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

#	Page	Line	Peer Reviewer Comment	EPA Response
1	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).	Text has been added to the introductory paragraph. In addition, the 1998 workshop is acknowledged and described (with reference) in Section 1.2. All individuals that participated in the Workshop, and their contributions, are provided in the Workshop report (EPA, 2001a)
2	1		The Introduction should acknowledge that there are other 'Ah' inducers (PAHs, flame retardants) that may contribute to dioxin-like toxicity but are not covered as part of this exercise. These are first mentioned on P11, 16 to 24.	The suggested change has been made; text added to introduction.
3	1	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).	The suggested change has been made; cumulative has been changed to "combined."
4	1		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 read 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.	Additional language has been added to the introduction concerning the target audience for this methodology, and references to EPA reports, including the 1998 Workshop Report (EPA, 2001a) have been added.

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5	**1**	**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.	The section has been revised and the comment is no longer applicable. However, it should be noted that in the Preface, it is stated that the focus of this framework is on the TEF methodology and that it is not a comprehensive guide to risk assessment involving dioxin-like chemicals. Accordingly, the methodological considerations associated with using the TEF methodology are presented in Sections 3.1 and 3.2 to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations of using the TEF methodology against other methods they may be considering. The type and number of other approaches to be considered will be specific to each ecological risk assessment (ERA); hence, it is outside the scope of this Framework to attempt to anticipate all possibilities. This activity is best conducted during the planning and problem formulation phases of the specific ERA.
6	**1**	**28**	'which should' to 'to'	The suggested change has been made.
7	**2**	**Fig 1**	Add chlorine atom symbol to both rings on left panels	The suggested change has not been made because the left panels are provided to illustrate the possible positions and numbering convention for chlorine atoms; the right panels illustrate the placement of chlorine atoms.
8	**3**		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.	No changes necessary.
9	**3**		Section 1.1. A very useful clarification of the plethora of terms, definitions, and acronyms related to this approach.	EPA concurs. This section was developed in direct response to the recommendations from the 1998 Workshop.

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10	**3**		Chapter 1.1. The extension of terminology with RPF is an appropriate one compared with those used by the WHO in 1997.	No changes necessary.
11	**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 definition of an RPF is essentially that of a TEF, but without the "consensus" opinion. That is, an RPF is to be based on one or more studies, after careful consideration. Relative potency determined in a single study that is used in risk assessment would be designated as an RPF in risk assessment, where the RPF = ReP. The RPF definition has not been changed to require two endpoints. The requirement of more than one endpoint may be too restrictive, i.e., more than one study on the same endpoint would be a corroboration and add strength, but it would be an RPF, not an ReP (if the document is to be consistent with the definition of ReP established by the WHO expert meetings).
12	**4**		The definition for TEC should be included in the list.	The definition has been added.
13	**3**		The second paragraph on page 3 (it begins with, "The WHO meeting report..") is very clearly written. I agree with the recommendation to use ReP rather than REP, since ReP is more grammatically correct.	No changes necessary.
14	**3**	**Text 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'.	The suggested acronyms have been added to Text Box 1.

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15	**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.	The Framework adopts the terminology clarified in the 1997 WHO Expert Meeting (Van den Berg, 1998). Additional clarifications, such as introduction of the term TEC, are made in response to recommendations from the 1998 EPA-DOE Expert Meeting (EPA, 2001a). The majority of other reviewers concurred with EPA's introduction of clarified terminology.
16	**5**	**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."	The paragraph referenced describes the state of knowledge during NATO/CCMS deliberations (circa late 1980s) and uses the past tense, i.e., "were known." To further clarify, the word "significantly" was added to the sentence. Likewise, in the second sentence referenced, the word "significantly" was added.
17	**6**	**15**	Delete the first "available"	The suggested change has been made.
18	**6**	**19&25**	reconcile 13 vs. 12 congeners	Text has been added to explain the changing number of PCB TEFs.
19	**6**		Chapter 1.2. 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.	Text has been added to the 7th paragraph of Section 1.2.
20	**6**	**32**	P6, 32: line ends in the middle of a sentence.	The formatting has been corrected.
21	**6**		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.	The contributions and reviews by Safe and co-workers are acknowledged in the 4th paragraph of Section 1.2.

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22	7	Table 2	Table 2 outlines the WHO TEFs, I would have a preferred some brief discussion here identifying the different endpoints used for derivation of TEFs in mammals, birds and fish. This is found later on in the document.	The purpose of this section is to provide an overview of the historical development of TEFs, not the underlying scientific basis. A discussion of the different endpoints used to derive the WHO TEFs would be quite lengthy, and as the reviewer notes, these details are found later in the document. As a "pointer" to the reader, references to the source of the WHO TEFs are provided as footnotes.
23	9		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.	Reference to details of the WHO scheme is made in Section 2.1. This point is further illustrated in the mammalian (mink) example in Section 3.3.2.3.
24	9		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.	Discussion and reference to the 2005 WHO conclusions has been added to Section 2.1. Discussion of and reference to similar conclusions of the NRC has been added to Section 2.1.
25	9	5	Underline "for each dioxin-like compound"	The suggested change has been made.
26	9	16	Change "estimates" to "estimate" Delete "a" and insert "it's" Insert "(TEC)" after "concentration"	The suggested changes have been made.
27	9	18	Delete "the" and insert "both"	The suggested change has been made.
28	9	19	Delete the second "the"	The suggested change has been made.
29	9	20	Delete "chemicals" and insert "congeners"	The suggested change has been made.
30	9	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.	The suggested change has been made.
31	10	1	Delete "It should be noted that" and insert "However"	The suggested change has been made.
32	10	2	Delete "however, that"	The suggested change has been made.
33	10	8	Delete "inhibition or synergy" and insert "antagonism or synergism"	The suggested change has been made.

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34	**10**	**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.	While this is a valid point, the Framework document is citing the 1997 and 2005 WHO expert workshops as the source of the "criteria" for inclusion. Neither of these reports included lack of response in AHR-null organisms as a criterion.
35	**10**	**23**	Insert "an" after "elicit"	The suggested change has been made.
36	**10**	**30**	Delete "seven" and insert "7"	The suggested change has been made.
37	**11**	**1**	Begin sentence with "For PCBs,	The suggest change has been made.
38	**11**	**9**	Chapter 2.1 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.	EPA agrees that the nature of non-dioxin-like effects of PCBs in wildlife is currently not well defined. However, it may be possible to discern these effects as more information is gathered on non-dioxin-like effects. Therefore, EPA has indicated ERA for both may be warranted, i.e., in the future. The references cited in this paragraph present approaches that could be explored for conducting a dual analysis to discern the critical endpoint(s).
39	**11**	**11**	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.	The composition and concentrations of PCBs will differ for each ERA. The text provided simply acknowledges that more than one MOA may be operative for PCBs and that the analysis and decision about which need be considered (i.e., dioxin-like, non-dioxin-like, or both) needs to be addressed during problem formulation of the specific ERA.
40	**11**	**12**	Delete "examples" and insert "references"	The text has been revised; the comment is no longer applicable.

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41	**11**	**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.	The reference has been added.
42	**11**	**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.	Text has been added to clarify that the list of compounds includes byproducts of flame retardants and combustion thereof.
43	**12**	**8**	Insert "RELATIVE" after "APPROPRIATE"	The suggested change has been made.
44	**12**	**10**	Section 2.2 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.	No changes necessary. EPA notes that while we appreciate the reviewers opinion on the relative frequency of use of WHO-TEFs vs. assessment-specific RePs or RPFs, the purpose of this document is to provide guidance on how to go about selecting or deriving assessment-specific relative potency factors when it has been determined that they will provide a better estimate risk for a specific ERA. Furthermore, the issues discussed in Section 3 relative to selecting or deriving relative potency factors also provide risk assessors with a framework for evaluating the applicability of and describing uncertainties associated with any relative potency factor, including the WHO-TEFs.
45	**12**	**17**	A number of toxicological endpoints are listed. These have not been defined. You could include these in the list of abbreviations.	The definition of each endpoint has been provided in the text and added to the list of abbreviations and the glossary.
46	**12**	**21**	Define CYP1A in a footnote.	The abbreviation has been defined in the text and the glossary.
47	**12**	**24**	Chapter 2.1 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.	EPA envisions that compounds that meet the criteria outlined in Section 2.1 could be assigned RePs or RPFs in ERAs. Reference to the WHO criteria for inclusion in the TEF methodology has been added to this paragraph.

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48	**12**	**27**	Start the sentence as follows: "Values of the TEFs WHO98" and delete "values"	The suggested change has been made.
49	**12**	**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.	The suggested change has been made.
50	**13**	**1**	Delete "relative potency factors" and insert "RePs"	The suggested change has been made.
51	**13**	**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.	The text has been revised.
52	**13**	**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.	The text has been revised.
53	**13**		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.	The existing text applies as is. BAFs are used when using tissue-based TEFs, i.e., concentration in an organism. However, when TEFs are based on studies of effects resulting from administered doses (e.g., most of the mammalian TEFs), then a BAF conversion is not needed.
54	**13**	**21-24**	Chapter 2.3. 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.	No changes necessary.

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55	**13**	**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.	Considerations for use of bioaccumulation factors are discussed in detail in Sections 3.3.1.3 and 3.3.1.4, as noted in the parenthetical statement at the end of the paragraph.
56	**14**	**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? [NOTE: Comment (1) is #5 in this compilation.] What is reference U.S. EPA 2001d?	Each of the guidance documents referenced contains compilations of exposure and effects information that may be pertinent to conducting an ERA for dioxin-like chemicals. The methodological considerations associated with using the TEF methodology are presented in Sections 3.1 and 3.2 to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations of associated with using the TEF methodology against other methods they may be considering. The type and number of other approaches to be considered will be specific to each ERA; hence, it is outside the scope of this Framework to attempt to anticipate all possibilities. As provided in Section 3.1, this activity is best conducted during the planning and problem formulation phases of the specific ERA. The reference to U.S. EPA 2001d has been changed to U.S. EPA 2001b.

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57	**14**	**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.	These issues are part of risk management and should be discussed in planning. The Considerations in Planning section (3.1) and Text Box 2 have been revised.
58	**14**	**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.	This section has been revised; the comment is no longer applicable.
59	**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?}	Section 3.1 has been extensively revised to address the issues raised. Text Box 2 has been revised to outline considerations to be made in the planning phase related to whether the TEF methodology is an appropriate choice for a particular ERA. Guidance for answering those aspects of the questions specifically related to the use of the TEF methodology is included in the Framework. However, specific methodological and cost-benefit choices will vary depending on the specific assessment (e.g., nature and extent of contamination; matrix of interest; receptors of concern) and on the data quality objectives defined for the assessment and hence, will need to be made on a case-by-case basis.

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60	**17**	**Text Box 2**	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?	The bullet has been revised.
61	**17**	**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.]	This comment now applies to Text Box 3. A partial revision has been made. Use of the term "receptor" for an ecological entity was not used in this document to avoid confusion with reference to the Ah receptor. Furthermore, EPA's Guidelines for Ecological Risk Assessment define an assessment endpoint to include an entity and an attribute.
62	**17**	**Text Box 2**	5th Check, L6 - Change "endpoints" to "endpoint" and then after the word "endpoint" insert "species."	This comment now applies to Text Box 3. The suggested change was not made; assessment endpoint is consistent with EPA's Guidelines for Ecological Risk Assessment, i.e., an assessment endpoint is the entity and attribute being protected. Another reviewer correctly pointed out that use of "assessment endpoint species" (on page 33) is inconsistent with EPA guidance.

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63	**18**	**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.	EPA agrees that relevance to risk management goals needs to be considered in planning and scoping as discussed in EPA's Guidelines for Ecological Risk Assessment (EPA, 1998). However, as stated in the Preface this Framework focuses on considerations for using a specific tool, the TEF methodology, within an ERA and is not a comprehensive guide on how to conduct an ERA for dioxin-like chemicals. Therefore, the discussion in this document regarding receptor is focused only on those characteristics that are relevant to applying the TEF methodology.
64	**18**	**9**	An updated version of Hahn 1998 is: Hahn (2002) Aryl hydrocarbon receptors: Diversity and Evolution, *Chem.-Biol. Interact.* **141:** 131-160.	The reference has been added.

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65	**18**	**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: 1) 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. 2) Karchner, S.I., Powell, W.H., and Hahn, M.E. (1999) J. Biol. Chem. 274: 33814-33824. 3) Abnet, C.C., Tanguay, R.L., Hahn, M.E., Heideman, W., and Peterson, R.E. (1999) J. Biol. Chem. 274: 15159-15166. 4) Hansson, M.C., Wittzell, H., Persson, K., and von Schantz, T. (2003) Gene 303: 197-206.	Section 3.2.1.1. has been expanded and the suggested references added.
66	**18**	**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.	The discussion of invertebrate AHR has been expanded.

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67	**18**	**14**	Change the word "demonstrate" to "conclude".	This section has been revised; the comment is no longer applicable.
68	**18**	**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.	The paragraph is specifically addressing relative sensitivity of one toxicity endpoint across different classes of organisms. Therefore, it would not be appropriate to split apart the 3 classes of organisms as suggested. The opening sentence has been revised to clarify.
69	**18**	**28**	Change "non-human primates" to "monkeys".	The suggested change has been made.
70	**18**	**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.	The reference has been added.
71	**18**		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.	Text has been added to the 4th paragraph of Section 3.2.1.1.
72	**19**	**6**	Insert ":" after "with"	The suggested change has been made.
73	**19**	**14-15**	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.	The text has been modified.

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74	**19**	**14-15**	Chapter 3 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.	The text has been modified.
75	**19**	**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.	The paragraph in Section 3.2.1.1. regarding amphibians, reptiles, and primitive fish has been revised.
76	**20**	**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."	The suggested change has been made.
77	**20**	**20**	I don't think you need to refer to the exposure assessment as "complicated". Simply state what needs to be considered.	The suggested change has been made.
78	**20**	**26**	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.	The suggested change was not made. All risk assessments do not have the same purpose. Determination of appropriate assessment endpoints for a specific ERA should be determined in the problem formulation phase, not in a guidance document. In addition, dioxin-like chemicals are toxicants, but not toxins.

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79	**20**	**28**	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.	Assuming commenter means through page 22, line 7. The text has been revised. The glossary of terms clearly defines both terms.
80	**20**	**30**	Eliminate the parenthetical phrase about equilibrium. This really does not add anything and can be misleading.	The text remains (without parentheses), but was revised in response to another reviewer.
81	**11**	**12**	This not is not completely correct, since the analysis by Giesy and Kannan, 1998 did use the proposed WHO TEFs.	The text has been revised; acknowledging that Giesy and Kannan used the 1998 WHO-TEFs.
82	**21**		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	NOTE: This comment applies to Table 3. Table 3 has been updated and revised extensively to include additional references. The Framework has been updated to include additional references suggested by peer reviewers and public commenters, recently published references pertinent to BAFs/BSAFs, and updated conclusions and recommendations from the WHO expert meeting in 2005 and the NRC report on TEFs published in 2006. Endocrine disruptor effects have not been incorporated, as they have not been established to be AHR-mediated. The WHO expert meeting did not consider these effects in revising the mammalian TEFs in 2005 either.

#	Page	Line	Peer Reviewer Comment	EPA Response
83	**21**	**Table 3**	First column on "Effect" Delete "21" from "Immunotoxicity" Add "embryo/" before "fetal" Consider adding "Cardiovascular Toxicity"	The suggested changes have been made.
84	**21**	**Table 3**	The word chicken needs to be fixed in the table. In addition, do fish have chloracnegenic effects?	Table 3 has been updated and revised extensively, including the references.
85	**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.	Table 3 has been updated and revised extensively, including the references.
86	**21**	**Table 3**	Second column on "Fish" Hyperpigmentation is not chloracne - consider deleting the "+"	Table 3 has been updated and revised extensively. The comment no longer applies.
87	**21**	**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. Porphyria 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.	Porphyria has been added to Table 3. The Kennedy et al. reference has been added to Table 3 and the References section. The edema reported by Gilbertson et al. in herring gulls cannot be attributed specifically to dioxin-like chemicals because several other chemical toxicants were also found in the birds.
88	**22**	**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.	The text has been revised.

#	Page	Line	Peer Reviewer Comment	EPA Response
89	**22**	**5-8**	This is an awkward sentence. Please clarify.	The sentence and paragraph have been revised.
90	**22**	**14**	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.	The 3rd paragraph of Section 3.2.1.2. has been rewritten to clarify.
91	**22**	**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?	The 3rd paragraph of Section 3.2.1.2. has been rewritten to clarify.
92	**22**	**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.	The sentence and paragraph have been revised.
93	**22**	**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.)	EPA disagrees with the comment, and has added a reference (Burkhard et al., 2003) to support the text regarding benthic versus pelagic food chain bioaccumulation.
94	**22**	**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?	EPA disagrees with the comment and has added a reference (Burkhard et al., 2003) to support the text regarding benthic versus pelagic food chain bioaccumulation.

#	Page	Line	Peer Reviewer Comment	EPA Response
95	**22**	**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".	The paragraph has been revised; the suggested change has been made.
96	**22**	**29-30**	Would suggest "...competing mechanisms of bioaccumulation and metabolism..." better captures the issue.	The paragraph has been revised; the suggested change has been made.
97	**23**	**7**	Consider a better way to refer to "opposing factors". These factors do not really oppose one another.	The paragraph has been revised.
98	**23**	**12**	What do you mean by "population vulnerabilities"?	The text has been changed to "population effects."
99	**23**	**16**	Do you mean Variations in the ***composition*** of dioxin-like compounds?	Yes; the text has been revised.
100	**23**	**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.	This comment appears to be objecting to characterization of relative sensitivity. The previous sentence sets out that this paragraph is addressing susceptibility, i.e., the integrations of sensitivity * exposure.
101	**23**	**18**	Insert "than fish" after "sensitive"	The suggested change has been made.
102	**23**		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.	Table 3 summarizes, with references, effects of dioxin-like chemicals on various species. In ERA effects in tested species are most often extrapolated to assessment-specific "target" species using scientific evidence and judgment. The "target" species is assessment specific, i.e., the ecological entity part of the assessment endpoint is ERA specific as defined in problem formulation. Therefore, it is not possible to identify a definitive list of possible target species and endpoints suitable for all ERAs.
103	**23**	**30**	Change "guild" to "community".	The text has been changed to "class".
104	**26**	**8**	I am not sure what is meant by, "Determination of theoretical or empirical measures of exposure".	The bullet has been rewritten and a parenthetical added per comments from another reviewer [comment #105].

#	Page	Line	Peer Reviewer Comment	EPA Response
105	26	8	number 2. Change to: "Determination of theoretical or empirical measures of exposure *(duration, frequency and intensity).*	The bullet has been rewritten and parenthetical added.
106	26	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.	The quotient method is a simple method, but it can be a defensible risk estimation method depending on the purpose and scope of the ERA. Nonetheless because a range of examples is not provided, reference to the quotient method alone has been removed.
107	26	11	A minor point, but a quotient method is not an estimation of "risk" per se, only an indication of exceedance of some threshold.	Reference to quotient method has been removed.
108	28	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.	Section 3.2.1.4 is on pages 23-24. It is unclear if the proper section has been referenced by the reviewer. Section 3.2.1.1 addresses species for which the methodology applies (i.e., those with AHR) and the relative sensitivity among species. The appropriateness of various TEFs, RPFs, and/or RePs is the subject of Section 3.3.2. Assuming the reviewer is referring to Section 3.3.2, this section has been substantially revised based on comments of other reviewers.

#	Page	Line	Peer Reviewer Comment	EPA Response
109	28	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.	This comment now applies to Text Box 4. The text referenced has been revised.
110	28	29-30	"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.	The suggested change has been made; the sentence has been deleted.
111	29		Para 1 - Define the term "congener-specific"	"Congener-specific" has been defined in the text.
112	29	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.	Discussion of the prerequisites, strengths, and limitations to be considered in applying the TEF methodology are provided in Sections 1, 2, 3.1, 3.1.1, and 3.2.2. Section 3.1 (Considerations in Planning) specifically raises the issue that costs and benefits need to be considered during the planning phase of an ERA. However, costs will vary depending on the scope and objectives of the ERA and will vary over time. Therefore it is not appropriate to provide a specific comparative analysis within the Framework document.
113	29		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.	The paragraph has been revised to reflect that the specific analytical methods may change over time and that which is deemed "sufficient" would be dependent on the goals and data quality objectives (DQOs) of the particular ERA and hence, should be determined during planning (Text Box 2) and problem formulation (Text Box 4).

#	Page	Line	Peer Reviewer Comment	EPA Response
114	30	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).	The text has been revised.
115	30	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.	The "average" text has been removed. EPA's approach to BAFs includes carbon normalization to minimize over estimation of bioavailability (EPA, 1995a, 2000c, 2003).
116	30	20	Chapter 3.3.1.2. I think the pattern on congeners in abiotic media usually does not reflect that found in biotic samples.	The text has been revised.
117	30	26	Insert underlined word: "to obtain predicted concentrations"	Text has been added.
118	30	31	Chapter 3.3.1.2. Besides administered dose, aspects of bioavailability (C-content and aging) could be mentioned.	Bioavailability is discussed in Section 3.3.1.3.
119	31	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.	The text has been shortened for clarity.
120	31	17	No real guidance was provided here. What do you expect the risk assessor to do?	The text has been changed to suggest that assessors "describe" the errors introduced.
121	32	5-27	Chapter 3.3.1.3. This is a good reflection of the actual situation.	No changes necessary.
122	32	13-16	This statement implies that estimation of tissue concentrations is a relatively straightforward and robust procedure – it is not.	This is an introduction/conceptual statement. Sections 3.3.1.3, 3.3.1.4, and 3.3.1.5 are dedicated to providing the details of performing such a procedure in a robust fashion.
123	32	17	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.	Clarifying text has been added, reflecting the current situation of mammalian TEFs largely based on administered dose.

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124	33		Chapter 3.3.1.4. See general comment earlier and remarks about validation.	A "validation" by modeling with actual sediment concentrations is in the reference Cook et al., 2003, as mentioned in Section 3.3.1.3. Additional text has been added to the end of Section 3.3.1.4 further discussing this publication, and the examples in Tables 4-6, as presented in Section 3.3.1.4, are an illustration of how this "validation" exercise was conducted.
125	33	4	Insert "s" after "PCDF"	The suggested change has been made.
126	33	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…".	The text has been revised.
127	36	Table 4,5,6	general question: Relative potencies used to generate TEFs are usually derived from <u>molar</u> ratios of TCDD potency and congener potency. However, TEC calculations usually apply these TEFs or RPFs to concentrations expressed as <u>masses</u> (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?	Molar ratios are commonly used for fish TEFs, but not for birds and mammals. The error is small, but may be calculated based on difference in molar weight.
128	36	Table 4,5,6	Column 4 consists of "Predicted" concentrations and should be edited to show this fact.	Tables 4, 5, and 6 have been revised.

#	Page	Line	Peer Reviewer Comment	EPA Response
129	36	Table 4,5,6	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.	A note has been added to the titles for Tables 4 - 6. The footnote to BSAFs indicates the specific lipid and organic carbon % used in normalizing them. Additional text and references to specific examples have been added. As indicated by the reviewer, the text indicates to the reader that decisions and assumptions were made in providing the examples and need to be made for any such exercise by answering questions in Text Box 5.
130	39	Text Box 4	symbols like C, fl and fsoc should be in italics exactly as they are portrayed in the formulas. Similarly P43, l7 & l8.	This comment now refers to Text Box 5. The suggested changes have been made.
131	40	1	"following two equations" Where does this sentence begin??	The formatting has been corrected.
132	40	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/BSAF models can be a major source of uncertainty and can grossly overestimate the concentrations of these compounds in aquatic organisms.	References have been added to peer-reviewed publications on the validation of the approach.
133	40	30-31	This line states that $D_{i/r}$ is the <u>difference</u> between P_{socw} values but text box 4 says "<u>ratio</u> between P_{socw} values for". Which is correct? Also, words are missing from bottom of text box.	This comment now refers to Text Box 5. The section has been revised to resolve the discrepancy. Missing text in Text Box has been corrected.

#	Page	Line	Peer Reviewer Comment	EPA Response
134	41	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.	This section has been rewritten to provide more explanations and references to peer-reviewed publications that provide the basis for the guidance.
135	41	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?	The BSAFs from Lake Ontario were used merely as examples for illustrative purposes. A note has been added to each table to make this clear. The text has been revised to clarify, and reference to the Lake Ontario BSAFs has been provided.
136	43	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.	Text has been added to clarify.
137	43	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.	Analytical methods are addressed in Section 3.3 (see Text Box 3 & Section 3.3.1.1); therefore, the text has been moved to Section 3.3.1.1.

#	Page	Line	Peer Reviewer Comment	EPA Response
138	44	Figs 7-9	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.	There exists much misunderstanding regarding applications of TEFs to media. Figures 7 to 9 are provided with the intent to illustrate the error that can be introduced if assessors inappropriately apply TEFs directly to abiotic media.
139	45	8	Change "insect" to "invertebrates".	The suggested change has been made.
140	45	22	Begin new paragraph at "Although".	The suggested change has been made.
141	45	14-18	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.	In lieu of highlighting or providing another case study, reference to a recent peer-reviewed publication (Burkhard, 2006a) that describes the basis and examples of extrapolation of BAFs/BSAFs across ecosystems has been provided. Further, the paragraph that follows the one referenced also refers to the Workshop Report (EPA, 2001a) that includes such an ecosystem case study.
142	46	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.	Additional references (e.g., Burkhard et al., 2004, 2006) have been added. These peer-reviewed publications include examples from not only the Great Lakes ecosystem, but also from the Hudson River, a lotic system.

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143	46	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.	A reference has been added that discusses the approach in detail (Burkhard et al., 2006). The text already includes an example of a food-web model that can be used, i.e., Gobas (1993). This model has been applied previously by EPA in establishing appropriate bioaccumulation factors for setting Water Quality Criteria for the Great Lakes (EPA, 1995) and developing EPA's Methodology for deriving National Human Health Water Quality Criteria (EPA, 2000). EPA is also developing additional guidance on developing site-specific BAFs.
144	46	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.	The text has been revised, and references to several peer-reviewed publications that describe and validate extrapolation of bioaccumulation factors have been added.
145	46	11	This statement needs to include some information relative to the quantification of uncertainties when using these models to estimate tissue concentrations.	The text has been revised, and references to several peer-reviewed publications that describe and validate extrapolation of bioaccumulation factors have been added.
146	46	15	Should end: "…total maximum daily load (TMDL) limits."	The text has been corrected.
147	46	18	It is probably better to say that TEFs and RPFs provide the means to convert exposure to a complex mixture into a singe dose metric for mixtures of ….(Note this is discussed nicely on P. 62, Line 7.)	This section was highly redundant with other sections and has been removed.
148	46	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.	This section was highly redundant with other sections and has been removed.
149	46	31-32	Seems to be a typographical error resulting in the repetition of part of the previous sentence.	This section was highly redundant with other sections and has been removed.

#	Page	Line	Peer Reviewer Comment	EPA Response
150	46	31-32	These lines are repeated on the next page.	This section was highly redundant with other sections and has been removed.
151	46	31-32	Lines repeated on p. 47.	This section was highly redundant with other sections and has been removed.
152	46		There appears to be some scrambling of text here.	This section was highly redundant with other sections and has been removed.
153	46	31-32	Delete	This section was highly redundant with other sections and has been removed.
154	46	31	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).	The purpose of the Framework is to educate and provide guidance on how to evaluate and select non-default RePs and RPFs when the decision has been made that relative potency factors that are more specific than the WHO-TEFs are necessary or desirable for the particular ERA. Therefore, Section 3.3.2 is critical to the purpose of the document and has not been moved to an appendix. However the introductory section was highly redundant with other sections and has been removed.
155	47	Text Box 5	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.	The text box poses questions that should be addressed when using the TEF methodology within the broader context of an ERA. The TEF methodology does not dictate what analytical method or detection limits need to be used. Additional text regarding considerations for analytical methods has been added to Sections 3.1, 3.1.1, and 3.1.2.

#	Page	Line	Peer Reviewer Comment	EPA Response
156	47	Text Box 5	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?*	This comment now refers to Text Box 6. The suggested text has been incorporated.
157	47	Text Box 5	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?	This comment now refers to Text Box 6. The following text was added: "*Have I considered implications of biomagnification for higher trophic level organisms?*"
158	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).	This comment now refers to Text Box 6. The text box has been moved up to the same page as the referenced text.
159	47	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.	This section was highly redundant with other sections and has been removed. Discussion of benefits and methodological considerations has been revised and moved to Section 3.1.
160	47	28-33	Consider re-working this sentence. The "benefits" are not made clear.	This section was highly redundant with other sections and has been removed. Discussion of benefits has been revised and moved to Section 3.1.
161	49	16-18	Replace "hierarchical" with "hierarchal"	The suggested change has been made.
162	49	27	Insert underlined word: "...suggest that <u>greater</u> species sensitivity..."	The section has been revised; the comment is no longer applicable.
163	49		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.	The examples provided in Section 3.3.2.4 reflect this current reality.
164	50	Fig 10	Fig. 10 legend, insert underlined words: "...how similar a reported dose <u>metric</u> is to the dose <u>metric</u> ~~of concern~~ <u>used to define TEFs and the TCDD dose-response relationship</u>.	This comment now refers to Figure 11. The legend has been revised to incorporate the suggestions.
165	50	Fig 10	The use of color made it difficult to see the words in the lower right box.	This comment now refers to Figure 11. The color has been adjusted.
166	50	7-9	Sentence needs clarification. It is unclear what this means.	Text has been added to clarify.

#	Page	Line	Peer Reviewer Comment	EPA Response
167	51	30	Insert underlined word: "When level 4 data <u>for some congeners</u> are in agreement..."	The text has been revised to incorporate the suggested emphasis.
168	54	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.	The suggested reference has been added.
169	54		Chapter 3.3.2.3. The given examples provide a good illustration of the problems associated with the suggested use of RPFs.	This comment now applies to Section 3.3.2.4. No changes necessary.
170	54	14-18	Change RPF(s) to ReP(s) on these lines	The suggested changes have been made.
171	54	23	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.	This comment now applies to Section 3.3.2.4. Assessors select to use TEFs vs. RePs or RPFs, and they select RePs to use alone or in combination (i.e., to derive an RPF). The text has been clarified to read "select ReP" or "derive RPF," as appropriate.
172	54	25	This sentence does not make sense and needs to be re-written.	The text has been revised.
173	55	1	Insert "mortality" after "stage"	The suggested change has been made.
174	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.	An example as suggested by the commenter, i.e., using real data from the literature, is provided in the the mink example. The three examples provided were developed to increase in realism; however, they commensurately increase in complexity. Therefore, EPA has kept the bird example somewhat generic to illustrate the concept more simply, i.e., without the complications of evaluating specific data.

#	Page	Line	Peer Reviewer Comment	EPA Response
175	55	17	Include the reason why this is so.	The text has been revised to include rationale.
176	55	21-23	This is an awkward sentence. Please clarify.	The sentence has been deleted.
177	55	33-59	(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.	This section has been rewritten to provide more clarity.
178	55	34	Was the source of liver tissue the mink dam or mink kit?	Text has been added to clarify.
179	56	4	Delete "an" after (A) and after (B). Change "ReP" to "RePs"	The suggested changes have been made.
180	56	5	Change "ReP" to "RePs" Delete ", which are" Delete "the"	The suggested changes have been made.
181	56	26	Move all text to L7	The suggested change has been made.
182	56	27	Move and center this title above the text inserted on L7	The suggested change has been made.
183	57	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.	The suggested change has been made.
184	57	8	Delete "female" and insert "dam" after "mink	The suggested change has been made.
185	57	9	Delete "female" and insert "dam" after "mink	The suggested change has been made.
186	57	21	Insert "dam" after "mink"	The suggested change has been made.
187	57	26	Can you state "would be advisable" more strongly? Don't you mean, "then exposure should be based on the".	The suggested change has been made.
188	57	28	Insert "dam" after "mink"	The suggested change has been made.
189	58	7	Delete "female" and insert "dam" after "mink"	The suggested change has been made.

#	Page	Line	Peer Reviewer Comment	EPA Response
190	58	12	"…closer to the H4IIE-RPFs than rat liver H4IIE-RPFs"?????	The text has been revised.
191	59	7	Line ends prematurely. Delete "return function" so text moves up to fill complete line	This section has been revised; the comment is no longer applicable.
192	59	33	Insert "vertebrate" before " the word "class"	The suggested change has been made.
193	60	Table 8	First column, bottom row, second box - Delete "female" and insert "dam" after "mink"	The suggested change has not been made.
194	61	5	Delete the first "a"	The suggested change has been made.
195	61	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.	This is a conclusion is drawn from working through the examples. The preceding sections were dedicated to outlining such dosimetry (exposure regime and purity) considerations.
196	61	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.	This is a conclusion is drawn from working through the examples. The preceding sections were dedicated to outlining such dosimetry (exposure regime and purity) considerations. As stated, the text highlights that endpoint alone should not be the only consideration.
197	61	15	This bullet is unclear. Please clarify.	Text has been added to clarify.
198	61	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.	The figure is a conceptual reference to the dose-response underlying TEFs, RPFs, and RePs. The figure itself is not material to the Framework document. The curve is "representative" of typical TCDD dose-response curves, but it is not derived from a specific study or curve; hence, no reference is needed. Description of methods for generating dose-response curves is beyond the scope of this document. Furthermore, generation of such dose-response curves is a common exercise in the field of toxicology, and there are many statistical approaches and even more software packages to do this.

#	Page	Line	Peer Reviewer Comment	EPA Response
199	61	30	Insert “cardiovascular and” before “endocrine”	The suggested change has been made.
200	61	29	Move “immunotoxicity” after “wasting syndrome;” on L30	The suggested change has been made.
201	61	34	Insert “in different fish bird and mammalian species” after “compounds”	The suggested change has been made.
202	63	13-16	Expand this to include non dioxin-like effects of PCBs as a consideration in risk assessment.	The suggested change has been made.
203	63	Text Box 6	The last question is missing a word “evidence?”	This comment now refers to Text Box 7. The text has been corrected.
204	63	Text Box 6	words missing at bottom (same true of some others).	This comment now refers to Text Box 7. The text has been corrected.
205	63	Text Box 5&6	Switch “Text Box 6" (P63) with “Text Box 5" (P64)	This comment now refers to Text Box 7. The text has been corrected.
206	64	11	'complete' for 'comlete'	The suggested change has been made.
207	64	1	“complete”	The suggested change has been made.
208	62	Text Box 5	Page 62 Text Box 5 is repeated here. [page 64]	The redundant text box has been deleted.
209	64	Text Box 5	This text box is a duplicate of that on page 47.	The redundant text box has been deleted.
210	64	Text Box 5	Text box 5 is repeated here (first appears on p. 47).	The redundant text box has been deleted.
211	64	Text Box 5	The text box 5 on this page is a repeat of the one on page 47.	The redundant text box has been deleted.

#	Page	Line	Peer Reviewer Comment	EPA Response
212	64	26-32	Chapter 3.4.2 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.	The reviewer's comments are consistent with the conclusions presented in the concluding paragraph in Section 3.1.2. Due to the current limitations mentioned, EPA will not make the conclusions recommendations at this time.
213	65	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.	The references have been added.
214	65	10-13	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, L26-L18)	The sentence has been revised. The point regarding other substances with dioxin-like activity is made in the next paragraph. The "false-positive" conclusion comes directly from the EPA/DOI expert workshop report. (Assuming reference is to line 16-18) This point is acknowledged in the next paragraph.
215	65	9-16	Bioassay approaches can be used in a TIE approach to demonstrate that PCDD/Fs account for a certain proportion of the TEC.	The section has been revised; the comment is no longer applicable.

#	Page	Line	Peer Reviewer Comment	EPA Response
216	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?	Monte Carlo is mentioned in Section 3.4.3.1.4, and additional text has been added to bullet 5 in Section 3.4.3.1.3. Yes, there are challenges to quantitation of uncertainty that are unique to the TEF methodology as described in point 4 of Section 3.4.3.1.4.
217	65	31	Insert "than" after "significant"	The suggested change has been made.
218	66	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.	Reports on non-additive effects are acknowledged (Van den Berg et al., 1998). Text has been added to elaborate on the point regarding the magnitude of non-additive effects.
219	66	29	Delete "Tillet" and insert "Tillitt"	The correction has been made.
220	67	14	What is meant by "multiple models"?	The text has been revised to clarify that biological models are being discussed.
221	67	20-21	Add one line space	The formatting has been corrected.
222	67	25	I suggest reephrasing this in terms of reducing the uncertainty associated with a derived RPF.	The suggested change has been made.
223	67	26	Insert "relative" after "true"	This section has been revised; the comment is no longer applicable.

#	Page	Line	Peer Reviewer Comment	EPA Response
224	67	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.	The statement says, "carried over into deriving TEFs" (see Henry et al., 2001). At this time there are no common practices for quantifying uncertainty in the TEFs or TECs. Therefore, while the Framework raises the issue that uncertainty needs to be acknowledged and discussed, it is limited to qualitative terms for the purposes of this Framework. Although there are no common practices at this time, additional text has been added referring to Haws et al. (2005), which addresses recent approaches and quantitative uncertainty analysis.
225	68	12	"in the report" What report?	The section has been revised; the comment is no longer applicable.
226	68	14	Insert "," after "sensitivity" Insert "and" after "field"	The suggested change has been made.
227	69	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.	EPA disagrees with this comment. The sentence referenced acknowledges the uncertainty, as stated "Hence, extrapolation of bioaccumulation factors (BAFs or BSAFs) from one ecosystem to another is a source of uncertainty." Furthermore, inclusion of text and examples regarding BAF/BSAF in Sections 3.3.1, 3.3.1.4, and 3.3.1.5 attests to the fact that the issue has not been dismissed.
228	69	11	How is uncertainty in the extrapolation characterized - qualitatively, quantitatively, other? Is this assumption of reduced uncertainty intuitive or empirical?	The reduction in uncertainty has been empirically demonstrated by Burkhard et al. (2006a). Whether uncertainties associated with an ERA-specific extrapolation are characterized qualitatively, quantitatively, or by other means is a decision to be made in during the ERA process, as part of planning and problem formulation.

#	Page	Line	Peer Reviewer Comment	EPA Response
229	69	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.	The reference has been published in a peer-reviewed scientific journal. The reference has been updated in the text and in the References section.
230	69	16	change "measuring" to "determining". Also mention the bioaccumulation models here as they can also be site-specific.	The suggested change has been made.
231	69	17-20	Water is an irrelevant matrix for determination or monitoring.	The sentence has been revised.
232	69	26	Delete "with" and insert "while"	This section has been revised; the comment is no longer applicable.
233	69	32	The last paragraph at Line 32 is confusing. Please clarify.	A new introductory sentence has been introduced to clarify. As referenced, this point was discussed in detail in Section 3.3.1.4.
234	70	14-15	Delete "toxicity equivalence factors" and insert "TEFs"	This section has been revised; the comment is no longer applicable.
235	70	16	Insert "vertebrate" after "deriving" Insert "-" after "class"	This section has been revised; the comment is no longer applicable.
236	70	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.	The section has been revised; the comment no longer applies.
237	71	1	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.	EPA has decided to retain the current organization of the framework, including the conclusions at the end. However, salient conclusions are presented in the Preface and Introduction.
238	71	2	I endorse this application of the use of a sensitivity analysis.	No changes necessary.
239	71	6	Insert "relative to 2,3,7,8-TCDD" after "potencies"	The suggested change has been made.
240	71	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…"	The suggested change has been made.

#	Page	Line	Peer Reviewer Comment	EPA Response
241	71	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.	Section 3.1 has been rewritten and re-organized. The suggested additions have been made to Sections 3.1, 3.1.1, and 3.1.2. EPA has decided to retain the current organization of the Framework, including the conclusions at the end. However, salient conclusions are presented in the preface and introduction.
242	71	32	Insert "relative" after "appropriate"	The suggested change has been made.
243	71	33	Insert "relative" after "selecting"	The suggested change has been made.
244	72	5	Insert "relative" after "new"	The suggested change has been made.
245	72	21	Insert "exposure to" after "from"	The suggested change has been made.
246	82	7-8	Delete the end of the sentence beginning on L7 with "binding of ..."	The suggested change has been made.
247	82	39	Insert "relative to 2,3,7,8-TCDD" after "congeners	The suggested change has been made.
248	84	3	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.	The suggested change has been made.

#	Page	Line	Peer Reviewer Comment	EPA Response
249	84	9-10	Delete the sentence beginning with "The concept of ..." 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."	The suggested changes have been made.