g., bottom of p. 5 and top of p. 11). This leaves open a question on how to best approach sites contaminated by a broad spectrum of PCBs. The document should provide clarification on this so that risk assessors can have a better understanding of how to use the TEF/TEQ approach in concert with other approaches for assessing risks associated with PCBs.

EPA Response: Section 2.1 has been revised to clarify that the Framework applies only to dioxin-like chemicals, and hence, only dioxin-like polychlorinated biphenyls (PCBs). The non-dioxin-like PCB discussion highlights that these PCBs may cause toxicity via mechanisms independent of the aryl hydrocarbon receptor (AHR), and because the TEF methodology will only account for dioxin-like activity of PCBs, non-dioxin-like PCBs would need to be assessed using another approach/analysis, just like any other chemical of concern that may co-exist with the dioxin-like PCBs. The Framework is not a comprehensive guide to conducting a risk assessment for dioxin-like or non-dioxin-like chemicals; hence, to elaborate further on the appropriate analysis for addressing risks of non-dioxin-like chemicals is outside the scope of the Framework.

B.4. Does the TEF/TEQ methodology require measurement or estimation of all dioxin-like compounds including dioxins, furans, and PCBs in order for it to be valid? There are numerous investigations underway in which PCBs are being analyzed on a congener-specific basis but where analyses are not being carried out for dioxins and furans. This is fairly typical for a site where PCBs are considered the main issue. Inclusion of chlorinated dioxins and furans can be accommodated but at a significant additional analytical cost. The document should be clear on this matter one way or the other and should include some discussion of the limitations (i.e., uncertainties) of including only PCBs in the approach.

EPA Response: The TEF methodology is a tool that facilitates cumulative assessment of any “dioxin-like” chemical, i.e. any that acts via the AHR. The “validity” or robustness of a risk assessment could be influenced by whether all chemicals acting via the AHR

have been cumulatively accounted for and whether only AHR-agonists are accounted for in the TEC; the TEF methodology allows one to include any or all AHR-receptor agonists. The chemicals of concern in any ecological risk assessment are site or assessment specific. Therefore, the decision regarding what chemical(s) should be included in the analysis plan for an ecological risk assessment should be discussed and decided upon during the planning and problem formulation phases of the ecological risk assessment.

B.5. Figure 6 can be modified (or additional figures generated) to illustrate for the reader the specific characteristics of dose response curves for fish, birds and mammals. This would make it so much easier for the ecological risk assessor who is uninitiated in the use of the toxicity equivalence methodology to grasp the concept. Thus, the addition of TCDD dose response curves for a sensitive, population-relevant endpoint for a representative fish, bird and mammal would be valuable additions. It would be helpful to designate, for teaching TEF methodology only, a "hypothetical" threshold or action level for TCDD for each species to which the calculated TECs could be compared.

EPA Response: A single, generic dose-response is provided in Figure 7 of the revised Framework. It is representative of the type of dose-response that is typical of fish, birds, and mammals for an endpoint that is sensitive and highly relevant in ecological risk assessment (i.e. early life stage mortality). Definition of a threshold or action level is an assessment-specific activity and is therefore not included in the generic figure.

EPA believes a generic illustration is appropriate for the Framework because the document is not intended to be a comprehensive guide to risk assessment of dioxin-like chemicals. In addition, the intended audience for the Framework is risk assessors who have a working knowledge of EPA's Guidelines for Ecological Risk Assessment (U.S. EPA, 1998) and are familiar with issues related to conducting risk assessments for dioxin-like chemicals. EPA has added text to the Preface and the Introduction clarifying the scope and intended audience for the Framework.

B.6. The document should be a little more critical of the existing WHO values. This might be handled with a text box in the Introduction. In this respect it should be mentioned that the eco-TEFs determined by WHO for fish and birds have often been determined with a minimum available data set. As such, this limitation certainly represents the observed difference between birds or fish versus mammals in TEFs. However, it should be realized that at the time no better choice could be made due to the limited information available. Thus, the eco-TEFs derived in 1997 should be considered as interim and preliminary values that definitely do not have the accuracy and detailed information that has been used for establishing the mammalian TEFs. The EPA should allow itself more to express this higher uncertainty in bird and fish TEFs where appropriate. Furthermore it could also be suggested that the database should be expanded and the 1997 WHO eco-TEFs being reviewed within the near future to obtain a higher degree of certainty. Such a

revision would likely be done within an international framework such as WHO-IPCS.

EPA Response: EPA's position that it is appropriate to use the World Health Organization (WHO) class-specific TEFs in ecological risk assessment is supported by the conclusions from two World Health Organization expert meetings (Van den Berg et al., 1998; 2006), an EPA-DOI expert workshop (U.S. EPA 2001a), and the recent National Research Council (NRC) report (2006). In each of these reports, the experts convened agreed that application of TEFs for the purpose of risk assessment is currently an appropriate and scientifically defensible approach. Furthermore, the participants in the EPA-DOI expert workshop on the application of the TEF Methodology in ecological risk assessment concluded that the uncertainty in the TEF approach is not greater than the overall uncertainty of the ecological risk assessment process (U.S. EPA, 2001a).

The Framework acknowledges that when applying the TEF methodology, one expected approach is to use the TEFs adopted by the WHO in 1998 and 2006 (TEF-$WHO_{98/05}$ values). However, a large proportion of the Framework (Chapter 3) is dedicated to describing a logical way in which risk assessors can organize relative potency data (including the WHO TEFs) according to species similarity, endpoint relevance, and dose relevance and consistency (Figure 10 and Tables 6 – 8) in order to understand the strengths, limitations, and uncertainties associated with such data and select the relative potency values that are most appropriate for a particular ecological risk assessment.

B.7. The table of BSAFs generated much discussion concerning the source(s) of these values as well as concerns that they might be viewed as default values. There was strong sentiment that they should not be portrayed as default values. The reviewers felt that the legend should be expanded to make that clear and that information should be given on where these values did come from.

EPA Response: All the values used as BSAFs, BAFs, or BCFs are illustrative examples. A note has been added to Tables 4, 5, and 6 to clarify that the data are for illustrative purposes only and are not recommended default values.

C. Responses to Charge Questions

Charge Question 1: A main goal of this document is to assist ecological risk assessors in applying the toxicity equivalence methodology correctly. Please comment on the overall effectiveness of the document in achieving this goal. Please discuss document organization, appropriateness of the level of detail, and usefulness of figures/tables.

C.1.1. Perhaps include a few sentences about risk methods that could be used in addition to the hazard quotient method. Examples are probabilistic and joint probability analysis.

EPA Response: Section 3.4.1 has been revised to acknowledge that the hazard quotient is but one simple risk estimation method and that other methods for risk estimation may be

used in an ERA. However, because the Framework is not meant to be a comprehensive guidance for conducting an ecological risk assessment for dioxin-like chemicals, the reader is referred to EPA's Guidelines for Ecological Risk Assessment (U.S. EPA, 1998) for more information on additional methods.

C.1.2. Move the "Conclusions" upfront and make it into an Executive Summary or part of the Introduction. Organizing the document in this manner will enable first-time readers to obtain an overview of the methodology, and important considerations associated with it, before they enter the detailed portion of the guidance.

EPA Response: EPA has retained the *Conclusions* section at the end of the Framework. However, broad conclusions have also been incorporated into the *Preface* and *Introduction*.

C.1.3. Check all figures and tables for complete legends so that the figure or table can stand alone. The text could make more use of the figures and tables, so it is worth another read to be sure that nothing has been overlooked.

EPA Response: Figure legends and Table titles have been modified as suggested.

C.1.4. A casual reference is made on P. 20 (Line 20) to the use of "uncertainty factors". These are often used for interspecies extrapolations. However, this is the only place the matter is discussed. Is this Framework suggesting the use of interspecies extrapolation factors for developing TECs? If so, that is an important aspect of the method. Either develop that a bit further or do not raise the issue only in this casual way.

EPA Response: EPA is not suggesting the use of uncertainty factors for developing TECs. The noted reference to "uncertainty factors" has been removed to prevent confusion.

C.1.5. Consider breaking out Table 3 to provide information for each major animal class. Each table would provide information on different species of fish, birds, and mammals. The reason for breaking Table 3 into three tables is that early life stage toxicity is a very relevant endpoint for ecological risk, yet the "profile of TCDD effects" that characterize early life stage toxicity in fish and birds, respectively, is not clearly illustrated in Table 3 or anywhere else in the document. The adverse developmental effects caused by exposure to TCDD in for example egg laying fish and related AhR agonists (edema, impaired jaw development, impaired heart development and function, reduced trunk blood flow, anemia, growth retardation, and mortality) needs to be captured in the mind of the reader of this document (along with the well known effects on enzyme induction). Table 3 simply does not accomplish this objective. If possible, it would help to give the reader a feel for the relative sensitivity of the endpoints. This might be done with a "+" to "+++" type approach.

EPA Response: Table 3 has been modified to provide information on each of the major animal classes as a group for endpoints that are unique or particularly relevant for characterizing dioxin-like toxicity. The broad endpoint of developmental and reproductive toxicity is now included in the table. However, detail about specific species and magnitude of effects is not included given that the Framework is not intended to be a comprehensive guide for conducting the effects characterization for an ecological risk assessment. The Table does include extensive references to guide risk assessors in their further investigation of the literature pertinent to conducting an effects characterization. In addition, EPA has previously published comprehensive reviews of available toxicity data for dioxin-like chemicals that are referenced in the document (U.S. EPA 1993).

C.1.6. Some qualification is needed in connection with information presented on mono-ortho PCBs (in particular consider the use of "less than" indicators as was originally provided by WHO). Specifically, it was noted that underlying research indicates that mono-ortho PCBs are not toxic to fish. Use of the upper range of the TEFs (i.e., 0.000005) for the mono-ortho substituted PCB congeners in fish will overestimate the TEC. At some points in 3.2.1.1 it might be useful to expand a bit more in the basic difference between the species sensitivity for dioxin like compounds and the relative potency differences e.g. observed between mammals and fish for MO-PCBs. It should be emphasized that in the future, risk assessment should more be based on internal dose/concentrations levels than administered dose/uptake is essential to obtain more information regarding differences in species sensitivity for AhR mediated mechanism.

EPA Response: The "less than" indicators associated with the WHO TEFs for fish for mono-ortho PCBs are present in Tables 2 and 4. Furthermore, a paragraph comparing the sensitivity of fish, birds, and mammals to mono-ortho PCBs is included in Section 3.2.1.1. Language describing how internal dose/concentration reduces the variability in toxic effects thresholds and the need for more approaches based on internal dose/concentration data has also been added to Section 3.2.1.1.

C.1.7. Section 3.3.1.3 discusses choices for exposure dose metric. It would be helpful to emphasize the importance of insuring a proper match of dose to effects as part of Planning. Look especially at the last paragraph on p. 32.

EPA Response: This discussion has been highlighted by repeating it in Section 3.1 *Considerations in Planning*.

C.1.8. Section 3.3.2.1 could be set up better. It needs a better introduction. Consider moving the second paragraph (P. 48 Line 11) to after the current third paragraph (at Line 29).

EPA Response: Much of Section 3.3.2.1 was redundant with other parts of the document. Therefore Section 3.3.2 has been extensively revised.

C.1.9. Section 3.4.2 needs a conclusion. It also has embedded within it various screening tests. Because these are not recommended as lines of evidence for risk characterization, do these belong in this section? Should these types of tests be given their own section, perhaps in an early tier where screening may be appropriate?

EPA Response: A conclusion has been added to Section 3.4.2. The experts at the EPA/DOI workshop concluded that although screening bioanalytical tools should not be used as an alternative to congener-specific analysis and the TEF methodology, they may be considered as additional lines of evidence in a risk characterization. Thus, EPA believes that such tests should be discussed in Section 3.4.2 (*Lines of Evidence*).

C.1.10. On P. 68, Lines 3 – 5, a method is suggested involving the use of ranges of RePs. Is this appropriate for this document? If there is a desire to evaluate uncertainties, perhaps an explicit discussion should be put together on how to quantify this.

EPA Response: EPA believes that it is appropriate to use alternative ReP values, when available, to describe the range of possible risk values. This approach is consistent with EPA's *Guidelines for Ecological Risk Assessment* and with the Office of Management and Budget's (OMB) 2007 memo on Updated Principles for Risk Analysis (http://www.whitehouse.gov/omb/memoranda/fy2007/m07-24.pdf).

Charge Question 2: The document proposes to resolve current inconsistencies in the scientific literature over terms such as "ReP" by establishing and using clearly-defined, unified terms. Please comment on the clarity and effectiveness of the terms used.

C.2.1. Why use the term ReP to represent Relative Potency? One should be able to represent two words with two letters (RP).

EPA Response: The ReP terminology, definition, and acronym were adopted directly from those agreed to at two World Health Organization international consultations (Van den Berg *et al*., 1998; 2006).

C.2.2. There was strong sentiment that the acronym (term) TEQ should be retained rather than TEC. The term 'TEQ" is so well entrenched in the literature that introducing the new term "TEC" would only add to the confusion.

EPA Response: EPA chose to retain the acronym **TEC** to represent **T**CDD (or **T**oxicity) **E**quivalent **C**oncentration because it more accurately represents the fact that the end product of applying the TEF methodology is a **concentration**, and it is more consistent with the construction of the acronyms derived for other terms associated with the TEF Methodology as established by the World Health Organization (Van den Berg *et al*., 1998; 2006). EPA reviewed the responses of each of the peer reviewers regarding this charge question. Six of the reviewers provided positive comments on the terminology section without reservations regarding the introduction of the term TEC to represent

TCDD (or **T**oxicity) **E**quivalent **C**oncentration. Four reviewers did not address the issue specifically. Only two of the twelve reviewers expressed concerns about introducing the term TEC.

C.2.3. Analogous acronyms to TEF have also been REP, RPF and RP. It was suggested that REP, RPF and RP be added in the table as analogous acronyms.

EPA Response: These terms have been added to Text Box 1 in the revised Framework.

C.2.4. Consider moving definitions on p. 4 to the beginning of 1.1. For a Framework document, it is most useful to present the definitions and then follow with the rationale for what is being proposed.

EPA Response: The suggested change had been made in the revised Framework.

C.2.5. Inconsistent use of other terminology currently in the document can lead to confusion. To avoid this EPA should consider having the document reviewed by people less familiar with the methodology. Members of the peer review group identified the following terminology issues and have suggested changes:

- **Readers of this document will find it confusing that the words: compound, chemical, and congener are used interchangeably. This is especially problematic when TEFs are listed for "congeners" and the type of chemical analysis required to measure exposure to PCDDs, PCDFs, and PCBs is referred to as being "congener-specific". It is suggested that the phrase "dioxin-like congener" or "dioxin-like compound" be used to insure clarity.**

  EPA Response: For consistency EPA chose to use the term dioxin-like chemical(s) when referring to the whole group of PCDDs, PCDFs, and PCBs throughout the document.

- **The symbol (IIsocw) used to describe the sediment-water concentration quotient appears unconventional. The use of the II in the symbol is not intuitive. Various symbols have been used to describe sediment water partitioning such as Kd or Kp or K**

  EPA Response: The term $\prod_{socw}$ is well established in the peer-reviewed literature (e.g., Burkhard, 1998, 2003; Burkhard et al., 2003a; 2008) and has been defined and used in previously reviewed and published EPA documents (U.S. EPA 1995a, 2000, 2003b).

- **Consider how confusion around the word "receptor" can be reduced. The term "receptor" is used both to refer to the aryl hydrocarbon receptor (AHR) and to "ecological receptors" (meaning target species, e.g. p.14, 28). The term "receptor" has a specific meaning in pharmacology, defined more than 100 years**

ago, and its use in reference to the AHR is consistent with that. Using "receptor" in the context of a target species, while common in ecological risk assessment, is potentially confusing. The term "target" or "target species" would be more descriptive and less ambiguous.

EPA Response: The term "receptor" is used exclusively to refer to the aryl hydrocarbon receptor (AHR) in the revised Framework. "Ecological receptors" are simply referred to as species.

- **Similarly, the term "stressor" is often used in the document in reference to the chemicals that act through the AHR (e.g. "AhR-mediated stressors", p. 14, 28). Why not simply say "chemicals"? (Note also that the chemicals are not AhR-mediated, their effects are.)**

  EPA Response: The term "ecological stressor" has been changed to "ecological endpoint," and "stressor" has been changed to "chemical stressor." The terminology "stressor-response profile" has been retained, since this term is consistent with EPA's Guidelines for Ecological Risk Assessment (U.S. EPA, 1998).

- **P. 1, Line 10. Add after the sentence ending with "situations." "In this document, the term "dioxin-like effects" and "dioxin-like compounds" are used to refer to those effects that are similar to those caused by 2,3,7,8-TCDD and for those compounds that exert such effects through binding with the Ah Receptor.**

  EPA Response: The Framework has been revised consistent with this suggestion.

- **The term "potency" should not be used as a stand alone word at any place in this document. The potency of every dioxin-like congener should always be mentioned relative to 2,3,7,8-TCDD as relative potency. In the vast majority of the framework document relative potency is used. However, there are a few places where "potency" only is used and where this occurs it needs to be corrected. The same comment applies to the use of "potency factor" in place of the correct term, "relative potency factor".**

  EPA Response: The suggested change has been made in the revised Framework.

Charge Question 3: Please comment on whether the advantages of using the toxicity equivalence methodology are adequately explained.

C.3.1. Provide a brief comparative discussion of the alternative methods. This might involve the preparation of a sub-section entitled "Advantages and Limitations for the TEQ Methodology" This might be placed in the Introduction. Two reviewers suggested giving an actual example that compared the methods (e.g., total vs. TEQ

vs Aroclor). This would serve to show how uncertainty is reduced through using the TEQ methodology. For amplification see comments of Hope, Hahn, and Menzie.

EPA Response: Discussion of the prerequisites, strengths, and limitations to be considered in applying the TEF methodology is provided in Sections 1, 2, 3.1, 3.1.1, and 3.2.2 of the Framework. Methodological considerations associated with using the TEF methodology are presented in Sections 3.1 and 3.2 to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations associated with using the TEF methodology against other methods they may be considering. The type and number of other approaches that could be considered for PCBs as well as other dioxin-like chemicals will be specific to each ecological risk assessment, such that comparisons of strengths and limitations is best conducted during the planning and problem formulation phases of the specific ecological risk assessment. Since strengths and limitations will vary depending on the alternatives available and the scope and objectives of a specific ecological risk assessment, EPA does not believe it is feasible to provide a specific comparative analysis within the Framework. However, EPA has inserted several references to a recent document addressing the benefits of PCB congener analysis (U.S. EPA, 2005). As appropriate, EPA Offices and Regions may consider strengths and limitations of all methods deemed available or feasible as they implement the Framework for their individual programs.

C.3.2. Point out that the method is applicable to vertebrates but not for invertebrates. Note that there are non-dioxin-like effects that can be important for invertebrates and that may need to be evaluated using a separate methodology. Consider changing the title of this document to reflect that the TEF/TEQ methods applies to fish and wildlife (to distinguish it from what might be needed for invertebrates.) See Adams comment on Daphnia.

EPA Response: Section 3.2.1.1 has been revised to include a paragraph addressing the insensitivity of invertebrates to dioxin-like chemicals and the recent findings that AHR analogs found in some invertebrates are unable to bind prototypical AHR agonists, thus, providing a mechanistic understanding of the relative lack of sensitivity in invertebrates. This paragraph also addresses the Adams comment on *Daphnia*.

The identification and functional characterization of AHR in a variety of species is an active area of research (Hahn et al., 2002a, b; Jensen and Hahn, 2001; Yasui et al. 2004, 2007). The suggested title change would exclude the applicability of the Framework to a whole class of organisms if the current understanding of invertebrate sensitivity to dioxin-like toxicity were to change as a result of on-going research. Therefore, EPA has not made the suggested change to the title of the Framework.

Charge Question 4: The framework emphasizes the importance of measuring or estimating chemical-specific PCDD, PCDF, and PCB concentrations in tissues in order to apply the methodology. Please comment on this and whether sufficient discussion of estimating concentrations in tissues is provided. Is the explanation of the application to the methodology to dietary exposure in mammals, as distinguished from fish and birds, adequate?

C.4.1. Consider providing a bit more guidance relative to the development of tissue concentrations estimated from sediment or dietary exposure. In those cases, it is imperative to consider the trophic transfer and biomagnification that occurs from fish to bird species. The use of a model such as that proposed by Gobas (1993) should not be thought to be optional.

EPA Response: The *Characterization of Exposure* section of the Framework (Section 3.3.1) has been reorganized and revised extensively. In the revised Framework, Sections 3.3.1.4 and 3.3.1.5 are dedicated to providing guidance and illustrative examples of how to derive tissue concentrations in fish (Table 4), tissue concentrations in bird (Table 5) and mammalian dietary concentrations from sediments using BSAFs (Table 6). The bird example (Table 5) illustrates how to calculate tissue concentrations in bird eggs, rather than through adult bird diet, because early life-stage toxicity is more relevant assessment endpoint for dioxin-like toxicity, and available TEFs for birds are largely tissue-based rather than based on dietary intake. Additional discussion has been added to Section 3.3.1.5, and questions have been added in Text Box 6 regarding how to consider biomagnification and extrapolation of BAF or BSAFs across sites using food-web models (e.g. Gobas, 1993 and Gobas et al., 1998). In addition, a reference (Burkhard et al., 2006) to a recent demonstration of a "hybrid modeling approach" using BAFs/BSAFs and food-web modeling to account for trophic transfer and biomagnifications has been added.

C.4.2. Comment C.4.2: The document should address the issue of non-detects. Consider developing a short section for the main portion of the document or, alternatively, treat this in the uncertainty section. Several reviewers felt this is an important issue with regard to the low levels of congeners that occur in some media. A source of uncertainty is the change in detection levels from one study to the next or at different times in the same study. (See de Fur and Giesy for further discussion.)

EPA Response: The issues of analytical methods and detection limits are overall risk assessment issues, not issues specific to the TEF methodology. In addition, the analytical methods and detection limits issues are specific to each ecological risk assessment and are therefore best addressed during the planning and problem formulation phases of the specific ecological risk assessment. While the TEF methodology does not dictate what analytical method or detection limits need to be used, methodological considerations associated with using the TEF methodology are presented in Sections 3.1 and 3.2. In section 3.3.1.1, it is explained that the best method for handling non-detects in a particular risk assessment should be determined during planning and/or problem

formulations phase(s) of the risk assessment. In section 3.4.3.2.2, uncertainties associated with characterization of exposure, including detection limits, are discussed. In both Sections 3.3.1.1 and 3.4.3.2.2 a reference to other EPA guidance that addresses this issue is provided.

C.4.3. One reviewer expressed concern about applying TECs in the diet. This concern is based in part on the fact that each congener not only has its own unique ReP or TEF, but also a unique BAF. Thus, the use of TECs in dietary items could lead to additional variability in the analysis. However, as long as the dietary item is not predicted the use of TECs in dietary items is appropriate. More discussions of the limitations of this use of TECs would be useful. This comment was not discussed further during our phone conversation.

EPA Response: The *Characterization of Exposure* section of the Framework (Section 3.3.1) has been reorganized and revised extensively. In the examples presented in Section 3.3.1.5 (and in equations 3-3 and 3-4), it is clear that bioaccmulation factors (BAFs or BSAFs) are congener-specific, that is, unique for each congener. EPA believes the least amount of variability and uncertainty in the TEC is achieved by using a congener-specific TEF and B(S)AF in calculating the TEC.

In the revised Framework, Section 3.3.1.3 stresses the need to for consistency in the dose metric in the exposure assessment and the effects assessment, which the peer reviewers raised as an important issue (see Charge Question C.1.7). Section 3.3.1.3 now also explains that although tissue concentrations are the preferred dose metric, since TEFs for mammals are largely derived from studies using administered dose, application of mammalian TEFs to the diet is a more accurate approach and will minimize variability in the analysis. Therefore, the mammalian diet example (Table 6) is prefaced with a discussion regarding the fact that although tissue concentrations are the most relevant dose-metric, it is often impractical or impossible to define dose on a tissue-specific basis for mammals.

Additional discussion of the limitations and variability associated with calculating TECs based on diet rather than tissue concentrations (i.e. internal dose) has been added to Sections 3.2.1.1 and 3.3.1.3 (see also response to charge question C.1.7).

C.4.4. The use of bioaccumulation factors to estimate tissue concentrations from environmental media (or to relate known tissue concentrations back to ambient levels) is described in section 3.3.1.4. This section is clearly written until the p. 35-p. 40 transition, at which it appears that some words are missing. In addition, the description of sediment water concentration quotients (IIsocw) and Di/r on pp. 40-41is somewhat cryptic.

EPA Response: The *Characterization of Exposure* section of the Framework (Section 3.3.1) has been reorganized and revised extensively to improve clarity. In addition, definitions of II_{socw} and $D_{i/r}$ have been added to Text Box 5.

Charge Question 5(a): The framework provides considerations for selection of relative potency factors that may be more specific for the species, endpoints, and doses of concern in individual ecological risk assessments than the international consensus TEFs. Please comment on the completeness and clarity of this discussion.

C.5(a).1. It is recognized that the WHO factors are starting points. From a management perspective, it would be useful to have more discussion about what situations "trigger" an assessment to develop assessment-specific RPF values. The text should be enhanced to show how to make these site-specific selections without being arbitrary and without simply adopting the selections that are easiest, favored by the entity that complains the most in the situation, or happen to be on the computer at the time of the calculation. Again, EPA needs to provide more text with guidance on how to make this decision to reduce the potential for arbitrary outcomes.

EPA Response: Whether to use the TEF methodology and whether to use consensus WHO-TEFs or RPFs are decisions specific to individual ERAs and hence need to be determined on a case-by-case basis during planning and problem formulation. Sections 3.1 and 3.2 discuss issues for risk assessors and risk managers to consider in the decision-making process. Section 3.3 and especially Section 3.3.2 (and the examples in Section 3.3.2.4), provide guidance on how to logically organize available ReP data to make site-specific selections that minimize uncertainties and maximize species similarity, endpoint and dose relevance, and consistency. Using this approach facilitates the transparent and defensible selection of relative potency factor(s) for use in risk assessments.

C.5(a).2. A suggestion was made that EPA consider the Bursian et al. (2003) paper along with the Tillitt paper for the example on mink. Giesy provides a rationale for this.

EPA Response: EPA believes the use of the Tillitt et al. (1996) paper in the mink example is adequate for this Framework, as the example is only intended to illustrate the procedure for organizing data and selecting relative potency values and is not intended to evaluate the toxicity or the TEF for mammals in general or for mink specifically.

C.5(a).3. A few of the reviewers found the examples for birds and for mammals unclear. It may be helpful to have these read over by someone unfamiliar with the methodology in order to identify how these examples can be made more understandable.

EPA Response: Section 3 in general, and the mink example in particular, has been revised to improve clarity.

C.5(a).4. It would be helpful to include a website address in the Framework Document for the 1997 TEF database. This database consists of all relevant toxicological data for dioxin-like compounds through 1997. It was used to establish the WHO98 TEFs for fish, birds, and mammals given in Table 2. It seems like there is more data available on RePs for different species of birds based on embryo toxicity than is referenced in the Framework Document. It would be helpful to update the bird RePs accordingly.

EPA Response: The 1997 ReP database created by the Karolinska Institute is available on EPA's web site along with the final Framework. To EPA's knowledge, the Karolinska Institute is not presently updating or maintaining this database. However, the database of mammalian RePs was reviewed and refined by Haws et al. (2006). This database was used in the 2005 WHO reevaluation of mammalian TEFs as described in van den Berg et al. (2006). The Haws et al. database is published in peer-reviewed literature (Haws et al., 2006).

Charge Question 5(b): The framework provides considerations for selection of relative potency factors that may be more specific for the species, endpoints, and doses of concern in individual ecological risk assessments than the international consensus TEFs. Are the matrix presented in Figure 10 and the examples used to illustrate the application of the matrix clear and adequately explained? Are there elements which should be added or removed from the matrix? Do you agree with their place in the tiers on the matrix? Please explain.

C.5(b).1. Some simple ways to clarify the discussion of the matrix include: a) just refer to it as the matrix (not the matrix model), b) refer to all categories as "levels" and not "tiers" in order to distinguish between these levels of information and tiers of risk assessment, c) P. 49, Lines 10 through 16. Simplify all of this by simply introducing the Matrix as a tool for guiding the selection of ReP values from which to derive a RPF.

EPA Response: The suggested changes have been made in the revised Framework.

C.5(b).2. The dose specificity axis of Figure 10 is an important part of the matrix. However, this axis actually combines two different components related to the dose metric (or exposure metric) used to determine RPFs. This is noted in the draft document [p. 52 lines 22-25] but the discussion of these two aspects could be clarified and additional guidance provided on how to balance these two components in the selection of RPFs. The first component is the degree to which the dose metric used to derive RPs is the same as the dose metrics used in the exposure assessment and in the effects assessment. The authors call this "consistency". The second component of this axis is the degree to which the dose metric used to derive RPs is relevant to the target tissue and effects of concern. It is this component that is actually reflected in the "tiers": dose in tissue, dose in

organism, administered dose, and nominal/predicted dose. The authors call this "specificity"; "relevance" may be a better term. In the presentation of example 3 (mink; pp. 55-58) the authors point out a situation in which a less relevant dose metric (administered dose) may be preferable when it is more consistent with the dose metric used for the effects assessment (TCDD dose-response curve). The authors could make a more explicit statement to provide additional guidance on how to balance these two considerations. For example, they might say that one should choose RPs generated using the most relevant dose metric that is also fully consistent with the dose metric used for the effects assessment (i.e. consistency is given priority over relevance).

EPA Response: The terminology used to label the z-axis of the Matrix has been changed in the revised Framework to "Dose Relevance and Consistency." Further, discussion of the z-axis has been re-written to clarify the two aspects of dose under consideration when selecting relative potency values to be used in deriving RPFs (see Section 3.3.2.3 entitled *RPF Dose Relevance for Effect and Consistency with Dose-Response Relationship*). To further this point, the Matrix and discussion now include a strategy for weighing each of the components by introducing a scale on the z-axis that facilitates summing of ReP dose relevance with dose-response consistency.

C.5(b).3. During the December 5th conference call there was a discussion of how the Matrix could be made more clear. During that call, Peterson recommended that the Matrix in Figure 10 be changed as highlighted below:

For the Y Axis, Endpoint Similarity the levels would be named:

1. **Toxic Effect of Concern in vivo**
2. **Other Toxic Effect in vivo**
3. **AhR-Dependent Biochemical Endpoint in vivo**
4. **AhR-Dependent Biochemical Endpoint in vitro**
5. **Other Biochemical Endpoints (AhR Binding)**
6. **Quantitative Structure Activity Relationships (QSAR)**

For the X Axis, Species Similarity, Level 3 would be Vertebrate Class-Specific "Consensus" TEFs

The Z Axis would be identified as Target Tissue Similarity / Dose Similarity

EPA Response: The y-axis levels have been revised, largely as suggested, although they have been abbreviated in some cases in order to fit into the figure. The x-axis change was not incorporated because the use of "Consensus TEFs" would imply this level would always be the WHO TEFs. However, EPA envisions that when additional data are available and relevant, a risk assessor may select among any existing RePs to derive RPFs and would not necessarily have to be constrained to selection of the consensus TEFs. The z-axis was revised as described above in response to comment C.5(b).2 to clarify and highlight the two aspects of dose similarity to be considered.

C.5(b).4. Following the conference call, Mark Hahn provided the following additional commentary on the Matrix:

The y-axis might best be called "Endpoint relevance" (referring to its relevance to effects of greatest concern).

The x-axis should be called "Species similarity" as suggested by Dick.

The z-axis should be called "Dose metric consistency and relevance" to reflect the two aspects of this axis, as discussed above.

EPA Response: Changes consistent with these suggestions have been made in the revised Framework.

C.5(b).5. On P. 59, Line 32, a key point is made that needs to come earlier in the section and certainly at the beginning of 3.3.2.4. That point is that you start with the TEFs and only become more site or species specific when there is very good reason. Further, as more information becomes available, the Matrix can be used to guide the development of new default TEF values.

EPA Response: Text expressing the expectation that WHO-TEFs can and will be used in ecological risk assessment has been added to the beginning of Section 3.3.2.

Charge Question 6: Please comment on whether the uncertainties associated with the application of the toxicity equivalence methodology are comprehensive and adequately explained.

C.6.1. The influence of detection levels on the uncertainty around risk estimates could be addressed in the uncertainty section.

EPA Response: The suggested addition has been made in the revised Framework.

C.6.2. It would be helpful to have a bit more information on the relative magnitudes and direction of uncertainties around estimates. It may be helpful to have discussion around the uncertainties associated with selection of BSAFs (or other methods for estimating bioaccumulation) relative to the uncertainties around TEF and RPF values. It may be helpful to encourage users of this document to use sensitivity analyses to guide the levels of effort they devote to the different components of applying the TEQ/TEF methodology. Not all aspects of the methodology have similar degrees of variability and uncertainty nor do they have an equivalent impact on the final outcome the TEF methodology.

EPA Response: The uncertainty associated with a risk estimate will be specific for each individual risk assessment and dependent on each component (e.g. chemical concentration, BAF or BSAF, RPF or TEF, TRV) of the risk estimate. Therefore it is not possible to provide a relative measure of uncertainty for BSAFs vs. TEFs or RPFs. The

selection matrix approach described in Section 3.3.2 is designed to guide risk assessors through evaluation of available relative potency data to, in part, identify where the greatest uncertainties in relative potency data lie. In addition, Section 3.4.3 has been re-organized in an attempt to prompt risk assessors to think about uncertainties associated with the use of the TEF methodology itself within the broader context of an ecological risk assessment (i.e. that the methodology serves as a framework for conducting a qualitative sensitivity analysis).

C.6.3. Section 3.4.3.1.1 suggests that there are non-AhR-dependent mechanisms of action, but is vague on the point. There are certainly non-AhR-dependent mechanisms known in the toxicology literature, and the section must point that fact out, give at least some mention of which ones (immune systems, neurological, developmental, estrogenic) are known and offer something more in the way of explanation. This uncertainty would underestimate the effects of these compounds. Section 3.4.3.1.2 refers to no known interactions, yet Cook et al. in Rolland et al., 1998 report synergistic responses in fish from exposure to TCDD and PCBs. Section 3.4.3.1.4 refers to the TEFs and RPFs as point estimates, yet fails to acknowledge that these point estimates were the result of a consensus meeting among scientists form different countries. Point estimates work with little uncertainty if there is a huge database to support them (and a low C.I.) or if they are set as protective, as in a barrier. However, these point estimates are neither. There is but a modest database and no attempt to set these as "not greater than" in regulatory terms. Therefore, one source of error/uncertainty is the greater response (or lesser) due to the biological differences among animals for the same species, or genus or family or even order. These basic biological differences could account for huge uncertainty and natural variation.

EPA Response: Language has been added to the *Preface* and the *Introduction* to clarify that the Framework is not intended to provide comprehensive guidance on conducting ecological risk assessment. The purpose of the Framework is to provide guidance on how to apply the toxicity equivalence factors for *dioxin-like* activity of chemicals within an ecological risk assessment for *dioxin-like chemicals*. Accordingly, the Framework does not include guidance on determining or accounting for ecological affects from other modes or mechanisms of action, whether from PCDDs, PCDFs, and PCBs or other chemicals.

Not accounting for all possible modes or mechanisms of action of PCDDs, PCDFs, and PCBs (or any other chemicals) will not necessarily result in an underestimate of effects or risks. The relative potency of the various modes/mechanisms of action relative to the exposure concentrations would need to be considered. For dioxin-like PCDDs, PCDFs, and PCBs, current evidence indicates that the greatest potential for effects on ecological endpoints of most concern (*e.g.*, growth, survival, reproduction) is from the AHR agonists (Giesy and Kannan, 1998; Rice *et al*., 2002). Nonetheless, determining the chemicals, modes/mechanisms of action, species, and endpoints of concern is assessment-specific and is therefore best performed during the planning and problem formulation phases of the individual risk assessment.

C.6.4. The methods used to estimate tissue levels are likely to have the greatest uncertainties associated with them. Because there are various methods by which tissue residues can be measured or estimated, the Framework should expand on this source of uncertainty in the application of the method. This is discussed further under Charge Question 8. Giesy, Metcalf, Kennedy, Hope and Menzie provide detailed discussion on this issue.

EPA Response: A discussion of uncertainties associated with the characterization of exposure, including both measuring and estimating tissue concentrations, is included in Section 3.4.3.2.2 of the revised Framework. In addition, additional discussion and references regarding the use and/or extrapolation of BAFs/BSAFs and food-web modeling have been added to Section 3.1.5.

C.6.5. One issue not addressed specifically concerns some of the uncertainties and complexities associated with the additivity assumption. For example, the issue of ligand "intrinsic efficacy" and how it (together with ligand affinity) contributes to the "potency" of AHR agonists is not mentioned. The issue may be too technical to treat in this Framework (e.g. on p. 10), but it is relevant to the additivity assumption in that compounds with lower intrinsic efficacy can act as "partial agonists" and thus inhibit the response to full agonists at certain dose ratios (Toxicol. Appl. Pharmacol. 168: 160). This has been shown both theoretically and experimentally, but the extent to which it occurs with environmentally relevant mixtures is not clear.

EPA Response: Additional text summarizing empirical data from both laboratory and field studies on ecological species that provide strong support for the additivity model has been added to Section 2.1. This section also includes reference to U.S. EPA, 2000a, as it has an extensive discussion regarding the empirical research and "receptor-based theory" underyling the additivity assumption. This information was not reiterated in the Framework in the interest of keeping the document clear and concise and focused on the application of the TEF Methodology. Discussion regarding "intrinsic efficacy" and the potential for partial agonists to act as antagonists at environmental concentrations has also been added to Section 2.1 and 3.4.3.1.2 by noting that Van den Berg et al. (1998; 2006) concluded that antagonistic effects are usually seen above environmentally relevant doses, such that the presence of chemicals that have demonstrated antagonist activity (primarily *in vitro*) is unlikely to result in large errors when antagonists are present.

C.6.6. The uncertainty section should include some discussion regarding the source information for derivation of RePs. RePs determined from NOAELs, LOAELs, and benchmark doses are not as accurate as those based on LC50s, EC50s, LD50s or ED50s.

EPA Response: Language consistent with this suggestion has been added to Section 3.4.3.1.3 in the revised Framework.

C.6.7. Bioanalytical tools are identified on P. 66, Line 16 as a means of reducing uncertainty. But earlier these tools were referred to as screening tools and not ready

for risk assessment. This may need further discussion with regard to how and when these tools can be used to address uncertainty.

EPA Response: The Framework notes that the experts at the EPA/DOI workshop concluded that such bioanalytical tools should not be used as an alternative to congener-specific analysis and the toxicity equivalence methodology. Rather these bioanalytical analyses are complementary tools that can be useful in providing additional lines of evidence. The referenced sentence has been revised to clarify that bioanalytical tools may reduce uncertainty by providing another line of evidence regarding whether dioxin-like toxicity risks are fully represented by the TEFs-$WHO_{98/05}$.

Charge Question 7: Are you aware of any essential references that have been omitted?

C.7. Reviewers typically provided suggestions for references within the context of specific comments. EPA should review these for contextual information.

EPA Response: Many of the suggested references have been incorporated in the revised Framework. In some cases, a more recent or more relevant reference on the topic was included rather than the one suggested.

Charge Question 8: Is the discussion of exposure and bioaccumulation sufficient for basic applications of TEFs and RPFs in ecological risk assessments? Please explain.

C.8.1. The types of methods by which exposures (in the diet or in the tissues) can be measured or estimated. The Framework restricts itself largely to discussing this in terms of "factors" such as BAFs and BSAFs. Such factors are one of several ways by which exposure information can be developed. The other two important means are direct measurement and the use of bioaccumulation and food-chain models. These might include steady state as well as kinetic models. During our conference call, it appeared that BSAF was being used to imply the use of all of these tools. However, this will lead to confusion on the part of practitioners who think of BSAFs as factors (e.g., taken from a table or derived to reflect steady state conditions). The use of measurements and models do not receive adequate discussion in the framework. The discussion of exposure within the Framework can easily be broadened to be inclusive of the various methods available for estimating exposures and doses and not to indicate that the method is exclusively related to selection of BSAF or BAF factors. See Menzie for suggestions on where changes can be easily made to accommodate this larger view. Also, during our conference call, Phil Cook indicated that there was some information that could be added to help the reader work through the proper selection of methods and/or to have confidence in certain values.

EPA Response: See response to comment C.4.1.

C.8.2. Many comments were made concerning the application of BSAFs and BAFs. These fall into several categories. Collectively the comments suggest that this part of the TEF/TEQ approach can use some careful re-working. This might be reduced as an issue if BSAFs are subsumed into a broader discussion of measuring and/or estimating body burdens. BSAFs then are but one tool that can be used and not the only tool.

EPA Response: See response to comment C.4.1.

C.8.3. There is no suggestion of a reliable, non-controversial source of universally applicable "generic" BSAF values which would allow this approach to be used in lieu of site-specific information. Much more needs to be said about where or how one obtains the BAFs/BSAFs essential to the application of this method. It also needs to be made clear whether the BSAF values in Tables 4-6 are intended as examples only or as de facto "generic" factors. The challenges associated with measuring BAFs/BSAFs are also understated here. The Group generally felt that "extrapolation" is a non-controversial way around any of these challenges.

EPA Response: The Framework does not suggest a source of universally applicable "generic" BSAF values because EPA does not advocate or support such an approach. EPA's approach for acquiring and using BAFs/BSAFs is summarized in Section 3.3.1 of the Framework and is based on many previously published peer-reviewed publications (Burkhard, 2003; Burkhard et al., 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001a; 2003b). Indeed, EPA's bioaccumulation approach includes extrapolation of BAFs/BSAFs when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001a; 2003b). This body of information is summarized rather than reiterated in detail in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to Sections 3.3.1.4 and 3.3.1.5. A note has also been added to Tables 4 – 6 to clarify they are not intended to be "default" values, and a note has been added to each table to clarify this point.

C.8.4. If the use of BSAFs is to be advocated, there should be more discussion of the assumptions of the technique and the range of expected values and the limitations of the technique.

EPA Response: EPA's approach for acquiring and using BAFs/BSAFs, as summarized in Section 3.3.1 of the Framework, is well established and based on many previously published peer-reviewed publications (Burkhard, 2003; Burkhard et al., 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001a; 2003b); all of which are referenced in Section 3.3.1. This body of information is extensive. In the interest of keeping the Framework concise and focused on the application of the TEF Methodology, the approach is summarized rather than reiterated in detail; however, additional references to these articles and guidance documents have been added Sections 3.3.1.4 and 3.3.1.5 in the revised Framework.

C.8.5. One reviewer suggested that the statements on the limitations of the use of TEC in the diet be made more apparent. While the discussion points out these limitations, it comes to the conclusion that this is an acceptable practice when additional information is not available. It is this reviewer's opinion that the concentrations in target tissues should be predicted with congener-specific BAF or BMF values and then the TEFs applied to calculate predicted tissue-specific TEC concentrations which can then be compared to toxicant reference values (TRVs). Because of associated uncertainty, it would be useful to highlight the value of multiple lines-of-evidence approaches.

EPA Response: EPA believes that it is appropriate, in some cases, to use diet-based TECs. The rationale and limitations for this approach for mammals is discussed in Sections 3.3.1.3, 3.3.1.5, and 3.3.2.4.3. Example 3 also discusses how multiple lines-of-evidence (tissue-based TRVs and diet-based TRVs) could be compared, when appropriate data are available.

C.8.6. There was a strong sentiment among reviewers that BAFs (water to tissue) would not be a reliable way to estimate tissue levels. For example, the report includes an admission that dioxins, furans and non-ortho PCBs would be present in water under most exposure scenarios at concentrations well below detection limits. Data are rarely available on the ng/L concentrations of these hydrophobic compounds in water, since this would require extraction of large volumes of water. While part of the concern relates to the ability to estimate or measure the concentrations of dioxin-like compounds in water, there is also a concern that empirical BAF values may be highly variable and contribute to substantial uncertainty in exposure estimates. Metcalfe and Giesy give detailed discussion of these concerns.

EPA Response: While it has historically been difficult to measure low concentrations of PCDDs, PCDFs, and PCBs in water, as acknowledged in the Framework, it is possible and increasingly feasible to perform such measurements given newer analytical techniques. In fact, high quality BSAFs have been measured in a number of ecosystems (U.S. EPA 1995a; Burkhard et al., 2004; see also EPA's BSAF data set at http://www.epa.gov/med/Prods_Pubs/bsaf.htm). As summarized in Section 3.3.1.4, EPA has developed extensive guidance for minimizing variability in BAF and BSAF measurements and when extrapolating BAFs and BSAFs across ecosystems with similar conditions (U.S. EPA 1995a; 2000; 2003b). Also included in Section 3.3.1.5 is a discussion of approaches (e.g. the use of food-chain models and/or the "hybrid modeling approach") that can be taken to adjust BAFs/BSAFs to decrease variability and increase accuracy when extrapolating across ecosystems.

C.8.7. With regard to BSAFs, there are several technical issues related to the application of BSAFs for predicting tissue concentrations that were not discussed in sufficient detail in the Framework.

- **The concentrations of chlorinated contaminants in sediments are typically very heterogeneous; both vertically with sediment depth and horizontally in river or lake ecosystems. The sediment concentration chosen for the risk analysis**

exercise will be critical to the outcome, but no guidance is provided on the solution to this challenge.

EPA Response: The Framework is not intended to be a comprehensive guide for conducting ecological risk assessment for dioxin-like chemicals. Sampling designs and collection methods are not issues specific to the use of the TEF methodology, but rather issues to be addressed in the analysis plan for a risk assessment of dioxin-like chemicals. Nonetheless, EPA's extensive guidance for minimizing variability in BAF and BSAF measurements (U.S. EPA 1995a; 2000; 2003b) is referenced in the section that summarizes EPA's approach for measuring and extrapolating bioaccumulation factors for PCDDs, PCDFs, and PCBs (Section 3.3.1.4).

- **The Framework currently suggests that BSAFs can be used to predict the concentrations of chlorinated contaminants in fish from concentrations in sediment. An example is provided using BSAF data for Lake Ontario. There may be enough data in the literature from various aquatic ecosystems to generate reasonable estimates of the sediment/fish BSAFs for the many of the dioxin, furan, and PCB congeners (although this is subject to debate). However, there are few data in the literature on BSAFs calculated from the ratio of contaminant concentrations in sediments and the eggs of fish-eating birds. The report provides BSAFs calculated from sediment and herring gull egg data for the Lake Ontario ecosystem, but applying these BSAFs to other ecosystems (e.g. rivers, shallow lakes, etc.) would/could introduce substantial uncertainty. With respect to this potential uncertainty, the report should identify other approaches for determining the residues of chlorinated contaminants including direct analysis of bird eggs.**

  EPA Response: The Framework highlights the strengths of measuring tissue concentrations (residues or internal dose) of PCDDs, PCDFs, and PCBs in Sections 3.2.1.1 and 3.2.1.2. Section 3.3.1.3 highlights the accuracy of using TECs based on measurements of PCDDs, PCDFs, and PCBs in tissues. Section 3.3.1.4 begins with a discussion highlighting the fact that the most straightforward way of calculating TECs is from measured concentrations of dioxin-like chemicals in tissues using Equation 2-1.

- **The limitations of applying a BSAF to estimate tissue residues have not been adequately described. The Framework does not address the variability and precision inherent in this approach relative to predictions of contaminant concentrations in flora and fauna within ecological systems or between ecological systems. Thus, the magnitude of potential errors generated in predicting contaminant concentrations in wildlife and plants can not be put into perspective relative to other sources of variability and uncertainty that are inherent in the TEF methodology. In part, this is due to the reliance of these models on lipophilicity as the only determinant of accumulation. However, studies have shown that this factor alone is not a sufficient predictor of bioaccumulation and**

in fact, accumulation is a function of many factors including molecular size, conformation, sediment characteristics and biological factors (feeding habits).

EPA Response: EPA's approach for acquiring and using BAFs/BSAFs, as summarized in Section 3.3.1 of the Framework, are well established and based on many previously published peer-reviewed publications (Burkhard, 2003; Burkhard et al., 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001a; 2003b). This body of information establishes that bioaccumulation of PCDDs, PCDFs, and PCBs is a function of many factors (e.g. trophic level, food web characteristics, sediment organic carbon, organismal lipid, and sediment-water concentration quotient) and provides in-depth discussion of the uncertainties associated with the use of BAFs/BSAFs for estimating tissue concentrations. However, this body of information is extensive and in the interest of keeping the Framework concise and focused on the application of the TEF Methodology, the uncertainties are summarized, rather than reiterated in detail, in Section 3.4.3.2.2 in the revised Framework.

- **If BMFs and BSAFs are used to predict concentrations of PCDD/DF in tissues, an upper and lower bound could/should be given for the concentrations of each congener and this range of values propagated through the calculation of the TECs in tissues. To this end, probability bounds may be a useful tool.**

  EPA Response: EPA agrees that providing a range of values (upper and lower bound) for the congener concentrations would be useful to illustrate the variability around these values. However, in the interest of keeping the example TEC calculations concise and simple for the purpose of illustration and to avoid the misperception that the values are anything other than hypothetical (a concern expressed by the reviewers regarding the values in Tables 4 – 6; see comment #B.2), a range of values have not been incorporated into Tables 4, 5, or 6.

C.8.8. One reviewer suggested including an approach (either a description of method or an example) that would serve to illustrate how the TEF/TEQ approach could be validated. He notes that while the examples are illustrative, he would prefer to see a kind of validation for this approach with real ecological situations indicating the feasibility and possible uncertainty. He suggests two exercises:

- **Model the transfer of dioxin-like compounds from actual sediment concentrations with the endpoint being a prediction of concentrations for species higher in the food chain. These data could than be compared with actual concentrations found in the relevant species for that specific environmental situation.**
- **The second validation could be done in a reverse way. In this case calculations should go back from TEC concentrations observed in an actual top predator species and calculate the possible concentration levels in species at lower trophic levels and the sediment.**

Both exercises should produce more clarity about the predictive power of the suggested EPA method described in chapter 3.3.1.4.

EPA Response: EPA has performed the first exercise (Cook et al., 2003). Reference to this peer-reviewed publication has been added in appropriate sections (Sections 3.3.1.3 – 3.3.1.5) of the revised Framework to demonstrate/clarify the predictive power of the TEF Methodology. Numerous studies have demonstrated the second type of "validation," i.e. correlations between effects of environmental mixtures in marine mammals and avian species and food-web dietary concentrations (Ross *et al*., 1996; Summer *et al*., 1996a, b; Giesy and Kannan, 1998; Restum *et al*., 1998; Shipp *et al*., 1998a, b; Ross, 2000). References to these studies have been added to Section 2.1 of the revised Framework.

References

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