

Final Peer Review Report

Peer Review of EPA's *Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment*

Prepared for:

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Summary of Comments From Peer Review Prepared by Charles A. Menzie, Ph.D.

Introduction

This peer review summary has been prepared by Charles Menzie (chair) and is based on written comments and the December 5th, 2003 conference call among reviewers. The intent is to highlight our general comments and responses to the charge questions. To that end, this document is structured as follows:

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

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

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

B. Overview of General Comments

The review group felt that the Framework document met its goals and objectives and there was general agreement that this document will be very helpful. Several reviewers raised comments concerning issues of clarification that relate to how the Framework may be used by managers and risk assessors in real world situations. The following comments and recommendations emerged from the review and the conference call:

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 and the costs (burdens) 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.
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).
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.

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.
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.
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.
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.

C. Responses to Charge Questions

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.

In general, the group felt that the document contains the information needed to understand and implement the TEF methodology. There were some differences of opinion on the value of particular tables and figures and some reviewers felt that improvements could be made in the clarity of the document. Some specific comments and suggestions include:

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. During discussions the Group felt that a brief mention was appropriate.
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.
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.
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.
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.

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.
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.
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).
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?
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.

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.

In general, the group found the terms - ReP, RPF, and TEF – well defined. A few reviewers noted some confusion about the relationships among RePs, RPFs, and TEFs in various parts of the document. This could be spelt out better; a good technical editing job would help there. There were some comments about specific aspects of these terms as well as other words used in the document:

1. Why use the term ReP to represent Relative Potency? One should be able to represent two words with two letters (RP).
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.
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.
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.
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.
 - The symbol (II_{socw}) 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 K_d or K_p or K .
 - 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. 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.)

- 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.
- 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”.

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

Reviewers felt that the advantages are well explained. Specific comments and suggestions include:

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.
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 method applies to fish and wildlife (to distinguish it from what might be needed for invertebrates.) See Adams comment on *Daphnia*.

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?

The issue of bioaccumulation is addressed in more detail under Charge Question 8. In general the reviewers felt that there was adequate explanation but several comments and suggestions are offered to help clarify:

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.
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.)
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.
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 (Π_{socw}) and $D_{i/r}$ on pp. 40-41 is somewhat cryptic.

5) 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.

a) Please comment on the completeness and clarity of this discussion.

Reviewers generally found this discussion complete. However, there were a number of comments and suggestions related to clarity and the need for some additional guidance:

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 happens 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.
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.
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.
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.

b) 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.

Reviewers found the matrix helpful although its description would benefit from clearer writing. Reviewers noted that the highest levels (highest quality information) would rarely be available. One of the values of the matrix is that it could guide research efforts and this should be noted. Other comments and suggestions regarding the matrix include:

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.
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).
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

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.

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.

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

The qualitative discussion of uncertainties was adequate. However, reviewers had a number of comments and suggestions regarding specific sources of uncertainty and the possibility of indicating magnitude and direction of uncertainty:

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

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 from 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.
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.
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.

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.
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.

7) Are you aware of any essential references that have been omitted?

Reviewers typically provided suggestions for references within the context of specific comments. EPA should review these for contextual information. The following list reflects some but not all of the references cited by reviewers.

References on Toxicity

An updated version of Hahn 1998 is: Hahn (2002) Aryl hydrocarbon receptors: Diversity and Evolution, *Chem.-Biol. Interact.* 141: 131-160.

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Hilscherova, K., M. Machala, K. Kannan, A.L. Blankenship and J.P. Giesy. 2000. Cell Bioassays for Detection of Aryl Hydrocarbon (AhR) and Estrogen Receptor (ER) Mediated Activity in Environmental Samples. *Environ. Sci. Pollut. Res.* 7:159-171.

Kennedy, S.W., Fox, G.A. Trudeau, S. Bastien, L.J. and Jones, S. P. (1998) Highly carboxylated porphyrin concentration: a biochemical marker of PCB exposure in herring gulls. *Mar. Environ. Research* **46**, 65-69.

Lorenzen, A., Shutt, J.L. and Kennedy, S.W. (1997). Sensitivity of common tern (*Sterna hirundo*) embryo hepatocyte cultures to CYP1A induction and porphyrin accumulation by TCDD, TCDF, PCBs and common tern egg extracts. *Arch. Environ. Contam. Toxicol.* **32**, 126-134

Peterson, et al. (1993) Developmental and Reproductive Toxicity of Dioxins and Related Compounds: Cross-Species comparisons. *CRC Crit. Rev. Toxicol.* 23: 283-335.

Powell-Coffman, J.A., Bradfield, C.A., and Wood, W.B., *Caenorhabditis elegans* orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator, *Proc. Natl. Acad. Sci. USA* **95**: 2844-2849, 1998.

Prasch, A.L., Teraoka, H., Carney, S.A., Dong, W., Hiraga, T., Stegeman, J.J., Heideman, W., and Peterson, R.E.: Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish. *Toxicol. Sci.* **76**: 138 - 150, 2003.

Tanguay, R.L., Andreasen, E.A., Walker, M.K., and Peterson, R.E., Dioxin Toxicity and Aryl Hydrocarbon Receptor Signaling in Fish. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 15, pp. 603-628, 2003.

Theobald, H.M., Kimmel, G.L., and Peterson, R.E., Developmental and Reproductive Toxicity of Dioxins and Related Compounds. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 9, pp. 329-431, 2003.

Villeneuve, D.L., A.L. Blankenship and J.P. Giesy. 2000. Derivation and Application of Relative Potency Estimates Based on In Vitro Bioassay Results. *Environ. Toxicol. Chem.* 19:2835-2843.

References on Exposure

While the methods and equations presented are adequate for someone familiar with the science, a few key references on partitioning theory for non-polar organics would be good. You also might reference the following:

DiToro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, C.C., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning, *Environ. Toxicol. Chem.*, 10, 1541, 1991.

Non-particle flux of PCBs from sediments to the water column has recently been found to be an important transport route (the Framework does not mention it). At the top of P. 30 there is an opportunity to cite to this recent literature because the current sentence suggests that only particle transport is important. Broadening this with a citation can be helpful. This work has been carried out by Joe De Pinto at Limnotech and others.

There was an EPA Risk Assessment Forum workshop on Problem Formulation for assessing risks of dioxin-like compounds to fish and wildlife species that was chaired by Bob Hugget in the 1990s. At that workshop, there were several useful products one of which was a list of fate and transport models (prepared by Joe De Pinto and Paul Rogers) that might be useful for evaluating the fate and transport of dioxin-like compounds. The list was in the order of complexity.

References on Statistics

These references may be helpful:

Newman, MC, Dixon, PM, Looney, BB, and Pinder III, JE. 1989. Estimating mean and variance for environmental samples with below detection limit observations. *Water Resources Bulletin* 25(4): 905-916.

WDOE. 1993. Analyzing Site or Background Data with Below-Detection Limit or Below-PQL Values (Censored Data Sets). Supplement S-6, Statistical Guidance for Ecology Site Managers, Washington Department of Ecology, Olympia, Washington.

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

Many reviewers felt that the discussion of bioaccumulation and exposure is not adequate. There are at least two aspects of this:

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.

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.

Selection and Application of BSAFs and BAFs

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.
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.

Limitations of the Use of TEC in the Diet

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

Estimating Dietary Exposures and Tissue Levels from Water

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.

Discussions of Limitations on the BSAF Approach

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.
 - 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.
 - 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).

- 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.

Validation

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 then 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.

9) Please provide any other comments or recommendations you may have.

See line-by line comments provided in each reviewer's individual comments.

Individual Peer Reviewer Comments.

**Review by
William J. Adams, Ph.D.**

Review Of:

Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment

By

William J. Adams, Ph.D.

General Impressions

I thought the authors did a very nice job in putting together this guidance document and it should be a big help to both risk assessors and risk managers. I particularly liked the examples that were included in Tables 4-6. One other aspect of the report that was quite good was that specific recommendations were made in several places that give good practical guidance. For example, at the bottom of page 59, “However, in the absence of more specific RFPs for the species and endpoint of concern, the class specific TEFs-WHO are expected in most cases to be used” and at the top of page 70, “it is highly recommended that concentrations in abiotic media be converted to concentrations in diet or tissue using bioaccumulation factors and models as discussed...”

Major Comments

I have three comments to offer as areas for improvement.

- (1) Uncertainty analysis: The document provides in-depth discussion on sources of uncertainty, which should be useful for risk assessors and risk managers. However, there was no discussion of what to do with the uncertainty or how to quantify it or incorporate in the risk characterization. Left totally unquantified one cannot be sure whether or not the outcome of the risk assessment would indicate no risk, little risk, or serious risk. The original assessment that the TEQ approach accurate to approximately one half order of magnitude, was primarily drawn from professional judgment. The extent of the accuracy of the TEC method is unknown! The document sites an EPA report (USEPA 200c) and indicates that Monte Carlo analysis is not recommended at this time. I would like to suggest that Monte Carlo analysis could be a very useful tool to perform sensitivity analyses and to help identify most likely scenarios for the risk conclusions.
- (2) The hazard quotient method is mentioned several place in the document as one way of characterizing risk. This approach has merit in that it requires a minimum of data. However, a better approach would be to perform a simple probabilistic risk analysis (PRA) integrating the full range of the dose-response relationship for effects with the exposure distribution. Calculation of the joint probability of exposure and effects overlapping gives a much better estimate of risk. This approach utilizes the full dose response and exposure distributions, not just data greater than the NOEC or LOEC. The Agency has developed guidance on the use of PRA and several software programs make the computations relatively easy.
- (3) The example data sets in Tables 4-6 clearly show the effect of bioaccumulation and trophic transfer of PCBs, PCDDs and PCDFs to aquatic-linked terrestrial

species (birds, mammals). As a result the use of BSAFs to estimate tissue concentrations in birds and mammals will always underestimate risk for dioxin-like chemicals. The solution to this problem is to recommend that models such as Gobas (1993) be utilized when using BSAFs to estimate tissue concentrations for aquatic-linked species other than fish. This approach was discussed in the report, but it came short of recommend using this as a standard approach to reduce uncertainty and improve accuracy.

Response to Charge Questions

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.

I thought the document was well prepared; the examples were very useful as were the recommendations in the report. The tables and figures were clear, and very helpful as were the text boxes. A suggestion or two is provided later to improve one or two of the text boxes and figures.

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.

I think the report helps standardize terms and provides a useful reference for risk practioners to look for definitions and cross references to other terms. Two minor comments. Why use the term ReP to represent Relative Potency? One should be able to represent two words with two letters (RP)? I found the symbol (II_{socw}) used to describe the sediment-water concentration quotient rather unconventional. The use of the II in the symbol is not intuitive. Various symbols have been used to describe sediment water partitioning such as K_d or K_p or K . The use of “K” leads the reader to recognize a partition or distribution coefficient is being described.

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

The advantage of the method is adequately described. However, an example showing a comparison between the results using total PCBs, PCDDs, and PCDFs and the toxicity equivalence method would be interesting.

The document indicates, that in general, invertebrates are insensitive to 2,3,7,8-TCDD induced toxicity including the “dioxin-like” PCB congeners (page 20). I would like to add that PCBs expressed as aroclors (1248, 1254, 1260) are quite chronically toxic to

many invertebrates such as *Daphnia magna* and the toxicity follows their solubility and octanol-water partition coefficients.

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?

The discussion on estimating tissue concentrations is critical to this report and the overall methodology. While the methods and equations presented are adequate for someone familiar with the science, a few key references on partitioning theory for non-polar organics would be good. You also might reference the following:

DiToro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, C.C., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning, *Environ. Toxicol. Chem.*, 10, 1541, 1991.

The explanation referencing the application to mammals is adequate. However, I thought you should make give 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.

Question 5. 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.

a) **Please comment on the completeness and clarity of this discussion.**

The concept is clear and referencing the data set of Tillitt et al. (1991) was quite helpful.

b) **Are the matrix presented in Figure 4 and the examples used to illustrate the application of the matrix clear and adequately explained? Are there elements that should be added or removed from the matrix? Do you agree with their place in the tiers on the matrix? Please explain.**

Figure 4 is helpful in terms of visualizing the use of BSAF / BAFs and relating concentration to tissue relationships. However, this figure might be better if it were set up to mimic the EPA risk assessment paradigm. As it exists now it shows the output of the exposure assessment flowing into the effects assessment, which is not the way risk assessments are performed. The Characterization of Exposure Box and the Characterization of Effects Box should be parallel with the

output of the former being the application of the TEF/RPF leading to the TEC summation as shown in Tables 4-6. The output of the effects characterization should be a dose response curve as shown in Figure 6. The exposure and effects data can then be integrated to provide a risk estimate.

The following is a minor point. Figure 4 starts at the top with “Dose Metric-Specific Calculation of TEC.” Why use the word dose? The calculations are all made in terms of concentrations?

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

I believe the uncertainties associated with the application of the toxicity equivalence methodology are comprehensive and adequately explained. However, there is a lack of guidance regarding what to do with the uncertainty in terms of using the output of the methodology. See my major point number 1 above.

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

No. I did a small literature search of recent literature, while I found many additional references, I think you have included most of the pertinent ones.

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

The discussion is adequate. I would like to see Section 3.3.1.5 include other examples where bioaccumulation data has been used to assess the potential for effects from “dioxin-like” substances. Two or three references in addition to the data extracted in the report from USEPA (1995a), Guiney et al. (1996 and Government of Canada (1991) would be helpful. What I want to provide are examples of the way the data were handled and estimates were made so the reader can gain experience with the methodology.

Minor points: Page 34, line 12; I suggest adding the following sentences after “all routes of exposure.” These values are usually obtained from field-collected organisms.

Page 34, line 18; “bioaccumulation factors must also be congener- and species-specific.” Inclusion of the words species-specific might need some rethinking. The methodology presented (equation 3-1) assumes that bioaccumulation can be predicted on the basis of lipid normalization, which gets around species-specific bioaccumulation. Use of lipid normalization should minimize species differences, at least between fish and bird species when eggs are utilized.

Question 9. Please provide any other comments or recommendations you may have.

I am including several minor comments for consideration.

Text Boxes (*Italics reflect word additions*)

Text Box 2. I suggest the following for the 5th question. Conceptual Model – Does the conceptual model describe the relationship *and linkages* between sources, fate and transport, ~~and~~ bioaccumulation of dioxin like compounds, and exposures to identified *receptor* assessment endpoints? [I want to emphasize the importance of linking the exposure to the receptor.]

Text Box 5 – page 47. I suggest the following for the 4th question. Have I selected appropriate methods for measuring or estimating the fraction of organic carbon in the sediment *at the site of interest*?

I suggest the following for the 5th question. Have I measure or selected appropriate BAFs or BSAFs that will be used to estimate concentrations of each chemical in the organism's tissue or diet? *Have I considered implications of biomagnification for higher trophic level organisms?*

Text Box 5 – page 64. This text box is a duplicate of that on page 47.

Text Box 6 – page 63. The last question is missing a word “evidence?”

Figure 10. The use of color made it difficult to see the words in the lower right box.

Specific comments

Page 5, line 20. “Only the seventeen 2,3,7,8- substituted TCDD congeners were known to bioaccumulate.”

While the emphasis of this statement is correct, it would be incorrect to indicate that other congeners “do not bioaccumulate.” They bioaccumulate, but to a much smaller degree. However, they can be detected and their bioaccumulation factors are not zero. Likewise on Page 22, 6th line from the bottom should read “do not *significantly* bioaccumulate in pelagic invertebrates.”

Page 13, line 5. I suggest you change the word “dose” to “exposure.” The following sentences all refer to expressions of dose as concentration. Strictly speaking, dose is usually expressed in terms of mass.

Page 20, line 7. I suggest adding the following at the end of the paragraph. “Note, it should be pointed out that PCBs measured as aroclors have been shown to be chronically toxic to daphnids at low ppb levels.”

Page 26, number 2. Change to: “Determination of theoretical or empirical measures of exposure (*duration, frequency and intensity*).

Page 28, 2nd line from the bottom. “The data models and procedures are similar...” In reality the models are most likely are not the same in all cases. Later in the report it is mentioned that the ability to model specific chemical substances requires modifications to the models. I don’t think one would not expect to model exposure for all dioxin like substances with no modifications to the model. The sentence could be deleted.

Page 31, line 17. No real guidance was provided here. What do you expect the risk assessor to do?

Page 46, last two lines. These lines are repeated on the next page.

Page 71, line 17. “Alternatively, assuming that all dioxin-like chemicals found in the environment have toxicity potency equal to 2,3,7,8-TCDD would *significantly* overestimate risk posed by...”

**Review by
Scott B. Brown, Ph.D.**

Scott Brown

Chief, Priority Substances Effects Project
National Water Research Institute
Environment Canada

November 8, 2003

Peer Review of EPA's TEF Framework for Eco Risk

I have read this document and find that it represents a scientifically sound, understandable and reasonable approach for the application of toxic equivalent methodology. I only have cosmetic suggestions for consideration. The document meets its intended goals of introducing the toxic equivalence methodology as well as providing considerations and examples for use. I agree that the weight of evidence so far supports that an additive approach for the various congeners is warranted. Selection of optimal RPF or TEFs can be a difficult job for risk assessment because presently there is not enough data to clearly associate the sensitivity to dioxin-like chemicals in many species. I would prefer the selection matrix to be more prescriptive. Given the general lack of available information I agree with the general hierarchy discussed for RPF selection. Overall, the report does a reasonable job of discussing the sources of variability. The report also emphasizes the need for determining the concentrations of dioxins, furans and PCBs in diet or tissues. I have a few minor comments and typos listed below.

Specific Comments

The Introduction should acknowledge that there are other 'Ah' inducers (PAH's, flame retardants) that may contribute to dioxin-like toxicity but are not covered as part of this exercise. These are first mentioned on P11, 116 to 124.

P1, 128: 'which should' to 'to'

P6, 119 & 125: reconcile 13 vs 12 congeners

P6, 132: line ends in the middle of a sentence.

Table 2 outlines the WHO TEFs, I would have preferred some brief discussion here identifying the different endpoints used for derivation of TEF's in mammals, birds and fish. This is found later on in the document.

P19, 114-115: Fish as less sensitive organisms to mono-ortho substituted PCBs is dependent on the endpoint of concern. This is certainly not the case for recent studies where P450 enzyme induction has been assessed in dietary exposure studies.

P20, 126: I feel that owing to biomagnification that any ecological risk assessment 'must' rather than 'should' include higher trophic level species for these strongly hydrophobic toxins.

P32, 117: This explanation needs to be clearer, I not sure that I agree with the 'more accurate' comment. My opinion is that if the same amount of information were available re tissue burdens in mammals for RFP that this would be the preferable dose metric to use.

P39. In text box 4, symbols like C, fl and fsoc should be in italics exactly as they are portrayed in the formulas. Similarly P43, 17 & 18.

Why are sediment based TEC's calculated for biota in Figures 7 to 9 when in reality there is a need to consider the effects of bioaccumulation? I understand comparative aspects but don't see the need to demonstrate it.

P45. The need to consider ecosystem specific factors for BAFs or BSAFs is critical to proper general application. So I recommend highlighting lines 14 to 18. I might also consider inserting another case study to directly illustrate extrapolation to another ecosystem.

P64, 11: 'complete' for 'comlete'

P65, 110 to 13: I disagree with the comments here. The same metabolism issue exists for other analytical techniques for PCB 77. There are also other substances that produce 'dioxin-like' activity. I believe that 'false-positive' is the incorrect term to use. These assays are definitely very useful screening tools to use and positive responses invite more detailed chemical analyses. (see p66, 126-118)

I would place the "Conclusions" with the 'Preface', this simply strengthens the reason for developing the 'Framework' and provides the reader with a good overall introduction.

**Review by
Peter L. deFur, Ph.D.**

**Comments of Peter L. deFur, Ph.D. on
Framework for Application of the Toxic Equivalency Methodology for Polychlorinated
Dioxins, Furans and Biphenyls in Ecological Risk Assessment**

Question 1: 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.

The document is well done, properly organized and thought out by EPA. The level of detail and the length of the document are about right for an audience of EPA staff who are familiar with the issues and the processes. The absolute novice will be a bit lost, but such a staff will be at the entrance level and in training, or perhaps moving from another area of expertise. In either case, background documents and reports exist to provide a reader with a more detailed account and background if needed.

The readers should gain a clear understanding of applying TEF's to ecological risk assessment situations. The figures and tables are clear and well done, easy to follow. That said, EPA needs to insure that all figures and tables have 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.

Figures 7, 8 and 9 need to have the units of measure inserted for the tables that are part of the figures, just to be sure that the reader knows these are the same numbers in the figures.

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.

I have never been confused by the terms in the first place, but this scheme makes sense and is easy to follow. The definitions does a good job of clarifying the issue of meaning. EPA needs to get with the human health folks to be sure that the terminology is used in all aspects of TEF use and application.

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

The advantages are explained well, insofar as EPA does not really recommend an alternative approach. Alternatives are not really as accurate and effective as the TEF approach, as noted in the Framework document. This issue is and will be whether there is a way to compare older data obtained without congener analysis to recent data with detailed congener-specific analysis.

This methodology has been the subject of discussion in the scientific literature and regulatory arenas for some time now, so this Framework cannot really be viewed as a dramatic alteration of practice.

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?

I assume this question refers to the text and tables in section 3.3.1.4 on bioaccumulation, with the accompanying tables 4 and 5. If I restate the question as to whether or not the section is clear—the answer is almost. The Framework needs to work an example with numbers. Take the equations on page 35, apply them into the data in tables 4-5 and show the reader how the plan works.

The one issue that I have encountered on this matter is the one of non-detects (censored data) and the Framework is silent on the matter. This Framework is the last place to punt the issue to the reader or the risk assessors and managers. Essentially, the Framework is saying that EPA HQ either does not know how to deal with non-detects, does not care, or believes it has not the will or authority to recommend how the user should deal with levels below quantification. This approach is wrong and will cause huge confusion. Every Region then has the authority to use a different approach or a different method. Sites and cases beside one another will be using different approaches if it suits the managers or assessors. EPA has to make a decision here and recommend how to deal with low levels that cannot be quantified.

5) 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.

a) Please comment on the completeness and clarity of this discussion.

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 avoid the arbitrary outcomes that can and are now plaguing EPA in the Regions.

b) Are the matrix presented in Figure 4 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.

I think the figure is fine, but the legend is not detailed enough. The figure legend also has to refer to the accompanying text for explanation.

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

I think this section is unnecessarily brief and should be expanded. Section 3.4.3.1.1 suggests that there are non-Ah mechanisms of action, but is vague on the point. There are certainly non-Ah 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 PCB's.

Section 3.4.3.1.4 refers to the TEF's and RPF's as point estimates, yet fails to acknowledge that these point estimates were the result of a consensus meeting among scientists from different countries with different political issues operating. No matter what anyone claims, I am sure that

there is some degree of politics in the outcome. 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. These point estimates were 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 may account for huge uncertainty and natural variation, for all we know.

7) Are you aware of any essential references that have been omitted?

Not yet.

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

I think it works for the known cases – that is for fish, birds and mammals of the type that make up the database for the Framework. Animals that do not easily and obviously fall into the biological life styles and physiologies represented by the animals studied thus far. I am not sure that marsupials will fit into the specific conditions, though I see no reason why the general aspects described in the Framework will not work with them.

When scientists figure out what is happening with some of the other animals, such as invertebrates, lesser known vertebrates, then we will find out if the general approach applies.

9) Please provide any other comments or recommendations you may have.

The Framework is not meant for the naïve reader, who is not familiar with ecological risk assessment and the basics of Ah receptor toxicology issues (for TCDD, TCDF, PCB). I recommend that EPA add a paragraph in the introduction to the effect that the reader who is new to both fields will get lost in the TEF woods in a hurry. This paragraph should also point the reader to readings where background information is found and the reader can red up on the issues and then come back to this. The Dioxin Reassessment and Workshop Report (from the Jan 98 workshop) are two key readings on the subject. Others include the Van den Berg and Birnbaum papers and the chapter on PCB toxicity on the new Handbook of Toxicology.

**Review by
John P. Giesy, Jr., Ph.D.**

Review Of:

**Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated
Dioxins, Furans and Biphenyls in Ecological Risk Assessment**

By

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General Impressions:

The document provides a detailed description of the rationale for the application use of the toxic equivalency methodology for the risk assessment of PCDDs, PCDFs and PCBs. The document is comprehensive and well organized and clearly written. In general, this document is very useful and a much needed improvement on previously available documents and guidance. The authors indicate that the document is not meant to be a comprehensive review or a complete guide to risk assessments for dioxin-like compounds. The goal of the document was to provide only a summary of current best practices. There are clearly places where “best professional judgment” is required and I endorse the flexibility built into the guidance such that new and potentially more relevant information can be used where appropriate. The application of best judgment should be a cornerstone of these kinds of documents. In the following sections, I outline and discuss the limitations of the TEF framework and make recommendations as to how guidance can be incorporated into the framework. So that it is not thought that I have been overly critical of a useful document, I want to point out that this is a very complex issue with continually improving knowledge of the basic mechanisms and continuously greater amounts of data. Furthermore, 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.

Response to Charge Questions:

Question #1.

In general the document is well organized and written. The background section is clear and concise and provides sufficient information for the reader to understand the rest of the document. Additional details can be found in the references given. All of the pertinent references are provided. The use of the text boxes is effective. The use of the three- dimensional matrix (Figure 10) is not particularly effective. I personally like Figure 10 and understand it, and fully understand what the authors are trying to present, I am not sure that it is particularly useful in practice. It is not wrong, but just not very effective. The examples selected were appropriate and the information presented in each example is necessary and sufficient. In general the number of tables and figures is appropriate and I would neither add, nor remove figures or tables. The tables are clearly presented and useful. The discussion of the uncertainties is appropriate as written. While it would be difficult to do so, some readers may wonder why weightings or uncertainty factors are not provided. Some discussion of this topic and reasons why they are not provided would be useful.

Question #2.

In general I agree with the definitions of terms. I agree with the terms ReP, RPF, and TEF and their proposed definitions. I feel that it is important to have well defined and uniform nomenclature and terminology that is accurate and succinct for each of the parameters used in the calculations. However, for terms such as TEQ, which have been long used in the literature, I think it is a problem to change them. I do not support the change of terminology from 'TEQ' to 'TEC'. I think the term 'TEQ' is so well entrenched in the literature that introducing the new term 'TEC' would only add to the confusion. Instead, the term 'TEF' should be adopted and defined as being equivalent to the other terms. However, for the purposes of my review, I have retained the use of "TEC".

I suggest that EPA consider defining a term for TEC concentrations derived from bioassays of extracts from environmental matrices containing complex mixtures of AhR-active compounds. Because the bioassay accounts for infra- and supra-additivity, due to interactions between and among the AhR-active and inactive components of the mixture, it gives a fundamentally different value than the TEC. In part these differences may be due to the fact that the bioassay based TEC concentrations may include other classes of compounds that contribute to the total TEC but are not part of the targeted chemical classes analyzed in samples used in a risk assessment. The terminology applied in the guidance document, which builds on that proposed by the WHO is appropriate and should make the field clearer. Recently, I have noticed a great deal of confusion in the literature and in presentations due to this point. Furthermore, I have seen TEC data misused in mass (potency) balances where TEFs were applied instead of the bioassay specific RePs. I would simply indicate that it would be useful to have a term defined to apply to TEC that are derived by a bioassay so that they can easily be differentiated from those calculated from concentrations of individual congeners and application of TEFs. This would obviate the need to

write out “bioassay derived TEC”. It is often confusing which equivalency concentration is being discussed. Since this guidance document endorses the possible use of bioassays, it would seem to be appropriate to coin a term for this special situation. My research group has applied the term ‘TCDD-EQ’ or some other distinctive term to refer to concentrations of TEC in complex mixtures of AhR-active substances in biotic or abiotic samples (Blankenship, 1999; Coady, 2001). While this may not be the most appropriate term, it is one that has been defined and applied in the literature for almost 20 years.

Question 3.

I think that the document makes it very clear that the use of congener-specific analyses and the application of the TEF/TEC methodology is appropriate and provides a more accurate and scientifically-defensible assessment of risk. An example of the decrease in uncertainty or increase in precision and accuracy would be useful.

Question 4.

The discussion of the need to make congener-specific quantifications in target tissues is quite clear. However, I am less optimistic 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.

Question 5.

The information presented in Figure 4 is clear and adequately explained. I agree with the presentation. It just needs to be reiterated that the use of BSAFs is very variable and of limited utility. This is because they are species- and system-specific, depend upon physical factors such as grain size and composition, and depend upon how well the surficial sediment concentrations have been characterized over the foraging range of specific organisms.

Endpoint/Threshold Selection

A second issue concerns the use of the paper by Tillitt *et al.* (1996) as an illustrative example in the prioritization and selection of ReP for RPFs and the emphasis in this section on the derivation of threshold TEC values. This paper and a companion paper (Heaton *et al.*, 1995) presented information from a study that exposed adult ranch mink to carp collected from Saginaw Bay, Michigan and evaluated potential effects on survival and reproductive performance of adults and kits. As part of that study, tissue residue data for Saginaw Bay carp, mink diets, and mink livers are presented and discussed. While the study was well designed and included chemical analyses of both diet and tissue samples, the use of carp in the diet that were collected from a contaminated area also exposed the mink to co-contaminants. However, the significance of each chemical group in the overall toxicity of the contaminated diet to mink is difficult to ascertain. For instance, of the total TEQs measured in the diet at the LOAEL, approximately 82.6% of the TEQs were attributed to PCDDs and PCDFs (Tillitt *et al.*, 1996) while only 17.4% were

attributed to PCBs. However, when total TEQs were compared to H4IIE bioassay equivalents measured in extract from the diet, only 20% of the total activity was accounted for by the presence of PCDDs, PCDFs and PCBs whereas over 80% of the activity was the result of unknown agents (Giesy *et al.*, 1997). If all of the TEC were attributed to PCDD/DF/PCBs, the resulting TRV would overestimate the toxic potency of this complex mixture by a factor of about 5. Thus, the assumption that the PCDD/PCDF/PCB equivalents were the sole source of toxic equivalents that contributed to the adverse effects observed in the study may have overestimated the contribution of these chemicals to the toxicity observed in mink fed carp from Saginaw Bay, MI. Thus, while this study is an important source of information, relative to evaluating the risk of contaminants to mink in the Saginaw Bay, the presence of other co-contaminants were likely present at toxicologically significant levels. As a result, the use of this study is not appropriate to derive TRVs because of potentially confounding impacts of other co-contaminants on mink that have been accounted for in the study.

My concern is that by highlighting this particular study in the framework document, it implies that this study is the best study from which to derive TRVs for TECs in mink. If the EPA retains this study as their example, they should add a disclaimer that states that EPA is not necessarily endorsing this study for the derivation of TRVs. Alternatively, EPA could compare the Tillitt study with that of Bursian *et al.* (2003) which is a better and more recent example that includes many of the same authors that were part of the Tillitt *et al.* (1996) study. The Bursian study is based on a study in which mink were fed fish from the Housatonic River, MA but unlike the Saginaw Bay study, the Housatonic River is not heavily industrialized and the contaminants are mostly PCBs, PCDDs and PCDFs.

Question 6.

In general the major uncertainties are well discussed. The greatest uncertainty that needs additional discussion is the application of the BSAFs or the use of BAF values. I think some examples of the ranges of values should be added to the document. Perhaps some average values could be provided on a congener-specific basis.

While the TEF framework addresses issues of variability and uncertainty of the TEF methodology, it never discusses the magnitude of each of these issues in relation to each other for each of the sections outline in the report. 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. I further suggest some type of sensitivity analysis should be employed within the framework to evaluate those parameters that may have the greatest effect on the final outcome in the TEF methodology. For instance, the selection of a species specific ReP over that of a more general value for a single congener may only change the final TEC value by 1%. In contrast, the incorrect prediction of chemical concentrations in tissue via the inappropriate use of a BAF model could change the result by several orders of magnitude.

I think that the sections on application of BAFs and BSAF values are the weakest part of the proposed methodology. The propagation of concentrations of TEC through the various compartments of the ecosystem is clearly inappropriate because each congener not only has a

unique ReP or TEF, but also a unique BAF or BMF. This is particularly true of BSAF values (Froese *et al.*, 1998). It is my opinion that these methods will result in the greatest amount of uncertainty in the entire approach. While the currently proposed guidance is a great improvement over previous methods where concentrations of TEC could be predicted in one compartment from multiplying concentrations of TEC in another compartment there are still significant limitations to the use of predictive models to predict movement of TEC among compartments. I agree that accumulation factors need to be applied to individual compounds and then the concentrations of TEC in tissues should be estimated by the additive model of relative potency. However, I would suggest some additional refinements. First, I would suggest a probabilistic assessment. On page 71 (line 2) it is suggested that sensitivity analysis be conducted based on TECs that result from the use of alternative RPFs. I would agree with this suggestion and would further suggest that such a sensitivity analysis and resulting range of values should be applied to the entire risk assessment process. This would provide risk assessors and risk managers with an inclusive range of potential risks so that effective decisions could be made. I endorse this application of the use of a sensitivity analysis. While the distributions describing these uncertainties may be currently unknown, I think that this sort of analysis would incorporate the various uncertainties and indicate where additional information is needed.

Another one of the greatest uncertainties in the application of the method is the selection of appropriate toxicity reference values (TRVs). I would suggest adding more information on these uncertainties and also providing some guidance on how TRVs should be derived when being applied in the TEC approach.

Non-detects and TEF methodology

Minimal guidance is provided on how to handle 'less than' values which invariably occur in PCDD/F and PCB data sets. There are numerous proposed methods of handling this data but is the industry standard (use ½ MDL for less than values) the preferred method? The use of ½ MDL can lead to some overestimation of TECs in samples that are essentially not contaminated. Since the MDL values determined in PCDD/F analysis are usually sample specific, systematic errors can occur which result in increased TEC concentrations in samples due to the presence of interferences, not necessarily PCDD/Fs. These occurrences can make statistical analysis of data sets comparing 'contaminated' and 'reference' samples difficult, this is especially true when evaluating potential gradients within the environment where the relative differences between sites is not great, but near some threshold for effects to a species being evaluated in a risk assessment. The framework needs to provide concrete guidance on how <MDL data should be treated.

Question 7.

I have added some references to this review that support points that I have made. These could be added to the document if they add to the discussion. In particular, if a section on bioassays is added as I have suggested, the two key references would be:

Hilscherova, K., M. Machala, K. Kannan, A.L. Blankenship and J.P. Giesy. 2000. Cell Bioassays for Detection of Aryl Hydrocarbon (AhR) and Estrogen Receptor (ER) Mediated Activity in Environmental Samples. *Environ. Sci. Pollut. Res.* 7:159-171.

Villeneuve, D.L., A.L. Blankenship and J.P. Giesy. 2000. Derivation and Application of Relative Potency Estimates Based on In Vitro Bioassay Results. *Environ. Toxicol. Chem.* 19:2835-2843.

Question 8.

In general, the discussion is sufficient. But, I would make the statements on the limitations of the use of TEC in the diet 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 my 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).

I do not think that BSAFs should be applied unless they are site- and congener-specific. I have surveyed the literature and found that BSAF values for individual congeners can vary by as much as three orders of magnitude (1000-fold), depending on the hydrologic situation and species investigated. If the use of BSAFs is to be advocated, I suggest adding a discussion of the assumptions of the technique and the range of expected values and the limitations of the technique.

I agree with the authors of the guidance document that it is most appropriate to use TECs based on concentrations of concentrations of individual congeners in specific, target tissues. I further agree that the TEC approach can be applied in risk assessments based on the diet, but only if no information is available on tissue concentrations. However, due to potential differences in bioavailability and or metabolism and tissue disposition among congeners, this will lead to greater uncertainty in the risk assessment. I suggest adding text that advocates multiple lines of evidence in which dietary exposures and target tissues are both evaluated when possible.

My greater concern revolves around the recommendations for monitoring and modeling concentrations of PCDD/Fs in water up into upper trophic level wildlife. It is my opinion that, at this time, the values available for general application of BAF and BSAF values are too variable (often by several orders of magnitude) to be of any use in a risk assessment. In general, if the species- and tissue-specific concentration information is available to derive site-specific BAF and BSAF values, it should be preferentially used in place of predicted or literature values. If this approach is applied, it should be done only with site-specific values.

As mentioned above, water is an irrelevant matrix for exposure to and regulation of PCDD/Fs since, in most circumstances, the primary vector of PCDD/Fs is via dietary intake and not through accumulation from water. In addition, in various sections (e.g. Page 40, lines 11-32) in the document, there is discussion of the use of freely dissolved water concentrations in the risk

assessment procedure. PCDD/Fs and coplanar PCBs are highly lipophilic and so their freely dissolved concentrations are highly variable and are dependent on a variety of conditions including temperature, pH and organic carbon (both particulate and dissolved). Numerous studies have demonstrated that water is only a minor source of these highly lipophilic compounds even in aquatic organisms that could bioaccumulate PCDD/Fs directly from water. Therefore, estimates of the dissolved concentrations of PCDD/Fs are of little practical value in risk assessment since water is not a relevant matrix for PCDD/F exposures. Thus, these predictions would be so variable as to be of little use in risk assessments. The bioavailability of the PCDD/DF would be the greatest uncertainty in these models (Luthy et al., 2003)

While the TEF framework includes the use of BAF/BSAF models as a means to estimate tissue concentration in receptors of concern from environmental concentrations, it does not outline the limitations of this approach. In particular, it 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) (Lyytikainen *et al.*, 2003). As a result, many of these models can lead to overestimates of concentrations of PCDD/DF in tissues and thus their related potential risks. If BMFs and BSAFs are used to predict concentrations of PCDD/DF in tissues, an upper and lower bound should be given for the concentrations of each congener and this range of values propagated through the calculation of the TECs in tissues. In conclusion, I agree with the authors of the guidance document when they say that “When TECs in organisms of concern are unknown, they may be calculated from chemical concentrations in water, sediment, or soil only if appropriate bioaccumulation factors are available to relate the concentrations of each congener in the media to concentrations in the organism or its diet”. However, I suggest adding that such an approach may result in considerable uncertainty spanning one or more orders of magnitude.

The section on “Selection of ReP and/or RPFs” is well done and presents a good background on the sources of uncertainty in the analysis and gives good guidance on how to select appropriate values. This section should, however, provide stronger guidance and decision making tools. The selection of the appropriate RPFs is critical to the success of the approach, and, as it stands, the reader is essentially given free license to mix and match different RPFs from different species and studies. For instance, RPFs between closely related species are not necessarily similar and while the example of two trout species may be appropriate, there are instances between similar species where there are large differences between RePs. As a result, this concept needs to be elucidated and expanded. However, we do agree with the concept that RPF rank orders should be transferable between species.

Question 9 Specific Observations:

Page 3, Text Box 1. Use of the term TEC throughout the document represents the introduction of another acronym to a field already replete with them. The term TEQ has been almost universally applied and accepted to describe the total concentration of equivalents. I see little reason to introduce TEC as a new acronym even if it does demonstrate adherence to EPA's standard procedures for abbreviation.

Page 11, lines 21-22. The text suggests the PBDDs and related compounds are used as flame retardants!? While PBDEs are used for this purpose, the other chemicals listed are not directly or intentionally used. It should be noted that the conclusion of the paper by Giesy and Kannan (1998) was that under the conditions examined, the AhR-mediated effects were the critical effects. That is, that they would occur at the lesser concentration of complex mixtures than would the non-AhR-mediated effects. Thus, while the other types of effects could occur, that the use of the TEF approach, based on TEQs derived from the AhR-mediated effects would be protective and thus, the most appropriate risk assessment. This paper provided support for the conclusions presented in the EPA guidance document.

Page 13, lines 21-24. This can be a major source of uncertainty and variability in estimating TEC concentration in animals from environmental media such as water, sediment and soils. In many cases, the models can either overestimate or underestimate actual tissue concentrations by orders of magnitude thus introducing considerable uncertainty into ecological risk assessment. This aspect of this approach needs to be included in the framework to better prepare assessors.

Page 21, line 12. This not is not completely correct, since the analysis by Giesy and Kannan, 1998 did use the proposed WHO TEFs.

Page 23, line 18. This statement should be referenced since it is not necessarily true and may be a consequence of the ratio of PCDD/F to PCB concentration in the environment or exposure of the organism.

Page 26, line 11. Using the "quotient method" may be an overly simplistic approach given the complexity and degree of uncertainties involved in the risk assessment of TEQs.

Page 28, line 5. Section 3.2.1.4 is the weakest section of the document. It contains useful information, but it is poorly organized and needs to be reorganized and rewritten so that it is better focused. It is unclear whether the discussion pertains to determining for which species it is appropriate to apply the TEC approach or if it is a discussion of the reasons for variation in sensitivity (responsiveness or relative responsiveness-meaning that different TEF or ReP or RPF values would be used for different classes or species.) Each of these issues is relevant and should be discussed, but under separate headings. First, a discussion of whether the TEC approach is appropriate, then, a discussion of the appropriateness of the various TEFs, as discussed by van den Berg et al., 1998, should be given. In this section, the issue or differences in relative potency should be undertaken. Finally, a section that discusses the relative sensitivities of species to TEC, not TEF, should be written. The entire issue of selecting the

proper species-specific threshold value or toxic reference value (TRV) is more difficult than the overall derivation of TEF values.

Page 32, lines 13-16. This statement implies that estimation of tissue concentrations is a relatively straightforward and robust procedure – it is not.

Page 40, line 26. This statement needs to include some statement relative to the accuracy of the predictions of the BAF/BASF models. For instance, how valid are the predictions of these models relative to measured values in cases where both approaches have been evaluated. The use of BAF/BASF models can be a major source of uncertainty and can grossly overestimate the concentrations of these compounds in aquatic organisms.

Page 41, line 13. Again, the framework should include some mention of the types of adjustments that need to be evaluated and included in the application of BAF and BASF models. At a minimum, additional references should be included that give examples of the types of adjustments needed to use the models.

Page 46, line 11. This statement needs to include some information relative to the quantification of uncertainties when using these models to estimate tissue concentrations.

Page 46, lines 31-32. Seems to be a typographical error resulting in the repetition of part of the previous sentence.

Page 54, line 25. This sentence does not make sense and needs to be re-written.

Page 61, line 5. This is not necessarily true in that differences in exposure regime and purity of chemicals can have a significant effect on results of the derivation of a ReP or RPF. All aspects of study design and implementation need to be evaluated prior to substituting one value for another.

Page 61, line 9. This statement is misleading in that it does not accurately portray the effect of study design, chemical purity, and other experimental parameters on toxicological endpoints other than induction.

Page 62 Text Box 5 is repeated here.

Page 65, lines 9-16. Bioassay approaches can be used in a TIE approach to demonstrate that PCDD/Fs account for a certain proportion of the TEC.

Page 66, lines 20-31. There is some evidence for non-additive effects but interactions are not a major source of variability. The statement as presented seems to indicate that interactive effects have been shown to not occur. This is not the case and the text needs to be modified to indicate this. While interactive effects do occur, the magnitude of the effects is generally negligible in the context of a TEF approach.

Page 67, lines 28-30. How should estimates of variability of REPs be carried over into the TEC calculation? While this aim is laudable there is a need to explain how this variability is incorporated into TEQ calculations and presented in the resultant TEQ estimations.

Page 69, lines 17-20. Water is an irrelevant matrix for determination or monitoring.

Page 70 lines 32-34. Non AhR mediated effects occur only at much higher concentrations and so are generally of less relevance than reproductive and developmental effects which may affect species populations.

Page 71, line 2. I endorse this application of the use of a sensitivity analysis.

Tables

Table 3. The word chicken needs to be fixed in the table. In addition, do fish have chloracnegenic effects?

Tables 4, 5, and 6. Column 4 consists of "Predicted" concentrations and should be edited to show this fact.

References:

Blankenship, A.L. and J.P. Giesy. 1999. Use of Biomarkers of Exposure and Vertebrate Tissue Residues in the Hazard Characterization of PCBs at Contaminated Sites: Application to Birds and Mammals. Chapter 9, pp. 153-180, In: G.I Sunahra, A.Y. Renoux, C. Thellen, C.L. Gaudet and A. Pilon. (Eds.) *Environmental Analysis of Contaminated Sites: Toxicological Methods and Approaches*. John Wiley and Sons, New York.

Bursian, S.J., R.J. Aulerich, B. Yamini, and D.E. Tillitt. 2003. Dietary Exposure Of Mink To Fish From The Housatonic River: Effects On Reproduction And Survival. Revised Final Report to USEPA, June 10, 2003. Available at: http://www.epa.gov/region1/ge/thesite/restofriver/reports/final_era/SupportingInformation%20and%20Studies%20for%20the%20HousatonicRiverProject/Dietary%20Exposure%20of%20Mink.pdf.

Coady KK, Jones PD, Giesy JP. 2001. 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents from tissue samples from the Rocky Mountain Arsenal Commerce City, CO. *Environmental Toxicology and Chemistry* 20(7): 2433-2442.

Froese, K.L., D.A. Verbrugge, G.T. Ankley and J.P. Giesy. 1998. Bioaccumulation of Polchlorinated Biphenyls from Sediments to Aquatic Insects and Tree Swallow eggs in Saginaw Bay, USA. *Environ. Toxicol. Chem.* 17:484-492.

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- Lyytikainen, M., Hirva, P., Minkkinen, P., Hamalainen, H., Rantalainen, A.L., Mikkelsen, P., Paasivirta, J., Kukkonen, J.V.K. 2003. Bioavailability of sediment-associated PCDD/Fs and PCDEs: relative importance of contaminant and sediment characteristics and biological factors. *Environ. Sci. Technol.* 37: 3926-3934.
- Tillitt, D.E., Gale, R.W., Meadows, J.C., Zajieck, J.L., Peterman, P.H., Heaton, S.N., Jones, P.D., Bursian, S.J., Kubiak, T.J., Giesy, J.P., and Aulerich, R.J. 1996. Dietary Exposure of Mink to Carp from Saginaw Bay. 3. Characterization of Dietary Exposure to Planar Halogenated Hydrocarbons, Dioxin Equivalents, and Biomagnification. *Environ. Sci. Tech.* 30:283-291.

**Review by
Mark E. Hahn, Ph.D.**

**Comments on EPA's Draft Document Entitled
*Framework for Application of the Toxicity Equivalence Methodology for
Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment***

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November 2003

General Impressions:

This document ("The Framework") provides a nice summary of the toxicity equivalence methodology and its application to ecological risk assessment. It is well organized, and for the most part clearly written. The document draws heavily on the results of the 1998 workshop (U.S. EPA, 2001a) and thus incorporates many of the scientific and methodological concepts and some of the written material produced in that workshop. The current Framework document expands upon and updates the previous work and provides it in a format that will be more useful for those wishing to understand the advantages and limitations of the toxicity equivalence approach and how this approach can be applied in ecological risk assessment.

Overall, the authors have done a fine job of organizing and presenting the information. Most of the ideas, methods, and concepts are clearly expressed, although there are places where clarity could be improved by modifying terminology or eliminating some of the risk assessment jargon (e.g. use "chemical" instead of "stressor"). The examples provided in the text, tables, and figures are particularly helpful in illustrating the methodology and its application in different circumstances. There may be places where additional examples would be beneficial.

Responses to Charge Questions:

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.

• The document is well organized and, overall, very effective. The figures and tables are quite useful, especially as they illustrate concepts discussed in the text or provide examples of the calculations and the application of the toxicity equivalence methodology. I found the "text box" questions (text boxes 2, 3, 5, 6) to be less valuable, although it is possible that risk assessors would better appreciate this aspect of the document.

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.*

- Most of the terms are clearly defined and well justified. The term “relative potency factor” (RPF), which is introduced here for the first time, initially puzzled me. However, its value became clear as I read the document. The term “relative potency” is used appropriately, although the abbreviation “ReP” should be simplified to “RP” (the “e” serves no purpose).

Other terms are potential sources of confusion. 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. 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.)

3) *Please comment on whether the advantages of using the toxicity equivalence methodology are adequately explained.*

- The advantages of the toxicity equivalence methodology are described primarily in sections 1.2 and 4. Although the advantages are mentioned and are clear to those of us working in the field, I’m not sure that someone without such experience would be able to easily extract that information from this document. It might help to have a specific section titled “Advantages of toxicity equivalence approach over other approaches for assessing the risks of dioxin-like chemicals” that clearly describes these in one place.

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?*

- The rationale for using tissue concentrations as the dose metric for exposure, TEF/RPF, and effects in risk assessments involving birds and fish is clearly described in section 3.3.1.3. 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 (Π_{socw}) and $D_{i/r}$ on pp. 40-41 is somewhat cryptic. Tables 4, 5, and 6 are very helpful in illustrating the methods and calculations

described in the text. The explanation (p. 32) of the application to the methodology to dietary exposure in mammals, as distinguished from fish and birds, is adequate.

5) 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.

a) Please comment on the completeness and clarity of this discussion.

- The selection of RPFs is described primarily in section 3.3.2 (p. 46ff). The potential benefits of using RPFs over TEFs are nicely described on p. 47. Overall, this discussion is very clear except as noted below.

- On page 49-50 the authors make an important point: that the issue of relative potencies is separate from that of species sensitivity to TCDD. They go on to discuss some of the data that support this idea, then somewhat confusingly conclude that “there are insufficient data at present to determine if there is any association between sensitivity to TCDD and relative potencies...” My view from looking at these data (and from a mechanistic understanding) is that present data suggest that relative potencies and sensitivity to TCDD are largely, if not completely, independent. In other words, if species A is more sensitive than species B to TCDD, then species A is also more sensitive than species B to other chemicals that act via the AHR, and the potencies relative to TCDD are similar in the two species. I agree that more data would help clarify this issue. But the way this paragraph is written is self-contradictory, with the topic sentence and concluding sentence reaching nearly opposite conclusions.

b) Are the matrix presented in Figure 4 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.

- I assume that this question refers to Figure 10 rather than Figure 4.

- The elements in the *endpoint specificity axis* of the matrix are modified from those put forward at the WHO workshop (van den Berg et al 1998); their selection and placement are well justified and clearly described (section 3.3.2.2.1.).

- The elements in the *species specificity axis* are modified from the matrix designed at the 1998 EPA/DOI workshop (U.S. EPA 2001a, pp. C-E-20ff). These also seem appropriate and clearly described (section 3.3.2.2.2.).

- The *dose specificity axis* is new in this document, and represents an important addition to the matrix. However, the axis and its description (section 3.3.2.2.3) are in need of clarification with regard to the following points:

- i) The tiers portrayed in the figure (tiers 1 through 4) are not matched by the tiers described in the text (p.53). Tier 2, for example, is listed as “measured in organism” in the figure but as “administered dose” in the text. I will refer to the tiers according to the figure.

- ii) The dose specificity axis is illustrated in Figure 10 and described in the text in terms of “Tiers”, with Tier 1 (dose measured in tissue) being portrayed as more

- desirable than Tier 3 (Administered dose). However, it is clearly more appropriate to use RPFs based on administered dose when administered dose is used also for the exposure and TCDD dose-response metrics. Perhaps the tiers on this axis should not represent specific dose metrics but rather the degree to which the dose metric used to generate RPFs is consistent with that used for the exposure and TCDD dose-response data. (Tier 1 = same dose metric used for all; tier 2 = dose metric for RPF one step removed, etc)
- iii.) The opening paragraph of section 3.3.2.2.3 (p. 52) is confusing.
 - iv.) There is a need for greater precision in the writing. For example, in several places in section 3.3.2.2.3 and the legend to Fig. 10 the words “dose” or “dose data” are used, whereas I think the more specific phrase “dose metric” is meant.

- The 3 examples used to illustrate the application of the matrix are extremely helpful.

Example 1 nicely illustrates the use of incomplete RPF data sets.

Example 2 considers how to balance uncertainties arising from species extrapolation with those from extrapolation across endpoints. This example also shows how the choice of RPFs must be made independently of the choice of TCDD dose-response curve to be used in the risk assessment. It might be possible to more clearly differentiate these two issues in the paragraph on p. 54.

Example 3 starts off with a very clear statement of the need for consistency in dose metric and provides a nice example (mink) of how this might be considered. Overall, this example is excellent. However, I am not sure I agree fully with the conclusion (p. 57 lines 28-30) that “the mink liver chemical residue data provide a more direct and precise measure of exposure than...dietary exposure”. The issue of using tissue measurements of dose is a complicated one because the tissue usually cited (liver) is not necessarily the target tissue for the effects of concern (reproductive toxicity). So does expressing doses as concentrations in liver really get us any closer to the effects of interest than expressing dose as concentrations in diet?

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

- Overall, the document captures most of the uncertainties quite well.

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 “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.

7) *Are you aware of any essential references that have been omitted?*

- There are a few important references that have been omitted, are inappropriate, or are out of date. These are noted in the specific comments below.

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

- Yes

9) *Please provide any other comments or recommendations you may have.*

Specific observations:

- p. vii, , The authors and contributors section should acknowledge the conceptual and written contributions of the participants in the 1998 workshop, since some of the writing was taken verbatim from the workshop report (which included written contributions from many of the scientists present at that workshop).
- p. 1, Line 16, The phrase “cumulative” effects is used to refer to the effects of mixtures of dioxin-like compounds. Does “cumulative” imply a time factor rather than a summing over many compounds? Would “integrated effects” (as used later - line 23) or “combined effects” be better? (See also page 15, line 21).
- p. 10, Line 23, It might be useful to mention the criteria for an effect being considered “AHR-mediated”: effect does not occur in AHR-null mice (or fish) or AHR-deficient cells.
- p. 11, Line 17, Statement about diverse structures of AHR ligands should cite Denison and Nagy (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals, *Annu Rev Pharmacol Toxicol* **43**: 309-334.
- p. 18, Line 9, An updated version of Hahn 1998 is: Hahn (2002) Aryl hydrocarbon receptors: Diversity and Evolution, *Chem.-Biol. Interact.* **141**: 131-160.
- p. 18, Line 9, It might be useful to provide more information regarding the species- and class-specific differences in AHR number and function, to illustrate the complexity of this issue. Dick describes the situation in zebrafish, which have two AHRs, only one of which (AHR2) is active. However, it needs to be made clear that the zebrafish results should not be generalized to all fishes. For example, in the Atlantic killifish (the species in which AHR2 was first identified [Hahn et al 1997; Karchner et al 1999]), both AHR1 and AHR2 are active. Moreover, in other fish species there are additional AHRs; for example, there are four in medaka and five in the pufferfish *Fugu* (our unpublished results). There are additional AHRs also in salmonids (Abnet et al 1999; Hansson et al 2003). In addition, there are two AHRs in some species of birds (our unpublished results). It is not yet clear whether these differences in AHR diversity and function play a role in species differences in sensitivity to toxicity.

References cited:

- Hahn, M.E., Karchner, S.I., Shapiro, M.A., and Perera, S.A. (1997) Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 13743-13748.

- Karchner, S.I., Powell, W.H., and Hahn, M.E. (1999) Identification and functional characterization of two highly divergent aryl hydrocarbon receptors (AHR1 and AHR2) in the teleost *Fundulus heteroclitus*. Evidence for a novel subfamily of ligand-binding basic helix-loop-helix Per-ARNT-Sim (bHLH-PAS) factors. *J. Biol. Chem.* **274**: 33814-33824.
- Abnet, C.C., Tanguay, R.L., Hahn, M.E., Heideman, W., and Peterson, R.E. (1999) Two forms of aryl hydrocarbon receptor type 2 in rainbow trout (*Oncorhynchus mykiss*): Evidence for differential expression and enhancer specificity. *J. Biol. Chem.* **274**: 15159-15166.
- Hansson, M.C., Wittzell, H., Persson, K., and von Schantz, T. (2003) Characterization of two distinct aryl hydrocarbon receptor (AhR2) genes in Atlantic salmon (*Salmo salar*) and evidence for multiple AhR2 gene lineages in salmonid fish. *Gene* **303**: 197-206.

- p. 18, Line 13, The invertebrate dioxin-binding proteins identified in Brown et al 1997 are unlikely to be AHR homologs. See Butler et al 2001 paper for cloning and binding analysis of invertebrate AHRs.
- p. 18, Line 30, “differences in exposure regimes.” An important reference for this is Peterson, et al. (1993) Developmental and Reproductive Toxicity of Dioxins and Related Compounds: Cross-Species comparisons. *CRC Crit. Rev. Toxicol.* **23**: 283-335.
- p. 21, , Table 3: Needs updating. For example, AHR has been found in guinea pig; binding of AHR complex to DRE has been shown in avian wildlife and marine mammals.
- p. 22, Line 16, “PCDDs etc ...do not biomagnify via diet in invertebrate food chains” Is this really true? Don’t lobsters (for example) accumulate these compounds from their prey?
- p. 30, Line 26, Insert underlined word: “to obtain predicted concentrations”
- p. 36, , Table 4 / general question: Relative potencies used to generate TEFs are usually derived from molar ratios of TCDD potency and congener potency. However, TEC calculations usually apply these TEFs or RPFs to concentrations expressed as masses (ng/kg). Is the error introduced by this of any significance? I expect not in the case of TEFs, which are half-order of magnitude estimates. But what about RPFs?
- p. 40, Line 1, “following two equations” Where does this sentence begin??
- p. 40, Line 30-31, This line states that $D_{i/r}$ is the difference between Π_{socw} values but text box 4 says “ratio between Π_{socw} values for”. Which is correct? Also, words are missing from bottom of text box.
- p. 46, Line 31-32, Lines repeated on p. 47.
- p. 49, Line 27, Insert underlined word: “...suggest that greater species sensitivity...”
- p. 50, , Fig. 10 legend, insert underlined words: “...how similar a reported dose metric is to the dose metric of concern used to define TEFs and the TCDD dose-response relationship.”
- p. 51, Line 30, Insert underlined word: “When level 4 data for some congeners are in agreement...”
- p. 54, Line 1, Better reference for PCDFs as contaminants in PCBs is: Goldstein, et al. (1978) 2,3,7,8-Tetrachlorodibenzofuran in a commercially available 99% pure

polychlorinated biphenyl isomer identified as the inducer of hepatic cytochrome P448 and aryl hydrocarbon hydroxylase in the rat. *Drug Metab. Dispos.* **6**: 258-264.

p. 58, Line 12, "...closer to the H4IIE-RPFs than rat liver H4IIE-RPFs"?????

p. 63, , Text box 6: words missing at bottom (same true of some others).

p. 64, , Text box 5 is repeated here (first appears on p. 47).

p. 65, Line 2, Other recent reviews on this topic: Giesy, et al. (2002) Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Mar. Poll. Bull.* **45**: 3-16.

Hahn (2002) Biomarkers and Bioassays for Detecting Dioxin-like Compounds in the Marine Environment. *Sci. Total Environ.* **289**: 49-69.

p. 68, Line 12, "in the report" What report?

**Review by
Barbara L. Harper, Ph.D.**

TEF Framework comments

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1. Overall effectiveness in applying the methodology correctly; organization, appropriate level of detail, usefulness of figures?.

Could be an exec summary to a larger doc, given the huge amount of studies. Overall, I think this should help with the consistency of PCB-dioxin eco risk assessments, and therefore standards and cleanups.

Overall purpose is not quite clear. I have to use this within a regulatory framework, so I need to start with an “official” TEF and BSAF table in order to be accepted by regulators, EPA included. Is Table 2 the “official” generic table that should be used if other species-specific TEFs are not developed? EPA has officially adopted the WHO 1997 TEF ratios for human risk assessment – is this supposed to be the same as the “mammalian” column in Table 2? The mono, di, tri, and tetra homologues also have TEFs for humans; why not for mammals? In fact, given the statement that every ecosystem is likely to contain a known sensitive species, why not simply use Table 2 values (presumably for the most sensitive mammal, fish, and bird) instead of going through all the species specific RPF or TEF factors? At least this would tend to err on the side of precaution and protection. (Actually, the very last page of the text does mention this – it would help to put it up front, too.)

How does this document relate to the dioxin reassessment? People are mammals, so will Table 2 be revised based on the new studies? The (more or less) official position of the courts with respect to relying on the dioxin reassessment is that EPA cannot rely on the reassessment until it is promulgated, but it can rely on the same studies and come to the same conclusion. So, since dioxin is 6-10 times more toxic than thought, does Table 2 reflect this finding? Or, does the “mammalian” term refer to mink as the most sensitive species and not to other mammals?

What prevents endless arguing about species specific TEFs that are less stringent than Table 2? How do we know when we have reached a ‘weight of evidence’? Is the burden on regulators and affected communities to find the studies that a PRP omits? Will there be a set of approved references for general application of this method, and if so, why not develop a more complete table of TEFs for more species and more endpoints? This would relieve the huge burden on individual practitioners and regulators to develop these from scratch. However, if the most sensitive endpoint should be used for a risk assessment, why can’t we pick that now? Yes, the biological relevance of some endpoints such as enzyme induction is not clear; an ecological reference dose should still be able to be picked. As a

practitioner, I can imagine spending a large amount time arguing about endpoints, too, as well as which endpoints are relevant to populations.

2. Does it help clarify terms and resolve inconsistencies in terminology?

TEC v TEQ? TEQ is in common use in human risk assessment – is TEC the ecological equivalent? Equation 2-1 looks like TEQ. Add TEC definition on page 4, and explain how it is different from TEQ in common usage.

3. Are advantages of using TEF methods explained?

Yes

4. Is the method for estimating tissue concentrations adequate? Does it explain any difference between mammals, fish, and birds?

Yes. But see comments below on whether Table 2 is a default table for the most sensitive mammal, fish, and bird for cases where it is not practical to collect a lot of expensive site-specific congener data.

5a. Is the TEF selection method for specific species, endpoints, and doses of concern complete and clear?

Yes

5b. Is Figure 4 and examples adequately explained? Are the tiers placed correctly?

OK with a little study and rereading the text a couple of times. However, I was looking for the dose-response section (lower tier in the figure), which is mentioned in 3.4.1, but no real guidance is provided.

6. Are the uncertainties explained completely and clearly?

They are discussed, but no real sensitivity or uncertainty analysis is possible generically.

7. Are there essential references that have been omitted?

Not my area of expertise (I'm not sure).

8. Is the discussion of exposure and BAF adequate for use with TEF methods in a ERA?

BAF 3.3.1.4 Include web addresses for official EPA BAFs, since this is where we have to start, or justify why we are not using them. It would help to show an example of bioaccumulation moving all the way up the food chain. If dioxins bioaccumulate a million-fold, it would help to include a reality check showing TEQ (or TEC) for each icon in one of the conceptual models.

3.2.3 Analysis plan. The list should include a method for BAF and a DQO so that data useful for BAF is collected.

9. Other comments.

It has been 5 years since the workshop; how has more recent data been included in Table 2? Are the endocrine disruptor effects incorporated? This needs to be added in section 3.2.1.1

This method requires congener analysis, such as 1668A which is not promulgated yet. 3.3.1.1 should mention 1668A as the method of choice.

Text Box 3. The question is asked whether I have “obtained bioaccumulation factors” but I don’t think that “obtained” is the right word – I either look them up somewhere, or develop them from literature, or collect site-specific data so I can calculate them.

**Review by
Bruce K. Hope, Ph.D.**

PEER-REVIEW COMMENTS by BRUCE HOPE
DRAFT Framework for Application of the Toxicity Equivalency
Methodology for ...

General Impressions

I thought the document was well organized, generally clearly written, and addressed key issues associated with the TEF methodology without undue detail. Although this is not my area of expertise, the discussion of the toxicology and adverse effects of PCDD, PCDF, and PCBs appeared succinct yet informative. In general, I have three areas of concern regarding this guidance in its current form (which are discussed in greater detail under the charge questions and specific observations below: (1) Reliance on hard-to-obtain BAFs/BSAFs as the sole means of exposure estimation, (2) Focus mainly on the advantages of the TEF methodology, with no balancing discussion of its disadvantages, and (3) Offering the possibility of site-specific RPFs in lieu of using consensus TEFs, without apparent recognition of how this might affect regulatory review.

Response to Charge Questions

(1) A main goal of this document is to assist ecological risk assessors in applying the toxicity equivalency methodology correctly. Please comment of 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.

Overall, I feel that this guidance is useful, as is, to practitioners, particularly those working large, well funded sites. I do feel, however, that it would be useful to a greater range of practitioners, and in a more equitable fashion, if it directly addressed both the benefits and the costs (burdens) 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.

While I'm not suggesting that this document should delve deeply into these non-technical issues, side-stepping them is ultimately not helpful to practitioners.

Organizationally, I would suggest adding a comparison of alternatives to the TEF methodology in its own section within the Introduction section so that the comparative benefits and costs of the method are readily available for review. I would also suggest moving all of Section 4 (Conclusions) to the front of the document as an "Executive Summary". 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.

(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.

I found this to be a very useful clarification of the plethora of terms, definitions, and acronyms that have become associated with the TEF methodology.

(3) Please comment on whether the advantages of using the toxicity equivalency methodology are adequately explained.

The document is heavily weighted toward listing and discussing only the advantages of the TEF methodology. I feel strongly that it is just not enough to extol the virtues of the TEF approach without providing information to help counter other, possibly less credible (but also less costly) approaches. It was suggested during the first teleconference that this guidance assumes that the decision to use the TEF approach had already have been made and therefore other approaches need not be discussed here. But if not here, then where? I think this is the place to include a more balanced assessment of the this

methodology including some of its disadvantages (such as the increased cost of congener-specific analyses) or an adequate discussion of what alternatives to the TEF a responsible project manager (RPM) may have to sort through.

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

While I fully agree that tissue concentrations are the appropriate place at which to apply the TEC, offering primarily BAFs/BSAFs for exposure estimation without a full appreciation for the significant challenges associated with obtaining these on a site-specific basis, is problematic. Conversely, 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. I also do not feel, based on experience, that “extrapolation” is a non-controversial way around any of these challenges.

(5) 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. (5a) Please comment on the completeness and clarity of this discussion (5b) Is the matrix presented in Figure 4 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 of the matrix? Please explain.

This guidance offers a well thought out process for developing site-specific RPFs in lieu of using consensus TEFs. No mention is made, however, of the additional burden such flexibility could potentially place on the regulatory review process or the opportunities such flexibility could afford for spurious manipulation of results. As is suggested at several points in the text, TEFs-WHO₉₈ are likely to be used at the great majority of sites. The benefits associated with having site-specific RPFs are, particularly at smaller, less well funded sites or jurisdictions, likely to be out-weighted by the greater benefits (ease of use, consistency, acceptability (lack of contention), and ease of review) associated with international consensus based TEFs.

(6) Please comment on whether the uncertainties associated with the application of the toxicity equivalency methodology are comprehensive and adequately explained.

The qualitative discussion of uncertainties associated with this method appears to be satisfactorily complete. It would be useful to include some discussion of where there are opportunities for quantitative assessment of uncertainties specifically associated with the TEF methodology.

(7) Are you aware of any essential references that have been omitted?

Two, as noted in Specific Observation (18).

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

The comments in Charge Question (4) apply here as well. I also have some definitional issues related to bioaccumulation that are detailed in the specific observations below.

(9) Please provide any other comments or recommendations you have.

See “Specific Observations” below.

Specific Observations

- (1) **Page 1, lines 22-24.** This text indicates that the TEF methodology is not the only tool for assessing integrated risks from PCDDs, PCDFs, and PCBs. The other tools described elsewhere in the document are essentially other data gathering techniques consistent with the TEF approach. But are there are other non-TEF approaches [alternatives are alluded to on **Page 65, line 32; Page 66, line 6; Page 68, line 23; Page 71, lines 20-26**] that may be used to make assessments of PCDDs, PCDFs, and PCBs? Before an RPM decides to use the TEF (congener-specific) approach, they are very likely to be offered other approaches by a regulated party who wishes to avoid the cost and effort associated with the congener-specific analyses required by the TEF approach. Because the “push-back” on this issue by the regulated community can be intense, it would be extremely helpful if this document provided some discussion (or as a table?) of the pros and cons of any alternative approaches (scientifically valid or otherwise) for assessment of PCDDs, PCDFs, and PCBs.
- (2) **Page 3, section 1.1.** A very useful clarification of the plethora of terms, definitions, and acronyms related to this approach.
- (3) **Page 12, Section 2.2, lines 10-11.** Conceptually, allowing for site-specific alternatives to the TEF shows flexibility and holds out the opportunity for lower uncertainty in the risk estimate. However, the extra effort (time, cost, expertise) needed by the regulated party to derive these, and for the regulator to evaluate and approve (or refute) them, is not mentioned. This

extra effort could be significant. Cost and time constraints, the need for regulatory consistency across sites, and the desire to avoid use of questionable alternatives all suggest that the TEFs-WHO₉₈ will be used as the default at the majority of sites, particularly those that are small, not overly complex, and/or poorly funded.

- (4) **Page 14, lines 11-13.** Reference is made to risk assessment guidance that addresses issues beyond the TEF methodology. Which of these guidance contains a specific discussion of the issue raised in Comment (1) above? If not these, then is there an extant guidance document that address this issue? What is reference U.S. EPA 2001d?
- (5) **Page 14, lines 23-25.** While it is important for risk managers to appreciate the points made here about the acceptance and usefulness of the TEF methodology, it is also necessary for them to understand the greater costs imposed on (and the resulting counter-reaction from) the regulated community by the need for congener-specific analyses. Risk managers also need to know that they will be presented, often quite forcefully, with what look like reasonable alternatives to the TEF methodology and will need to consider how to respond. These are clearly issues of strategy and cost-benefit that are not inappropriate to address, even if only cursorily or by reference, in what is essentially a framework document.
- (6) **Page 17, Text Box 2.** This comment follows along with Comment (1) above. These are good questions, but where is there guidance on how to answer them (particularly the first one) specifically for a TEF-based assessment? For the first question under “Planning”, for example, what criteria should a risk assessor and/or RPM use to answer this question one way or the other? Congener-specific analysis for dioxins/furans are usually challenged primarily for cost, while that for PCBs typically challenged both for cost and interpretation of toxicity at the congener level. What specific risk management objectives might an RPM have that would make them force the issue of congener-specific analyses? *{Is there a references to text box 2 in the text itself?}*

- (7) **Page 18, lines 1-3.** This focus on the particular characteristics of dioxin-like chemicals does not justify the complete separation of ecological and management relevance when selecting an assessment entity. A useful entity is one that embodies both ecological and societal/political (management) relevance. There may be a number of potential assessment entities that, while relating well to the chemical characteristics, hold little social and/or political relevance for risk managers. This lack of an ecological ~ management connection is continued in Section 3.1.2.4 (**Page 23, line 23**), which (if read out of context) could suggest that one is free to select on the basis of ecology alone. However, without societal relevance, it may be difficult to justify the effort (particularly the extra cost) required to investigate, and perhaps ultimately remediate, such dioxin-like chemicals.
- (8) **Pages 20, line 28 - Page 21, line 7.** The term “bioaccumulation” is apparently being used to describe both a process (uptake from all exposure routes) and a state (tissue concentrations at dis-equilibrium with (higher than) those external to an organism). The term “biomagnification” appears to be defined as a state of bioaccumulation existing at a higher trophic level. The statement (**Page 22, lines 2-3**) that “...biomagnification causes...higher concentrations in tissues than in fish,...” does not convey the multi-trophic level process required to generate this outcome. All of this is confusing. It seems clearer to keep with the idea of bioaccumulation and biomagnification as two processes which lead to the state of higher tissue concentrations.
- (9) **Page 22, lines 14-22.** This section is somewhat confusing - possibly because it tries to distill what is a fairly complex set of issues into a few lines. The first sentence seems unconnected with what follows. The distinction between pelagic and benthic invertebrates is not made until the last sentence. Where concentrations in contaminated sediment exceed equilibrium conditions is not clear: pore water or solids? The last sentence might be all that need be said here.

- (10) **Page 22, lines 29-30.** Would suggest “...competing mechanisms of bioaccumulation and metabolism...” better captures the issue.
- (11) **Page 26, line 11.** A minor point, but a quotient method is not an estimation of “risk” *per se*, only an indication of exceedance of some threshold.
- (12) **Page 29, line 8.** This comment follows along with Comments (1) & (6) above. This is the first mention of alternatives (homolog groups, total PCB) to the congener-specific TEF approach. Much is said about the benefits of the TEF approach but what about its costs, and its costs and benefits relative to other approaches? The TEF approach may now be the only scientifically credible way to approach the issue of dioxin-like chemicals but some sort of comparative analysis is required.
- (13) **Page 30, line 13.** What method are you thinking of here? Please provide a reference. Why the average concentration? It should be acknowledged that such estimation can involve considerable uncertainty and availability (and resulting water concentrations) may be overestimated.
- (14) **Page 31, lines 1-17.** This section is overly wordy and hard to read. Since it appears to be giving specific suggestions on how to proceed under certain circumstances, a bulleted or outline format may make the message easier to extract.
- (15) **Page 33, line 5.** Another minor point, but if U.S. EPA is going to create new definitions, it behooves us to use them. So, “...an assessment entity...” should replace “...assessment endpoint species...”.
- (16) **Pages 36-38, Tables 4-6.** These were very useful in explaining the calculation and summation of TEC values. The site and sediment data are hypothetical (although this is not apparent in the table itself but should be). However, the BSAF values appear real but their source is not referenced in the tables (later [**Page 41, lines 23-25**] we find that they are derived from the Great Lakes). The gross misapplication of tables of numbers in guidance documents is such a common practice that it is almost unnecessary to mention that, unless U.S. EPA intends otherwise (as is suggested on **Page 45, lines 16-17** and **Page 46, lines 1-11**), it

needs to be absolutely clear in both the tables and the text that these Great Lake BSAF values are offered here only as an example. Otherwise, these values will begin to appear as U.S. EPA-sanctioned, generic, default BSAF values for dioxin-like chemicals in risk assessments at sites far removed from the Great Lakes or even freshwater ecosystems.

(17) **Page 41, line 24.** Why are these BSAF values only “...roughly based on...” the data sets mentioned? What does this caveat imply? That the BSAF values in the tables are modified so as to be useful as examples only?

(18) **Page 43, lines 24-30.** The choice of how to address undetected chemicals is not statistically neutral but rather is driven by how much relative error one is willing to accept in the estimate of the mean and standard deviation of a sample. If this issue should be addressed during Problem Formulation (as it should), why not move this discussion to Section 3.2 and provide references to specific guidance on how to do so? Suggest adding to key references for this issue:

- a. Newman, MC, Dixon, PM, Looney, BB, and Pinder III, JE. 1989. Estimating mean and variance for environmental samples with below detection limit observations. *Water Resources Bulletin* 25(4): 905-916.
- b. WDOE. 1993. **Analyzing Site or Background Data with Below-Detection Limit or Below-PQL Values (Censored Data Sets)**. Supplement S-6, Statistical Guidance for Ecology Site Managers, Washington Department of Ecology, Olympia, Washington.

(19) **Page 46, lines 1-11.** This whole discussion finally (but tacitly) acknowledges that it can be very challenging (both economically, technically, and politically) to obtain site-specific BSAF values. For this reason, extrapolation and model adjustment are attractive ideas but ones constrained by numerous caveats, not all of which are listed here, regarding comparability of conditions. Development of this section may have been conditioned by experience within the Great Lakes ecosystem, where comparable conditions are more like to occur across different sites. However, on a national scale, truly comparable conditions are more likely

- the exception, as **Page 46, Line 7** acknowledges. If extrapolation is going to be offered as a method applicable on a national scale, then there should be a much more extensive and emphatic discussion of the caveats and limitations that apply.
- (20) **Page 46, line 8.** If conditions are not comparable, the suggestion is to adjust BAFs or BSAFs (who's source is unspecified) in accord with site conditions. More details are required (possibly a worked example in an appendix) of how one would adjust BAFs and BSAF with a basic food chain model to increase accuracy. Unless U.S. EPA supplies specific guidance on this issue, it may, given the vast number of models available, be hard to achieve any consensus on the efficacy of this approach or which (if any) models might be used to implement it.
- (21) **Page 46, lines 10-11.** While agreeing with the case study suggestion, it is clear that "...validate these extrapolation approaches..." clearly underscores the somewhat speculative nature of the extrapolation and model adjustment approaches. If case studies are to be used for validation, it is imperative that they be drawn, to the extent practicable, from a range of aquatic ecosystems within the U.S.
- (22) **Page 46, line 15.** Should end: "...total maximum daily load (TMDL) limits."
- (23) **Page 46, lines 18-32.** This section is overly wordy and hard to read. It's not clear what lines 18 to 28 have to do with (or lead to) "Thus, the first step..." in line 28. Suggest re-writing to simply state what you're trying to accomplish here.
- (24) **Page 46, line 31 - Page 47, line 1; Page 59, lines 32-33.** This comment follows along with Comment (3) above. As these lines suggest, TEFs-WHO₉₈ are likely to be used in the great majority of cases. The benefits associated with having site-specific RPFs are in many jurisdictions, particularly at smaller, less well funded sites, likely to be out-weighted by the greater benefits (ease of use (see "...minimizes the effort..." on **Page 47, line 14**), consistency, acceptability (lack of contention), and ease of review) associated with international consensus based TEFs. For this

- reason, it might be better to move the text between **Page 47, line 18 and Page 61, line 19** to an appendix and then state, early in Section 3.3.2, that, although the TEFs-WHO₉₈ are typical default values, there is a more elaborate process in the appendix for deriving site-specific RPFs if you have the resources to do so (and the regulators seem responsive to you doing so).
- (25) **Page 47, Text Box 5, last question.** This is a good question, but should be more closely linked to the text on **Page 46, lines 1-11**. More importantly, answering it is not a trivial exercise (see Comment (19) above). *{Why is Text Box 5 repeated on Page 64?}*
- (26) **Page 47, line 28.** There may be benefits associated with use of this method, but there should be a balanced discussion of the “...increased effort...” that is noted only in passing. Please elaborate on these extra efforts so as to provide a practitioner with a balanced view of this method.
- (27) **Page 61, lines 26-28.** It would be more helpful to have a separate figure for the dose-response curve, one in which the curve itself is larger and where the figure is closer to this text. Please provide a reference to the source of the dose-response curve shown in the figure (assuming it’s based on real data) and also a reference to methods for generating such curves.
- (28) **Page 65, Section 3.4.3 et seq.** It would be useful, if possible, to have the places in this discussion of uncertainty where it is thought amenable to quantitative characterization (including Monte Carlo). For example, many part of an ecological exposure assessment (Section 3.4.3.2.1) can be thus quantified, as can aspects of the dose-response relationship (Figure 6 & Section 3.4.3.2.2). Are there any challenges to quantitation of uncertainty that are unique to the TEF methodology?
- (29) **Page 69, line 11.** How is uncertainty in the extrapolation characterized - qualitatively, quantitatively, other? Is this assumption of reduced uncertainty intuitive or empirical?
- (30) **Page 69, lines 14-15.** Is the adjustment mentioned here the same as that mentioned with respect to Comment (20) above? The reference Burkhard

et al. 2003 has not actually been published yet and is thus not accessible for review.

- (31) **Page 71, lines 20-29.** This is a good start on the comparison of alternatives to the TEF methodology. This discussion (similar parts scattered throughout the text - see Comment (1) above) should be moved to its own section within the Introduction section so that the comparative benefits and costs of the method are readily available for review. It would also be helpful to have all of Section 4 (Conclusions) moved to the front of the document as an Executive Summary. Organizing the document in this manner will enable readers to obtain an overview of the methodology, and important considerations associated with it, before they enter the detailed portion of the guidance.

**Review by
Sean W. Kennedy, Ph.D.**

General Impressions

My general impression is that this is an important document. The authors have, in general, done a very good job of preparing a document that will be of use to risk assessors.

However, as indicated below, a few areas are in need of improvement and/or clarification

Response to Charge Questions

Question 1

In general, I think that this document *will become* an excellent document for assisting ecological risk assessors in applying the toxicity equivalency methodology correctly. The document does a good job in several places to make some very important points that are too often overlooked (at least, that has been my experience) when risk assessors attempt to apply the toxicity equivalency methodology. For example, the document clearly and correctly indicates six important factors that must be considered in the risk assessment (page 26), and a similar list of important things to consider is presented in Text Box 3 (page 28).

However, several areas need clarification (details are provided below under ‘Specific Observations’).

Question 2

An excellent start, but see my comments under “Specific Observations’, below.

Question 3

I think that the advantages of using the toxicity equivalency methodology are adequately explained.

Question 4

Some editing is required to clarify this important point. For example, see my minor comment about Chapter 2 below. Perhaps the point would be made more clearly by presenting an example (using made-up, or real data) to show the impact on the TEC when one does the analysis the correct way vs. the wrong way.

Question 5

- a) As indicated below, the bird section could be improved. Also as indicated below, I found the mink section somewhat difficult to follow.
- b) The matrix is a good idea, and easy to understand.

Question 6

It would be helpful to see some real data to show how large the uncertainties can be. Then, right at the beginning of an assessment, the risk assessor would have a better appreciation of the types of issues that he/she will have to deal with.

Question 7

The herring gull porphyria paper cited below should be included (and the table needs to indicate that porphyria has been reported in birds (there is a strong correlation between porphyrin concentrations and PCBs 105 and 118). The common tern paper (cited below) should be included if the authors choose to use a specific data to modify the bird section (see below).

Question 8

This section is sufficient, but once again, perhaps it could be improved by using illustrative examples.

Specific Observations

Page 3, Box 1

Analogous acronyms to TEF have also been REP, RPF and RP. This is a problem that was identified in 1998 WHO report. So, I suggest that REP, RPF and RP should be added in the table as analogous acronyms. Reason – it should be made very clearly to the reader that definitions and inconsistencies with usage have been somewhat of a ‘dog’s breakfast’.

Page 4

The definition for TEC should be included in the list.

Page 4

I think that there may be an error (and, therefore, confusion for the reader) in the definitions of ReP and RPF. As defined in this document, an RPF could also be a ReP since an RPF could be based on “**one**” study (as can and ReP). Having said this, I think that the authors may be on to something of value. A suggested distinction between ReP and RPF --- consider allowing an RPF to require *at least 2 endpoints* AND “*careful scientific judgment*”. In this way, it will allow the use of the most appropriate measure of relative potency for a particular study. Thus, an RPF would be somewhat like a TEF (because scientific judgment would be required to assess which of the two or more RePs are more important), but it would not yet be “sanctioned” by the WHO or some other organization.

The second paragraph on page 3 (it begins with, “ TheWHO meeting report..”) is very clearly written. I agree with the recommendation to use ReP rather than REP, since ReP is more grammatically correct.

Chapter 1

With exception with some concerns that I have with the definitions of ReP and RPF (see below), I think that this is an excellent introduction to the topic. I think that the history of the development of TEFs and TECs is recorded accurately and in sufficient detail to be useful to risk assessors and managers.

Chapter 2

Sections 2.1 and 2.2 are fine. However, I am not sure if the message in section 2.3 is clear. An equation to calculate TEC using concentration of a congener *n* in an organism (i.e., tissue or whole-body concentration) *or* in its food is presented. The sentence after the equation states that an appropriate bioaccumulation factor must be used if one is going to use the TEC equation. I agree, but I think that the wording needs to be altered to make it explicitly clear that that one **must** use bioaccumulation factors if food concentrations are used.

Chapter 3

Page 19, lines 14-15

This sentence is not correct. Fish are not, “generally more sensitive to PCDDs and PCDFs relative to birds”. The chicken is at least as sensitive as rainbow trout, and the ring-necked pheasant is only 5-10 times less sensitive than the chicken (*in vivo* work by Peterson and colleagues and cell culture work by Kennedy and colleagues). As stated correctly in the previous paragraph, there is at least a 50-fold difference in sensitivity of fish species to TCDD, and birds also differ similarly (or, even more) in sensitivity.

Table 3

The authors should read:

Kennedy, S.W., Fox, G.A. Trudeau, S. Bastien, L.J. and Jones, S. P. (1998) Highly carboxylated porphyrin concentration: a biochemical marker of PCB exposure in herring gulls. *Mar. Environ. Research* **46**, 65-69.

Porphyrin should be added to the table under Avian Wildlife.

Edema was reported in herring gulls in the Great Lakes in the early 1970s by Gilbertson and colleagues, and the cause was thought to be due to exposure to dioxins and/or dioxin-like PCBs.

I also suggest that the authors should see if there are any recent papers by Keith Grasman on immunotoxic effects that are associated with dioxins or dioxin-like PCBs in birds.

Table 5

The material presented in this table is not within my area of expertise. If not already done, the authors may wish to consider having Dr. Ross Norstrom, formally of the Canadian Wildlife Service, peer-review this section. He is an expert on bioaccumulation model(s) of dioxins and PCBs in herring gulls.

Page 55, first full paragraph

The logic here seemed reasonably clear (after I re-read it a few times). It might be easier for readers to understand this section if the illustration were made a bit more specific by using real data to illustrate the point. For example, there are EROD-inducing potency values for common tern hepatocyte cultures (Lorenzen, A., Shutt, J.L. and Kennedy, S.W. (1997). Sensitivity of common tern (*Sterna hirundo*) embryo hepatocyte cultures to CYP1A induction and porphyrin accumulation by TCDD, TCDF, PCBs and common tern egg extracts. *Arch. Environ. Contam. Toxicol.* **32**, 126-134). In some cases, the relative potencies are quite different than those found in chickens.

Pages 55 -59 (The mink example)

I found this section to be very confusing, and I am still not sure what 'the bottom line' is. I will re-read this again prior to the peer-review meeting to try to see if we need to discuss the section.

**Review by
Charles A. Menzie, Ph.D.**

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MEMORANDUM

To: David Bottimore
From: Charlie Menzie
Subject: Review of *Framework for Application of the Toxicity Equivalency Methodology*

A. General Impressions

I believe this Framework includes the technical information needed to implement the TEF/TEQ Methodology.

Improvements can and should be made on the clarity of the document. As this is intended to provide a Framework it is especially important that the document use clear language, that the examples be easily understood, and that terms used be clear and consistent. The document could benefit from improvement in these areas and I have provided specific suggestions.

While the methodology does describe how to implement the TEF/TEQ methodology for dioxin-like compounds including about a dozen PCBs, it does not provide sufficient information or guidance on two matters:

1. 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? I raise this issue because 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. I recommend some discussion of the limitations (i.e., uncertainties) of including only PCBs in the approach.
2. The document makes various references to other methods for doing PCB risk assessment (specifically arochlors 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 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. My suggestion is to use a combined congener/homologue analytical strategy that allows for both the determination of key congeners and also allows for the estimate of PCB totals by homologue group. This type of strategy satisfies investigations needs with respect to both risk assessment and the determination of extent of contamination.

I believe the Framework with suggested improvements by the external peer-review group will be an important document for guiding the assessment of ecological risks associated with mixtures of dioxin-like compounds.

B. Responses to Charge Questions

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.

The document contains the information needed to understand and implement the TEQ methodology. Its effectiveness can be increased by improving the clarity of writing and by being more consistent in the use of terminology. The level of detail is probably adequate. However, I suggest that the extended discussion on the use of ReP values to derive RPFs is a bit more detailed and perhaps could be placed in an appendix. Given the extended discussion given to this subject, I feel it is out of balance with other portions of the document.

The document (and the title) should be specific to the types of receptors for which it applies. Those receptors are fish and wildlife. I think it would be helpful to indicate this in the title. The document does not relate to invertebrates.

On P. 20, Line 20, A casual reference is made 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.

Section 3.3.1.3 discusses choices for exposure dose metric. I think 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.

Section 3.3.2.1 could be set up better. It needs a better introduction. I would also move the second paragraph (P. 48 Line 11) to after the current third paragraph (at Line 29).

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?

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.

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.

Three key acronyms – ReP, RPF, and TEF – are defined on p. 4. I recommend moving these definitions to the beginning of 1.1. For a Framework document I feel it is most useful to present the definitions and then follow with the rationale for what is being proposed. This is true for most of the document that tends to build a rationale and then end with the outcome. It is easy to get lost in these rationales if you don’t know where they are headed. So, start with the definitions.

There is still some confusion in the document over the relationship between RPF and TEF. At times I got the sense that the TEF could be the RPF and elsewhere the RPF is identified as an alternative to the TEF. A risk assessment could use some combination of TEFs and RPFs depending on the species being evaluated. I suggest that this be clarified and that the text be checked for how these terms are used.

There are other terms used in this document that can lead to unnecessary confusion on the part of the reader and can easily be changed to insure clarity:

- The term “congener” is used fairly loosely and I suggest it not be used as it can lead to confusion. Congeners can include compounds that have dioxin-like properties as well as compounds that don’t (e.g., there are 209 congeners of PCBs). Instead, the document will have greater consistency if it relies on the term “dioxin-like compound” for the discussion of the methodology. This should not be a problem since this term is used at least as much as “congener”. The use of “dioxin-like compound” is specific to the intent of this document.
- “AhR” and “Ah Receptor” are both used throughout the document. Choose one.
- 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.

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

The advantages are well explained. However, as noted in my general comments the document should provide the reader with a broader perspective on planning inasmuch as PCB risk assessments can not rely completely on the TEF/TEQ approach and the assessment of the extent of contamination may require analytical methods that are more comprehensive than those associated with the analysis of specific congeners. A short section on these issues would be very helpful because they are common issues at numerous sites.

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?

I believe this is adequately explained but there are a few areas of clarification I suggest. These are given in the detailed comments by page.

5) 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.

a) Please comment on the completeness and clarity of this discussion.

I believe the discussion is complete but this section lacks clarity and is difficult to read. I found the examples particularly difficult to read. I knew the points being made but someone less familiar with those points will find the examples hard to understand. I give specifics in Section C of my comments.

b) Are the matrix presented in Figure 4 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.

The matrix is useful. However, I suggest the following small changes when referring to it: 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.

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.

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.

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

The uncertainties are adequately explained. A few comments are offered:

- The tone seems defensive. Is that necessary?
- 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.
- I don't agree with the simple declaration on P. 68, Lines 24 – 26. Some fate and transport models like to work with a select group of model compounds and infer from that. This is a matter of practicality that should at least be acknowledged.

7) Are you aware of any essential references that have been omitted?

I am not aware of any essential references that have been missed. There are some additions that should be considered:

- Non-particle flux of PCBs from sediments has recently been found to be important. At the top of P. 30 there is an opportunity to cite to this recent literature because the current sentence suggests that only particle transport is important. Broadening this with a citation can be helpful. This work has been carried out by Joe De Pinto at Limnotech and others.
- On p. 30, Line 12 a statement is made concerning estimating average concentrations in water from sediment values. This is not an easy thing to do but there are ways this can be accomplished. Citations to general approaches would be helpful.
- There was a workshop on assessing risks to fish and wildlife species that I attended and that was chaired by Bob Hugget in the 1990s. At that workshop, there were several useful products one of which as a list of fate and transport models (prepared by Joe De Pinto and Paul Rogers) that might be useful for evaluating the fate and transport of dioxin-like compounds. The list was in the order of complexity. I did not see a reference to this workshop or to a few of the useful products that were developed for it.

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

The discussion of bioaccumulation and exposure is not adequate. The current discussion focuses exclusively on using BSAFs or BAFs and that is not general enough and excludes bioaccumulation and food chain models that do not employ these “factors”. I suggest some changes to improve clarity and to make the Framework more general:

- On p. 13 Line 21 there is a sentence talking about “appropriate bioaccumulation factors”. Here and elsewhere there is the impression that bioaccumulation factors are an essential part of the methodology. While these are indeed useful, they are really part of a broader process that involves estimating the bioaccumulation of dioxin-like compounds. That can be accomplished in two ways: a) through use of bioaccumulation factors, and b) through various bioaccumulation and food-chain models. I think here and elsewhere, the Framework should adopt the more general process of determining accumulation and resultant body/tissue burdens and not appear to limit that process to bioaccumulation factors. For the sentence on p. 13, I suggest the phrase “if appropriate bioaccumulation factors” be replaced with “if appropriate bioaccumulation factors or bioaccumulation models”. This is important because bioaccumulation models that rely on toxicokinetics do not simply use bioaccumulation factors as a means of generating a body or tissue burden of the chemicals.
- Figure 4 on p. 27 includes BAF/BSAF as a step. I suggest this step and adjacent box be changed to Bioaccumulation to make it more general and not to seemingly exclude toxicokinetic and other models that will be needed to account for time and space issues as these do not simply use BAF/BSAF values.
- Text Box 3 refers only to bioaccumulation factors and this can be broadened to simply be estimates of bioaccumulation (from bioaccumulation factors or models)
- Section 3.3.1 is well written and an example of how exposure should be described. In contrast, other parts of the discussion of bioaccumulation are more confused.
- In Section 3.3.1.1 there should be some discussion of the use of bioaccumulation, food-chain, and toxicokinetic models as tools for estimating body or tissue burdens.
- Section 3.3.1.4 should be expanded to identify the range of tools available for estimating bioaccumulation. I suggest organizing that re-write into direct measurements, use of BAFs/BSAFs, and models. This is important because exposure estimates will often be derived by direct measurement. Also, there will be models that rely on dynamic processes or steady-state conditions and not BAF or BSAF factors. If the object is to estimate bioaccumulation then that should be separated from the idea that the object is to get to a BSAF.
- Include models in the discussion at the top of P. 46.

9) Please provide any other comments or recommendations you may have.

On p. 22, Line 20. A statement is made that food chains beginning with benthic invertebrate will result in the greatest exposures to fish and wildlife. This is too simple and can be misleading. For example, non-particle PCB flux from sediments appears to be a very important pathway that links sediment contamination with body burdens in fish and wildlife. This pathway does not depend on ingestion of benthic invertebrates. I suggest broadening the sentence to include both ingestion of benthic invertebrates as well as exposure of water column organisms to chemicals released from sediments (e.g., non-particle flux of PCBs from sediments.)

C. Edits and Suggested Areas Where Clarity Could be Improved.

P. 9, line 33. It is more accurate to say, “Dioxin-like compounds exert effects by binding with AhR (references) ...” Some dioxin-like compounds (e.g., some PCBs) may also exert toxic effects that do not involve binding to the AhR.

P. 11, line 1. Begin sentence with “For PCBs,

P. 12, Line 17. A number of toxicological endpoints are listed. These have not been defined. You could include these in the list of abbreviations.

P. 12, Line 21. Define CYP1A in a footnote.

P. 12, Line 28. The sentence These TEFs are considered is unclear. Simply state that the TEF values were derived from available RePs and rounded up or down to the nearest half-order of magnitude.

P. 13, Line 6. A concentration in the diet is not a dose; I suggest changing “the primary expression of dose” to “used to determine the dose” to make the sentence accurate.

P. 14, Line 27. Add the phrase “dioxin-like” before PCDFs to make this sentence more accurate. There are PCBs that are not dioxin-like and therefore may need to be evaluated in a different way.

P. 18, Line 14. Change the word “demonstrate” to “conclude”.

P. 18, Line 18. The opening sentence is awkward as is the paragraph. This can be reworked to read more clearly. Break the paragraph into either a set of bullets or spit apart the discussion of mammals, birds, and fish.

P. 18, Line 28. Change “non-human primates” to “monkeys”.

P. 19, Line 19. This paragraph gives various perspectives on whether or not dioxin-like effects occur in amphibians and reptiles. I found it a bit confusing to read. The clarity and main point of this paragraph should be improved.

P. 20, Line 20. I don’t think you need to refer to the exposure assessment as “complicated”. Simply state what needs to be considered.

P. 20, Line 30. Eliminate the parenthetical phrase about equilibrium. This really does not add anything and can be misleading.

P. 22, Line 1. This is an awkward sentence. I would say “Because spatial and temporal scales of species can vary in relationship to the temporal and spatial patterns of exposure, care must be taken when characterizing exposure regimes and when estimating body burdens.

Bioaccumulation and food-chain models that account for the spatial and temporal patterns of species can be useful for estimating exposures in these situations.

P. 22, Lines 5 - 8. This is an awkward sentence. Please clarify.

P. 22, Line 19. The reference to the equilibrium relationship between sediments and surface water is a bit confused. Simply state that surface waters are often not at equilibrium with sediments. This is really not unusual for these compounds or for any other compound.

P. 22, Line 23. This paragraph is confusing and should be clarified. Also, the phrase “sufficient to significantly reduce bioaccumulation” should be changed to “it results in significantly less bioaccumulation”.

P. 23, Line 7. Consider a better way to refer to “opposing factors”. These factors do not really oppose one another.

P. 23, Line 12. What do you mean by “population vulnerabilities”?

P. 23, Line 16. Do you mean Variations in the *composition* of dioxin-like compounds?

P. 23, Line 30. Change “guild” to “community”.

P. 43, Line 8. Change “insect” to “invertebrates”.

P. 43, Line 13. Begin new paragraph at “Although”.

P. 46, Line 18. It is probably better to say that TEFs and RPFs provide the means to convert exposure to a complex mixture into a single dose metric for mixtures of(Note this is discussed nicely on P. 62, Line 7.)

P. 46 bottom and top of 47. There appears to be some scrambling of text here.

P. 47, Lines 28 – 33. Consider re-working this sentence. The “benefits” are not made clear.

P. 50, Lines 7 – 9. Sentence needs clarification. It is unclear what this means.

Section 3.3.2.3. The discussions in the examples can be improved to make them read more clearly. RPFs are “derived” not “chosen”. Isn’t that correct? See Line 23 on P. 54.

P. 55, Line 17. Include the reason why this is so.

P. 55, Lines 21 – 23. This is an awkward sentence. Please clarify.

P. 57, Lines 6 – 9. To avoid confusion split the bullets into two groups: diet based TECs and tissue-based TECs so that the reader recognizes that the units differ for these four values.

P. 57, Line 26. Can you state “would be advisable” more strongly? Don’t you mean, “then exposure should be based on the”.

P. 61, Line 15. This bullet is unclear. Please clarify.

P. 63, Lines 13 – 16. Expand this to include non dioxin-like effects of PCBs as a consideration in risk assessment.

P. 64. Text box 5 is repeated.

P. 67, Line 14. What is meant by “multiple models”?

P. 67, Line 25. I suggest rephrasing this in terms of reducing the uncertainty associated with a derived RPF.

P. 69, Line 16. change “measuring” to “determining”. Also mention the bioaccumulation models here as they can also be site-specific.

P. 69. The last paragraph at Line 32 is confusing. Please clarify.

**Review by
Christopher D. Metcalfe, Ph.D.**

Peer Review of EPA's Framework for Application of the Toxicity Equivalence Methodology
for Polychlorinated Dioxins, Furans and Biphenyls
in Ecological Risk Assessment

Submitted by Chris Metcalfe
November 7, 2003

Response to Charge Questions:

- 1) Effectiveness of document: The document provides a thorough and comprehensive evaluation of the Toxicity Equivalence Methodology, including a description of TEFs and their applicability to environmental risk assessments. However, the document is weak in terms of the description and evaluation of the methods for estimating concentrations of persistent chlorinated compounds in the tissues of receptor organisms. This shortcoming of the report will be discussed in detail below. Overall, I felt that the document was focused on toxicity issues and did not provide an adequate framework for applying toxicity equivalence factors in risk assessment scenarios.
- 2) Resolving inconsistencies in the literature: The terms, ReP, RFP, TEF and TEC are appropriate and were adequately described in the report.
- 3) The advantages of the methodology: The advantages of the toxicity equivalence methodology were adequately explained.
- 4) Estimating concentrations: My primary criticism of the report was the description used on pages 33-40 for applying BAFs and/or BSAFs to estimate concentrations of persistent chlorinated contaminants in the tissues of receptor organisms:
 - a) With regard to BAFs, the report indicates that bioaccumulation factors applied to concentrations in water can be utilized to predict tissue concentrations in fish and in bird eggs. However, 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. In my opinion, the report should not make any suggestion that BAFs can be applied to data on the concentrations in water to provide estimates of tissue concentrations and this material should be removed from the report.
 - b) With regard to BSAFs, there are several technical issues related to the application of BSAFs for predicting tissue concentrations that were not addressed in the report. First, 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. In the report, it is recommended 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 introduce unacceptable levels of uncertainty. In my opinion, the report should make a recommendation that chlorinated contaminants be analyzed directly in bird eggs, since analysis of bird eggs is a relatively non-invasive sampling technique that has gained acceptance for risk assessment applications. The recommendation in the report to use data on contaminant concentrations in forage fish to estimate concentrations in fish-eating mammals is appropriate and is explained adequately.

- c) The report indicates on page 45 that much the same methodologies used for risk assessment in aquatic ecosystems can be applied in terrestrial ecosystems, including using BSAFs to estimate tissue concentrations. This is not a valid recommendation and in my opinion, there should be no indication provided in the report that these risk assessment techniques can be applied to terrestrial ecosystems.
- 5) Selection of RPFs: The report provides a comprehensive discussion of the potential for applying RPFs that may be more appropriate for specific receptor species. The decision making matrix provided in this section is understandable and complete.
- 6) Uncertainties: There should be some guidance provided on the sensitivity of the risk assessment process to applying alternate RPFs. For instance, choosing an alternate RPF for PCB congener 77 (a major non-ortho congener in fish), may influence the outcome of a risk assessment more than choosing an alternate RPF for the mono-ortho PCB congener 105. There should be some direction provided on what scenarios warrant the selection of specific RPFs, as opposed to just using the recommended TEFs.
- 7) References: The reference section is very comprehensive with respect to the literature on TEFs for chlorinated dioxins and related compounds.
- 8) Exposure and bioaccumulation: The discussion of exposure and bioaccumulation was adequate. However, as described above, the discussion of the application of bioaccumulation factors to risk assessments was weak and was not helpful in addressing real-life risk assessment scenarios.

9) Other comments:

- a) Text Box 2 (Page 17): What is meant by the bullet, “Are the assumptions inherent in applying the toxicity equivalence methodology valid for the specific situation at hand?” What would be an example of a scenario in which the assumptions were not valid?
- b) Page 22, lines 20-22: A more important determinant of exposures in aquatic organisms is food chain length. Whether the organisms are directly linked to contaminated sediments is of lesser importance. Is there a reference for the point made in this sentence?
- c) Page 26, line 8: I am not sure what is meant by, “Determination of theoretical or empirical measures of exposure”.
- d) Page 30, line 4: PCBs are not more volatile than PCDDs and PCDFs, but they do tend to partition from water to air to a greater extent (function of Henry’s Law constants).
- e) Page 43, lines 9-21: After reading this section a few times, I was able to understand what was being calculated for “TECs calculated for eggs versus sediment” (see Figures 7 and 8), but this calculation and the concept was poorly explained.
- f) Text box 5 (Page 47): Re the bullet on how to handle chemicals with concentrations below detection limits, some guidance should be provided. There are basically 3 choices: i) Consider the concentration as 0, ii) Use the detection limit as the concentration, iii) Randomly select values between 0 and the detection limit.
- g) Page 64: The text box 5 on this page is a repeat of the one on page 47.
- h) Page 69, lines 9-10: As discussed above, the report appears to be dismissive of the fact that, “extrapolation of bioaccumulation factors from one ecosystem to another is a source of uncertainty”. In my opinion, the uncertainty of this extrapolation greatly exceeds uncertainties related to selection of TEFs.

**Review by
Richard E. Peterson, Ph.D.**

Reviewer Richard E. Peterson, University of Wisconsin

US EPA June 2003 External Review Draft

Document Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment

Comments

Pvi, P3, L5-6 It would be useful to EPA scientists in learning how to use the TEF methodology to include the results of a TCDD dose response study for a sensitive, population-relevant response for a prototype fish (lake trout embryo mortality), bird (eagle embryo mortality), and mammal (mink fetus and pup mortality) in the document.

The omission of such TCDD dose response results is problematic in the document when one reaches the point where exposure to dioxin-like PCDDs, PCDFs, and PCBs expressed as a single TEC value is to be compared to an adverse population relevant effect of TCDD for the purpose of assessing risk. The present document does a poor job of illustrating in a transparent fashion how this is done.

While this reviewer understands that the purpose of the document is not to provide a step-by-step ecological risk assessment approach for TCDD and related compounds, the inclusion of this information 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 help to overcome a major deficiency in the current document. Also 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. **# 1**

P20, L14 Expand this section by stating, for illustration purposes only, “possible” action levels expressed in appropriate dose units for each vertebrate class: fish - TCDD concentration in eggs, bird - TCDD concentration in eggs, and mammal - TCDD concentration in diet. **# 1**

Also see comments in Pvi, P3, L5-6 (above) and P42, Fig 6 (below).

P1, L14 Update Hahn (1998) reference with Hahn (2003)

Hahn, M.E., Evolutionary and Physiological Perspectives on Ah Receptor Function and Dioxin. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 14, pp. 559-602, 2003. # 7

- P2, Fig. 1 Add chlorine atom symbol to both rings on left panels # 9
- P6, L15 Delete the first “available” # 9
- P9, L5 Underline “for each dioxin-like compound” # 9
- P9, Sec. 2 For this section and for the document in general I believe 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”. Yet when the toxic equivalence methodology is applied and the necessary steps to be followed are explained in Section 2 the term “congener” is not used at all. Instead “chemical” and “compound” are used and I am concerned that some readers may not understand that these terms are intended by US EPA to mean the same thing as “congener” for the purpose of this document. It is recommended that the word “congener” be used instead of “chemical” or “compound” in Section 2 because it is such a pivotal section for understanding the methodology and it provides a better linkage to Tables 4, 5, and 6 where TEFs are given for individual PCDD, PCDF, and PCB “congeners”. # 2
- P9, L5 Change “compound” to “congener” # 2
- P 9, L7 Change “chemical” to “congener” # 2
- P9, L9 Insert “of the congener” after “estimates” # 2
- P9, L11 Insert “of the congener” after “estimate” # 2
- P9, L13 Insert “of the congener” after “concentrations” # 2
- P9, L16 Change “estimates” to “estimate” # 9
Insert “of the congener” after “estimate” # 2
Delete “a” and insert “it’s” # 9
Insert “(TEC)” after “concentration” # 9
- P9, L18 Delete “the” and insert “both” # 9
- P9, L19 Delete the second “the” # 9

- P9, L20 Delete “chemicals” and insert “congeners” # 2
- P10, L1 Delete “It should be noted that” and insert “However,” # 9
- P10, L2 Delete “however, that” # 9
- P10, L8 Delete “inhibition or synergy” and insert “antagonism or synergism” # 9
- P10, L9 Delete “chemicals” and insert “dioxin-like congeners” # 2
- P10, L23 Insert “an” after “elicit” # 9
- P10, L30 Delete “seven” and insert “7” # 9
- P11, L12 Delete “examples” and insert “references” # 9
- P11, Para 2 It is recommended that the documented existence of other AhR agonists, some of which occur naturally in animal tissues, but are not persistent and do not bioaccumulate, be acknowledged for the sake of completeness. Including a citation to a recent review article by Dr. Michael Denison on this specific subject would be appropriate.
- Denison, M.S. and Nagy, S.R., Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu. Rev. Pharmacol. Toxicol.* 43: 309-334, 2003.
6, #7
- P12, L8 Insert “RELATIVE” after “APPROPRIATE” # 9
- p12, L16-19 RePs determined from NOAELs, LOAELs, and benchmark doses are not as accurate as those based on LC50s, EC50s, LD50s or ED50s. This point needs to be added to the information presented. # 6
- P12, L27 Start the sentence as follows: “Values of the TEFs WHO98” and delete “values” # 9
- P12, L29 Insert “relative to 2,3,7,8-TCDD” after “congeners” # 6
- P13, L1 Delete “relative potency factors” and insert “RePs” # 9
- P15, L7 Define the term “congener-specific”. Otherwise some readers may not realize that the concentration of each one of the 29 dioxin-like PCDD, PCDF, and PCB congeners is determined with this approach. # 6
- P17 Test Box 2, 5th Check, L6 - Change “endpoints” to “endpoint” and then after the word “endpoint” insert “species.” # 9

P18, L9 Delete "Hahn, 1998" and replace with "Hahn, 2003"

Hahn, M.E., Evolutionary and Physiological Perspectives on Ah Receptor Function and Dioxin. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 14, pp. 559-602, 2003. # 7

Also add three new sentences as follows:

"While there is one form of AhR in mammals, two AhRs that are the products of separate genes, AhR1 and AhR2, are expressed in fish (Tanguay et al., 2003). This raises a question as to which one of the AhRs in fish is required for TCDD to cause toxicity. In zebrafish TCDD developmental toxicity is mediated entirely by AhR2, AhR1 is not involved (Prasch et al., 2003)." # 6

Tanguay, R.L., Andreasen, E.A., Walker, M.K., and Peterson, R.E., Dioxin Toxicity and Aryl Hydrocarbon Receptor Signaling in Fish. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 15, pp. 603-628, 2003.

Prasch, A.L., Teraoka, H., Carney, S.A., Dong, W., Hiraga, T., Stegeman, J.J., Heideman, W., and Peterson, R.E.: Aryl hydrocarbon receptor 2 mediates 2,3,7,8-tetrachlorodibenzo-p-dioxin developmental toxicity in zebrafish. *Toxicol. Sci.* 76: 138 - 150, 2003. # 7

P 18, L21 After "fish" insert "(Theobald et al., 2003)."

Theobald, H.M., Kimmel, G.L., and Peterson, R.E., Developmental and Reproductive Toxicity of Dioxins and Related Compounds. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 9, pp. 329-431, 2003. # 7

P19, L6 Insert ":" after "with" # 9

P19, L12 After "1998" insert "; Tanguay et al., 2003.)"

Tanguay, R.L., Andreasen, E.A., Walker, M.K., and Peterson, R.E., Dioxin Toxicity and Aryl Hydrocarbon Receptor Signaling in Fish. In: Dioxins and Health, 2nd edition, (Schechter, A. and Gasiewicz, T.A., eds.) John Wiley & Sons, Chapter 15, pp. 603-628, 2003. # 7

P20, L7 One of the first reports that invertebrate AhR homologs do not bind AhR agonists was conducted in *C. elegans* by Powell-Coffman and coworkers. Since it was one of the first publications of this finding it should be cited.

Powell-Coffman, J.A., Bradfield, C.A., and Wood, W.B.,
Caenorhabditis elegans orthologs of the aryl hydrocarbon receptor and its heterodimerization partner the aryl hydrocarbon receptor nuclear translocator, Proc. Natl. Acad.Sci. USA 95: 2844-2849, 1998. # 7

P21, Table 3 First column on “Effect”

Delete “21” from “Immunotoxicity” # 9

Add “embryo/” before “fetal” # 9

Consider adding “Cardiovascular Toxicity” # 9

Second column on “Fish”

Hyperpigmentation is not chloracne - consider deleting the “+” # 9

P21 Add a new table that focuses solely on TCDD effects associated with early life stage toxicity in fish and birds. The reason for including this new table 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) 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. # 1

Accordingly, it is recommended that this deficiency be corrected by adding a new table and possibly a representative photograph of a TCDD-exposed lake trout larva and a chicken hatchling illustrating the hallmark effects of the TCDD developmental toxicity syndrome in fish and birds.

If this is not done, the possibility exists that other developmental toxicity syndromes (caused by exposure to non-AhR agonists that result in an entirely different profile of developmental effects that nevertheless culminate in early life stage mortality) may be confused with TCDD.

Therefore, early life stage toxicity in fish and birds should be highlighted in its own table. This table would not only identify the different types of adverse early life stage toxicity effects associated with exposure to potent AhR agonists such as TCDD and PCB 126 in fish and bird embryos,

larvae, and hatchlings, but importantly, it would also list the various species of fish and birds in which these types of effects have been observed. This would be analogous to the current Table 3 where results for 9 different species of mammals are shown, but effects in birds and fish are under represented.

Taken together, the proposed new table and accompanying photographs will provide greater support for the fundamental concept that TCDD and related AhR agonists cause early life stage toxicity in several different species of fish and birds. Table 3 clearly falls short in this respect. # 1

- P23, L18 Insert “than fish” after “sensitive” # 9
- P25, Fig 3 Bottom of figure is missing. # 1
- P27, Fig 4 Under the heading BAF/BSAF - Change “chemical” to “congener” # 2
Under the heading TEF/RPF - Change “chemical” to “congener” # 2
- P29, Para 1 Define the term “congener-specific” # 9
- P33, L4 Insert “s” after “PCDF” # 9
- P42, Fig 6 While the intent of Figure 6 is good it would be far more helpful to EPA scientists who are learning the TEF approach, if it was focused to a greater extent on: TCDD dose response curves, “possible” choice of TEC threshold action level, and lastly a transparent illustration of exactly where the TEC value determined for each species falls on the TCDD dose response curve with respect to the “possible” TEC action level selected for that species. The present document “dances around these issues” without bringing them all together in one Figure. Yet this type of Figure is exactly what is needed for EPA scientists to fully comprehend and use the TEF approach to risk assessment of dioxin-like PCDDs, PCDFs, and PCBs. # 1

The current major focus of Figure 6 on food chain exposure to dioxin-like PCDDs, PCDFs and PCBs should be down played because it is already covered adequately in Figures 3 and 5. Accordingly it is recommended that Figure 6 be changed dramatically. # 4

The new Figure 6 should highlight a representative TCDD dose response curve for each vertebrate class and show exactly where the TEC calculated for each representative species (lake trout, bald eagle, and mink) falls on each curve. The ordinate in these TCDD dose response curves should designate more specifically the exact TCDD response being assessed (lake trout - increased sac fry mortality; bald eagle - increased embryo and hatchling mortality; mink - decreased litter size) and the TCDD dose shown on the abscissa for each species (lake trout - TCDD egg

concentration; bald eagle - TCDD egg concentration; mink - TCDD concentration in diet) should be in the same units as the TECs being calculated. The TECs calculated for lake trout eggs, bald eagle eggs, and mink diet should be designated directly on the TCDD dose response curve for each species along with the “possible” choice of TEC action level for each species.

Thus, by placing greater emphasis on the TCDD dose response curve for a sensitive, population relevant endpoint, by showing three separate TCDD dose response curves for a representative fish, bird, and mammal, and by illustrating where on the respective TCDD dose response curves the TECs and “possible” choice of TEC action level for each species fall will not only underline far more effectively the utility of the TEF approach but it will bring the entire process to closure in a logical fashion that is more readily understood by the first time reader of the document. In short, including the proposed “new Figure 6” will better enable EPA scientists who are considering using TEF methodology to see the final outcome of their effort. The current document falls short in this respect and it is strongly recommended that this weakness be corrected. # 1

P42, Fig 6 Figure 6 is misleading in that the arrow from each type of TEC calculated should not point to the same TCDD dose response curve, but to separate TCDD dose response curves that are specific for that species. This presentation format is confusing and needs to be changed.

Also a more accurate description of the exact type of response being assessed on the ordinate is needed. Percent sac fry mortality in fish, percent embryo/hatchling mortality in birds, and percent decrease in litter size in mammals is better. Also a more accurate description of the TEC dose on the abscissa is needed - the present one is confusing because TEC in eggs will be used in fish and birds but in mammals dietary exposure will be used. The current abscissa does not distinguish between these important differences.

Thus, by “short cutting” these important species differences for the sake of brevity of presentation in Figure 6 robs the reader of this document an opportunity to understand better exactly how TECs determined in a particular species will be used in a TEC risk assessment for that species. Figure 6 needs to be changed accordingly. # 1

P43, L4 The upper range of the TEFs (i.e., 0.000005) for the mono-ortho substituted PCB congeners in fish will overestimate the TEC.

What the TEF values of 0.000005 represent is the highest concentration of the mono-ortho substituted PCBs tested by injection into newly fertilized

rainbow trout eggs that caused no evidence of dioxin developmental toxicity.

For all of the other TEFs (PCDDs, PCDFs, and nonortho-substituted PCBs) the TEFs were based on egg concentrations of a particular congener that caused 50% early life stage mortality. The TEFs of 0.000005 for the monoortho-substituted PCBs were associated with 0% early life stage mortality. **# 3 and # 6**

- P46, L31-32 Delete **# 9**
- P49, L16/18 Replace “hierarchical” with “hierarchal” **# 9**
- P49, L26-29 Vague. Rewrite and clarify. These sentences seem to suggest that there is a “meaningful association” between species sensitivity to TCDD and RPFs of certain dioxin-like PCB congeners when the opposite is intended. These sentences need to be rewritten or dropped because they confuse the issue rather than clarifying it. In contrast the next sentence starting with “Two species ...” is good. **# 6**
- P50, Fig 10 The 3-dimensional matrix model approach for selection of RPFs or TEFs is excellent. Missing from the document is the notion of it being a useful guide for identifying future TEF methodology research needs. **# 5b**
- P54, L14-18 Change RPF(s) to ReP(s) on these lines **# 9**
- P54, L27-29 Add references for TCDD dose response studies, based on early life stage mortality, in chickens and pheasants.
- Please check the current peer-reviewed scientific literature to see if TCDD dose response studies, based on early life stage mortality, in other bird species have been published.
- This reviewer thought such a study had been done on kestrels. If so, kestrels (and any other bird species and any other bird species on which such a study has been done) should be added to the chicken and pheasant in this particular sentence along with the appropriate reference for each. **#1**
- P55, L1 Insert “mortality” after “stage” **# 9**
- P55, L34 Was the source of liver tissue the mink dam or mink kit? **# 9**
- P56, L4 Delete “an” after (A) and after (B).
Change “ReP” to “RePs” **# 9**
- P56, L5 Change “ReP” to “RePs”

- Delete “, which are”
Delete “the” # 9
- P56, L26 Move all text to L7 # 9
- P56, L27 Move and center this title above the text inserted on L7 # 9
- P57, L8 Delete “female” and insert “dam” after “mink” # 9
- P57, L9 Delete “female” and insert “dam” after “mink” # 9
- P57, L21 Insert “dam” after “mink” # 9
- P57, L28 Insert “dam” after “mink” # 9
- P58, L7 Delete “female” and insert “dam” after “mink” # 9
- P59, L7 Line ends prematurely. Delete “return function” so text moves up to fill complete line # 9
- P59, L33 Insert “vertebrate” before “ the word “class” # 9
- P60, Table 8 First column, bottom row, second box :
Delete “female” and insert “dam” after “mink” # 9
- P61, L5 Delete the first “a” # 9
- P61, L26 Revise Figure 6 to include dose response results for a representative fish, bird, and mammal, respectively. See above comment (P42, Fig 6) for justification. # 1
- P61, L29 Move “immunotoxicity” after “wasting syndrome;” on L 30 # 9
- P61, L30 Insert “cardiovascular and” before “endocrine” # 9
- P61, L31 Add a new sentence that identifies TCDD effects that characterize early life stage toxicity in the embryo, larva or hatchling of fish and birds (i.e., in TCDD exposed fish larvae some of the endpoints include pericardial, yolk sac and meningeal edema, impaired jaw development, impaired heart development and function, reduced trunk blood flow, anemia, hemorrhage, growth retardation, and mortality) # 1
- P61, L34 Insert “in different fish bird and mammalian species” after “compounds” # 9

If a new Table is added that identifies the “profile of TCDD effects” that characterize early life stage toxicity in fish and birds, respectively, it should be cited along with Table 3. **# 1 and # 9**

- P63, L7 Vague. What are “secondary ecological effects”? Give a few examples to clarify this point. **# 1**
- P63, L9 It is recommended that a new Figure 6 be included that shows TCDD dose response curves for a representative species in each vertebrate class. See above comment (P42, Fig 6). **#1**
- P63-64 Switch “Text Box 6” (P63) with “Text Box 5” (P64) **# 9**
- P64, L1 “complete” **# 9**
- P65, L31 Insert “than” after “significant” **# 9**
- P66, L29 Delete “Tillet” and insert “Tillitt” **# 9**
- P67, L20-21 Add one line space **# 9**
- P67, L26 Insert “relative” after “true” **# 9**
- P68, L14 Insert “,” after “sensitivity”
Insert “and” after “field” **# 9**
- Comment 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”. **# 2 and # 5a**
- P68, L14 Insert a “,” after “sensitivity” **# 9**
Insert “and” after “field” **# 9**
- P69, L26 Delete “with” and insert “while” **# 9**
- P70, L14-15 Delete “toxicity equivalence factors” and insert “TEFs” **# 9**
- P70, L16 Insert “vertebrate” after “deriving” **# 9**
Insert “-“ after “class” **# 9**

- P71, L6 Insert “relative to 2,3,7,8-TCDD” after “potencies” # 9
- P71, L32 Insert “relative” after “appropriate” # 9
- P71, L33 Insert “relative” after “selecting” # 9
- P72, L5 Insert “relative” after “new” # 9
- P72, L21 Insert “exposure to” after “from” # 9
- P74, L18 Delete “accepted for publication” and insert volume and page numbers # 7
- P82, L7-8 Delete the end of the sentence beginning on L7 with “binding of ...” # 9
- After “but” on L7 insert:
- “... sustained activation of AhR signaling, by persistent, bioaccumulative xenobiotics that are AhR agonists binding to the AhR, is known to disrupt normal development and functioning in fish, birds, and mammals.” # 1
- P82, L35 Insert the definition of “Congener-Specific”. # 2
- P82, L39 Insert “relative to 2,3,7,8-TCDD” after “congeners” # 9
- P84, L3 Insert after “TCDD” “, it is the congener to which all other dioxin-like congeners (dioxin, furan, and PCB) are compared to determine their ReP for producing a particular AhR-mediated toxicity or biological effect. When this is done, the ReP of 2,3,7,8-TCDD is assigned a value of 1.0. # 2
- P84, L9-10 Delete the sentence beginning with “The concept of ...” # 9
- Add the following sentences:
- “The concept of translating the concentrations of dioxin-like congeners (dioxin, furan, and PCB) in fish, birds or mammals to a 2,3,7,8-TCDD equivalence concentration. This is done by multiplying the vertebrate class-specific and congener-specific RPFs or TEFs by whole body or tissue concentrations of the individual dioxin-like congeners in a fish, bird, or mammal, respectively, to give a corresponding 2,3,7,8-TCDD equivalence concentration for each congener. These concentrations are then summed for all dioxin-like congeners present in the fish, bird, or mammal to yield a total 2,3,7,8-TCDD equivalence concentration.” # 4
- P84, L19 After “(TEF)” insert “or relative potency factor (RPF)” # 9

Comment A website address should be included 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. # 5a

It would be prudent for the database to be maintained by the US EPA, in addition to the Karolinska, so it can be updated in the future. Tim Kubiak, DOI, has a copy of the database.

Comment 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. # 5a

**Review by
Martin Van den Berg, Ph.D**

Comments on the EPA document “Framework for the Application of the Toxicity Equivalency Methodology for PCDDs, PCDFs and PCBS in Ecological Risk Assessment”

My general impression is that of a carefully written document, which could definitely serve its purpose and being a guide for ecotoxicological risk assessment for these compounds. The report is a significant, valuable and realistic extension to the approach that was chosen in 1997 by the WHO-IPCS to develop ecotoxicological TEFs. The document presents an in-depth analysis of the problems that an ecotoxicological risk assessor encounters when he/she needs to determine the risk of these type of compounds in the environment. Besides a number of minor, often technical, comments that will be given later I have two major comments regarding the report and its suggested approach to work with “eco-TEFs/RePs/RPF”.

The first is that the authors more or less suggest that the eco-TEFs determined by WHO are more or less the default values, when a more precise risk assessment with e.g. RePs or RPFs can not be performed. 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 should to my opinion be done within an international framework for which the WHO-IPCS is the most logical one. My second comment relates to the approach suggested when kinetic factors are

combined with TEFs or related parameters. All the formulas and relationships described in that part of the report are scientifically sound and clearly illustrate the problems associated with TEFs when abiotic and biotic compartments with more trophic levels are present. The report presents three examples of an estimation of TECs in fish, birds and wild mammals in comparison with an abiotic compartment. These examples by it self are correct, but I would also prefer to see a kind of validation for this approach with real ecological situations indicating the feasibility and possible uncertainty. To validate this EPA approach I think two exercises with real environmental data can be very useful. The first one could be a modelling with 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. Personally, I am not fully convinced that the available physico-chemical data for all relevant congeners is sufficient to perform such an exercise, but such a validation should give more clarity about this. I guess that if such a calculation would come close to the real life situation within a factor 2 to 5 the practical feasibility is shown, given the range of differences found in concentrations found in wild life populations from one area.

More specific comments:

Chapter 1.1. The extension of terminology with RPF is an appropriate one compared with those used by the WHO in 1997.

Chapter 1.2. p.6.

The deletion of the di-ortho PCBs from the WHO TEF scheme in 1997 is not mentioned, but is a relevant one in view of the obvious absence of AhR mediated mechanisms by this group.

The major reason for WHO to develop eco TEFs was not because of availability of data itself, but the recognition by its experts that there were extensive differences in sensitivity between the distinguished classes.

I think that the extensive reviews by Steve Safe in CRC Crit Rev Toxicol in 1990 and 1994 that describe the SARs, possibilities and limitations for TEFs should get more credit in the report.

Chapter 2.

In its evaluation WHO obviously gave priority to (semi)chronic in vivo studies, but unfortunately these were almost exclusively available for the mammalian studies.

Chapter 2.1.

WHO also states that non additive effects observed in several studies play a minor role in the use of TEFs compared with other uncertainties e.g. the large differences in species sensitivity, which are observed between classes.

p.11. I.9. I wonder if these effects could ever be separated for the two groups of compounds. For PCB cancer risk in humans there might be observable differences between the two groups of congeners based on laboratory studies, but for wildlife this is merely a theoretical situation in view of the lack of distinct information for both group of congeners in wild animals.

Chapter 2.2.

p.12. I.24. Which other type of compounds has EPA in mind for RPFs? Some realist suggestions for future inclusion in the TEF concept might be useful to direct future research.

Chapter 2.3.

p.13, l.21-24. This a very important statement. The more and more common use of in vitro assays for detecting TECs in the abiotic compartments illustrates the importance of this statement with respect to ecotoxicological risk assessment.

Chapter 3.2.1.1.

At some points in this chapter 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 e.g. MO-PCBs. Especially the approach 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.

Chapter 3.2.1.4.

It would be advisable to identify possible target species and most sensitive endpoints for ecotox risk assessment in one table.

Chapter 3.3.1.2.

p.30, l.20. I think the pattern on congeners in abiotic media usually does not reflect that found in biotic samples.

p.30, l.31. Besides administered dose, aspects of bioavailability (C-content and aging) could be mentioned.

Chapter 3.3.1.3.

p.32, l.5-27. This is a good reflection of the actual situation.

Chapter 3.3.1.4. See general comment earlier and remarks about validation.

Chapter 3.3.2.2. The presented three dimensional matrix for selection is a good one, but for real life situations the upper left part of the dimension will seldom be reached.

Chapter 3.3.2.3. The given examples provide a good illustration of the problems associated with the suggested use of RPFs.

Chapter 3.4.2.

p. 64. 1.26-32. The statements about the use of bioassays could be expanded some more with a conclusion that e.g. a fish cell line would be the more appropriate tool to identify levels in the aquatic environment. Mammalian cell lines should be used for those situations that involve mammalian or human exposure. Furthermore it should be realized that very few of these genetically modified in vitro assays that are presently used for determining TECs have adequately been validated for the in vivo situation in the same species.

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Summary of Conference Call on October 16, 2003

**Minutes of October 16, 2003 Conference Call on
Peer Review of *Framework for Application of the Toxicity Equivalence Methodology
for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment***

David Bottimore of Versar opened the call by reviewing the agenda and purpose for the kick off conference call. He provided an overview to the goal of the peer review and described the process that is being used to augment the individual peer reviews with discussion via conference call. Included in his introduction were ground rules, which stated that the review would not seek consensus. Rather, each reviewer is asked to voice their individual opinion which will be presented in the peer review report and summarized by the Chair in the synthesis/executive summary. He finished by asking the peer reviewers to introduce themselves with short descriptions of their expertise related to developing or implementing toxicity equivalence methods for dioxins and PCBs in ecological risk assessment (ERA). Members of EPA's technical panel that produced the document also participated in the call, providing background information and answering reviewers' questions. The list of attendees is presented at the end of this document, along with the agenda.

Charles Menzie, the Chair for the peer review, introduced himself and stated that his responsibility is to ensure that all reviewers provide input and that their opinions be reflected in the executive summary. Organizing the peer review report will be greatly facilitated if reviewers follow the charge questions and other instructions for preparing their individual written comments. He reiterated that the goal is not to reach consensus, however if there is general agreement on a topic, that it would be reflected in the summary. Similarly, divergent opinions will be noted. He completed his opening remarks by asking each reviewer to use the time on the conference call to ask clarifying questions about the scope of the document and the charge questions for the peer review.

Tala Henry, from EPA/NHEERL, presented background information on EPA's framework document. During her presentation she reflected on the history of the development and use of the toxicity equivalence method (TEM) as well as the recommendations which led to preparation of this framework. She described the purpose of the document as well as the intended audience. During her presentation she emphasized the need for a framework that clarifies terminology and definitions and introduces the TEM approach for application in ecological risk assessments for dioxins, furans, and PCBs. This framework is a supplement to the ecological risk assessment guidelines, providing a description of how the TEF approach fits within ERA, but it is not guidance on how to conduct a risk assessment for these compounds. The TEM approach is based on several assumptions including the fact that these chemicals have similar mechanisms of action but have different potency factors for different species. One of the distinguishing aspects of the document is the clarification of terminology, such as toxicity equivalence factors (TEFs), which applies to international consensus values, versus relative potency factors (RPFs), which are alternate values that can be derived from species-, endpoint-, or site-specific criteria to improve the accuracy of assessments. Also mentioned during her presentation was that TEFs or RPFs should be applied only to

tissue or dietary media; use of this method for other media (e.g., soils sediment, water) calls for the use of bioaccumulation factors. She reviewed several illustrations intended to help the risk assessor in the application of these approaches. Her presentation was completed with a restatement of the framework's purpose, which is to provide ecological risk assessors with an understanding of the TEM methodology and its proper application.

Following EPA's background presentation, Charles Menzie facilitated a question and answer session among the reviewers and the EPA authors to help clarify the purpose, scope, and content of the document. He posed the first question, about the boundaries of the report and its use in risk management decisions. He asked if there would be associated work products to help the user to better understand the utility of the document in guiding management decisions. He raised a few examples, such as in deriving TMDLs or conducting a risk assessment of contaminated sediments. He wondered if there would be additional guidance documents that support this framework. Tala Henry responded that the document is organized according to ERA paradigm and provides an umbrella for use of the approach in the planning/problem formulation and analysis phases. She stated that it is a stand-alone document but noted that references are provided to the ecological risk assessment guidelines, previous workshop reports, and other documents that could help the reader to understand how the procedure can be applied. Pat Cirone added that EPA wanted to avoid being too prescriptive; the framework document affirms the approach but does not prescribe how it should be used in every instance. Phil Cook added that one of the issues that reviewers might consider is whether the document provides adequate context and references to related reports.

Bruce Hope posed another question about the use of homologue PCB data in the TEM approach because he is encountering many organizations that advocate collection of homologue data rather than congener-specific data. Considerable discussion followed about the use of homologue data, with reviewers and EPA providing examples where homologue data might be collected for screening purposes. Several EPA staff reiterated that use of homologue data would introduce large uncertainties into assessments. Congener-specific data are the preferred analytical method for use with the TEM and this document makes a clear case on the strengths of using such data. Reviewers agreed that this document should encourage the collection of congener-specific data for risk assessments using the TEM approach.

Charles Menzie continued the discussion by asking the reviewers if they had any questions about the charge questions. Reviewers found the questions to be straightforward and clear. Mark Hahn asked about secondary data, one of the issues contained in Versar's peer review instructions. David Bottimore clarified that reviewers, as they evaluate the framework, should consider how EPA used secondary literature sources in the document. This could include attention to the data collection procedures, level of peer review, and overall utility of data used in this document. Also, if reviewers have additional references that they feel should be cited, they should consider data quality issues that might effect the applicability of such data in the document. Charles Menzie returned to the charge questions and reiterated that reviewers organize their thoughts according to the questions, which will help in producing the summary. David Bottimore

closed the call by reviewing the next steps and schedule for submitting the individual reviews, holding the second conference call, and preparation of the Chair's summary. He asked reviewers to complete their evaluations by about November 7. The individual reviews will be distributed to all reviewers and the Chair, who will complete a draft summary by about November 24. The second conference call will be scheduled to discuss the summary and other issues that reviewers would like to raise. Reviewers were asked about their general availability in early December. A tentative date of December 5 was discussed and most reviewers believed that they would be available for a call on that date. Peter deFur suggested that the date be set as soon as possible, so it would be set on reviewers' schedules. David Bottimore offered to send emails to reviewers in the next few days to confirm their availability in early December for the second conference call, with December 5 as the tentatively preferred date. Charles Menzie thanked all attendees for participating and closed the call.

Summary of Conference Call on December 5, 2003

Summary of Minutes of December 5, 2003 Conference Call on Peer Review of *Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment*

This document summarizes the discussion from the December 5, 2003, conference call, highlighting the major recommendations and suggestions provided during the call. It complements the Chair's summary of comments prepared following the call, which contains more details. Furthermore, the individual reviewers' comments should be consulted for even greater detail on some of the issues raised during the call..

Introduction

David Bottimore of Versar opened the call by reviewing the agenda and purpose for the conference call. He thanked the reviewers for submitting thoughtful comments and acknowledged Charlie Menzie for preparing the draft summary. Following introductions and a brief review of the purpose and process for the call, he turned over the floor to the Chair, Charlie Menzie, to lead the discussion.

Prior to the conference call, a summary of comments was prepared by Charles Menzie (Chair) based on written comments, which was intended to stimulate discussion during the call. The summary was structured according to (1) key issues identified in the reviews, (2) overview of general comments, and (3) responses to charge questions.

Key Issues and General Comments

There was broad agreement that the document met its major goals and objectives, so the comments focused on how the Framework can be made more useful to the intended audiences. The major issues addressed in the comments and during the call included: management-related considerations including how to judge the strengths and limitations of the TEM approach, the need for clarification of text and consistent use of terminology, issues related to estimating bioaccumulation, dose response, and uncertainties (including possible use of probabilistic methods).

The reviewers discussed these issues in detail, which resulted in several suggestions and recommendations for the document. Discussion might be added to the Executive Summary or Introduction that gives the reader a more complete view of the pluses and minuses of the method (e.g., increased costs onto regulated parties or greater review burdens onto regulators). Several reviewers suggested that the document provide an illustrative example(s) of the application of the method. The reviewers debated whether this example(s) should use real numbers or simply illustrate the process. There was 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. It was felt that any use of examples should be caveated to make sure the readers were aware that these were intended only for illustration. Similarly, the table of BSAFs generated discussion concerning the source(s) of the values as well as concerns that they might be viewed by readers 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 come from.

There was 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 the suggestion that EPA either include an example or some discussion of the value of sensitivity analysis (perhaps in the Uncertainty section). The document makes various references to other methods for doing PCB risk assessment (specifically for aroclors and homologues [totals]). The document should provide clarification on this so that risk assessors can have a better understanding of how to use the TEM approach in concert with other approaches for assessing risks associated with PCBs. Part of this discussion addressed whether the TEM methodology requires measurement or estimation of all dioxin-like compounds including dioxins, furans, and PCBs in order for it to be valid. Several reviewers voiced their opinions that 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 and there is no evidence that dioxins and furans are of concern (no known source or findings of elevated concentrations in biota). The document should be clear on this matter and should include some discussion of the limitations (i.e., uncertainties) of including only PCBs in the approach.

It was strongly suggested that Figure 6 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 easier for the ecological risk assessor who is uninitiated in the use of the toxicity equivalence methodology to grasp the concept. One reviewer also suggested that the document should not overemphasize the accuracy of the WHO values. This might be handled with a text box in the Introduction, noting that eco-TEFs determined by WHO for fish and birds should be considered as interim and preliminary values.

Responses to Charge Questions

The remainder of the discussion during the call addressed the reviewers' responses to the charge questions. Charlie Menzie led the panel through each question, summarizing the major points that had been made in the written reviews and asking individuals to add their thoughts.

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.

In general, the reviewers felt that the document contains the information needed to understand and implement the TEF methodology. There were some suggestions for clarifying certain points, and some differences of opinion on the value of particular tables

and figures. A suggestion was made to move the "Conclusions" section upfront and make it into an Executive Summary or part of the Introduction. Organizing the document in this manner would help readers to obtain an overview of the methodology before they enter the detailed portion of the guidance. One reviewer's comments suggested that a few sentences be added to mention risk methods that could be used in addition to the hazard quotient method, such as probabilistic and joint probability analysis. While some reviewers felt that such topics are overly complex for this document, others believed that a brief mention would be appropriate. A suggestion was made to check all figures and tables for complete legends so that the figure or table can stand alone. Similarly, a few definitions weren't clear and terminology is sometimes used in manners that are inconsistent. For example, on page 20 the term "uncertainty factor" is used but is not adequately explained. One reviewer suggested that EPA either develop that concept further or not raise the issue.

Discussion addressed the WHO TEF values, which are a key foundation to the TEM approach described in EPA's document. While some reviewers felt that the presentation of the WHO values and how a practitioner could "move away from these values" was too long, other reviewers clarified by saying that the approach actually highlights the potential for "refining values" for more site- and species-specific application. A few reviewers contemplated that this discussion could be shortened by moving some of it to an appendix, but most reviewers felt that it was essential to the document and should be retained in the body of the Framework. A comment was made that some of the WHO values should be examined in more detail, or additional discussion should be added to the document, because of different species' sensitivities. Specifically, it was noted that the mono-ortho PCBs are not toxic to fish. Another reviewer added that EPA's document might be expanded a bit more in the basic difference between the species sensitivity for dioxin-like compounds and the relative potency differences observed between mammals and fish for mono-ortho PCBs.

The final discussion of charge question 1 considered breaking out Table 3 to provide information for each major animal class. Each table would provide information on the toxic responses for 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. This change would help to give the reader a feel for the relative sensitivity of the endpoints.

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.

In general, the peer reviewers found the document's use of terminology to be clear, though there are some issues and areas of inconsistency. It was recommended that the document receive a good technical editing, preferably done by someone who is not familiar with the content (and would pick up on these inconsistencies). There were some comments about specific aspects of these terms as well as other words used in the

document. For example, the authors should consider the following issues on use of acronyms. The document could use RP, instead of ReP, to represent Relative Potency. In addition, there was strong sentiment that the acronym (term) TEQ should be retained rather than TEC. TEQ is so well entrenched in the literature that introducing the new term TEC would only add to the confusion. 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. Reviewers suggested that EPA consider moving the definitions on page 4 to Section 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.

Clarification of the terminology and usage would help to tighten the document. For example, the terms compound, chemical and congener are used interchangeably. One reviewer felt that the symbol (IIsocw), used to describe the sediment-water concentration quotient, appears unconventional, and that other recognized symbols have been used such as Kd or Kp or K. Another reviewer noted that the word "receptor" is used in several different contexts, which might cause confusion. Another minor comment was related to the term "potency," which should be changed to "relative potency" when discussing a compound's potency relative to 2,3,7,8-TCDD. The same comment applies to the use of "potency factor" in place of the correct term, "relative potency factor."

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

Reviewers felt that the document does a good job of explaining the advantages of the TEM, but felt that the document could be improved. As mentioned in the earlier general comments, several reviewers felt that the document should help the risk assessor (and risk manager) to understand the scientific benefits as well as potential costs of using this approach, in comparison to other available methods (e.g., homologue-specific approach). Providing this discussion, possibly entitled "Advantages and Limitations for the TEQ Methodology," in the Introduction might help the reader to see how uncertainty is reduced by using this approach. It was also pointed out by a few reviewers that the method is applicable to vertebrates but not for invertebrates. As a result, it was suggested that the document clearly 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. EPA might consider changing the title of the document to reflect that the TEM approach method applies to fish and wildlife.

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?

In general the reviewers felt that there was adequate explanation but several comments and suggestions were discussed that might help to clarify the document. It was also noted

that the issue of bioaccumulation is addressed in more detail under Charge Question 8. Several reviewers felt that the introduction to the document should emphasize that the TEM approach is intended to be used for upper trophic levels, based on measured or modeled tissue levels of dioxin-like compounds in biota. As mentioned earlier, the document might benefit from the presentation of an illustrative example (either using real or hypothetical data). In this, EPA should consider providing 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. Several reviewers voiced the opinion that the document should also address the issue of non-detects, because it is a science-policy issue that is important to the use of the TEM in an ecological risk assessment, due to the low levels of congeners that occur in some media. EPA might consider developing a short section for the main portion of the document or, alternatively, treat this in the uncertainty section. One reviewer expressed concern about applying TECs in the diet. This concern was 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 type would be useful.

5) 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.

a) Please comment on the completeness and clarity of this discussion.

Reviewers generally found this discussion complete, however, there were a number of comments and suggestions related to clarity and the need for some additional guidance for interested users of the TEM. One reviewer felt that it would be useful to have more discussion about what situations "trigger" an assessment to develop assessment-specific RPF values. While it is recognized that the WHO factors are starting points, the document should be enhanced to show how to make these site-specific selections without being arbitrary. One reviewer suggested that EPA provide more text or possibly a decision tree on how to make this decision to reduce the potential for arbitrary outcomes. Another reviewer suggested that EPA consider the Bursian et al. (2003) paper along with the Tillitt paper for the example on mink, because this study may not be appropriate for derivation of TRVs because of potentially confounding impacts of other co-contaminants on mink that have been accounted for in the study. Discussion also questioned the application of the method for dose-response assessment to the target organ/tissue levels versus concentrations from the diet. Reviewers felt that it would be advantageous to examine as many different tissue levels as possible when applying the TEM approach. It would be helpful to include a website address in the Framework Document for the 1997 TEF database (Tim Kubiak). 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.

b) 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.

Reviewers found the matrix helpful, although its description would benefit from clearer writing. First, it should be clearly stated that the matrix is a tool for guiding the selection of ReP values from which to derive a RPF. While reviewers noted that the highest levels (highest quality information) would rarely be available, the matrix could guide future research efforts, which should be noted. One reviewer stated that the dose-specificity axis is a new and important addition. Extensive discussion addressed the terminology used to describe the matrix axes, with several resulting suggestions from different reviewers. Overall, it was suggested that “matrix model” be simplified to just call it the matrix. Also, it would be clearer to refer to categories as "levels" and not "tiers" in order to distinguish between these levels of information and tiers of risk assessment.

Several suggestions were provided for renaming the axes, as well as the levels on each axis (see comment summary for more detail). With respect to the axes, there was discussion that the term "specificity" might be changed to “similarity” or "relevance." Some of the specific suggestions are noted below:

- 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.

Furthermore, it was suggested that for the y-axis, Endpoint Similarity, the levels could 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)

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

The reviewers generally agreed that the qualitative discussion of uncertainties was adequate, though a few reviewers would like to see more quantitative tools for assessing uncertainty. Most acknowledged that it would be acceptable to include discussion regarding specific sources of uncertainty and the possibility of indicating magnitude and direction of uncertainty. 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. Sensitivity analyses could be used to guide the levels of effort they devote to the different components of applying the methodology.

As discussed earlier, the detection limit issue is an issue that might be addressed in this section, with respect to the potential impact of different assumptions to the risk estimate. Reviewers also discussed the relative uncertainties of other assumptions inherent in the TEM approach, such as the additivity assumption. While it might be too detailed for this Framework document, one reviewer felt that there is some evidence of antagonism among congeners, which might be noted in the discussion of the underlying basis for the TEM approach. Finally, several reviewers pointed out that 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.

7) Are you aware of any essential references that have been omitted?

Discussion of this charge question was brief, stating that select references are suggested in individual reviewers' comments (and the comment summary). Three categories of references were provided, addressing toxicity, exposure, and statistics. It was suggested that EPA review these for contextual information.

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

Many reviewers felt that the discussion of bioaccumulation and exposure is not adequate and should be improved. It is important that the document introduce to the reader the different possible approaches, with direct measurement of tissue levels as having the greatest certainty. 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. It appears 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 should 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. In addition to input from the reviewers, EPA (Phil Cook) acknowledged that there is additional information that could be added to help the reader work through the proper selection of methods and/or to have confidence in certain values. Reviewers concluded this discussion by suggesting that EPA expand this section to note that 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. Furthermore, there was a strong sentiment among reviewers that BAFs (water to tissue) would not be a reliable way to estimate tissue levels

9) Please provide any other comments or recommendations you may have.

This charge question was not discussed during the call. It is recommended that EPA consult each reviewer's specific comments for additional detail.

Conclusion

The conference call ended with reviewers congratulating EPA on producing an excellent document that should be very useful. It was reiterated that the Framework met its major goals and objectives, so the comments provided by the reviewers focused on how the Framework can be made more useful to the intended audience.