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

The U.S. Environmental Protection Agency (EPA) appreciates the many thoughtful comments submitted on the draft Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment ("the Framework"). This summary provides the Agency's response to the major comments received. Many of the comments submitted on the Framework had common themes and have been grouped accordingly, along with the Agency's responses. A number of narrowly focused and editorial comments were also offered. These suggested changes are not detailed here, but many were incorporated into the final Framework. Comments were received from the following parties:

- Earth Tech
- Minnesota Chippewa Tribe
- Integrated Waste Services Association
- BBL Sciences
- General Motors Corporation
- American Chemistry Council & Utility Solid Waste Activities Group
- Chlorine Chemistry Council
- U.S. Army
- American Forest & Paper Association
- Arcadis G&M, Inc.
- Frank Ossiander

1. Comments Concerning the Advantages and Disadvantages of the TEF Methodology Relative to the Total PCB Approach

Several reviewers commented on the advantages, disadvantages, and uncertainties of the TEF methodology, particularly as it compares to the total PCB approach. Representative comments, along with the Agency's responses, follow:

A. The TEF methodology appears valid for extrapolation of relative risk to fish. The Framework provides an excellent description of the dioxin TEF/TEC approach that, until now, has been used for ecological risk assessments but never explained in this detail. Technically, the guidance provides the most scientifically valid approach that exists for evaluating mixtures of dioxins and dioxin-like compounds for risks to fish. This document provides additional guidance for the proper use of these TEFs within ecological risk assessment. Although there are several weaknesses in the approach as acknowledged, it is currently the most scientifically valid of the approaches that exist.

EPA Response: EPA agrees with this characterization of the document.

B. While the Framework states that the TEF methodology is "not the only available tool for assessing the integrated risks of PCDDs, PCDFs, and PCBs" and that it is "one of several tools within the broader context of ecological risk assessment," this message should be made clear to the reader so that the document is not construed as a regulatory guidance document with the intent of prescribing a single universal method for assessing risks from these compounds to ecological receptors.

EPA Response: The *Preface* highlights that the Framework is intended for guidance only and that it does not establish any substantive "rules" under the Administrative Procedure Act or any other law and will have no binding effect on EPA or any regulated entity. The *Introduction* explains that the Framework is a general guidance to provide EPA risk assessors and managers with the most current science policy. The *Introduction* also clarifies that while EPA believes that the Framework provides a sound, up-to-date presentation of a method for use in conducting risk assessments involving dioxins and dioxin-like chemicals and serves to enhance the application of the best available science, EPA and others may conduct risk assessments for such chemicals using approaches and methods that differ from those described in the document. *Section 3.1 – Considerations in Planning* and *Section 3.2 – Considerations in Problem Formulation* provide guidance for determining whether to use of the TEF Methodology in a particular ecological risk assessment.

C. At most PCB sites the total PCB approach produces similar, or somewhat more conservative, risk estimates than the TEF methodology. Comparisons of the toxic potency of environmental PCB mixtures have shown that their potencies are similar or lower than the potency of Aroclor 1254 (the basis of most toxicity thresholds in ecological risk assessment). These data suggest that the total PCB approach can be used to predict ecological risks of PCBs through environmental exposures. This has been confirmed by reviews of ecological risk assessments for PCB sites conducted on

both a total PCB and a TEQ basis. These reviews indicate that total PCB-based risk estimates are generally comparable to or more conservative than TEQ-based risk estimates, and that the TEQ approach has no advantage over the total PCB approach.

EPA Response: The Framework acknowledges that the TEF methodology is not the only available tool for assessing the integrated risks of PCDDs, PCDFs, and PCBs. The type and number of other approaches to be considered will be specific to each ecological risk assessment. *Section 3.1 – Planning* and *Section 3.2 – Problem Formulation* provide guidance for use in determining whether to use the TEF Methodology in a particular ecological risk assessment.

D. The TEF methodology provides no marked improvement over current ecological risk assessment technologies. The Framework fails to provide sufficient evidence that the TEF methodology will significantly improve how ecological risk assessments are currently conducted, improve the past record of remediation activities, or impact the recovery of impacted ecosystems.

EPA Response: The Framework acknowledges that the TEF methodology is not the only available tool for assessing the risks of PCDDs, PCDFs, and PCBs. The type and number of other approaches to be considered will be specific to each ecological risk assessment. *Section 3.1 – Considerations in Planning* and *Section 3.2 – Considerations in Problem Formulation* provide guidance for use in determining whether to use the TEF Methodology in a particular ecological risk assessment. Specifically, the benefits and methodological considerations associated with using the TEF methodology are discussed extensively to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations of using the TEF methodology relative to other potential methods.

E. The TEF methodology can present problems when one is attempting to remediate a site with multiple sources of contamination or to derive TMDL or NDPES permit limits for an aquatic ecosystem with multiple dischargers. TEQ concentrations measured in tissues of biota may be the result of uptake of PCDD/PCDFs and/or PCBs from multiple sources. Because many higher trophic level receptors, which are the species likely to have the highest levels of contamination, may have large feeding ranges and may bioaccumulate congeners from many different sources, it is very difficult to determine the impact that remediation of one source will have on total bioaccumulation.

EPA Response: The Framework was developed in response to recommendations from the EPA/DOI Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalence Factors to Fish and Wildlife (U.S. EPA, 2001). The report from this workshop includes two case studies illustrating problem formulations that include the use of the TEF methodology in two different ecosystems and regulatory scenarios. One case study describes how the TEF methodology could be incorporated into a prospective ecological risk assessment with the risk management goals to (1) control discharge of toxic pollutants through the

National Pollutant Discharge Elimination System (NPDES) permit program and (2) establish a total maximum daily load (TMDL) to ensure that both human health and fish and wildlife populations are protected from the cumulative effects of exposure to dioxin-like chemicals.

F. There is an over-reliance on data and information from studies conducted on the Great Lakes for nearly all of the ecological application examples provided in the document, despite the fact that this methodology is being applied in risk assessments dealing with various ecosystems throughout the world.

EPA Response: All of the examples are hypothetical illustrations of how to use the TEF methodology. A note has been added to Tables 4, 5, and 6 to clarify that the data are for illustrative purposes only and are not recommended default values. Additional examples of incorporating the TEF methodology into ecological risk assessments for a lentic and a lotic system are provided in the Workshop Report on the Application of 2,3,7,8-TCDD Toxicity Equivalence Factors to Fish and Wildlife (EPA, 2001; see previous comment). Additional references have also been added to the Framework to peer-reviewed publications that include examples from not only the Great Lakes ecosystem, but also from the Hudson River, a lotic system (*e.g.*, Burkhard *et al.*, 2004, 2006).

G. Although the Draft Framework mentions that PCBs may have "dioxin-like" (e.g., Ah receptor-mediated) and "non-dioxin-like" effects, it does not explain how the "non-dioxin-like" effects might be accounted for in applying the TEQ approach. The Framework should be revised to address in more detail how a risk assessor using the TEQ approach should account for any "non-dioxin-like" effects, and the Framework should acknowledge that Aroclor-based toxicity studies reflect the effects of both dioxin-like and "non-dioxin-like" congeners. The Framework should be revised to clarify EPA's intent regarding a "dual" approach to PCB ecological risk assessment in those cases where such an approach might be needed.

EPA Response: In the *Introduction*, EPA clearly states that the Framework and the TEF methodology on which it is based specifically apply to those effects of dioxin-like chemicals that are mediated by the aryl hydrocarbon receptor (AHR) and that meet the criteria set forth by the World Health Organization (WHO) Expert Meetings (Van den Berg *et al.*, 1998; 2006). *Section 2.1* clarifies that the Framework applies only to dioxin-like chemicals, and hence, only dioxin-like polychlorinated biphenyls (PCBs). Current evidence indicates that the greatest potential for effects on ecological endpoints of most concern (*e.g.*, growth, survival, reproduction) from exposure to PCB mixtures is from the AHR agonists (Giesy and Kannan, 1998; Rice *et al.*, 2002). However, the Framework also notes that these PCBs may cause toxicity via mechanisms independent of the AHR. Because the TEF methodology will only account for dioxin-like activity of PCBs, non-dioxin-like PCBs would need to be assessed using another approach/analysis, just like any other chemical of concern that may co-exist with the dioxin-like PCBs. A number of references are provided in the Framework for studies in which a dual analysis of risks based on total PCBs and on toxicity equivalence for dioxin-like PCBs was conducted to assess PCB mixtures (*i.e.*, Beltman *et al.*, 1997; Brunstrom and Halldin, 2000; Finley *et al.*, 1997; Giesy and Kannan, 1998; U.S. EPA 2005). The Framework is not a

comprehensive guide to conducting a risk assessment for dioxin-like or non-dioxin-like chemicals; hence, to elaborate further on the appropriate analysis for addressing risks of non-dioxin-like chemicals is outside the scope of the Framework.

2. Comments Concerning Framework Discussions of Uncertainty

Several comments suggested that the Framework needs to undertake a more rigorous treatment of uncertainties associated with application of the TEF methodology, and one commenter provided an analysis using published data for mink as a basis for evaluating assessment approaches for PCBs. Representative comments, along with EPA's responses, follow.

A. The Framework claims that the uncertainties associated with the TEF methodology are less significant than those associated with the total PCB approach, and that the TEF approach is more accurate, but these statements are not supported by any quantitative analysis. The primary argument in support of the claim that the total PCB approach involves uncertainty is based on the fact that the congener composition of PCBs in the environment weather. However, the Framework offers no discussion of weathering and no evidence that weathering increases the potency of PCB mixtures. Since the total PCB approach uses the "unweathered" PCBs to predict risk, the uncertainty related to weathering is the extent to which the total PCB approach will overestimate risk. This is an uncertainty that weighs in favor of using, not discarding, the total PCB approach to evaluate ecological risks as a screening tool. Moreover, review of the available data indicates that, if anything, weathering decreases the potency of environmental PCB mixtures.

EPA Response: The weathering of PCBs, coupled with differential PCB bioaccumulation, considerably alters the mixture of PCB congeners present in biota. With the passage of time, the PCB congener profiles that initially comprised commercial PCB formulations are less likely to resemble the PCB congener profiles found in biota. Hence, significant uncertainty may be introduced in assessing exposure and effects by assuming that congener profiles present in commercial mixtures used in toxicity tests (*e.g.*, Aroclors) are representative of PCB profiles in environmental samples. This uncertainty may result in either an over- or under-estimation of risk, depending on the pattern of change in the PCB profile. Therefore, EPA maintains that use of high quality congener-specific measurements of PCB concentrations and bioaccumulation and/or congener-specific bioaccumulation modeling to estimate exposures will result in more accurate exposure estimates. To aid risk assessors in finding more information about this issue, a reference to a recently published EPA report on the benefits of PCB congener-specific analysis for ecological risk assessment has been added in several places in the Framework (U.S. EPA, 2005).

B. A quantitative uncertainty analysis needs to be conducted to support Framework conclusions about the relative certainty and accuracy of the total PCB and TEF approaches. Assessments of PCBs conducted using the TEF approach are not "more accurate" and "more certain" than assessments conducted using the total PCB

approach. Whereas the TEF approach may be an appropriate and useful approach for ecological risk assessments of PCDDs and PCDFs (or mixtures of PCBs with substantial amounts of PCDDs and PCDFs), the TEF approach should not be the default approach nor should it take the place of the total PCB approach for assessing ecological risks at all sites where PCBs are present.

EPA Response: The Framework (*Section 3.1*) acknowledges that the TEF methodology is not the only available tool for assessing the risks of PCDDs, PCDFs, and PCBs. The type and number of other approaches that should be considered, as well as the results of a quantitative uncertainty analysis of each, will be depend on a number of factors specific to each ecological risk assessment (*e.g.*, the risk management goals, site-specific contaminants, assessment endpoints, etc.). It is outside the scope of the Framework to attempt to anticipate all possibilities. However, *Section 3.1 – Planning* and *Section 3.2 – Problem Formulation* provide guidance for use in determining whether to use the TEF Methodology in a particular ecological risk assessment.

C. A quantitative analysis of published results from studies testing the effects of PCB exposure on mink reproduction as a basis for evaluating various ecological risk assessment approaches for PCBs indicates that accounting for the bioaccumulative potential of ingested PCBs is far more important than accounting for the concentrations of "dioxin-like" PCB congeners in explaining the observed variation in reproductive success. This conclusion holds regardless of whether homologue or congener concentrations were used to estimate bioaccumulative potential. The evaluation of PCB congeners did not reduce uncertainty compared to the evaluation of total PCBs. Rather, toxicity estimates were more uncertain based on dietary toxicity equivalence concentrations (TECs) than dietary total PCBs, and the prediction of toxicity was more uncertain based on whole-body TECs than whole-body total PCBs. Based on this analysis, the Framework should be revised to remove statements indicating that a congener-based approach to ecological risk assessment is less uncertain, more accurate, or preferable to a homologue-based approach. The merits of a homologue-based approach should be acknowledged so that regulators do not mistakenly conclude that PCB congeners must be analyzed to properly assess ecological risks at PCB-contaminated sites. The draft Framework should also be revised to decrease the recommended reliance on toxicity reference values for TCDD in cases where congener-based assessments are undertaken.

EPA Response: EPA has recently published a report summarizing the benefits of using PCB congener-specific analysis in ecological risk assessment. To aid risk assessors in finding more information about this issue, a reference to this report has been added in several places in the Framework (U.S. EPA, 2005).

D. The 1998 Workshop participants stated that the development of relative potency estimate (ReP) and TEF values needed to be "adequately documented (including specific citations) in order to support the use of these values in regulatory risk assessment." The workgroup specifically noted that the World Health Organization panel did not provide adequate documentation for the selected mammalian TEFs

and that this omission was a "major limitation on the use of the document for risk assessment purposes" (EPA, 2001; p. 7-8). These deficiencies have not been adequately acknowledged or addressed in the Framework. In addition, specific information is not available to ecological risk assessors concerning the decision-making process that was used in assigning specific TEF values based on multiple studies. Although the Framework encourages risk assessors to consider the appropriateness of the individual TEFs to their site-specific conditions, without adequate documentation, risk assessors cannot reliably evaluate the appropriateness of the proposed TEFs to site-specific conditions, species, or endpoints of concern. Consequently, risk assessors are largely faced with either accepting the TEFs as presented, without consideration of their appropriateness, or conducting their own research into toxicological studies to select alternative values. Although acceptance of the existing TEFs without consideration of site specific factors may be inappropriate, the time and resources associated with having individual risk assessors independently evaluate the available data makes the alternative approach unrealistic. EPA should provide risk assessors with adequate background documentation for the selection of TEFs so that they can determine whether such TEFs appear appropriate to their species and endpoints of interest. While the Framework includes a link to the WHO database of available studies, this database lists the studies but provides no insight into the quality of the studies or the way that the studies have been considered in deriving the TEFs. Thus this database is not particularly helpful to risk assessors who need to evaluate individual studies for their quality and/or the species and endpoints of concern. In addition, because the database is limited in the information that it includes, it provides no insight into the uncertainties associated with the toxicological parameters that are derived from them, making it difficult for risk assessors to evaluate potential uncertainties associated with their selection, as recommended in the Framework.

EPA Response: The specific passage quoted from the 1998 workshop pertains only to the mammalian WHO TEFs. However, on page 12 of the Workshop Report (EPA, 2001), EPA added a footnote explaining that a review following the workshop indicated that the basis for establishing the mammalian TEFs had been better documented than initially concluded.

A large proportion of the Framework (*Section 3*) is dedicated to providing risk assessors with guidance on how to evaluate and select RePs and derive 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 ecological risk assessment. This guidance is equally applicable to evaluating the applicability of and describing uncertainties associated with the WHO-TEFs.

The purpose of the Framework is to provide guidance on how to apply the TEF methodology within ecological risk assessment and not to review the extensive data and methods on which WHO-TEFs are based. This information is available from other sources, all of which are referenced in the Framework:

- Van den Berg *et al*. (1998) contains criteria and methods used by the WHO expert panel to select studies and derive the 1998 WHO-TEFs.

- A 1997 ReP database created by the Karolinska Institute is available on EPA's web site along with the final Framework (http://www.epa.gov/osa/teffframework/). To EPA's knowledge, the Karolinska Institute is not presently updating or maintaining this database.
- Van den Berg *et al*. (2006) contains criteria and methods used by the WHO expert panel to select studies and derive the 2005 WHO-TEFs.
- The database of mammalian RePs that was used by the WHO expert panel to select studies and derive the 2005 WHO-TEFs was reviewed and refined by Haws *et al.* (2006).

E. It is critical that detailed guidance be established for the development of ReP/TEF values, and that this guidance be peer reviewed prior to the Draft Framework being finalized. Without such guidance, risk assessors will likely be faced with blindly accepting the proposed TEF values, despite the fact that they may not be appropriate for individual circumstances.

EPA Response: Criteria and methods used by the WHO expert panel to select studies and derive the 1998 and 2005 WHO-TEFs have been published in peer-reviewed scientific journals (Van den Berg *et al.*, 1998, 2006; Haws *et al.*, 2006). The Framework *is* a detailed, peer-reviewed guidance for organizing, reviewing, and selecting ReP data and developing RPFs if/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 ecological risk assessment.

3. Comments Concerning Screening-Level versus Higher-Tiered Risk Assessments

Several reviewers commented that application of the TEF methodology should be limited to use as a screening-level assessment tool, primarily at sites dominated by complex mixtures of PCDDs, PCDFs, and PCBs because it only predicts risk to individual animals and has not been validated for estimating population-level effects. Representative comments, along with the Agency's responses, follow.

A. EPA's 1997 *Ecological Risk Assessment Guidance for Superfund* indicates that risk assessments at the individual level can only be used as a screening tool to determine whether further investigation is warranted and that screening level ecological risk assessments should not be used as a basis to set cleanup standards. Although the Framework acknowledges that ecological risk assessment involves evaluation of population-level effects, it does not recognize that the TEF approach is an individual-level approach. The Framework implies that an ecological risk assessment could be based solely on the TEF approach and, in fact, mentions field studies as a possible, but not required, line of evidence for ecological risk assessment. The Framework should stress that where the screening level risk assessment indicates the possibility of unacceptable ecological risk, the definitive risk assessment should place the greatest emphasis on field studies designed to determine if adverse effects are actually occurring.

EPA Response: The Framework is not intended to be a comprehensive guide for conducting ecological risk assessment for dioxin-like chemicals. The TEF Methodology is a tool for facilitating the cumulative effect of all the dioxin-like chemicals to which a species may be exposed. The TEFs or RPFs are used to translate concentrations of individual dioxin-like congeners into a common "currency," the TCDD-equivalent concentration (TEC). The issue regarding how well concentrations in biota (usually measured in individuals or pools of individuals) predict or project population-level concentrations is not specific to the use of the TEC as an exposure metric or to the TEF methodology. Likewise, the issue concerning how well individual-level effects benchmarks (like those from which TEFs are derived) can predict population-level risks is a general risk assessment issue that is not specific to the TEF methodology. Nevertheless, reference to a peer-reviewed publication (Cook *et al.*, 2003) that illustrates how the TEF methodology can be applied in a retrospective population-level assessment has been added in appropriate sections (*Sections 3.3.1.3 – 3.3.1.5*) of the final Framework.

B. The TEF methodology has not been validated for estimation of population-level field effects, which is a widely accepted technique for conducting ecological risk assessments. Nonetheless, the Framework incorrectly claims: "Use of the toxicity equivalence methodology results in more precise characterization of AhR mediated stressors and their potential effects in ecological receptors." This statement is not supported by appropriate references and thus goes beyond the scientific evidence linking TEF tissue concentrations modeled with biomagnification factors and with adverse population effects. Further, the Framework addresses only endpoints for individuals rather than populations. This approach is in direct contrast to the strategy set forth in EPA's guidelines for ecological risk assessment. EPA should stress that the TEF methodology, as outlined in the Framework, is a screening tool that assesses potential risks to individuals, rather than populations.

EPA Response: The particular sentence quoted has been removed from the document. See also the response to comment 3.A.

C. The Framework should place greater emphasis on site-specific studies designed to determine the presence of adverse population effects and state that actual population studies using measurements of tissue dioxin and dioxin-like activity are more valid.

EPA Response: The Framework is not intended to be a comprehensive guide for conducting ecological risk assessment for dioxin-like chemicals. Sampling designs and collection methods are not issues specific to the use of the TEF methodology, but rather issues to be addressed in the analysis plan for a risk assessment of dioxin-like chemicals. However, the Framework acknowledges that site-specific data (*e.g.* BAFs, BSAFs), used in conjunction with the TEF methodology (*i.e.*, to calculate the TEC), can reduce uncertainty in the exposure estimates. In addition, the majority of *Section 3* is dedicated to factors to consider in selecting relative potency values that are most appropriate for site-specific risk assessments. Finally, *Section 3.3.1.3* in the final Framework highlights

how uncertainty can be reduced by using a tissue-based approach when applying the TEF methodology.

D. The Framework fails to identify the fact that the TEF approach is a screening level tool that assesses theoretical risks to individuals but does not provide insight into the potential impacts to site-specific populations. While the Framework acknowledges that ecological risk assessment should involve the evaluation of population-level effects, it does not acknowledge that the proposed TEQ approach only provides a rough prediction of the potential for individual effects and provides no insight into the probability that population-level effects will be elicited. Because the TEQ approach is based largely on cellular and molecular level responses, it cannot approximate the impact of site-specific conditions and organism-specific compensatory mechanisms that may result in no impact or reduced toxicity in the wild.

EPA Response: The Framework is not intended to be a comprehensive guide for conducting ecological risk assessment for dioxin-like chemicals. The decision regarding whether a particular risk assessment should address individual- vs. population-level effects is not specific to the use of the TEF methodology, but rather is an issue to be addressed in the analysis plan for a risk assessment of dioxin-like chemicals. See also response to comments 3A-C.

4. Comments Concerning Dose-Additivity of Mixtures of Dioxin-Like Chemicals

Several reviewers commented that the TEF methodology is based on the incorrect assumption that doses are additive for mixtures containing dioxins, furans, or co-planar PCB congeners. Representative comments, along with the Agency's responses, follow.

A. The TEQ approach assumes that the toxicities of all individual congeners in a mixture are additive. This assumption is not borne out in the scientific literature and is likely incorrect. Currently the National Toxicology Program (NTP) is conducting research to test the validity of the additivity assumption. This ongoing research has not been acknowledged or discussed in the draft Framework but its results may be critical for an important assumption upon which the proposed Framework is based. It is important that the methodology proposed in the draft Framework not be adopted until NTP has released its findings that may confirm or refute the key underlying assumption of the TEQ approach that the toxic effects of TEQ mixtures are additive.

EPA Response: The National Toxicology Program (NTP) studies to validate the additivity assumption have been completed (Walker *et al.*, 2005). The results of these studies further support and confirm the additivity assumption underlying the TEF methodology. Additional text has been added to *Section 2.1* discussing and referencing studies supporting the additivity assumption, including the recent results from the NTP. Furthermore, the additivity assumption has been evaluated and accepted by two meetings of international scientific experts convened in 1997 and 2005 by the WHO International

Programme on Chemical Safety (Van den Berg *et al.*, 1998, 2006). In addition, the National Research Council of the National Academies (NRC) review of EPA's draft *Exposure and Health Reassessment of 2,3,7,8-TCDD and Related Compounds* included an evaluation of the additivity assumption. The NRC concluded that "from an overall perspective, this assumption appears valid, at least in the context of risk assessment" (NRC, 2006). Empirical evidence, including the most recent data supporting the additivity assumption, is described in *Section 2.1* and *Section 3.4.3.1.2*.

B. Although EPA identifies the possibility of non-additivity as a concern, it brushes this off by reference to Section 9.4 of the September 2000 version of the draft Dioxin Reassessment which purportedly provides a comprehensive review of the studies supporting the assumptions that form the basis for the toxicity equivalence methodology. The issue of additivity warrants more than a reference to a review of supporting studies in an outdated draft EPA document that is marked "Do Not Cite, Quote or Reproduce," especially since the current version of the draft Reassessment is about to undergo review at the NAS, with specific attention to be paid to the application of the TEQ approach to PCBs. Non-additivity is more than a concern. Several groups that have investigated interactions of PCB mixtures and congeners with TCDD and other aryl hydrocarbon receptor (AhR) agonists have reported less than additive interactions (Walker et al. 1996; Bannister et al. 1987; Haake et al. 1987; Biegel et al. 1989; Davis and Safe 1988,1989, 1990; Morrissey et al. 1992; Harper et al. 1995; Zhao et al. 1997a,b; Tysklind et al. 1995; Harris et al. 1995; Keys et al. 1986; Bosveld et al. 1995; Aarts et al. 1995). A few studies have shown some synergistic interactions (Schmitz et al. 1995; Van Birgelen et al. 1996; Bager et al. 1995), or both antagonistic and synergistic interactions, depending on the specific response and species (Silkworth et al. 1984, 1989a,b). Safe (1993) reviewed the available data on additivity, and concluded that "the TEF approach may significantly overestimate the TEQs for environmental extracts containing PCB, PCDD, and PCDF mixtures in which concentrations of the PCBs were >100-fold higher than the PCDDs and PCDFs." Collectively, these data indicate that the additivity assumption is not correct for all responses, species, and mixtures.

EPA Response: See response to comment 4.A. In addition, empirical data that support the additivity model specifically for ecological species is discussed on page 22 – 25. A large body of empirical research, both laboratory and field studies, on ecological species provides strong support for the additivity assumption. This information was not reiterated in the Framework in the interest of keeping the document clear and concise and focused on the application of the TEF Methodology. Nonetheless, additional text has been added to *Section 2.1* discussing and referencing studies supporting the additivity assumption.

C. Dose additivity can only occur when substances have the same mechanism of action. Using a less stringent standard for dose additivity, EPA assumes that substances share a common mechanism of action or a "common mechanism of toxicity" when they "share major steps leading to an adverse health effect following interaction of a substance with biological targets" (U.S. EPA, 1999; 2000). A chemical that binds to the Ah receptor and causes any effect is termed an agonist. Conversely, a chemical

that binds but has no effect or otherwise inhibits the occurrence of an effect is called an antagonist. Chemicals that bind to a receptor with no adverse effect compete with agonists for sites on receptors. Thus, while an antagonist occupies the receptor site, an agonist does not occupy it and therefore has no effect. Although the Ah receptor is capable of binding with a variety of molecules, the configuration of a chemical molecule determines whether binding occurs, as well as the strength of that bind. Should a chemical bind weakly to the Ah receptor it may be displaced by a competing chemical capable of creating a stronger bond with that receptor. Therefore, Ah receptor binding may be as competitive as it is additive. Furthermore, the Framework states "empirical data support the use of the additivity concept." This empirical evidence is insufficient particularly when there are significant financial impacts of the TEF-derived risk estimates on remediation activities and costs. The Framework should recommend further experimental studies of mixture effects, preferably on populations, to determine if the TEF methodology is appropriate and valid rather than stating, "A substantial effort has been made to test the assumptions of additivity and the ability of the toxicity equivalence methodology to predict the effects of mixtures of dioxin-like chemicals."

EPA Response: Overall, the occurrence of either antagonism or synergism is ratio and dose dependent, with antagonism occurring at higher doses (Van Birgelen *et al.*, 1996). Since antagonistic effects are seen at higher doses, usually above environmentally relevant doses, the use of additivity in the TEF concept is unlikely to result in large errors in predicting concentrations of TEQs when antagonists are present (Van den Berg, 1998). See also response to comments 4.A and 4.B.

5. Comments Concerning Inter-Species Variability

Several reviewers commented that there are significant differences in species responsiveness to AHR-mediated effects and data gaps and uncertainties across species that contribute to errors in TEF values. Representative comments, along with EPA's responses, follow.

A. The assumption that the sensitivity of broad classes of animals can be represented by point estimate TEFs is highly uncertain. As the Framework recognizes, the varying sensitivity of species to AhR-mediated effects represents one of the single largest sources of uncertainty associated with a TEQ approach. The Framework notes that "[t]he relative sensitivity to dioxin-like toxicity among species that possess the Ah receptor varies greatly, even within taxonomic class," and cites Hoffman et al. (1996) for the finding that TCDD-induced mortality varies by about 200-fold in bird species. Id. In fact, ranges of TEFs for dioxins, furans, and PCBs among different species and endpoints can be even larger – TEF values for a specific congener can vary by more than four orders of magnitude (Ahlborg et al. 1994). As noted by a Workshop participant, "interspecies differences in sensitivity to TCDD are so large they might in fact dwarf the uncertainties associated with the TEF approach" (USEPA, 1998a, at 23). See also USEPA (1998a) at 22, 45, 46, 49 & 60.

EPA Response: As explained in *Section 3.3.2*, analysis of available data indicates that the issues of species, endpoint, or dose metric differences in ReP data are separate from the issue of species differences in sensitivity to 2,3,7,8-TCDD. Two species that differ widely in their sensitivity to 2,3,7,8-TCDD can have relatively similar RePs for most congeners. For example, chickens are 119-fold more sensitive than ducks to *in vitro* effects of 2,3,7,8-TCDD, yet for TCDF and PCB congeners 126 and 81, the *in vitro*-based RPFs differ less than 5-fold between these species (Kennedy *et al.*, 1996). Similarly among fish, salmonids (most sensitive fish species tested) and zebrafish (least sensitive species tested) differ by more than 40-fold in their sensitivity to the early life stage toxicity caused by 2,3,7,8-TCDD (Elonen *et al.*, 1998). However, RePs based on zebrafish *in vitro* endpoints (*i.e.*, CYP1A induction in liver) are generally within 5-fold of RePs determined in a variety of rainbow trout *in vitro* systems when the same endpoint in the same tissues are compared (Henry *et al.*, 2001). Hence, differences in relative potency are less than proportional to the differences in species sensitivity. Analysis of rainbow trout and zebrafish RePs suggests that uncertainties surrounding application of the toxicity equivalence methodology are likely to be greater when applying TEFs-WHO_{98} values or RPFs across tissues or endpoints than across fish species (Henry *et al.*, 2001). Furthermore, Cook *et al.* (1997; 2003) used independent sets of TEFs to predict TECs for lake trout eggs from Lake Ontario. The TEC analyses differed by less than a factor of 10 even though different species and effects were used to measure the TEFs.

If data are available for a specific species that has significantly different sensitivity to these chemicals, this information may be used to support a species-specific analysis. The majority of the Framework (*Section 3*) is dedicated to describing how to conduct such an analysis to minimize this type of uncertainty.

B. Limited studies exist for TEF derivations for fish and birds. A vast majority of the evidence supporting TEF application in fish has been done using Salmonids and injection of eggs as a method of chemical dosing. Despite this limited scientific basis, the Framework clearly intends for the TEF values to be applied to a wide variety of species. The available data demonstrate a range of sensitivities that should be evaluated according to species's sensitivity distributions. This need is clearly articulated in the following statement: "The relative sensitivity to dioxin-like toxicity among species that possess the Ah receptor varies greatly, even within taxonomic class."

EPA Response: A large proportion of the Framework (*Section 3*) is dedicated to providing risk assessors with guidance on how to evaluate and select RePs and derive 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 ecological risk assessment. This guidance is equally applicable to evaluating the applicability of and describing uncertainties associated with the WHO-TEFs. Also see the response to comment 5.A regarding sensitivity vs. relative potency.

C. The Framework claims that the TEF approach will strengthen ERA while reducing uncertainty, yet on page 20 the Framework admits: "Given the known differences in

sensitivity among species and endpoints, risk assessors should consider the uncertainty introduced when extrapolating from a species or endpoint for which sensitivity has been established to a species or endpoint of unknown sensitivity." Hence, this Framework should clearly articulate the relative newness of the TEF approach and its need for substantial improvement that only further experimental studies can provide.

EPA Response: The cited sentence provides risk assessors with guidance that is generic to any inter-species or endpoint-to-endpoint extrapolation; it is not specific to the TEF methodology. The Framework does discuss the uncertainties associated with the toxicity equivalence methodology for estimating risks from mixtures of these chemicals as it would be appropriate to do for any methodology. Use of the TEF methodology in risk assessment is not new; it has been used in human health risk assessment since the 1980s and has been recommended for use in ecological risk assessments since at least the mid-1990s (Van den Berg *et al.*, 1998; U.S. EPA, 2001).

D. Elonen et al. (1998) have reported 32- to 100-day chronic fish early life-stage no-observed effect concentration (NOEC) values for the following species exposed to 2,3,7,8-TCDD:

Species	NOEC	LOEC	LC_{10}/LC_{50}
Lake herring	175	270[a]	509/902
Fathead minnow	235	435[a]	293/539
Channel catfish	385	855[a]	429/644
Zebrafish	424	2000[a]	1610/2610
Japanese medaka	455	949[a]	656/1110
White sucker	848	1220[b]	1590/1890
Northern pike	1190	1800a	1530/2460

[a] Significant decrease in survival compared to controls ($p < 0.05$)
[b] Significant decrease in growth compared to controls ($p < 0.05$)

Fish species other than Salmonidae have been examined for dioxins and dioxin-like compound toxicity, including zebrafish, fathead minnow, bullhead and channel catfish, mosquitofish, guppy, bluegill, largemouth bass, and yellow perch. Collectively, these studies demonstrate the general susceptibility of fish to dioxins, but differences in exposure protocols (flow-through, static, static renewal, egg injection, and dietary), life stages tested (adult, juvenile, larval, embryo, and egg), the toxicological endpoints examined (pathology, growth, mortality, enzyme induction, and development) and the general lack of tissue-specific congener data, have indicated a lack of an integrated understanding of various aquatic species and life stage sensitivity. The current TEF values reflect the limited dataset upon which development of these values is based.

EPA Response: The referenced studies in using various fish species and exposure regimens are the body of scientific evidence that provides an integrated understanding of fish sensitivity to dioxin-like chemicals. The cumulative body of scientific evidence provides an integrated understanding that fish early life stages and developmental endpoints (growth, development, mortality) are the most sensitive life stage to dioxin-like toxicity, *i.e.*, the adult and juvenile studies were some of the first conducted, which led to

later findings that earlier life stages, *e.g.*, embryos, are the most sensitive. Likewise, the fact that a number and variety of fish species have been tested for sensitivity to TCDD provides understanding of fish relative sensitivity and a rationale for basing TEFs on salmonids, the most sensitive species tested.

EPA agrees that the WHO-TEFs for fish are based on a limited set of data. However, derivation of relative potency from early life stage toxicity in salmonids and via egg injection studies was considered by the WHO expert panel as the most desirable, because: (1) salmonids are the most sensitive fish species to dioxin-like toxicity (of the ~20 species tested to date), (2) exposure at the egg stage represents responses to embryos, which are the most sensitive life stage for dioxin-like toxicity and, (3) egg injection provides a tissue-based dose-metric for measurement of the relative potency that is not confounded by maternal pharmacokinetic issues.

EPA's position that it is appropriate to use the World Health Organization (WHO) class-specific TEFs in ecological risk assessment is supported by the conclusions from two World Health Organization expert meetings (Van den Berg *et al.*, 1998; 2006), an EPA-DOI expert workshop (U.S. EPA 2001a), and the recent National Research Council (NRC) report (2006). The Framework describes a logical way in which risk assessors can organize relative potency data (including the WHO TEFs) according to species similarity, endpoint relevance, and dose relevance and consistency in order to understand the strengths, limitations, and uncertainties associated with such data and select the relative potency values that are most appropriate for a particular ecological risk assessment (*Figure 11* and *Tables 6-8*).

E. When discussing interspecies differences in sensitivity to TCDD itself, participants in the 1998 Workshop indicated concern that, while it is assumed that the effects reported in laboratory studies will result in population effects in wildlife, this assumption has not been substantiated with either laboratory studies of wildlife species or field studies (EPA, 1998). One Workshop participant stated that "[t]his lack of knowledge produces a level of uncertainty that dwarfs any presented by the TEF/TEQ approach. The current ecological risk assessment process is seriously compromised by the inability of the 'best available science' to accurately predict effects." (EPA, 1998; C-E-20). To address this concern, EPA (2003, p. 20) suggests "risk assessors should consider the uncertainty introduced when extrapolating from a species or endpoint for which sensitivity has been established to a species or endpoint of unknown sensitivity." However, as a practical matter, if interspecies extrapolation is necessary, it is not likely that there are adequate data available for the untested species or endpoint to be able to adequately "consider" the uncertainty, as recommended. Without any real data, it is impossible for risk assessors to make an educated guess about the degree or direction of uncertainty associated with the extrapolation. Thus, such a recommendation, while appropriate under optimal circumstances, is meaningless in data deficient situations.

EPA Response: The quoted reference continues in the Framework: "This uncertainty… should be handled in a manner similar to any other chemical for which interspecies extrapolations need to be performed (*e.g.* consideration of taxonomic relatedness)."

Sections 3.3.1.3 and *3.2* of the Framework provide a detailed presentation of the considerations to be made to select TEFs-$WHO_{98/05}$, RPFs, or RePs that introduce the least amount of uncertainty when incorporating the toxicity equivalence methodology into a risk assessment. Furthermore, the three-dimensional matrix introduced in the Framework (*Figure 11*) provides an approach for careful selection of the ReP, RPF, or TEF-$WHO_{98/05}$ based on the most appropriate studies. Gaps encountered in the matrix illustrate the areas where species-specific data or additional research may be needed to reduce uncertainty.

F. Methods and data available to usefully quantify *non-carcinogenic* risk from tetra-chlorinated dioxin (TCDD), congeners, and other specific co-planar polychlorinated biphenyls (PCBs) are lacking and the adverse effects across vertebrate classes are too variable for use, presently. This variation *cannot* be explained by affinity to the Ah receptor, Arnt, or other ligand bonding or enzymatic relationships. Given the extreme variability in response in laboratory animals, the TEF approach should not be used across all species until a more robust evaluation of adverse effects across taxa is completed. The Van den Berg et al (1998) paper does not address adverse effects specifically.

EPA Response: See the response to comments 5.A and 5.E.

6. Comments Concerning TECs and Dose-Response Curves

A. The shape of the dose-response curve may not be consistent for all congeners. Because TEFs are used to equate the toxicity of individual congeners to that of TCDD (at any dose or concentration), the approach also necessarily assumes that the dose-response curves for all endpoints and congeners are parallel to the dose response curve for TCDD. There are indications that this is not correct (Pohjanvirta et al., 1995; Putzrath, 1997). In addition, the doses required to produce toxic effects vary considerably among congeners and endpoints. Safe (1990) evaluated the relative dose response for various dioxin and furan congeners in terms of the potencies associated with different endpoints and found that the relative potencies varied by more than an order of magnitude depending on the endpoints considered.

EPA Response: TEFs are used to convert concentrations of dioxin-like chemicals to TCDD-equivalent concentrations. These concentrations are then used to determine toxicity from a TCDD dose-response curve. EPA recognizes that relative potencies can vary across endpoints; this possibility is discussed in *Section 3.3.2*. The Framework explains that when relative potency values are compared on the basis of the same species, tissues, and endpoints, the variability for a given congener is greatly reduced (Henry *et al.*, 2001). The Framework (*Section* 3) thus guides risk assessors in selecting relative potency factors that best match the species, endpoint, and dose metric to those of the toxicity reference value(s) that will be used in a particular ecological risk assessment.

B. The proposed use of the Toxicity Equivalence Concentration (TEC) is questionable because the Van den Berg et al. (1998) paper indicates that the biological meaning of

this value is obscure. It is understood that the TEC is used to try to assess potential additive effects of a mixture, but this does not appear appropriate for all ecological receptors given our lack of knowledge on how toxicity is occurring.

EPA Response: The referenced statement is incomplete as presented in the comment. The Van den Berg *et al*. (1998) paper says (emphasis added), "TEFs and TEQs are used for risk characterization and management purposes, e.g., to help prioritize areas of concern for clean-up. *However, in relation to the use of TEFs for abiotic compartments, the biological meaning of these values is obscure*." The Framework provides a discussion of the issue of misapplication of TEFs to abiotic media (*Section 3.3.1.2*), and Figures 8, 9, and 10, and their accompanying text, are provided to illustrate the error that can be introduced by such misapplication.

7. Comments Concerning the Use of BAF and BSAF Values

Several comments were provided to the Agency concerning the derivation and use of bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs), particularly as they relate to uncertainty and variability among sites, over time, and in trophic position. Representative comments, along with EPA's responses, follow.

A. The use of BSAF values in the Framework incorrectly assumes that these values do not vary with location, time, or concentration and that an overall average BSAF value can be calculated for a food web position. Based on the long list of confounding variables (e.g., habitat, location, species (Muir et al., 1992, Lake et al., 1990)), the data suggest that BSAF values may be useful for screening-level risk assessments, but not for higher tier assessments, as significant errors in estimated chemical residues in biota may result. The range or uncertainty of BSAF values should be considered in addition to the average value in interpreting risk calculations, as this will place any calculated organism residues in a proper context.

EPA Response: The Framework does not assume BSAFs do not vary across ecosystems, locations, food-webs, or species. As summarized in *Section 3.3.1* of the Framework, EPA's bioaccumulation approach includes extrapolation of BAFs/BSAFs when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). EPA's approach for acquiring and using BAFs/BSAFs is based on an extensive body of peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). This information is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

B. The Framework states that site-to-site extrapolation is appropriate if adjustments are made to reflect differences in lipid levels between species. There is, however,

some indication that lipid content may not be the only key factor in determining uptake of PCDD/PCDFs. Miller and Schram (2000) assessed the uptake of organochlorine compounds by Lake Trout and compared the observed results to a number of morphometric parameters. Using their total PCB results as a surrogate for the similarly recalcitrant PCDD/Fs, the authors reported a positive correlation between total PCB levels in flesh with length (r2=0.69) and age (r2=0.71), but no correlation (r2=0.03) with lipid content. This finding is significant and contributes substantial uncertainty to EPA's approach for site-to-site extrapolation because the correlation between lipid content and TEQ accumulation is a key underlying assumption of the proposed approach.

EPA Response: EPA's approach for acquiring and using BAFs/BSAFs is based on an extensive body of peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). This body of information establishes that bioaccumulation of PCDDs, PCDFs, and PCBs is a function of many factors (*e.g.* trophic level, food web characteristics, sediment organic carbon, organismal lipid, and sediment-water concentration quotient) and provides in-depth discussion of approaches for extrapolating BAFs/BSAFs when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). This information is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

C. The Framework places an inappropriate emphasis throughout the document on the use of published or generic bioaccumulation factors (e.g., BAFs, BSAFs), rather than a strong emphasis on the need to collect site-specific bioaccumulation data.

EPA Response: The Framework does not suggest "generic" or default BSAF values because EPA does not advocate or support such an approach. EPA's approach for acquiring and using BAFs/BSAFs is based on an extensive body of peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). Indeed, EPA's bioaccumulation approach includes extrapolation of BAFs/BSAFs when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). This information is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

D. The Framework recognizes that the feasibility of the TEF approach for ecological risk assessment relies on the assumption that BAFs and BSAFs are available (or can be calculated on a site-specific basis) to accurately predict concentrations of PCDD, PCDF, and PCB congeners in tissues of receptors or their prey from concentrations

measured in water or sediment. Moreover, the Framework advocates the use of site-specific BAFs and BSAFs due to the substantial uncertainties associated with extrapolation from other sites. However, notwithstanding EPA's recognition of the importance of using site-specific data to derive BAFs and BSAFs, the Framework inexplicably concludes that direct extrapolation of BAF/BSAF data from one location to another is acceptable. The Framework should more accurately and thoroughly describe the uncertainty associated with extrapolation of BAFs and BSAFs.

EPA Response: The Framework does not recommend extrapolating BAFs/BSAFs directly from one ecosystem/species/tissue to another because EPA does not advocate or support such an approach. Recognizing that site-specific BAFs/BSAFs may not always be available or feasible to determine, EPA's bioaccumulation approach does include approaches for extrapolating BAFs/BSAFs, but only when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). EPA's approach for acquiring and using BAFs/BSAFs is based on an extensive body of peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). This information is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

E. EPA acknowledges that BAFs and BSAFs are "the essential connectors of concentrations of PCDDs, PCDFs, and PCBs in the environment with concentrations in the diet or relevant tissues of organisms of concern which are then used to calculate ECs." (EPA, 2003; p. 43). The draft guidance does not, however, adequately discuss the uncertainties associated with the derivation of BAFs and BSAFs.

EPA Response: As summarized in *Section 3.3.1.4*, EPA has developed extensive guidance for minimizing variability in BAF and BSAF measurements and reducing uncertainties when extrapolating BAFs and BSAFs across ecosystems with similar conditions (U.S. EPA 1995a; 2000; 2003). Also included in *Section 3.3.1.5* is a discussion of approaches (*e.g.* the use of food-chain models and/or the "hybrid modeling approach") that can be used to adjust BAFs/BSAFs to decrease variability and increase accuracy when extrapolating across ecosystems. A discussion of uncertainties associated with the characterization of exposure, including both measuring and estimating tissue concentrations, is included in *Section 3.4.3.2.2* of the final Framework.

Uncertainties associated with EPA's approach for acquiring and using BAFs/BSAFs is discussed in detail in an extensive body of peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). In the interest of keeping the Framework concise and focused on the application of the TEF Methodology, EPA's bioaccumulation approach is summarized, rather than reiterated in detail, in the Framework.

F. The draft Framework states that BAFs and BSAFs are "determined and applied for conditions that approximate steady-state of the organism with respect to water and sediments, respectively." (EPA, 2003; p. 34). While BSAFs and BAFs are assumed to reflect steady state relationships among contaminant concentration in biota, water and sediments, often this is not the case. Changes in water and sediment concentration over time as a result of changing hydrologic conditions or changes in the concentration of compounds in discharges along with seasonal movements of fish can all preclude the establishment of a true steady-state. In river systems where anadramous fish are species of concern, the fish may never reach steady state with the ecosystem.

EPA Response: EPA's approach for acquiring and using BAFs/BSAFs assumes that bioaccumulation of PCDDs, PCDFs, and PCBs is a function of many factors (*e.g.* trophic level, food web characteristics, sediment organic carbon, organismal lipid, and sediment-water concentration quotient). Extrapolating BAFs/BSAFs is only appropriate when specified conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). *Section 3.3.1.4* discusses the sediment-water quotient ($\prod_{socw}$) and an approach for "normalizing" or "adjusting" BAFs/BSAFs in systems where sediment, water, and biota concentrations are not at equilibrium. This approach is based on an extensive body of peer-reviewed publications (Burkhard, 1998, 2003; Burkhard *et al.*, 2003a; 2008) and EPA guidance documents (U.S. EPA, 1995a; 2000; 2003). EPA's bioaccumulation approach is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

G. For sites where site-specific data on BSAFs or BAFs are not available, the Framework recommends that the factors used for deriving ambient water quality criteria be used. These values were based on sediment and biota sampling in one Great Lake. While it was questionably appropriate to apply these factors to other Great Lakes waters, they certainly do not provide an adequate substitute for site-specific bioaccumulation data across the entire nation.

EPA Response: The Framework does not suggest a source of universally applicable "generic" BSAF values because EPA does not advocate or support such an approach. *Section 3.3.1.4* highlights the preference for site-specific BAFs/BSAFs, while recognizing that site-specific BAFs/BSAFs may not always be available or feasible to determine. Indeed, EPA's bioaccumulation approach includes extrapolation of BAFs/BSAFs when appropriate conditions are met and appropriate normalizing factors are incorporated (U.S. EPA, 1993; 1995a, b, c; 2000; 2001; 2003). Furthermore, high quality BSAFs have been measured in a number of ecosystems (U.S. EPA 1995a; Burkhard *et al.*, 2004; see also EPA's BSAF data set at http://www.epa.gov/med/Prods_Pubs/bsaf.htm).

EPA's approach for acquiring and using BAFs/BSAFs is based on many peer-reviewed publications (Burkhard, 2003; Burkhard *et al.*, 2003a, b; 2004; 2006) and EPA guidance documents (U.S. EPA, 1993; 1995a, b, c; 2000; 2001a; 2003b). This body of information is summarized in *Section 3.3.1* in the interest of keeping the Framework concise and focused on the application of the TEF Methodology. However, additional references to previously published peer-reviewed articles, EPA guidance, and EPA's BSAF data set have been added to *Sections 3.3.1.4* and *3.3.1.5*.

H. While food web models, such as those recommended by EPA when there is need to extrapolate from one location to another, provide a means of validating BSAF and BAF data, they depend on adequate characterization of the site-specific bioaccumulation process in order for them to produce reliable results.

EPA Response: Included in *Section 3.3.1.5* is a discussion of approaches and references thereto (*e.g.* the use of food-chain models and/or the "hybrid modeling approach"), that can be used to adjust BAFs/BSAFs to decrease variability and increase accuracy when extrapolating across ecosystems.

8. Comments Concerning Analytical Testing Methods Limitations

The Agency received comments suggesting that the Framework does not provide sufficient or accurate information for analyzing non-detects for a risk assessment. These comments, along with the Agency's responses follow.

A. The draft Frameworks states that the "analytical detection levels for congeners should be lower than concentrations at which important biological effects may occur." It goes on to conclude that Method 1668 is acceptable for PCBs and the Methods 8290 or 1613 are acceptable for PCDD/Fs. The draft Framework states that when analytical detection limits for individual chemicals are "too large to allow measurement of concentrations which would significantly add to the TEC", risk assessors may set concentrations at zero, half the detection limit, or at the detection limit. It goes on to say that "the best method for handling non-detect in a particular risk assessment should be determined through consultation between risk assessors and risk managers early in the risk assessment process." This approach is highly subjective and can result in vastly different estimates of TEQ, depending upon the way in which non-detect concentrations are handled. The draft Framework should instead make specific recommendations concerning how to handle non-detect concentrations so that the approach is applied in a uniform way from site to site.

EPA Response: The issues of analytical methods and detection limits are overall risk assessment issues, not issues specific to the TEF methodology. In addition, the analytical methods and detection limits issues are specific to each ecological risk assessment and are therefore best addressed during the planning and problem formulation phases of the specific ecological risk assessment. While the TEF methodology does not dictate what analytical method or detection limits need to be used, methodological considerations associated with using the TEF methodology are presented in *Sections 3.1* and *3.2*. In

Section 3.3.1.1, it is explained that the best method for handling non-detects in a particular risk assessment should be determined during the planning and/or problem formulations phase(s) of the risk assessment. In *Section 3.4.3.2.2*, uncertainties associated with characterization of exposure, including detection limits, are discussed. In both *Sections 3.3.1.1* and *3.4.3.2.2* a reference to other EPA guidance that addresses this issue is provided.

B. For post-remedial risk evaluation, existing methods (i.e., Method 1613) approved by EPA may not have sufficient resolution to quantify low levels of TEQ congeners in media of concern. In addition, the same issues of surrogate species and steady state, which impact the bioaccumulation impacts, come into play when attempting to determine whether post-remedial concentrations in water or sediment are acceptable.

EPA Response: While it has historically been difficult to measure low concentrations of PCDDs, PCDFs, and PCBs in water, as acknowledged in the Framework, it is possible and increasingly feasible to perform such measurements given newer analytical methods. In addition, *Section 3.3.1.4*, discusses the use of the sediment-water quotient ($\prod_{socw}$) for 'normalizing' or 'adjusting' BAFs/BSAFs in systems where sediment, water, and biota concentrations are not at equilibrium (*e.g.*, post-remedial conditions). This approach is based on a number of peer-reviewed publications (Burkhard, 1998, 2003; Burkhard *et al.*, 2003a; 2008) and EPA guidance documents (U.S. EPA, 1995a; 2000; 2003). Also see the response to comment 8.A.

C. The Framework does not provide sufficient or accurate information for analysing non-detects for a risk assessment. The substitution procedures described in the Framework or other replacement procedures result in biased estimates of the mean and variance. These procedures are not related to the sampled probability distribution and their use does not adjust for the loss of information. The procedures given in the statistical monographs by Cohen (1991) and Schneider (1986) contain methods which correct for bias and account for information loss.

EPA Response: See the response to comment 8.A.

D. The TEF Framework may lead to confusion among environmental regulators. Testing procedures and protocols for these compounds lack the precision and accuracy to measure coplanar PCBs and similar compounds at the levels emitted by waste-to-energy facilities. In many cases, testing of waste-to-energy facilities will result in non-detection of the pollutant with a very high level for the detection limit. Without a true measure of emissions, regulators and risk assessment specialists may use the detection limit as a conservative measure of an emission level. Unfortunately, such an assumption could trigger an ecological risk assessment when none is necessary.

There is only one commercial laboratory is capable of testing for coplanar PCBs at levels in the pg per sample range, yet waste-to-energy facilities often reach levels

below that detection limit.... We ask the EPA to inform states that ecological risk assessments should not be done when dioxin, furans, and coplanar PCBs are not detected at detection limits considered too high for use in the risk assessment analysis. We further ask that EPA recognize the fact that there are very few commercial laboratories available that can perform the emissions tests at very low levels seen at waste-to-energy facilities.

EPA Response: See the response to comment 8.A.

9. Comments Concerning Cost

Several reviewers expressed concerns about the cost of the TEF methodology and commented that a cost-benefit analysis was warranted. Specific comments, along with the Agency's responses follow.

A. The TEF approach is not inexpensive. The TEF approach requires that all samples taken as part of the ecological risk assessment be analyzed for PCB congeners. An informal poll of commercial analytical laboratories indicated that high resolution PCB congener analysis currently costs at least an order of magnitude more than total PCB analysis. Further, in the opinion of Mr. Andy Beliveau of EPA Region I, EPA Method 1668a should be the high resolution PCB congener method of choice because it enables laboratories to quantitate all 209 congeners in water, air, soil, sediment and biota samples. Mr. Beliveau further remarked that the analytical costs for a Method 1668a analysis are approximately $1,000 per sample, compared to approximately $100 per sample for total PCB analyses. Moreover, EPA staff recently estimated that the cost of analyzing dioxin and "dioxin-like" chemicals in biosolids could be as high as $2,000 per sample. Memorandum from Charles E. White, Economic and Statistical Analysis Branch to Alan Hais, Associate Director of Health and Ecological Criteria Division, Subject: Cost Associated with Regulation Dioxins, Furans, and PCBs in Biosolids, at 5 (December 15, 1999). Because the total PCB method generates ecological risk estimates that are similar to, but somewhat more conservative than, risk estimates generated by the TEQ method - and are less costly -, the total PCB approach should generally be favored for risk screenings at PCB sites. The Framework should be modified to recommend that the TEF approach be used primarily at sites dominated by complex mixtures of PCDDs, PCDFs, and PCBs, while the total PCB approach be used to screen for ecological risk at most PCB sites, and that the TEF approach should be available for site screening in unusual cases where it is suspected that the total PCB approach substantially over-predicts risk.

EPA Response: Methodological considerations associated with using the TEF methodology are presented in *Sections 3.1* and *3.2* to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations associated with using the TEF methodology against other methods they may be considering. The type and number of other approaches that could be considered will be specific to each ecological risk assessment, such that comparisons of costs and benefits are best

conducted during the planning and problem formulation phases of the specific ecological risk assessment. Additional discussion in this regard has been added to the Framework. Specifically, *Section 3.1* raises the issue that costs and benefits need to be considered during the planning phase of an ecological risk assessment. However, since costs and benefits will vary depending on the scope and objectives of a specific ecological risk assessment and will also vary over time, EPA does not believe it is appropriate to provide a specific comparative analysis within the Framework. As appropriate, EPA Offices and Regions may consider costs (monetary as well as other resource requirements) as they implement the Framework for their individual programs.

B. TEFs lack the precision necessary for making important and sound economic decisions with respect to remediation. The rounding effect for deriving the final TEF estimate can lead to substantial, and possibly unwarranted, remediation costs. EPA should quantify the uncertainty for TEFs so that the uncertainty is apparent in any ecological risk assessment that relies upon TEF methodology. Risk assessment is not a precise science, and different clean-up levels may be driven by or considered by the public as artifacts of the application of uncertainty factors. Because of the limited budget for environmental clean-up, overprotection at one site may result in lack of funds for another site where the resources are needed. For every environmental pollutant, health risks, clean-up benefits, and economical feasibility must be carefully evaluated" (Pohl et al., 2002). Basing an ecological risk assessment on TEF values fraught with multiple uncertainties and unproven assumptions can greatly alter the standards to which a site must be cleaned.

EPA Response: Methodological considerations associated with using the TEF methodology are presented in *Sections 3.1* and *3.2* to allow those conducting risk assessments for dioxin-like chemicals to consider the strengths and limitations associated with using the TEF methodology against other methods they may be considering. The type and number of other approaches that could be considered will be specific to each ecological risk assessment, such that comparisons of costs and benefits is best conducted during the planning and problem formulation phases of the specific ecological risk assessment. Additional discussion in this regard has been added to the Framework. Specifically, *Section 3.1* raises the issue that costs and benefits need to be considered during the planning phase of an ecological risk assessment. Furthermore, a large part of the Framework (*Section 3.3.2* and *Figure 11*) is aimed at guiding risk assessors in selecting relative potency factors that provide the least amount of uncertainty and the most precise estimate of TEC for a particular ecological risk assessment.

C. EPA needs to conduct a cost/benefit analysis associated with changing from a total PCB approach to a TEQ approach for assessing ecological risks. EPA also needs to conduct a quantitative uncertainty analysis to support its conclusion about the relative uncertainty and accuracy of the Total PCB and TEQ approaches.

EPA Response: See the response to comment 9.A.

D. The additional analytical costs associated with the use of the TEQ approach in site characterization can be substantial. While such additional costs might be justified if the results of the risk assessment based on the approach were substantially different and clearly superior to the results of an assessment based on total PCB analysis, it does not appear that this is likely to be the case at most sites. At PCB-dominated sites, the TEQ may have little, if any value, and will substantially increase the costs of risk assessment and, if cleanup objectives are expressed in terms of TEQ, the costs associated with post-remediation confirmation sampling. While such a cost might be justified if the TEQ approach were a stand-alone methodology (i.e., if field verification of population effects were not necessary), it is not clear that the additional costs provide adequate additional benefit when the methodology must be field verified.

EPA Response: See the response to comment 9.A.

10. Comments Concerning the EPA Dioxin Reassessment

Comments were received regarding the EPA Dioxin Reassessment. A representative comment along with the Agency's responses follows.

A. The Agency should not at this time issue guidance calling for use of the TEF method to evaluate the ecological risks that might be presented by PCBs and other dioxin-like compounds, until the National Academy of Sciences (NAS) has completed its review of the draft dioxin reassessment, including its application of the TEQ approach to PCB congeners. The Framework states "the methodology is well accepted in the scientific community, in the international risk assessment community, and within EPA for human health risk assessment" (EPA, 2003; p. 14) and implies that because of this general "acceptance", it is also appropriate to apply the methodology to ecological risk assessment. There is, however, considerable disagreement within the scientific community about the appropriateness of the approach, even for human health risk assessment. Application of the approach to ecological risk assessment is even more controversial because of the extremely limited data available for specific ecological receptors and endpoints, and the enormous differences among species, in terms of their sensitivity to these compounds, their metabolic functions, their potential for uptake, and their susceptibility to specific toxic endpoints. These important issues need to be addressed before the methodology can be adopted as an acceptable approach for evaluating the ecological risks of these compounds.

EPA Response: EPA agreed with this comment and delayed release of the final document until the National Research Council of the National Academies (NRC) completed their review of EPA's draft *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds*. As a result of the review, the NRC concluded that even with the inherent uncertainties, the toxicity equivalence methodology provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative potency of dioxin-like compounds (NRC, 2006). Hence,

the NRC has confirmed the conclusions of two World Health Organization expert meetings (Van den Berg *et al.*, 1998; 2006), and the EPA-DOI expert workshop (U.S. EPA 2001a) on the validity of using the TEF methodology in ecological risk assessment.

11. Comment Concerning the Information Quality Act and Guidelines

A. The Framework fails to comply with the requirements of the federal Information Quality Act ("IQA") and Office of Management and Budget ("OMB") and EPA information quality guidelines (collectively referred to as the "IQA Guidelines"). EPA has: (1) failed to comply with the "objectivity" requirement of the IQA Guidelines, which mandates that information be presented in a clear and complete manner; (2) failed to adhere to the statement in EPA's IQA Guidelines that the presentation of information on environmental risks is to be comprehensive, informative, and understandable; and (3) given short shrift to the thrust of the "transparency" requirement, which is designed to ensure a higher degree of disclosure of various assumptions employed and data and analytic methods applied to reach conclusions set forth in disseminated information.

EPA Response: EPA has followed standard procedures for external peer review and public involvement in the development of the Framework. EPA believes that this document conforms to all applicable guidelines on information quality.

References

Beltman, D; Anderson, M; Barron, M. (1997) Assessment of PCB ecological effects: congener-based vs. Aroclor-based approaches. *Society of Environmental Toxicology and Chemistry, 18th Annual Meeting Abstracts*, p. 119.

Brunstrom, B; Halldin, K. (2000) Ecotoxicological risk assessment of environmental pollutants in the Arctic. *Toxicol Lett* 112-113:111-118.

Burkhard, LP. (1998) Comparison of two models for predicting bioaccumulation of hydrophobic organic chemicals in aquatic food webs. *Environ. Toxicol. Chem.*17:383-393.

Burkhard, L.P., D.D. Endicott, P.M. Cook, K.G. Sappington, and E.L. Winchester. 2003. Evaluation of two methods for prediction of bioaccumulation factors. Environ. Sci. Technol. 37:4626-4634.

Burkhard, L.P, P.M. Cook, and D.R. Mount. 2003. The relationship of bioaccumulative chemicals in water and sediment to residues in fish: A visualization approach. Environ. Toxicol. Chem. 22:2822-2830.

Burkhard, LP. (2003) Factors influencing the design of BAF and BSAF field studies. *Environ Toxicol Chem* 16:1677-1686.

Burkhard, LP; Cook, PM; Lukasewycz, MT. (2004) Biota-sediment accumulation factors for polychlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans in southern Lake Michigan lake trout (*salvelinus namaycush*). *Environ Sci Technol* 38:5297-5305.

Burkhard, LP; Cook, PM; Lukasewycz, MT. (2006) A hybrid empirical/modeling approach for extrapolating BSAFs across species, time, and/or ecosystems. *Environ Toxicol Chem* 25: 1946-1952.

Burkhard, LP; Cook, PM; Lukasewycz, MT. (2008) Organic Carbon -Water Concentration Quotients (ΠIsocs and ppocs): Measuring Apparent Chemical Disequilibria and Exploring the Impact of Black Carbon in Lake Michigan. Environ. *Sci. Technol.* in press.

Burkhard, LP; Cook, PM; Mount, DR. (2003a) The relationship of bioaccumulative chemicals in water and sediment to residues in fish: a visualization approach. *Environ Toxicol Chem* 22:2822-2830.

Burkhard, LP; Endicott, DD; Cook, PM; Sappington, KG, Winchester, EL. (2003b) Evaluation of two methods for prediction of bioaccumulation factors. *Environ Sci Technol* 37:4626-4634.

Cook, PM; Robbins, J; Endicott, DD; Lodge, KB; Walker, MK; Zabel, EW; Guiney, PD; Peterson, RE. (2003) Effects of aryl hydrocarbon receptor mediated early life stage toxicity on lake trout populations in Lake Ontario during the 20th century. *Environ Sci Technol* 37:3864-3877.

Cook, PM; Zabel, EW; Peterson, RE. (1997) The TCDD toxicity equivalence approach for characterizing risks for early life stage mortality in trout. In: Rolland, R; Gilbertson, M; Peterson R, eds. *Chemically-Induced Alterations in the Functional Development and Reproduction of Fishes*. SETAC Technical Publications Series. Pensacola, FL: SETAC Press, pp. 9-27.

Elonen, GE; Spehar, RL; Holcombe, GW; Johnson, RD; Fernandez, JD; Erickson, RJ; Tietge, JE; Cook, PM. (1998) Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to seven freshwater fish species during early-life-stage development. *Environ Toxicol Chem* 17:472-483.

Finley, BL; Trowbridge, KR; Burton, S; Proctor, DM; Panko, JM; Paustenbach, DJ. (1997) Preliminary assessment of PCB risks to human and ecological health in the lower Passaic River. *J Toxicol Environ Health* 53:95-118.

Giesy, JP; Kannan, K. (1998) Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBS): implications for risk assessment. *Crit Rev Toxicol* 28:511-569.

Haws, LC; Su, H; Harris, M; Devito, MJ; Walker, NJ; Farland, WH; Finley, B; Birnbaum, LS. (2006) Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. *Tox Sci* 89:4-30.

Henry, TR; Nesbit, DJ; Peterson, RE. (2001) Relative potency values (REPs) for polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners based on induction of cytochrome P4501A mRNA in a zebrafish liver cell line (ZFL). *Environ Toxicol Chem* 20:1053-1058.

Kennedy, SW; Lorenzen, A; Jones, SP; Hahn, ME; Stegeman, JJ. (1996) Cytochrome P4501A induction in avian hepatocyte cultures: a promising approach for predicting the sensitivity of avian species to toxic effects of halogenated aromatic hydrocarbons. *Toxicol Appl Pharmacol* 141:214-230.

NRC (Committee on EPA's Exposure and Human Health Reassessment of TCDD and Related Compounds, National Research Council of the National Academies). (2006). Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC.

Rice, CP; O'Keefe, P; Kubiak, TJ. (2002) Sources, pathways and effects of PCBs, dioxins, and dibenzofurans. In: Hoffman, DJ; Rattner, BA; Burton, GA, Jr; Cairns, J, Jr, eds. *Handbook of Ecotoxicology.* 2nd ed. Boca Raton, FL: CRC Lewis Publishers. pp. 499-571.

U.S. EPA. (Environmental Protection Agency). (1993) *Interim Report on Data and Methods for Assessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Risks to Aquatic Life and Associated Wildlife.* Office of Research and Development, Duluth, MN; EPA/600/R-93/055.

U.S. EPA. (Environmental Protection Agency). (1995a) *Great Lakes Water Quality Initiative: technical support document for the procedure to determine bioaccumulation.* Office of Water; EPA/820/B-95/005.

U.S. EPA. (Environmental Protection Agency). (1995b) *Great Lakes Water Quality Initiative: Criteria Documents for the Protection of Wildlife: DDT, Mercury, 2,3,7,8-TCDD, PCBs.* Office of Water; EPA/820/B-95/008.

U.S. EPA. (Environmental Protection Agency). (1995c) *Great Lakes Water Quality Initiative: Technical Support Document for Wildlife Criteria.* Office of Water; EPA/820/B-95/009.

U.S. EPA. (Environmental Protection Agency). (2000) *Methodology for deriving ambient water quality criteria for protection of human health.* Office of Water; EPA/822/B-00/004. Available from: http://www.epa.gov/waterscience/criteria/humanhealth/method/complete.pdf.

U.S. EPA. (Environmental Protection Agency). (2001) *Workshop report on the application of 2,3,7,8-TCDD toxicity equivalence factors to fish and wildlife.* Risk Assessment Forum, Washington, DC; EPA/630/R-01/002. Available from: http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=23763.

U.S. EPA. (Environmental Protection Agency). (2003) *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health 2000. Technical Support Document Volume 2: Development of National Bioaccumulation Factors.* Office of Water, Washington, DC; EPA/822/R-03-030. Available from: http://www.epa.gov/waterscience/criteria/humanhealth/method/tsdvol2.pdf.

U.S. EPA (Environmental Protection Agency). (2005) *Memorandum: Response to Ecological Risk Assessment Forum Request for Informationon the Benefits of PCB Congener-Specific Analyses.* Office of Research and Development, Cincinnati, OH; NCEA-C-1315/ERASC-002F. Available from: http://www.epa.gov/oswer/riskassessment/pdf/1315-erasc-002f.pdf.

Van Birgelen, AP; Fase, KM; van der Kolk, J; Poiger, H; Brouwer, A; Seinen, W; van den Berg, M. (1996) Synergistic effect of 2,2',4,4'5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic porphyrin levels in the rat. *Environ Health Perspect* 104(5):550-557.

Van den Berg, M; Birnbaum, L; Bosveld, ATC; Brunstrom, B; Cook, P; Feeley, M; Giesy, JP; Hanberg, A; Hasegawa, R; Kennedy, SW; Kubiak, T; Larsen, JC; van Leeuwen, FX; Liem, AK; Nolt, C; Peterson, RE; Poellinger, L; Safe, S; Schrenk, D; Tillitt, D; Tysklind, M; Younes, M; Waern, F; Zacharewski, T. (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106(12):775-792.

Van den Berg, M; Birnbaum, LS; Denison, M, DeVito, M, Farland, W, Feeley, M; Fiedler, H; Hakansson, H; Hanberg, A; Haws, L; Rose, M; Safe, S; Schrenk, D; Tohyama, C; Tritscher, A; Tuomisto, J; Tysklind, M; Walker, N; Peterson, RE. (2006) The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. *Toxicol Sci* 93:223-241.

Walker, NJ; Crockett, PW; Nyska, A; Brix, AE; Jokinen, MP; Sells, DM; Hailey, JR; Easterling, M; Haseman, JK; Yin, M; Wyde, ME; Bucher, JR; Portier, CJ. (2005) Dose-additive carcinogenicity of a defined mixture of dioxin-like compounds. *Environ Health Perspect* 113:43-48.