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REPORT
from the
WORKSHOP ON THE APPLICATION OF 2,3,7,8-TCDD TOXICITY EQUIVALENCY FACTORS TO FISH AND WILDLIFE

Chicago Hilton & Towers
Chicago, Illinois

January 20-22, 1998

Submitted to:

Risk Assessment Forum
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Submitted by:

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02173-3134

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NOTE

This report was prepared by Eastern Research Group, Inc., an EPA contractor, as a general record of discussion during the workshop. As requested by EPA, this report captures the main points of scheduled presentations, highlights from the group discussion, and a summary of comments offered by observers attending the workshop; the report is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. This report will be used by EPA as a basis for additional study and work on the application of toxicity equivalency factors (TEFs) in ecological risk assessments. Except as specifically noted, none of the statements in this report represent analyses or positions of EPA.

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PREFACE

This workshop was developed by a joint planning group from the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Interior under the aegis of EPA's Risk Assessment Forum. One role that the Risk Assessment Forum plays within EPA is to promote consensus on risk assessment issues and to ensure that this consensus is incorporated into appropriate Agency risk assessment guidance. In the past, the Forum has issued guidance on the use of toxicity equivalency factors (TEFs) for assessing the human health risks associated with exposures to complex mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans (EPA/625/3-87/012 and EPA/625/3-89/016). This workshop was convened to examine the applicability of recently developed World Health Organization TEFs for assessing risks to fish and wildlife from polychlorinated dioxins, furans, and biphenyls.

I. INTRODUCTION

Many individual members of the family of chemicals known as polyhalogenated aromatic hydrocarbons have been shown to produce toxic effects that are similar to those associated with exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Among the classes of environmental contaminants falling into this general category are polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs), and dibenzo-*p*-dioxins (PCDDs), all of which are believed to exert their toxic effects at least in part as a result of their binding to the aryl hydrocarbon receptor (AhR).

Based both on their mechanistic similarity to TCDD and on the fact that these chemicals often exist as complex mixtures in the environment, efforts have been made to derive toxicity equivalency factors (TEFs) that can be used to express the toxicity of individual PCB, PCDF, and PCDD congeners relative to the toxicity of TCDD. In two previous workshops, convened by the World Health Organization (WHO) in August 1996 and June 1997, scientific experts reviewed the available relative potency data and developed consensus TEF values for use in risk assessments involving dioxin-like compounds. In addition to updating the existing mammalian TEFs, the WHO group developed consensus TEFs for birds and fish.

To examine issues associated with the application of TEFs and the related toxicity equivalents (TEQs) to ecological risk assessments, Eastern Research Group, Inc. (ERG), in consultation with the U.S. Environmental Protection Agency (EPA) and the U.S. Department of the Interior (DOI), assembled a group of experts to consider two hypothetical case studies: a prospective case study involving a risk assessment for a hypothetical point source requiring a water quality permit and a retrospective case study focusing on a hypothetical freshwater ecosystem in which reproductive effects have been observed and a remediation effort is being considered.

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II. OPENING PRESENTATIONS

To begin the workshop, Dr. Menzie introduced Ms. Christine Boivin, who welcomed workshop participants on behalf of EPA's Risk Assessment Forum, and Mr. John Blankenship, who extended a welcome on behalf of the DOI Fish and Wildlife Service. Following these introductions, Dr. Menzie provided an overview of the overarching goal of the workshop, which he described as exploring the extent to which a TEF/TEQ approach can be used in risk assessments that have progressed beyond the screening stage. As such, the focus of the workshop would be on the application and use of this particular tool rather than on the broader range of issues associated with the performance of ecological risk assessments. During the course of discussions, the group would attempt to identify, document, and compare the uncertainties associated with the derivation of individual TEF values—including both the uncertainties related to statistical variability and those related to a lack of knowledge—and to assess the impact of these uncertainties on ecological risk assessments.

Noting that risk assessment almost by definition occupies a position at the interface between science and policy, Dr. Menzie indicated that it would be most useful for discussions to remain as focused as possible on the more technical implications of gaps in the TEF knowledge base. Thus, he anticipated that discussions over the next few days would center on issues such as the relative contribution of TEF-related uncertainties to the overall uncertainty of an ecological risk assessment, additional data requirements and analytical support that might be needed to implement a TEF approach as opposed to other approaches that might be considered, and the ability and/or need to support a TEF approach with other lines of evidence. To consider these and related issues in a real-

world context, workshop participants would be asked to apply the TEF/TEQ methodology to the two case studies developed by the EPA/DOI Planning Group. For each of these cases, the goal would be to see how application of the TEF/TEQ methodology might impact the uncertainties associated with the exposure assessment, the effects assessment, and the overall characterization of risk.

Following Dr. Menzie's opening remarks, the experts heard a series of formal presentations designed to establish a common frame of reference for subsequent discussions. Brief summaries of these presentations are provided below.

Synopsis of the WHO Stockholm Meeting

Dr. Martin van den Berg, Chair of the WHO Working Group on TEFs

Dr. van den Berg began by noting that his presentation would provide an overview of the issues addressed and the decisions agreed to at the WHO-sponsored meeting on the derivation of TEFs for dioxin-like compounds in humans and wildlife, which was held in Stockholm, Sweden, in June of 1997. In contrast with earlier TEF meetings, which had addressed only mammalian and human TEFs, the Stockholm meeting also undertook an evaluation of TEFs for birds, fish, and wild mammals. Participants included approximately two dozen experts in wildlife toxicology and/or in the laboratory determination of TEFs, including several of the experts and Planning Group members who are also participating in this workshop. The Stockholm meeting was divided into two sessions—one dealing with human and mammalian TEFs derived from laboratory experiments, and the other dealing with TEFs for wild mammals, fish, and birds. The human/mammalian session was chaired by Dr. Linda Birnbaum, who is a member of the EPA/DOI Planning Group, and the wildlife session was chaired by Dr. Richard Peterson, who is one of the experts at this meeting. Rapporteurs were Drs. Mark Feeley and Sean Kennedy, who is also an experts at this meeting. Dr. van den Berg served as organizer and overall Chair of the Stockholm meeting.

Prior to the Stockholm meeting, criteria for including a compound in the WHO TEF scheme had already been established. To be included in the TEF scheme, a compound must:

- be structurally related to PCDDs and PCDFs;
- bind to the Ah receptor;
- elicit dioxin-specific biochemical and toxic responses; and
- be persistent and accumulate in the food chain.

In its deliberations, the WHO group discriminated between TEFs and relative effect potencies, or REPs. As defined by WHO, a TEF is an order-of-magnitude estimate of the toxicity of a compound relative to the toxicity of TCDD that is derived using careful scientific judgment after considering all available data. An REP, in contrast, is derived from the results of a single *in vivo* or *in vitro* study, which may be either a biochemical or a toxicological study.

In preparation for the Stockholm meeting, the Karolinska Institute assembled a database containing the results of thousands of published studies comparing the biochemical or toxicologic profiles of individual congeners with a reference compound (either TCDD or PCB 126). When PCB 126 was used as the reference compound, a REP of 0.1 was assumed. To be included in the database, a published study had to meet the following three criteria:

- At least one PCDD, PCDF, or PCB congener and a reference compound must be included in the study.
- The reference compound and the congener(s) must be included in the same experiment or studied with the same experimental design and by the same authors in separate experiments.
- The relevant endpoint should be affected by the congener as well as the reference compound.

Regarding the determination of relative potency values for inclusion in the Karolinska database, Dr. van den Berg indicated that there were several methods used. If a relative potency value was reported in a published study, that REP was included in the database without modification. If no REP was reported, one could be derived by any of the following methods:

- calculated by comparing dose-response curves or by using linear interpolation of log doses, comparing the same effect level;
- determined from the ratio of reported ED_{50}, LD_{50}, or EC_{50} values; or
- calculated from tumor promotion indices, K_d values for Ah receptor binding, or directly estimated from graphs.

The Karolinska database is now part of the public domain and can be accessed by anyone who applies to use it at the WHO European Center of Environmental Health.

Based on the wide range of REPs reported in the literature, workgroups at the Stockholm meeting proposed human, wild mammal, bird, and fish TEFs for each individual congener. These proposed values were then the subject of extensive discussion during a plenary session, and on the last day of the meeting consensus values were derived for each compound.

Turning specifically to the derivation of the human and mammalian TEFs , Dr. van den Berg noted that meeting participants decided that there was no scientific reason to assign TEFs for wild mammals that would differ from those derived for humans and laboratory mammals. He then outlined the criteria used to weight different types of experimental data. In evaluating toxicity data, meeting participants agreed that *in vivo* data should be given precedence over *in vitro* data, which in turn should be given precedence over data from quantitative structure-activity relationship (QSAR) studies. When more than one *in vivo* study was available, those involving chronic exposures were given the highest priority, and progressively lower priority was given to those involving subchronic, subacute, and acute exposure scenarios. Among studies using Ah receptor endpoints, toxicity studies were given greater weight than biochemical studies.

Because mammalian TEFs had previously been assigned by WHO on the basis of work done by Ahlborg et al. in 1994, participants at the Stockholm meeting had to decide under what conditions they would incorporate an existing TEF into their scheme. They agreed that if the available information was insufficient to warrant a change, the existing TEF value for PCDDs, PCDFs, and PCBs would be adopted. The major changes to existing mammalian TEFs agreed to at the Stockholm meeting are summarized in Figure 1. Notably, meeting participants agreed that the di-*ortho* PCBs,

REVISED MAMMALIAN TEFs

Congener	Old TEF	New TEF	Explanation of Change
1,2,3,7,8-PeCDD	0.5	1	CYP1A1/A2, tumor promotion
OCDD	0.001	0.0001	misinterpretation of earlier data; exposure versus tissue concentration
OCDF	0.001	0.0001	similarity to OCDD
PCB 77	0.0005	0.0001	EROD induction
PCB 81	--	0.0001	similarity to PCB 77
PCB 170	0.0001	--	*in vivo* data (CYP1A1, repro) do not support *in vitro* observations
PCB 180	0.0001	--	*in vivo* data (CYP1A1, repro) do not support *in vitro* observations

Figure 1.

which were assigned TEF values in the earlier WHO effort, should no longer be included in the TEF scheme. This decision was based on the fact that *in vivo* data, which includes both enzyme induction and reproduction studies, do not support the *in vitro* observations upon which the initial TEF values were based.

In evaluating the data for fish and birds, the WHO groups used a four-tier approach. In decreasing priority, the tiers were:

- Tier 1: overt toxicity observed in developing embryos (endpoint = LD_{50});

- Tier 2: biochemical effects observed in developing embryos (endpoint = CYP1A);
- Tier 3: biochemical effects observed in *in vitro* systems (endpoint = CYP1A); and
- Tier 4: estimates from QSAR studies.

To simplify matters for risk assessment and management purposes, participants at the WHO meeting attempted to harmonize the TEFs across the different taxonomic categories. This was not possible, however, because of clear taxonomic differences in the effects of various congeners. As an example of these differences, Dr. van den Berg mentioned the responses of fish and mammals to mono-*ortho* PCBs.

Another aspect of the harmonization effort involved a decision about whether to report the consensus TEFs as distinct individual values or to round them as had been done previously. For conformity with the existing TEF values, some of which were adopted by the WHO, new TEFs were rounded to a value of either 1 or 5. In this rounding procedure, Dr. van den Berg said that a conservative approach was used to provide optimal protection of fish and wildlife.

The consensus TEFs for dioxins, furans, non-*ortho* PCBs, and mono-*ortho* PCBs are listed in Figure 2. In general, fish and birds tend to be less sensitive to hexachloro- and heptachlorodioxins than are mammals, but there were not enough data to determine the relative sensitivity of either fish or birds to octachlorodioxins. The most notable taxonomic distinction for the dibenzofurans is the generally greater sensitivity of birds than either fish or mammals to TCDF and the two pentachlorodifurans. Among the planar PCBs, birds tended to be more sensitive than fish, particularly to PCBs 81 and 126. However, PCB 169 was less toxic to fish and birds than to mammals. For the mono-*ortho* PCBs, the group felt that it was not possible to establish TEFs for fish; to accommodate the fact that some regulatory agencies might require some number to be used, the group decided to assign an upper limit value to the TEFs for fish. In most cases, these compounds were also determined to be slightly less toxic to birds than to mammals.

WHO CONSENSUS TEFs FOR MAMMALS, FISH, AND BIRDS

	HUMANS/ MAMMALS	FISH	BIRDS
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	1	1	1
1,2,3,4,7,8-HxCDD	0.1	0.5	0.05
1,2,3,6,7,8-HxCDD	0.1	0.01	0.01
1,2,3,7,8,9-HxCDD	0.1	0.01	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.001	<0.001
OCDD	0.0001	-	-
2,3,7,8-TCDF	0.1	0.05	1
1,2,3,7,8-PeCDF	0.05	0.05	0.1
2,3,4,7,8-PeCDF	0.5	0.5	1
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01
OCDF	0.0001	0.0001	0.0001
3,4,4',5-TCB (81)	0.0001	0.0005	0.1
3,3',4,4'-TCB (77)	0.0001	0.0001	0.05
3,3',4,4',5-PeCB (126)	0.1	0.005	0.1
3,3',4,4',5,5'-HxCB (169)	0.01	0.00005	0.001
2,3,3',4,4'-PeCB (105)	0.0001	<0.000005	0.0001
2,3,4,4',5-PeCB (114)	0.0005	<0.000005	0.0001
2,3',4,4',5-PeCB (118)	0.0001	<0.000005	0.00001
2',3,4,4',5-PeCB (123)	0.0001	<0.000005	0.00001
2,3,3',4,4',5-HxCB (156)	0.0005	<0.000005	0.0001
2,3,3',4,4',5'-HxCB (157)	0.0005	<0.000005	0.0001
2,3',4,4',5,5'-HxCB (167)	0.00001	<0.000005	0.00001
2,3,3',4,4',5,5'-HpCB (189)	0.0001	<0.000005	0.00001

Figure 2.

Overview of the Retrospective Case Study

Dr. Donald Tillitt, EPA/DOI Planning Group

Dr. Tillitt began his presentation by thanking the experts for the excellent job they did in the premeeting comments they had submitted prior to the workshop. The goal of the workshop exercises, he said, was to apply the TEF methodology to a couple of hypothetical cases that are broadly representative of situations in which the method might be applied, and in so doing to gain a more complete understanding of the strengths and weaknesses of the approach.

Dr. Tillitt acknowledged, as some of the experts had pointed out in their premeeting comments, that the retrospective case study was not a true risk assessment, in that it did not address the full range of stressors on the system of interest. This limited focus was intentional, however, as the Planning Group had tried to confine its description of the case only to those elements that might be relevant to use of the TEF methodology. For the same reason, the Planning Group had provided a detailed description of mechanisms involved in the transfer of contaminants up the food chain. By establishing this type of information at the outset, the Planning Group hoped to steer participants away from discussions about what the correct values might be so that they could focus more directly on issues associated with application of the TEF methodology.

The site for the retrospective case study was Oneofakind Lake, a mesotrophic/oligotrophic freshwater system located in the northern United States (Figure 3). There are no industrial sources of contamination around the lake. At one time, there were some eutrophication problems in the lake, but those are now largely resolved. The source of dioxin-like contamination was a spill that occurred in the Yuckymuck River and subsequently moved into Oneofakind Lake. Currently, sediments and biota are known to be contaminated with PCBs and furans from the spill, and temporal sampling of the sediments has suggested a first-order loss of these compounds which is believed to be occurring primarily through sediment burial. Dioxin and furan loading to the lake is believed to occur mainly via atmospheric inputs. Previous logging activity around the lake included the use of DDT for insect control, but no logging has occurred for 30 years.

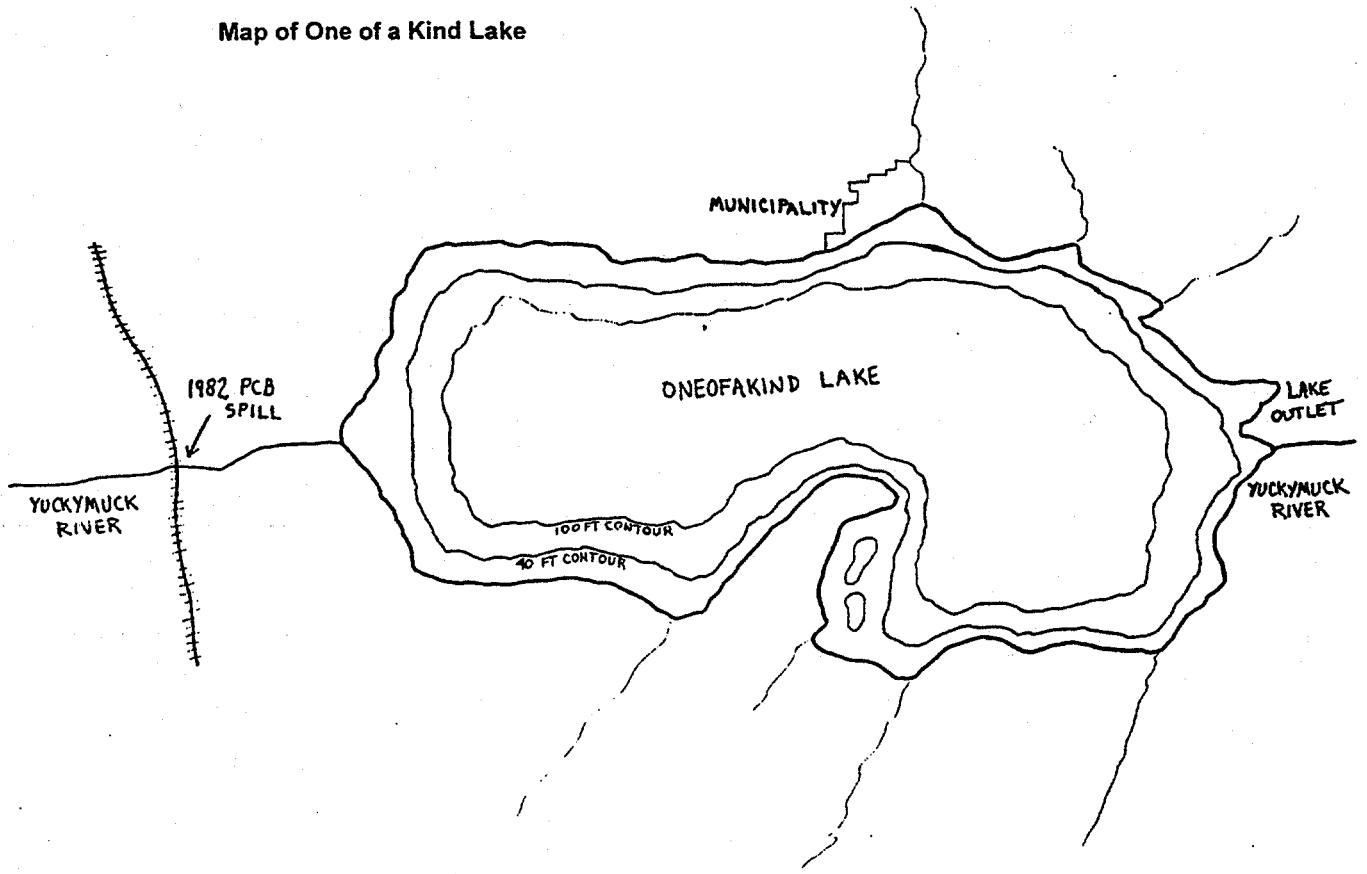


Figure 3.

Components of the aquatic ecosystem include lake trout, Atlantic salmon, largemouth bass, catfish, crappie, and bluegills; the forage fish are emerald and spottail shiners. The waterbird population is normal for this type of lake; the species that may be of concern to state agencies include herons, gulls, and terns. The three types of evidence suggesting some sort of disruption of the ecosystem are decreased Caspian tern reproduction, decreased lake trout recruitment, and anecdotal reports from trappers that the otter population is declining. For this case study, the Planning Group selected a tissue residue assessment approach. The target organ for dioxin-like effects is the developing embryo in the case of birds and fish, and the developing fetus in the case of mammals.

Figure 4 illustrates the simplified food chain model developed for this case study. Contaminated sediments are the primary load to the system. Biota sediment accumulation factors (BSAFs) are used to estimate the trophic transfer of contaminants from the sediments and up through the food chain and to predict tissue concentrations in the forage and piscivorous fish. Biomagnification factors (BMFs) are used to estimate the transfer of contaminants from fish to piscivorous birds and mammals, and to predict tissue and egg concentrations in the piscivorous species. Assessment endpoints for this study are lake trout recruitment, Caspian tern reproduction, and the size of the otter population.

Compartmental Model and Simplified Pathways of Chemicals in Oneofakind Lake.
BSAF- and BMF-Related Compartments are Bracketed.

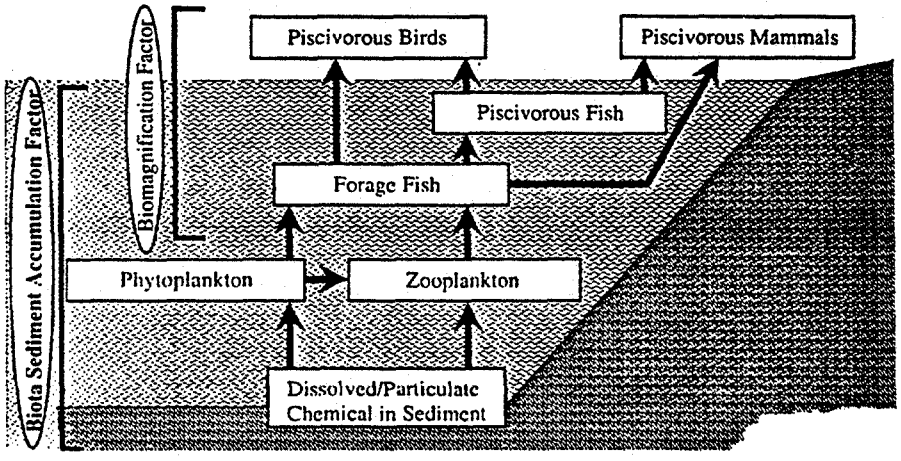


Figure 4.

Dr. Tillitt concluded his presentation by noting that, in the workshop exercise, participants were being asked to apply the TEF/TEQ methodology to determine how contaminant levels in the species of interest compare to hypothetical no-effect thresholds for fish eggs, bird eggs, and mink liver. In particular, he said, the Planning Group would

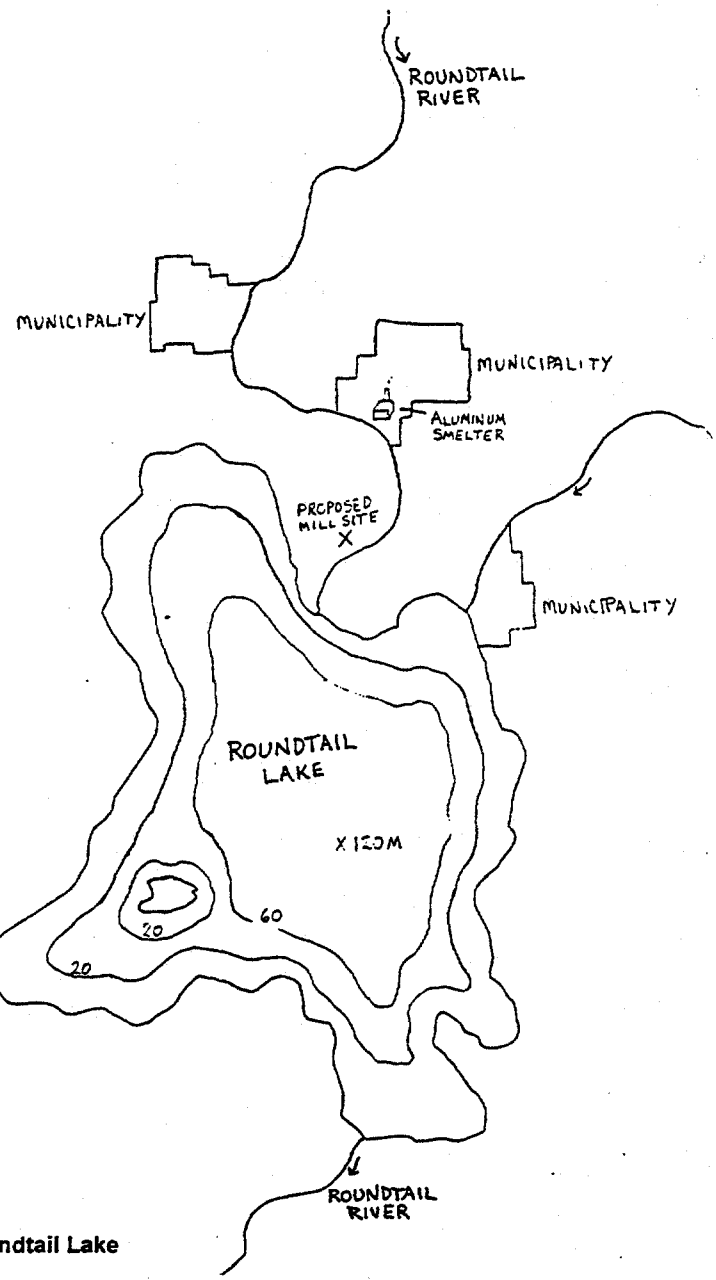
be interested in the experts' thoughts about how a risk assessment based on the use of the TEF model would compare with an assessment based either on TCDD alone or on total PCBs.

Overview of the Prospective Case Study

Dr. Steven Bradbury, EPA/DOI Planning Group

Dr. Bradbury began by noting that both the retrospective and prospective case studies were designed to explore whether it might be possible to move beyond the traditional use of TEFs, which has been exclusively for screening-level assessments. In the retrospective scenario, for example, it has already been established that an AhR agonist situation exists, and the question is whether the TEF methodology can be used to inform a decision about remediation. In the prospective scenario, the situation is that dioxins and furans are going to be released into an environment that already contains some PCBs, and the question is whether the TEF approach can be used to inform a permitting decision. In this sense, he noted, one goal of the workshop is to determine whether the state of the science is sufficiently advanced to support a different application of the TEF methodology than has been used in the past.

Regarding the specifics of the prospective case study, Dr. Bradbury noted that the setting for this case is a lake in the northwestern United States (Figure 5). A new paper mill has been proposed, and the mill is likely to discharge dioxins and furans into the system. The engineers associated with the plant may have some flexibility in manipulating the mix of congeners that will be released, but they need to know what targets they should be aiming to meet. There are already PCBs in the system, due to atmospheric deposition and other background sources.



Map of Roundtail Lake

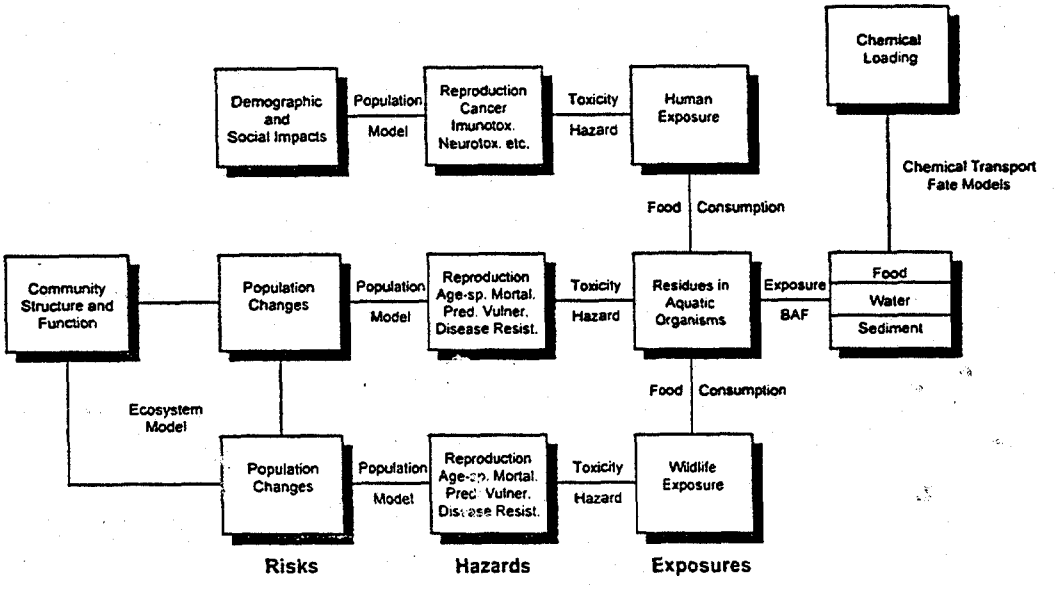
Figure 5.

In issuing a permit for the new paper mill, the state has decided to use a total maximum daily load (TMDL) approach. Accordingly, the regulators want to determine the total load of AhR agonists the system can tolerate and still maintain the productivity of fish, birds, and mammals in the ecosystem. Based on the current loading of the system from background sources, they will then be able to decide how much the new plant will be allowed to contribute and how much of the maximum load to set aside both to provide a margin of safety and to accommodate future demands on the system.

Among the aquatic species present in the ecosystem are salmon, lake trout, and bull trout. The bull trout is of particular concern to the risk managers, since it has recently become a listed species. A variety of piscivorous birds use this system for foraging, relying on Lake Roundtail for roughly half of their diet and on other lakes and rivers in the area for the remainder. River otter and mink are found in the system, but there is some question as to the home ranges of these populations.

Possible risk assessment endpoints for the prospective scenario include the productivity of birds, fish, and mammals, and the assessment could focus on the most representative, the most highly exposed, or the most sensitive species. Although population-level effects are clearly of concern, the bull trout's status as an endangered species also introduces a need for at least some attention to individual-level effects. As in the retrospective case study, the Planning Group provided hypothetical standards for protection of the species of concern, in this case the bull trout, bald eagle, and river otter.

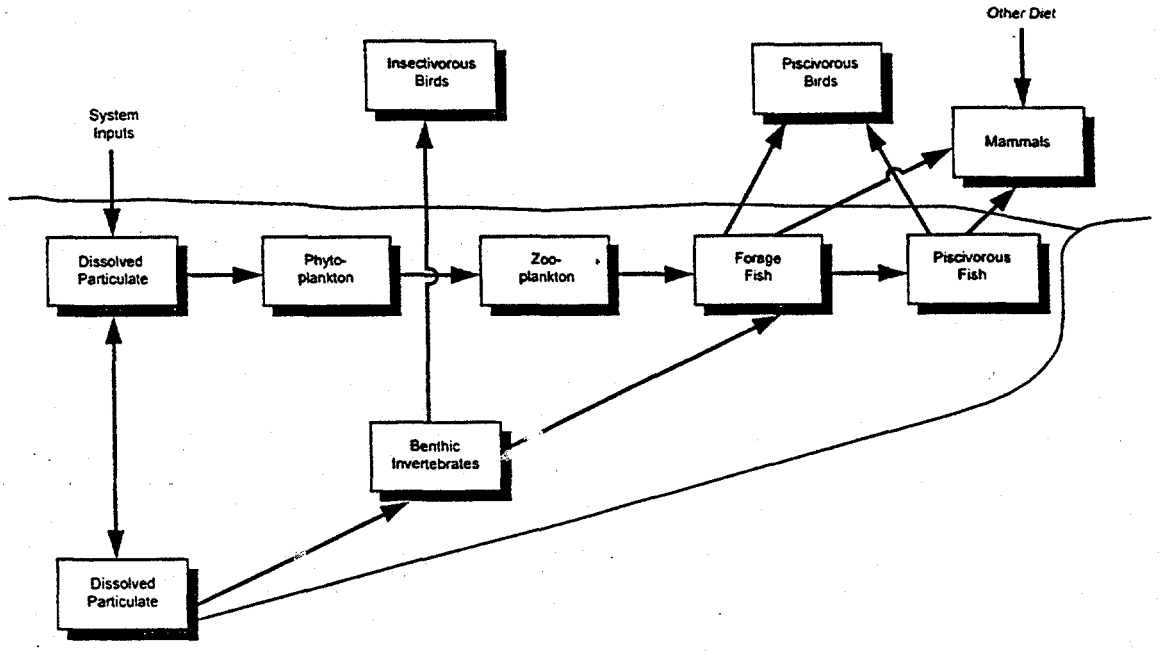
As Figure 6 illustrates, the conceptual model for the prospective case study is similar to that used in the retrospective case, except that it relies on either freely dissolved or total concentrations in the water as a predictor of residues in the organisms and therefore of expected effects. This approach is necessary because of the prospective nature of the assessment and the fact that loading of the system is the variable for which the permit is to be written.



. Conceptual Model for Risk Assessments and Criteria Development Involving Determination of Safe Loadings of Bioaccumulative Chemicals to Aquatic Systems

Figure 6.

Potential routes of exposure to the contaminants of concern are illustrated in Figure 7. As in the retrospective case study, movement of these chemicals through the various trophic levels of the ecosystem will determine the doses received by the organisms of concern. Thus, also as in the previous case, bioaccumulation and biomagnification factors will have to be used to work through the various exposure scenarios.



Pathways of TCDD Accumulation in Roundtail Lake

Figure 7.

To conclude his presentation, Dr. Bradbury presented a methodology the Planning Group had devised to address the various issues likely to arise in a prospective risk assessment tied to a TMDL model (Figure 8). The first step, he suggested, is to relate the total concentration of dioxin-like chemicals in the water to the concentrations of individual congeners, keeping track of both their TEFs and their bioaccumulation potential relative to TCDD. By using TCDD to standardize both the effect and exposure metrics, it should be possible to determine the maximum load an individual congener could contribute to the system and not exceed the water quality threshold. Repeating this process for each congener and for each of the species-specific threshold values will generate a matrix of values that are all normalized to TCDD. When the regulator decides on the percentage of the maximum load that will be allocated to the plant, the same fraction can be applied to all elements of the matrix. At the same time, the discharger can use the matrix both to see which congener is driving the assessment and to determine whether particular combinations of congeners will or will not exceed the permit level.

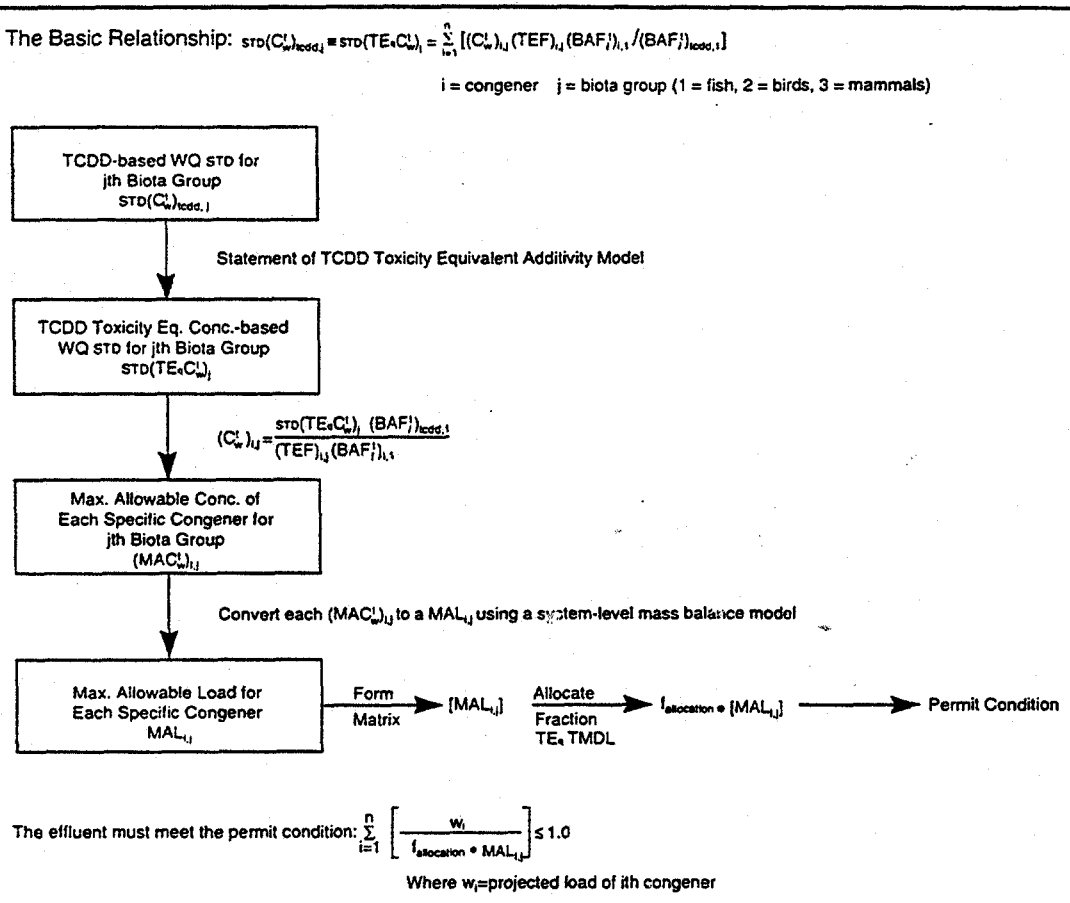


Figure 5. Process Diagram for Prospective Waste Load Allocation

Figure 8.

At the conclusion of Dr. Bradbury's presentation, Dr. DePinto pointed out that the rationale for using this approach has to do with the fact that each congener has a different fate and transport profile in the system. As a result, it is virtually impossible to model the TCDD toxic equivalency concentration as a single entity. The purpose of the matrix is to account for the differing fate and transport properties of individual congeners from discharge all the way to the endpoint or endpoints of concern. This is especially useful in complex ecosystems, since the suite of congeners released by the paper mill may be very different from the mix already present in the system, and both will likely differ from the mix of congeners entering the system from some other source. With the matrix, however, the issue is reduced to one of simple additivity.

In response to a question from one of the experts (deFur), Dr. Bradbury indicated that alternatives to using the TEF methodology are tracking TCDD alone and basing the permit on that determination, or issuing separate permits for each of the individual congeners. If workshop participants had other ideas about how to approach the problem, however, Dr. Bradbury encouraged them to explore these approaches and present them to the Planning Group.

A member of the Planning Group (Henningsen) questioned the case study's emphasis on daily loading, when the toxicity of these chemicals is usually more chronic and the sensitivity of the target organisms varies over different life stages. Dr. Bradbury noted that the TMDL model has a regulatory underpinning, and indicated that it would be just as useful for the group to think about total maximum load over some other time frame for risk assessment purposes.

Workshop Structure/Summary of Premeeting Comments

Dr. Charles Menzie, Workshop Chair

After the two case studies had been presented, Dr. Menzie reviewed the proposed agenda for the workshop (Appendix B). He noted that the workshop was designed to follow an iterative process in which small work group meetings would alternate with plenary sessions at which the group as a whole would have an opportunity to discuss the various approaches taken and lessons learned in the smaller work groups. To begin this process, workshop participants had been assigned to one of three expertise groups:

- Toxic Equivalency Factors, chaired by Dr. Richard Peterson;
- Fate and Transport, chaired by Dr. William Adams; or
- Risk Assessment and Population Modeling, chaired by Dr. Menzie.

The purpose of these groups, Dr. Menzie said, would be for individuals with specific expertise in each of these areas to come to a common understanding of what the issues are and how they might be addressed in the context of the two hypothetical case studies. Once this was done, members of the expertise groups would fan out among the three work groups in which the case studies themselves were to be reviewed. Thus, each work

group would contain some individuals from all three expertise groups. In this sense, the work group portion of the workshop could be thought of as a replication effort to see how three more or less similar groups might address the issues posed by each of the case studies. Then, in plenary sessions, the efforts of each work group would be discussed by the group as a whole to identify areas of agreement where they exist and to illuminate the reasons for any differences of opinion in areas where agreement could not be reached.

Having provided this overview of the workshop structure, Dr. Menzie noted that in its charge to the experts, the Planning Group had emphasized that the primary objective of the workshop was to identify, document, and compare the uncertainties associated with the use of the TEF/TEQ approach and to consider the impact of these uncertainties on ecological risk assessments. Toward this end, the Planning Group had posed a series of questions and issues to focus the experts' deliberations. Prior to the workshop, each of the experts submitted written comments outlining their individual responses to these questions (Appendix C). To provide a sense of the range of views experts had coming into the workshop, Dr. Menzie offered a general summary of the commonalities and differences he had noticed in his own review of the premeeting comments. His observations related to selected charge questions are summarized in the paragraphs that follow.

- Charge Question I-1: The WHO consensus TEF values are reported as point estimates and generally rounded off to the nearest order of magnitude. For the risk assessment case studies, additional background information used in the derivation of the TEF values is provided. Does this additional information enhance the means of evaluating uncertainties in the assessments? If so, how? If not, why?

In general, Dr. Menzie said, most experts agreed that the additional information was an enhancement. A number of experts indicated that the WHO tier system offers a useful framework for identifying at least the sources of uncertainty. Some felt that additional background regarding the derivation of specific TEF values would also be helpful, in that it would allow uncertainties to be carried along through the risk assessment in a more quantitative way. One person thought that this information was particularly important for the compounds that were driving a particular case study, while

another suggested that it would be very useful for someone to take on the task of developing a single document that addresses the uncertainties associated with the derivation of each of the consensus TEFs.

With respect to the rounding procedure used by WHO, Dr. Menzie noted that various opinions were expressed, but most experts agreed that rounding is probably not an important contributor to the overall uncertainty in the assessment. The general feeling seemed to be that the uncertainty associated with rounding would be less than half of an order of magnitude, and at least one expert noted that this question could be readily addressed by performing a model sensitivity analysis.

Finally, Dr. Menzie noted, various commenters had offered specific cautions related to use of the consensus TEF values. One expressed the view that it is not possible to quantitatively evaluate the available data and assign valid, comparable uncertainty rankings, and that qualitative assessment may be possible but may also be misleading. Another suggested that probabilistic methods could be used to examine uncertainties and limit the illusion of certainty associated with a point estimate.

- Charge Question I-2: Some TEFs were determined from several studies, endpoints, and exposure routes, while other TEFs were based on a single study and endpoint. Given the range of knowledge associated with specific compounds, should all TEFs be considered to have similar uncertainties? Why? Or why not?

In reviewing the individual responses to this question, Dr. Menzie noted that the overwhelming sense of the group was that uncertainties associated with the TEFs should not be considered similar, and that the level of uncertainty is related to the weight of evidence used to derive each of the individual TEF values. Several people noted that uncertainties tended to be largest for the least potent and most easily metabolized compounds, which are also the compounds least likely to drive a risk assessment. One expert wondered whether it might be possible to develop a sliding scale to capture the uncertainty associated with the individual TEF values. Others raised the possibility that uncertainty in the TEFs could be addressed by adopting an uncertainty factor similar to those employed to deal with other types of uncertainty in the risk assessment process.

Regarding the uncertainty associated with use of the TEF/TEQ approach, some experts felt that probabilistic methods could be used to determine the impact of TEF-related uncertainties on the overall uncertainty associated with the assessment, but others wondered whether even this level of quantification would be possible using the available data. One person expressed the view that uncertainties associated with individual TEFs will not be quantifiable until there is a common experimental basis for derivation of these values, and that attempts to partially quantify uncertainty could impart a false sense of accuracy. Another expressed particular concern about the TEFs for birds, which were derived mainly from in vitro assays using endpoints that are only peripherally related to the effects of interest.

- Charge Question I-3: The TEF values provided were based on endpoints that ranged from *in vitro* biochemical responses (e.g., induction of cytochrome P450 1A1) to *in vivo* early life stage mortality. To what extent can these endpoints be extrapolated to the measures of effects that are relevant for the assessment endpoint for each case study?

Dr. Menzie noted that in responding to this question a number of experts mentioned that uncertainty increases as the experimental evidence strays farther from the endpoint of interest. Many TEFs, however, are based on biochemical effects rather than toxic injuries, and these endpoints are poorly linked to survival, growth, and reproduction. In this regard, experts cautioned that particular care should be taken in applying TEFs derived from *in vitro* data unless the laboratory endpoint has been closely correlated to a toxic effect in a relevant species. As an example, one person commented on the questionable relationship between ethoxyresorufin-o-deethylase (EROD) induction and mortality in bird eggs, since *in vitro* enzyme induction assays do not take metabolism into account, and since the shape of the dose-response curve for EROD induction varies from one congener to the next. Another factor that may complicate the use of TEFs is the paucity of information about compensatory mechanisms that may mitigate the effect of dioxin-like compounds at the population level.

- Charge Question II-1. What are the implications, both quantitatively and conceptually, of assuming no dose-additivity or no interaction among the components of the mixtures described in the case studies? To what extent would the risk assessment conclusions differ if stressor response analyses were based on total PCBs or 2,3,7,8-TCDD alone?

Although some experts disagreed, the majority opinion was that the assumption of non-additivity would require a procedure for evaluating each compound separately. If such a procedure were used, however, the lack of toxicity data for many compounds would complicate an assessment of overall risk, which would normally be done by summing the hazard quotients for individual compounds. Some experts noted that the assumption of additivity was more likely to result in an overestimation of risk than the TEF/TEQ approach was to result in an underestimation of risk. Also, most experts felt that assessments based on total PCBs or on TCDD alone would typically give lower estimates of risk than would the TEQ approach. However, some noted that differences among the three approaches would largely disappear if the results of the assessment were to be judged against an established criterion or other benchmark value.

- Charge Question II-2. Many TEFs are based on LC_{50} or EC_{50} values. To what extent should TEF values derived at a median response level be used in risk assessments where a no adverse effect level is being employed?

Responses to this question covered a broad range of opinions, most of which had to do with the shape of dose-response curves for the endpoints of interest. A number of experts felt that the use of median response values was acceptable, since the goal was to determine relative rather than absolute potencies. Also, some pointed out that LC_{50} and EC_{50} values tended to be more stable measures within the dose-response curve than either NOAEL or LOAEL values. Other experts disagreed, however. One suggested using an effect level that is more relevant to the protection of ecological endpoints, and another suggested that it would be more appropriate to use a no adverse effect level, particularly for screening-level assessments. A third felt that this issue was relatively unimportant, since differences between the various metrics would probably be lost in the noise.

- Charge Question II-3. The TEF values provided were typically based on a single or limited number of mammal, bird, or fish experiments. To what extent can class-specific TEFs be directly extrapolated to the species identified within each case study?

The issue of interspecies extrapolation generated a variety of opinions, Dr. Menzie said, and most experts believe that this is a matter of substantial concern. In general, the experts felt more comfortable applying TEFs to organisms that are closely related to the species in which the TEF was derived, and less comfortable as the taxonomic distance

between the reference species and the species of interest in the risk assessment increased. In the prospective case study, for example, most people felt that it was appropriate to apply the fish TEF to the bull trout, since the data from which the TEF was derived were from another salmonic species; if largemouth bass had been the species of concern, however, use of the fish TEF would have been more problematic. A similar situation arises when TEFs based on data collected in chickens are used to predict the effects of exposure to dioxin-like compounds on eagles. One expert suggested that if data for the species of interest were available, those data should be used in lieu of the more generic TEF values.

Regarding the uncertainty associated with this aspect of the TEF/TEQ approach, some experts felt that a traditional uncertainty factor could be applied to account for differences between the reference species and the species of concern. One person pointed out that interspecies differences in sensitivity to TCDD are so large that they might in fact dwarf the uncertainties associated with the TEF approach. Dr. Menzie noted that this observation is particularly germane to the case studies, since the threshold for TCDD toxicity is itself a variable rather than a fixed value.

- Charge Question III- 1: To what extent does the TEF approach present challenges, introduce new uncertainties, or modify old uncertainties associated with modeling the exposure of AhR agonists? To what extent does the availability and quality of congener-specific physicochemical data limit the means of employing fate and transport or food chain models?

In general, Dr. Menzie noted, experts were in agreement that the TEF methodology poses a number of challenges for modeling, most of which are logistical problems that have to do with ways of accounting for the differing fate and transport properties of individual congeners and carrying these differences through the modeling effort. Some experts felt that this problem could be minimized if the model is focused on those compounds that are driving both the exposure and the risk.

A number of experts cautioned that uncertainties will be magnified in attempts to model exposure over more than two levels of the ecosystem. As an example, one person noted that uncertainties would be great in an approach that attempted to model avian

exposure on the basis of sediment levels, since contaminants would be moving through many different trophic levels of the system and uncertainties would be introduced at each step along this pathway.

- Charge Question III-3: To what extent does the TEF approach require a more rigorous analytical design in quantifying sediments, soil, and biota AhR agonist concentrations than is apparent in other methods which aggregate stressors (e.g., total PCBs)?

In their responses to this question, most experts agreed that the TEF methodology requires a more rigorous analytical design than other methods, and that analytical costs would probably be greater as a result of the need to quantify individual congeners.. Others, however, felt that this might not be the case, since congener-based analytical methods are now routinely used by many agencies and organizations.

- Charge Question IV-1: In evaluating the case studies, are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments? Do the uncertainties associated with TEFs limit the means of performing the assessments, or do the other areas of the effect and exposure characterization contribute similar or greater levels of uncertainty?

In general, experts did not feel that uncertainties associated with the TEF methodology would be any more problematic than other types of uncertainty in the risk assessment process. Indeed, one person suggested that the TEF-related uncertainties may actually be less problematic, since people have already worked through them. Others, however, felt that this question could not be answered *a priori,* noting that someone would have to go through a TEF exercise and really think through the issues to make any reasonable statement about the relative magnitude of the associated uncertainties.

- Biologically-based TEQ assays on environmental samples could be employed as an alternative to the TEF-based approach. What would the strengths and weaknesses of such an approach be? To what extent could these approaches be integrated?

Responses to this question were mixed. Several individuals pointed out the advantages of these methods, which include their ability to focus on an integrated response to a mix of chemicals in the environment and their lower cost in comparison with chemical-based approaches. Others, however, focused on the limitations of these methods: they do not account for metabolism; they can be confounded by other compounds; and they may not identify the most important compound for control purposes. In general, biologically-based TEQ assays were viewed primarily as a research tool at present, with a lack of regulatory acceptance. Some experts felt that these methods could be very useful, however, particularly as screening tools, and several suggested that these methods could be used in concert with the TEF/TEQ approach.

Observer Comments

At the end of his presentation, Dr. Menzie opened the floor to comments from those attending the workshop as observers. The only observer to take advantage of this opportunity was Dr. Angelique van Birgelen, who identified herself as a toxicologist with the National Institute for Environmental Health Sciences (NIEHS). Dr. van Birgelen noted that while it is rewarding to see how much progress has been made in the development and now the application of TEFs for dioxin-like compounds, it is also important not to lose sight of other ways in which the TEF approach can be improved. Toward this end, she suggested that there are three additional compounds or classes of compounds that should be assigned TEF values and included in the WHO scheme: 3,3',4,4'-tetrachloroazobenzene (TCAB); hexachlorobenzene (HCB); and several of the polychlorinated naphthalenes (PCNs).

According to Dr. van Birgelen, all of these compounds have been shown to bind to the Ah receptor, all have been shown to produce dioxin-like effects, and all have been shown to accumulate or to have a long half-life in certain species. Moreover, each may account for a substantial fraction of the total TEQ in some environmental settings.

Dr. van Birgelen provided the group with an extensive body of published data related to these three compounds/classes of compounds, which she summarized by briefly

describing the AhR binding properties, effect profiles, physicochemical characteristics, and estimated annual discharge for each compound or class. Based on this information, she urged the group to consider recommending that these compounds be included in the TEF scheme, and offered to provide further information if that would be useful.

III. WORKSHOP PROCEEDINGS

The second day of the workshop began with concurrent meetings of the Expertise Groups. Discussions in these groups were organized around question lists assembled by the Planning Group to raise issues of relevance to the various expertise areas. Each group included a notetaker from the Planning Group, whose job it was to capture the key points of the discussion. Appendix D of this report contains a list of Expertise Group assignments and the discussion summaries prepared by the notetakers.

Review of the TMDL Model

Before adjourning into breakout groups to discuss the prospective case study, workshop participants heard a brief presentation by Dr. Philip Cook, of the EPA/DOI Planning Group, who reviewed key aspects of the TMDL model and worked through a series of calculations related to that model. Dr. Cook began by discussing some elements of the flow chart originally presented during the opening plenary session by Dr. Steven Bradbury (see Figure 8, above). He noted that one can set a water quality standard based on the toxicity of TCDD, and that this standard may be based on effects observed in birds, fish, or mammals. Such a standard is represented in the uppermost box of the flow chart, where C represents concentration, the subscript w indicates that water is the medium of interest, and the superscript t refers to the fact that the standard deals with the total concentration of the contaminant of interest, in this case TCDD. In the second box, the same standard is expressed in terms of dioxin toxicity equivalents. Based on the additivity assumption, this standard can also be expressed in a third way, as the sum of the toxicity equivalence concentrations of individual congeners.

To determine toxicity equivalence concentrations for individual congeners in the system of interest, each congener's concentration in water must be adjusted to reflect both its toxicity relative to TCDD and its bioaccumulation potential relative to that of TCDD. This is done by taking the product of the congener-specific water concentration, the congener-specific TEF, and the congener-specific bioaccumulation factor, divided by the bioaccumulation factor for TCDD. When this process is completed for each congener, the toxicity equivalence concentrations for all congeners can be added together to determine the total toxicity equivalence concentration for the system, and this value can be compared with the standard to determine whether the system is or is not in compliance.

These same relationships underlie the TCDD Toxicity Equivalence Waste Load Allocation Model selected for the prospective case study. In this model, it is assumed that the ecosystem has a definable assimilative capacity for chemicals which, if not exceeded, will provide the desired level of protection. To facilitate waste load allocation for complex mixtures of AhR agonists, maximum allowable concentrations in water (MAC_ws) and maximum allowable loads (MALs) to the water body are calculated on the basis of each individual chemical's TEF, bioaccumulation factor, and fate/transport properties. Because each chemical is modeled individually, each MAC_w is equal to the toxicity equivalence concentration of that chemical in water.

Because of these relationships, the accuracy of the approach depends on how well the relationships between chemical sources and organisms of interest are modeled for each individual congener in the ecosystem. An important step in the modeling process, for example, involves relating the concentration of a contaminant in fish tissues, which can be measured, to a concentration of concern in water. Ideally, this conversion is achieved by applying a bioaccumulation factor that is both congener- and organism-specific. Similarly, fate and transport properties determine the relationship between a mass loading of the chemical to the system and its ultimate concentration in water, and these properties, too, are congener-specific.

The purpose of the MAC_w calculation is to determine the maximum concentration each congener could have in the water of this system if none of the other congeners were present, based on its toxicity profile. The MAL, in turn, relates this concentration to the loading of the congener into the system, based on its fate and transport characteristics. Because MAC_ws and MALs are normalized values, they can be manipulated to assess the combined impact of different mixtures of congeners on the system of interest.

To illustrate the application of this methodology, Dr. Cook worked through an example that showed how the TMDL approach would be applied to the two-chemical mixture described in Figure 9, assuming fish to be the organisms of interest.

VARIABLES USED IN A SAMPLE TMDL CALCULATION
FOR A TWO-CHEMICAL MIXTURE

Chemical	TEF	BAF	log K_{ow}	Projected Load
X (TCDD)	1.0	10^7	7	0.1 g/day
Y	0.1	10^6	6.5	20 g/day

Figure 9.

The two chemicals considered in this example, TCDD and a related congener Y, have different TEFs, different bioaccumulation factors, and different lipid solubilities. In the example, the proposed loading of dioxin is 0.1 g/day, and the proposed loading of congener Y is 20 g/day, and the water quality standard for TCDD has been set at 0.02 pg/L.

By definition, the MAC_w for TCDD is equal to the standard, or 0.02 pg/L. To determine the MAC_w for congener Y, the standard must be multiplied by the bioaccumulation factor for TCDD (10^7) and divided by the congener-specific TEF (0.1) and bioaccumulation factor (10^6). This calculation yields a maximum concentration of 2 pg/L for congener Y, which is, as one would expect given the lower potency of congener Y, many times higher than the maximum concentration for TCDD. Using a system-specific

mass balance model, the details of which are irrelevant to this example, the MAC_ws convert to MALs of 2 g/day for TCDD and 500 g/day for congener Y.

In the final stage of the TMDL methodology, the total load represented by the two compounds in the mixture is compared with the load allocated to the discharger under the permit condition, which in this example is defined as 10% of the total MAL. This is done by dividing the projected load of each chemical by both the allocation factor and its individual MAL, and summing the resulting values for all congeners present in the discharge. As long as this sum is equal to or less than 1, as it is in this case, the discharger is in compliance. Importantly, this is true regardless of the precise congener composition of the discharge; as long as the sum of their individual adjusted loads is less than or equal to 1, the permit condition is being met.

In response to a question from one of the experts, Dr. Cook indicated that the greater difference between the MALs than MAC_ws for these two chemicals has to do with physicochemical differences that affect their individual fate and transport profiles. Another expert asked whether water quality standards are typically based on dissolved or total concentrations of TCDD, and Dr. Cook said that there are currently no national water quality criteria for protection of fish and wildlife from the effects of dioxin. Based on what he has seen within EPA, however, Dr. Cook said that he would expect such standards to focus on the total concentration of chemical in the water. A third expert said that the example made it clear how to determine MAC_ws for chemicals in the case study, but that it was not clear how the associated MALs would be derived. Dr. Cook indicated that this had been a topic of discussion in the Fate and Transport Expertise Group, and that people from that group would be prepared to address questions about MAL derivation within the context of each breakout group's analysis of the case.

At the conclusion of Dr. Cook's presentation, workshop participants reported to their respective breakout groups for discussion of the prospective case study. The three breakout groups were chaired by Drs. Peter deFur, Janet Burris, and Charles Menzie. On the final day of the workshop, the same groups met to discuss the retrospective case study. Appendix E of this report contains a list of breakout group assignments and the

detailed summaries prepared by each of the workgroup facilitators at the conclusion of the workshop. Following each of the individual workgroup meetings, participants met in a plenary session to discuss the results of their deliberations.

Plenary Session: Discussion of the Prospective Case Study

Group #1. Dr. deFur noted that his group began its deliberations by addressing the use of more general as opposed to site-specific bioaccumulation factors (BAFs) for risk assessment purposes. The group agreed that site-specific BAFs would be a vast improvement over the more generic BAFs proposed for use in the case study. At a minimum, the group felt that some effort should be made to determine whether trophic conditions in the system of interest were or were not similar to those assumed in the derivation of the generic BAFs. If they were not, various methods could be used to generate more site-specific values. One method that was suggested was to develop a site-specific model that would incorporate published data more relevant to the site; another involved the collection of field data that could be used to develop more site-specific values.

Regarding uncertainties associated with the use of BAFs, members of the group identified numerous sources of variability in these values. In general, the group agreed that BAFs are most applicable in the system where they were developed, and that their reliability decreases as they are applied to systems that are progressively more different from the original system in terms of their size, biological and physical complexity, and scope. Indeed, group members felt that the relationship between the bioaccumulative behavior of TCDD and other congeners was likely to be more stable than the behavior of TCDD in different systems. As a result, they concluded that it would be more useful to improve understanding of the bioaccumulative behavior of TCDD than to improve understanding of the relationships between BAFs for TCDD and other dioxin-like compounds.

Throughout their discussions, Dr. deFur's group encountered a number of issues that highlighted differences in the European and American approaches to assessments of

dioxin-like compounds. The most striking of these was the fact that in Europe chemical analyses are seldom if ever done for a single congener, so it simply would not be the case that TCDD would be measured alone. As a result, environmental concentrations of the individual congeners are known, and it is usually possible to determine BAFs for the full suite of dioxin-like congeners. Given the obvious importance of BAFs to the TMDL model, the group agreed that wider adoption of the European practice would substantially reduce the uncertainty associated with TMDL-based regulatory and management decisions.

Turning to the question of dose-response relationships, the group discussed problems associated with relying on TEFs that are derived at the cellular or molecular level to predict effects at the population level. While recognizing that regulatory and management decisions are often constrained by the legal, policy, or even cultural context within which those decisions are made, group members felt that the level of uncertainty associated with these types of extrapolations is large and that this aspect of the assessment paradigm needs to be addressed. Particularly when attempting to set regulatory limits such as MACs, information about population dynamics is a critical component of the knowledge base. Like BAFs and other elements of the TMDL approach, population data will be most useful if collected on a site-specific basis, focusing on density-dependent as well as density-independent factors.

Another element of the group's discussion focused on the relationship between TEQ- and TEF-based approaches. In general, the group felt that these approaches are complementary, in the sense that TEQ-based bioassays might serve as a reality check for a TEF-based analyses. If the results obtained via both methods were concordant, confidence in the TEF-based analysis would certainly increase. Even non-concordance might be useful in highlighting specific areas where further investigation is needed.

The group also spent a fair amount of time discussing how the uncertainties associated with application of the TEF methodology compare to those associated with other elements of the risk assessment process, including the uncertainty in BAFs, uncertainty in population dynamic models, and uncertainty in environmental

measurements. In addition, the group discussed the many places within the TMDL model that errors were likely to be propagated and perhaps even magnified. At the end of this discussion, there was general agreement that no single source always generates the greatest amount of uncertainty, and that the relative contribution of individual sources of uncertainty varies from site to site.

At the end of his summary, Dr. deFur asked whether other group members would like to comment on any additional issues that came up during the group's deliberations. One member of the group noted that toward the end of the session there had been some discussion of the need to identify the uncertainties associated with various elements of the TMDL model, including but not limited to the uncertainties associated with the derivation of TEFs, and to find appropriate ways of carrying these uncertainties through the risk assessment process. Although presented as point estimates, all of the numbers in the case study exercise have some variance associated with them. To determine the relative contribution of individual uncertainties, therefore, one could use a Monte Carlo or other probabilistic method to see how each of these uncertainties affects the values generated via the TMDL process.

In response to a question from one of the other experts, Dr. deFur elaborated on the role that bioassay-based approaches might play within the TMDL framework. One way that bioassays could be useful, he said, was in screening-level analyses—for example, to see whether contaminants actually do accumulate at the predicted rate. Later in the process, bioassays could be used to determine how rates of enzyme induction, for example, compare with those predicted at one level of the TMDL model. In this setting, observed values should be fairly close to predicted values, or there should at least be some way of explaining disparities between the two approaches. He also noted that the group recognized the difference between their around-the-table discussion and the circumstances under which management decisions generally need to be made. In this sense, it might not always be possible for confirmatory bioassays to be run, due to both resource and logistic constraints. The group nevertheless felt that in some situations bioassays could provide a useful complement to a TEF-based approach.

Group 2. Ms. Burris began by noting that her group spent a good portion of the session discussing the uncertainties associated with the derivation of TEFs and the effect of these uncertainties on their application within the prospective case study. Based on this discussion, the group agreed that a hierarchical approach should be used to select the TEFs applied to a particular risk assessment. If a species-specific value is available, for example, that value should be used in lieu of the WHO consensus TEF. Also preferable to the consensus TEF would be a value derived for a more closely related species than that used to derive the WHO value. However, a sensitivity analysis should be performed to determine whether uncertainty would actually be reduced by the use of species-specific values.

Group members felt that more information about the methods used to derive consensus TEFs would have been helpful, since it would have allowed the uncertainties to be better understood and carried through the analysis. Their impression was that the process used to derive consensus values was not consistent from one congener to the next, and that this made it difficult to have even a qualitative sense of the uncertainties introduced by using the consensus TEFs. Rounding, in particular, seemed to be a quantifiable source of uncertainty, but information about the rounding process was too scant to allow a more detailed consideration of this issue.

Despite its shortcomings, the group concluded that the TEF approach is more valid than approaches using either total PCBs or TCDD alone. However, they thought that there would still be a need for total PCB-based approaches, since some of the effects of these compounds are not mediated by the Ah receptor.

Turning to the prospective case study, the group decided to use the consensus avian TEF for the bald eagle, but to look at the effects of rounding and not rounding the TEF value. In general, group members were comfortable extrapolating from the endpoint used in deriving the TEF to the reproductive endpoint in the assessment. For the bull trout, the group elected to use TEFs derived from rainbow trout data, and they thought that early life stage mortality was the appropriate endpoint. For the otter, they chose to use the WHO consensus TEF, but there was some discomfort about extrapolating from the TEF endpoint to the assessment endpoint.

Group members did not feel that the use of median values for deriving TEFs was a significant source of uncertainty, since the median values tended to be more stable and were probably more appropriate for looking at relative toxicity.

Moving on to the exposure assessment, the group felt that use of the TEF approach for this particular fate and transport modeling exercise was really no different than the use of any other chemical-specific model. The challenge, however, was in modeling the many different congeners and in having the data available to complete the modeling exercise.

Looking at the measurements of individual congeners in sediment and fish tissue, the group felt that the greatest uncertainties were in water measurements, due mainly to limit-of-detection issues. From a physicochemical perspective, the group had high confidence in the log K_{ow} values, but the K_{oc} data and Henry's Law constants were considered suspect. Biotransformation and metabolism of the individual congeners were not as clearly understood; in some cases there was no knowledge, and in others it is known that there are changes in the composition of congeners as they move between the different species. PCB 126 is enriched, for example, during transfers from fish to wildlife species, and this needs to be considered. In general, however, we have a better understanding of the transfer within fish than we do from fish to wildlife. In order to be able to appropriately model or understand the fate and transport of various congeners within the food chain, we need to know more about what the organisms are consuming, since the composition of congeners is species-specific and will therefore vary from one species to another.

In general, group members felt reasonably confident that they would be able to complete a worthwhile modeling exercise if they had more information about transfers from sediment to the sediment-water interface and about sediment transport within the system. Without this information, however, the modeling exercise would be extremely uncertain. Some members of the group thought that it would be a good idea to advise the risk managers to substitute a better-characterized model for the one proposed in the case study, but there was a divergence of opinion on this issue.

In terms of the analytical requirements to implement a TEF approach, group members agreed that the TEF approach would be more costly than the total PCB or TCDD-only approaches, since the discharger would have to analyze many different congeners. This might turn out to be beneficial, however, since a better understanding of the toxicity associated with specific congeners might give the discharger more flexibility in altering the composition of the discharge.

Overall, group members agreed that the uncertainties associated with the exposure profile and with projecting exposures in the future under these conditions were at least as great and possibly greater than those associated with the stress response profile or the use of TEFs. To gain a better understanding of relative uncertainties, the group recommended a sensitivity analysis focusing on TEFs, Koc values, and biomagnification factors. Regarding the latter, group members parenthetically noted that the same dose metric should be used for BMFs and TEFs.

Regarding the use of biological assays, group members felt that these really were not applicable to a prospective case study, since it is not yet clear which chemicals will be present in the system. However, biological assays could be used to document background conditions in the system before the discharge occurs, particularly since it is already known that PCBs are present.

When the group discussed errors associated with the application of a TCDD-based water standard, two potential problems were raised: the enrichment of PCB 126 from fish to wildlife and the observed loss of chlorinated dibenzofurans in some species of birds.

The group concluded its discussion by talking about ways the assessment for this site might be done better or differently. Group members agreed that it might be useful to put together a more site-specific model, but there would be no way of knowing whether such a model would be predictive. Other existing food chain models could be used, but these would have to be modified to address metabolism issues. Everyone was more comfortable using the TEF/TEQ approach than using either of the default approaches, but most

thought that the assessment would generate a range of risk estimates that would be perplexing to the risk manager. It was agreed, however, that this may be the best we can do given the current state of the science.

Following Ms. Burris' presentation of the group's findings and recommendations, there was considerable discussion of the role that bioassays might play in a prospective case scenario. In response to a question about how they came to their decision that bioassays would not be useful, a member of the group explained that there was some concern about how the results of bioassays could be misleading if appropriate extraction and fractionation steps were not included. Another member of the group mentioned studies of Canadian paper mills in which bioassays were applied directly to the effluent, resulting in a gross overestimation of discharge toxicity. The questioner agreed that these issues need to be taken into account, but suggested that the wording of the group's conclusion was overly strong. He noted that there are many different types of bioassays, and that some would be very useful in a prospective setting. As an example, he suggested a bioassay that is able to predict the relative potencies of various congeners for relevant endpoints in a fish species of concern. Such a bioassay could be used to test both how sensitive that system is to different compounds and how the sensitivity of the target species compares with that of other organisms in the system. This information, in turn, might be extremely useful in a prospective assessment of the impact that further loading of the system might have on the species of concern.

In response to a question from the Chair, Ms. Burris confirmed that the group's sense had been that uncertainties associated with the use of TEFs are no greater than those associated with exposure or response assessments, although the group did not have enough information to quantify these different types of uncertainty. The group also felt that uncertainties were less manageable in the context of a prospective case study, since a prospective scenario does not lend itself to the sorts of approaches that can be used to reduce uncertainty in a retrospective assessment.

Group 3. Dr. Menzie indicated that the results of his group's deliberations would presented by group members Donald Tillitt and Wayne Landis. Dr. Tillitt began by

noting that Group 3 had begun its analysis of the case study where the other groups had left off, in that this group had focused almost exclusively on how the various sources of uncertainty might be addressed in a risk characterization for the prospective case study. For purposes of this exercise, the group identified five major sources of uncertainty: the derivation of TEFs, the derivation and use of BAFs, extrapolation of TEFs between species, exposure modeling, and derivation of the threshold values themselves. For each of these sources of uncertainty, the group developed specific criteria that could be used to rank degrees of uncertainty on a scale of 1 to 4, which was chosen because of its rough correspondence to the tier system used in the derivation of TEFs at the Stockholm meeting.

Dr. Landis added that the group's intent in developing these criteria was to move from "feelings" and "senses" of relative uncertainty to a more quantitative expression. While recognizing that the ranking system is not quantitative in a statistical sense, it does provide a way of assigning relative values to the differing degrees of qualitative uncertainty that most people would agree exist in different interspecies extrapolations or in different types of gaps in the congener-specific data. In addition, this approach allows the uncertainty rankings to be manipulated arithmetically in ways that provide additional information about the system as a whole.

To illustrate the results of the group's deliberations, Dr. Landis showed the matrix reproduced as Figure 10. For each cell in the matrix, the group attempted to rank the uncertainty associated with a particular variable in either species- or congener-specific terms. For example, they felt that the uncertainty associated with application of a TEF derived in rainbow trout or lake trout to bull trout was considerably less than the uncertainty associated with applying a TEF derived in chickens to bald eagles; as a result, the group gave the TEFs for bull trout an uncertainty ranking of 1 and the TEFs for bald eagle an uncertainty ranking of 4. In considering BAFs, the group felt that these were less uncertain for fish than for either birds or mammals, and rankings were assigned accordingly. Similarly, because the exposure model was developed around fish, its application resulted in less uncertainty if a fish rather than a bird or mammal was the species of concern. Also, because of their migratory potential, birds and mammals are much more likely to have exposures outside the system than are fish.

Relative Uncertainties in the Ecological Risk Assessment Including Us of TEF Values

Ranks for uncertainty Species/Congener										
	TEFs	BAFs	Species Sens./Extrapola	Exposure Mod	Threshold concentration				Total	
Bull trout	1	2	2	2	2		Species specific	9	30	Bull Trout
1		2	1				Congener specific	21		
2		2	1							
3		2	1							
4		2	1							
5		2	1							
6		2	1							
7		2	1							
Bald Eagle	4	3	4	4	4			19	55	Bald Eagle
1		3	1					36		
2		3	1							
3		3	1							
4		3	4							
5		3	3							
6		3	3							
7		3	2							
River Otter	3	3	3	4	3			16	48	River Otter
1		3	1					32		
2		3	1							
3		3	1							
4		3	2							
5		3	2							
6		3	2							
7		3	2							
Criteria are described in the text. This approach and these values are presnted for illustration only.										

Figure 10.

Once these individual rankings were completed, the group summed all of the species- and congener-specific values to see how each contributed to overall uncertainty. From this summation, it became clear that the species-specific uncertainty was greatest for bald eagle, slightly less for the river otter, and much less for the bull trout. One of the encouraging conclusions that can be drawn, therefore, is that uncertainty is relatively low for the species that is endangered. In addition, the group concluded that the species most likely to drive the lower limit would be the river otter, for which uncertainty was the greatest.

Another way the group used this matrix was to identify the sources of greatest uncertainty in the assessment. To a large extent, Dr. Landis said, overall uncertainty was driven by uncertainty in the modeling. For individual species, however, it was possible to identify specific areas in which uncertainty was due to a lack of knowledge about the properties and effects of different congeners. In this sense, the matrix could also be used to identify ways of reducing the uncertainty in these assessments. For both the bald eagle and river otter, for example, additional information about species-specific TEF and BAF values would substantially reduce the uncertainty of the assessment. In this way, Dr. Landis suggested, use of this matrix would allow the risk assessor to answer a variety of questions that are vitally important to stakeholders, including how the situation might be improved. In addition, the group felt that this matrix might be a useful tool in communicating the results of the assessment to risk managers.

One caveat that the group identified in considering possible uses of the matrix is that the relative rankings are specific to the system under consideration. Because the rankings reflect relative rather than absolute measures of uncertainty, different values would have to be generated for different systems, and the results of site-specific analyses could not be directly compared.

Following these presentations, one of the experts from a different work group expressed some concerns about using a matrix such as this to identify the areas in which additional research is most needed. The reason for his concern was that the matrix does not address the relative sensitivity of the model as a whole to specific elements of the matrix. Depending on the model, it could be more important to reduce the uncertainty in one variable from 2 to 1 than to reduce the uncertainty in a different variable from 4 to 2. Dr. Landis agreed with this observation, noting that it would be necessary to combine the matrix with a more conventional sensitivity analysis to determine precisely where additional research would have the greatest impact on overall uncertainty. However, he thought that the matrix enables assessors and managers to better understand those aspects of the uncertainty problem that are not typically addressed in a sensitivity analysis. A member of the Planning Group suggested that it might be possible to combine these two approaches by weighting different cells in the matrix to reflect the results of a sensitivity analysis.

At this point in the discussion, another member of Group 3 noted that the group was unable to identify any place in the process diagram where this and other information about relative uncertainties could be incorporated into and carried through the TMDL process. He thought that this would be an important issue for the modelers to address, since the ultimate value of quantifying the uncertainties depends on there being a way to bring this information to bear on the decisionmaking process. One way to do this, he thought, would be to go back and reframe the question that the model was designed to answer in a way that includes specific attention to the impact of various types of uncertainty.

In response to a request from Dr. Menzie to describe the group's thoughts about use of the TEF approach as opposed to one of the defaults, Dr. Tillitt said that there was an agreement that the use of TEFs does not contribute disproportionately to overall uncertainty, and that the TEF approach reveals some useful information that would not be apparent if other approaches were used. As a result, the group felt that something important would be lost if one of the defaults were used.

One of the experts noted that it is important to be cautious when using a semi-quantitative method as a decisionmaking tool. The reason for his concern was that the weighting of different variables may reflect subjective biases, and this subjectivity could be obscured by the quasi-mathematical nature of the method. If this occurred, the method would simply be validating a conclusion that was essentially predetermined. Dr. Landis agreed, and noted that this is why it is important for the ranking criteria to be established *a priori,* before the method is applied to specific sites. Another group member noted that the ranking criteria themselves would certainly be open to debate, and might even change over time, as more information becomes available. Continuing along these same lines, another expert suggested that it would be an interesting test of the method this group used to see how different groups given the same a priori criteria and the same data set would rank the relative uncertainties. Finally, a member of the Planning Group urged that, in the workgroup's more detailed report of its deliberations, members of the group try to more clearly describe the ranking scheme they used to construct their matrix, since these *a priori* criteria represented such a key element of the process.

Summary. To conclude the plenary session, Dr. Menzie provided a brief summary of what he thought were the major conclusions that could be drawn from the group's consideration of the prospective case study. In general, all three workgroups felt that the TEF approach could be applied to a prospective case scenario, but that this approach might be more costly than the other alternatives. All three groups felt that there needed to be a way to track uncertainties through the risk assessment process, but that uncertainties associated with the application of TEFs are no greater than those associated with other elements of the TMDL model, and that they may in fact be smaller. As a result, all three groups concluded that use of the TEF-based approach is preferable to use of the traditional TCDD-based methodology, which in comparison might underestimate risk. There was some discussion of the usefulness of biological assays in supplementing the TEF approach, and a divergence of opinion regarding the applicability of these methods to a prospective case scenario. Finally, the group had discussed the need for better ways of incorporating what we do know about different sources of uncertainty into the TMDL model and for communicating the results of the assessment to risk managers.

At the end of Dr. Menzie's summary, one of the Planning Group members asked if any of the groups had addressed the aspect of the TMDL approach that has to do with issuing a permit that is based at least in part on chemicals that are not in the discharger's wastestream. One of the experts noted that this had been addressed to some extent in the comment that a TEF-based approach might in some cases actually turn out to be beneficial to the discharger, since only the subset of AhR agonists would be driving the assessment and therefore the permitting process. The questioner noted that this is a departure from the chemical-specific approach that EPA has traditionally used in regulating environmental contaminants, since it directs the regulator to mode of action or ecological effect rather than to chemical identity. One of the experts suggested that if the goal is truly environmental protection, then this is an appropriate re-focusing of the regulator's attention. Another expert disagreed, suggesting that further ground-truthing is needed before TEF-based approaches can reasonably be applied in a regulatory setting.

Plenary Session: Discussion of the Retrospective Case Study

Group #1. Dr. deFur began by noting that his group's approach to the retrospective case study differed in two important respects from their approach to the prospective case. First, the group attempted to be as quantitative as possible in addressing the retrospective scenario, as opposed to the largely conceptual approach they had taken to the prospective case. In addition, in accordance with guidance the facilitators had been given by members of the Planning Group, the group agreed to try to make a decision about the site described in the retrospective case study.

After reviewing the features of the site, the group first talked about what the decision was that they were trying to make. Rather than a decision about whether to remediate or not to remediate, the group elected to try and decide whether the data were sufficient to support a regulatory or management decision. In particular, they agreed to focus on whether the TEF/TEQ approach offered any advantages over approaches based on total PCBs or on TCDD alone.

The group's quantitative analysis centered on a graph that one of the members drew to summarize how the data from the site would look from both a TEQ and total PCB perspective (Figure 11). In this figure, the left-most bars in each graph represent the species-specific TEQs for the site, broken down to reflect the contribution of various classes of compounds to the total TEQ. The vertical line to the right of this bar represents the threshold range for the species of concern. In the right half of each graph, a similar method is used to depict the site-specific values and threshold ranges for total PCBs.

Interpretations of this graphic covered a fairly broad range. Some people felt that conclusions drawn on the basis of the TEQ data would differ from those drawn using total PCBs, but others felt that there would be no difference in the bottom-line conclusions as to whether exposures do or do not reach threshold. The group did not try to reach an agreement on this issue, since it seemed important to note that these data could be interpreted one way by some people and differently by others.

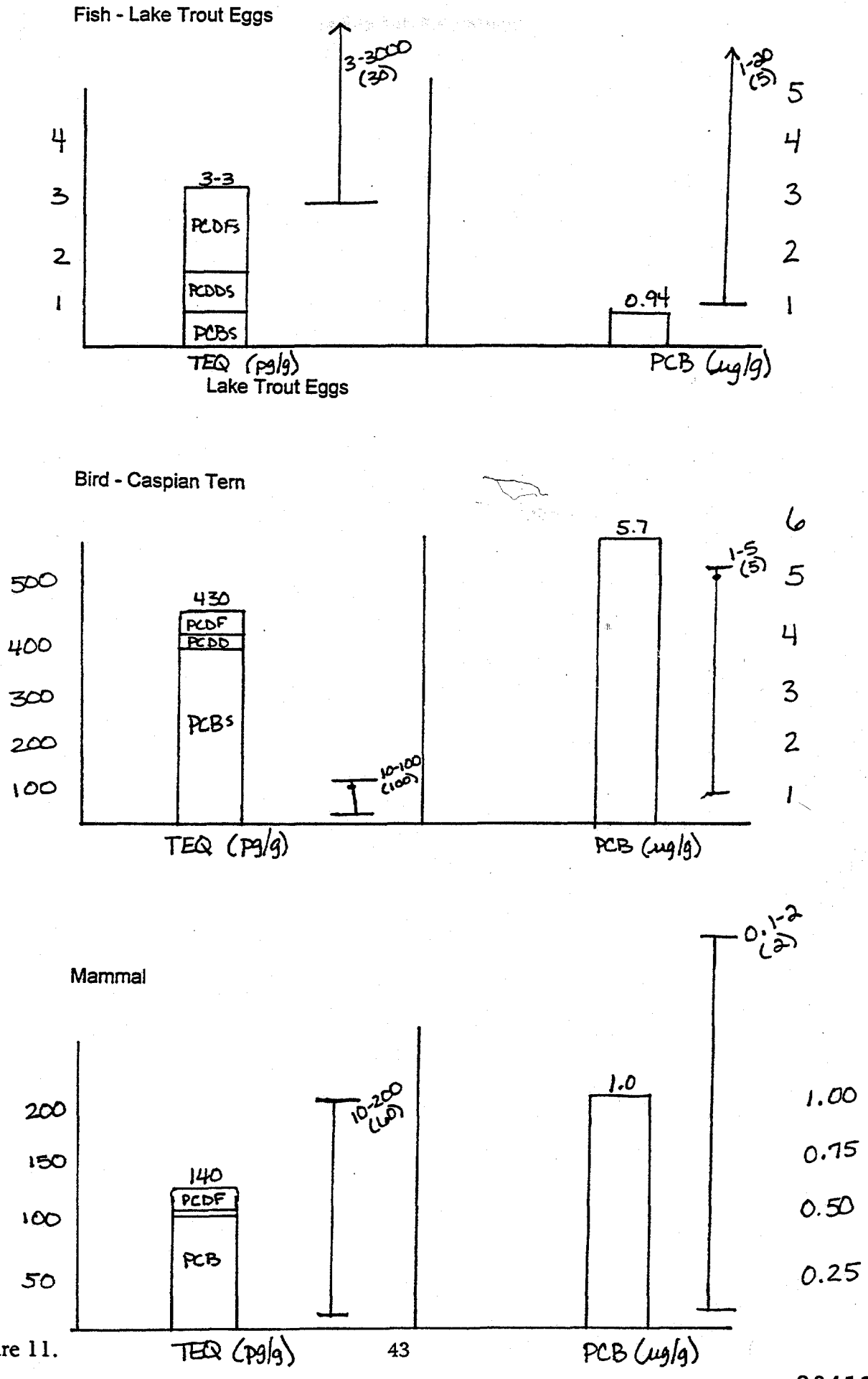


Figure 11.

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The group noted that in two of the three species, PCBs were the main contributors to total TEQ; TCDD for the most part made a relatively small contribution to the total TEQ, and furans were similarly minor contributors, except in fish. Clearly, the contribution of various classes was more obvious using the TEQ approach. Group members felt that this was important, since it increased people's comfort level about the range of conclusions that could be drawn about the site. Everyone agreed that the results of the TEQ analysis were sufficient to support screening-level decisions. Opinions began to diverge, however, as application of the TEQ approach moved closer to the regulatory arena.

Group members concluded that the amount of additional information revealed by application of the TEF approach depends on the mix of congeners present in the system. In at least one case, moreover, the group agreed that reliance on TCDD alone would alter the outcome of the risk analysis. In this case as in the prospective case study, group members who were not accustomed to dealing with the U.S. regulatory system were surprised that anyone would actually go out and measure TCDD alone, as opposed to the full suite of dioxin-like congeners, and even more surprised that a regulatory decision might be based on TCDD alone. Group members agreed that this approach is scientifically unsound.

The group engaged in an extended discussion of uncertainty, and members agreed that it is important to identify and put bounds on the various sources of uncertainty in the TEQ-based analysis. In particular, it is important to recognize that some uncertainties are quantitative, having to do with statistical variability, while others have to do with gaps in the knowledge base. Different analytical tools should be used to address these differing types of uncertainty and different analytical approaches are required to carry them through the assessment.

When it came to the actual decision the group had agreed to make, there was a divergence of opinion about whether the TEF approach is sufficient. Some people felt that the approach provided enough information to move forward, and others did not. Everyone agreed that the approach provides useful information about where the key gaps in the data are, and for that reason alone there was agreement that the approach should not be turned down. However, some people felt that the results of the TEF approach would have to be supplemented with more information on population dynamics and on the relationship between the biochemical or molecular endpoints on which the TEFs are based and effects at the population level before the approach could be used to decide whether to move forward into a regulatory decisionmaking mode.

Differences in opinion about the sufficiency of the TEF approach were based mainly on the paucity of information about the uncertainties associated with individual TEF values. Although group members uniformly felt that the underlying data was probably very robust, some nevertheless felt that TEF values could not legitimately be used in a risk assessment until and unless the associated uncertainties were expressed quantitatively and carried through the analysis. In particular, group members were concerned about uncertainties associated with the derivation of TEFs, with species differences in responsiveness to the various congeners, and with the ability of TEF-based methods to predict population-level effects.

At the end of their deliberations, Dr. deFur's group attempted to identify data gaps that seemed particularly critical in the context of the retrospective case study. Research efforts that might be useful in addressing these gaps included:

- testing of the Caspian terns themselves to develop species-specific BAF and BMF values;
- performing ground-truthing exercises to get a better sense of the relationship between exposure levels and responses in the tern population;
- gathering population data for the three species of concern;
- examining sediment core samples from the lake as opposed to the river to get a better sense of the distribution of chemicals in the system as a function of both time and space;

- determining deposition rates and inputs from sources other than the site of the prior spill; and

- performing ground-truthing exercises to assess the predictive capability of the TEF/TEQ approach at sites for which there is already a good body of data.

In response to a question from Dr. Menzie, who asked whether the group had identified any specific types of uncertainty in the TEF approach that were particularly problematic, Dr. deFur indicated that the three major concerns of the group had to do with differences between the species used to derive the TEF values and the species of concern in the risk assessment, with the statistical uncertainty in the derivation of a TEF from multiple REP values, and with the statistical uncertainty in the REP values themselves. Another group member pointed out that the reason for this concern was that group members were unsure whether the uncertainty in TEF/TEQ values was high enough to impact conclusions about whether observed levels of contaminants did or did not exceed the threshold value.

Another member of the expert group commented that the group's reticence to recommend that the results of the TEF analysis be used as a basis for risk management decisions seemed to include some presumptions about what those decisions might be. Noting that there was a similar reticence in his own group, this expert suggested that assessors should be sure they are not attempting to do the risk manager's job, since the decision could just as easily be whether to spend an additional $100,000 on research as to embark on a $1 billion remediation effort. If experts believe the method sufficient to support the former decision -- which most seem to -- then it was not clear to him why it wouldn't be sufficient to support the latter, since the validity of the method would not have changed. The task of the assessor, he noted, is to present the facts and associated uncertainties in a way that will inform the risk manager's decision, not to determine which decisions should or should not be made on the basis of the available data. Dr. deFur responded that there had been some discussion of this in the group, and that no one wanted to go on the record as recommending remediation even for a fictitious site.

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million cleanup, industry scientists would have a strong motivation to fill some of these gaps in the understanding of uncertainty, precisely because they would not want to be caught in the position of having to comply with management decisions that were based on back-of-the-envelope risk calculations that failed to take uncertainty into account. He went on to note that even he and the other people who were calling for better characterization of the uncertainties like the TEF approach, because it does have the advantage of bringing different congeners together in an integrated model. The only question is whether the method is sufficiently well developed to support definitive, quantitative risk management decisions. Without more precise information about the error in these values, it is simply not possible to answer this question.

In response to this comment, one of the experts expressed the opinion that uncertainties in the method do not mean that the method cannot or should not be used. He noted that decisions are made every day on the basis of incomplete information; if a decision needs to be made tomorrow, this incomplete method may represent the best that we can do. Another expert suggested that, at least from a risk management perspective, the question can also be framed in terms of the need to select between three different methods that are all incomplete in some way. From this perspective, he thought that most people would agree that despite its limitations, the TEF methodology offers important advantages over those based on total PCBs or on TCDD alone.

Group 2. Ms. Burris noted that her group began its deliberations by discussing the effects portion of the analysis, working through each of the species of concern to determine which TEF they would use and what level of uncertainty was associated with these selections.

For lake trout, the group decided to use both a TEF derived from the rainbow trout data (0.005) and an REP for PCB 126 in lake trout (0.003). The group felt that extrapolation from the trout data to other, non-salmonic species in the lake would introduce uncertainty, but that the magnitude of this uncertainty is unknown because the data needed to quantify it are not available.

For the Caspian tern, the group chose to adopt the WHO TEF, mainly because the information used to derive it was of better quality than the species-specific data that were available. Based on an EROD assay of PCB 126, the TEF derived for the Caspian tern using species-specific data was 0.03, and the WHO consensus value was 0.1. Therefore, use of the WHO value increased the TEQ from 185 to 426.

At this point in their deliberations, the group briefly discussed whether the risk assessor should be allowed to select a species-specific TEF from the available REPs, or whether that decision should be left to individuals with a better understanding of the literature. The group did not reach an agreement on this point, but they did feel that it was important for the assessor to have the flexibility to use a species-specific value if one was available.

For the mink, the group elected to use the WHO value. There was some discussion of the endpoints used in the derivation of this value, but the information needed to resolve this issue was not available.

Because of the difficulties they had in selecting TEFs for the species of interest, the group had a general concern about the lack of transparency in the WHO consensus TEF values. The group also felt that it would be more useful if these values were expressed as ranges, since management decisions are frequently not based on point estimates. Ranges would also help to quantify the uncertainty associated with a particular TEF, which would increase overall confidence in the results of the analysis.

Looking more closely at the issue of using TEFs other than those set forth by the WHO, the group attempted to develop a TEF selection hierarchy. In decreasing order of preference, the hierarchy they developed was as follows:

- a TEF derived using the endpoint of interest in the species of concern;
- a TEF derived on the basis of *in vivo* toxicity data in the species of concern;
- a TEF derived using the endpoint of concern in a related species;

- a TEF derived on the basis of *in vivo* toxicity data in a related species;
- a TEF derived from a Tier 2 REP for the species of interest; and
- the WHO consensus TEF.

The group also discussed whether uncertainty in the assessment could be reduced by performing a full food chain modeling exercise. They decided that such an effort would be problematic both because of the heterogeneity in the system and a possible lack of equilibrium. Members agreed that a full modeling exercise was probably not necessary, but that a partial modeling exercise could be useful in developing site-specific BSAFs and BMFs. These values, in turn, would allow the risk manager to examine the tissue level reductions that could be expected to occur in target species under different management scenarios. However, the model could probably not be used to predict concentrations over time.

The group's approach to the risk characterization was similar to that followed by Dr. deFur's group, and they noted that the TEF methodology yielded a higher estimate of risk that either the total PCB or TCDD-only methodologies.

A question that came up during the group's discussion of this case was how to account for the fact that, as a migratory species, the terns might be getting some of their exposure at another site. After some discussion, members agreed that the assessor could use a weight of evidence approach to evaluate the relevant scientific literature and develop an opinion about whether and to what extent tissue concentrations in the birds should be attributed to the site.

The group developed hazard quotients for individual organisms in each of the species of interest. In general, these values were borderline. Use of a TEF for common tern data as opposed to a TEF derived from the Caspian tern data altered the hazard quotient by less than an order of magnitude. There was some concern within the group about how hazard quotients should be translated to effects at the population or community level. Because the stated goal of the assessment was protection at the population level, the

group felt that a separate modeling exercise would be required to better understand the relationship between hazard quotients and the assessment endpoint. Without this information, some members of the group were concerned about the advisability of basing a management decision on the results of the TEF-based analysis.

Regarding issues that should be addressed in the risk characterization, one person suggested that it would be useful to try to describe how the system might look in one, five, and ten years if no action was taken. Some members of the group thought that PCB concentrations would decrease over time, eventually reaching a level that is lower than the action threshold. Others suggested that a hundred-year flood scenario should be included in the characterization, and that there should be some discussion of the decrease in reproduction required to produce a population effect. In view of the borderline condition of the system, some group members also felt that attention should be focused on the potential effect of additional inputs to the system that might occur in the future.

When a vote was taken, two members of the group voted for action and four voted for no action. In the event that the risk manager decided to pursue a cleanup, the group agreed that the otter would be the species of concern in setting cleanup levels. The reason for this choice had to do with the fact that the otter is considerably more sensitive to dioxin-like compounds than the reference species, so there is reason to believe that the true threshold for toxic effects would be at the low end of the range established for the mink.

To follow up on this latter point, one of the other members of the group noted that the range in the threshold for fish covers three orders of magnitude, and that this is a TCDD-based threshold. Given that the uncertainty in the threshold value for a single congener, particularly TCDD, is so great, this person wondered how much the estimated order-of-magnitude uncertainty in TEF values would actually add to the overall uncertainty of the assessment.

Another group member elaborated on the decision not to recommend a food web model for this system. First, group members had concluded that it would be difficult to

obtain credible water concentrations for the individual congeners, since they are present at such low levels. It would also be difficult to estimate sediment values, since the distribution of these compounds in sediment was likely to be heterogeneous. As a result, group members thought that development of species-specific BAFs and BMFs would be sufficient to reduce the uncertainty without introducing such formidable analytic challenges.

A member of the expert group raised a general issue related to the use of Ah receptor agonist levels in the liver as a marker of exposure, since there is a tendency for these chemicals to accumulate in the liver, and accumulation is itself dependent on the level of exposure. One of the Planning Group members pointed out that studies addressing this issue have shown no effect on the BMFs for the various congeners.

Another member of the Planning Group questioned the workgroup's use of a 50% reduction as a more or less universal population effect of concern, rather than tailoring this threshold to the local population. He thought that for bald eagles or nesting pairs, for example, a different metric might be more appropriate. The group member who had originally proposed the 50% value agreed, and said that historical records of reproductive performance might also be useful if the number of individuals or nesting pairs in the system was small. A different member of the Planning Group suggested that another way to approach this issue would be to simply use exceedance of the standard as a surrogate for population-level effects, since standards are developed to protect the most sensitive members of a population.

Group 3. Dr. Menzie said that his group began by revisiting a couple of the topics they had addressed previously, during consideration of the prospective case study. One member of the group, for example, had developed a concern that the uncertainty associated with the derivation of TEFs might be greater than was reflected in the matrix the group presented at the previous day's plenary session. The group therefore decided that it was important to stress that the matrix was intended to illustrate a conceptual approach, rather than to present hard and fast descriptions of the uncertainty in this particular system.

The group also revisited the issue of uncertainty in the water quality standards. Initially, the group had thought about the uncertainty in these values as having mainly to do with the interspecies extrapolations required in the application of these values. Subsequently, however, group members realized that there are probably other uncertainties associated with these values as well. The lesson, Dr. Menzie suggested, is that it is important to think about uncertainties on the exposure as well as the effects side of the analysis.

Like the previous group, Dr. Menzie's workgroup was able to trace the origin of the WHO consensus TEFs for fish and birds, but not for mammals. The group understood that this information does exist, but for purposes of this risk assessment the associated uncertainties were not quantifiable. Given the importance of uncertainty information to the risk assessment process, the group decided to recommend that some organization make an effort to provide that level of documentation for the consensus TEF values, so that risk assessors could have a better understanding of where those values come from.

One of the lessons the group learned from the case study exercise had to do with the availability of site-specific measurements in this case study. The group discussed the uncertainties associated with the measurements themselves, and concluded the need for measuring a large number of congeners in the TEF approach did not add appreciably to the overall uncertainty of the assessment. Assuming that appropriate analytical methods are used, the group thought that errors in these measurements would fall in the 5% to 30% range. The effect of these uncertainties might be substantial, however, if there was reason to question the analytical methods themselves.

Another point of discussion had to do with the potential for uncertainties related to detection limits for the individual congeners. In some situations, the detection limits of an analytical method might be well above levels of a congener that are of importance for risk assessment purposes. Because of this, risk assessors involved in a TEF/TEQ analysis must recognize the importance of achieving detection levels that correspond to the needs of the assessment process.

Dr. Menzie noted that the group talked a little bit about whether there are any sampling issues that are specific to the TEF/TEQ approach. Although they recognized sampling as an important element of the risk assessment process, group members did not think that sampling issues associated with the TEF/TEQ approach are any different than those associated with other methodologies.

Group members thought that the cost of the TEF approach would probably be greater than the cost of other methods, since the need for multiple-congener analysis translates to a higher price per sample. Some members predicted, however, that the cost of multi-congener analyses will decline as this methodology becomes more widely used.

The group also discussed how a risk assessor might use the TEF approach in dealing with a partial data set -- for example, one in which data were available only for PCBs. The group decided that in such a case it would be very valuable to analyze at least some samples for the full suite of congeners to get some sense of the relative importance of the different congener groups and to confirm that the compounds for which data are available are actually the congeners driving the assessment.

As a longer-term improvement to the methodology, the group felt that it might be useful to see if there is a reliable way of identifying, on a site-specific basis, a simpler measurement that could be used as a surrogate for TEQs. If so, this surrogate could be used to more cost-effectively monitor the effects of a remediation effort over time.

To address the quantitative aspects of the retrospective case study, Dr. Menzie's group used a process that was similar to those used in the other two groups, and they arrived at essentially the same conclusions. One caveat that the group thought it important to mention, however, is that there could be effects on endpoints other than reproduction that are not specifically being addressed in the risk assessment, particularly with regard to PCBs.

Regarding the issue of whether the TEF methodology was robust enough to support a regulatory decision, the group first agreed that the decision might involve a range of options rather than simply focusing on whether or not to dredge. In thinking about the

Dr. Menzie noted that the group talked a little bit about whether there are any sampling issues that are specific to the TEF/TEQ approach. Although they recognized sampling as an important element of the risk assessment process, group members did not think that sampling issues associated with the TEF/TEQ approach are any different than those associated with other methodologies.

Group members thought that the cost of the TEF approach would probably be greater than the cost of other methods, since the need for multiple-congener analysis translates to a higher price per sample. Some members predicted, however, that the cost of multi-congener analyses will decline as this methodology becomes more widely used.

The group also discussed how a risk assessor might use the TEF approach in dealing with a partial data set—for example, one in which data were available only for PCBs. The group decided that in such a case it would be very valuable to analyze at least some samples for the full suite of congeners to get some sense of the relative importance of the different congener groups and to confirm that the compounds for which data are available are actually the congeners driving the assessment.

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the spill. Given its borderline status, some members felt that a recommendation to simply monitor the system might be appropriate, while others thought that it would be preferable to formally model what the system was likely to look like in years to come.

Additional lines of evidence that the group thought might be brought to bear on the remediation decision include more extensive field observations of the current state of the population, with attention to whether effects predicted by the TEF/TEQ approach are actually occurring at the individual level. Similarly, they thought that it would be useful to obtain a more precise understanding of the distribution of contaminants within the sediments, so that remediation efforts can be directed where they are most needed.

A final point of discussion within the group had to do with the need for a top-down, population-level analysis of this system. In general, Dr. Menzie said, group members' sense of the urgency of this need tended to reflect their individual areas of expertise and familiarity with specific tools. Thus, toxicologists were more comfortable with the idea of collecting and working with toxicity data, while the population biologists were more comfortable with the use of specific metrics to describe what is going on in the system at a population level. During the course of this discussion, however, all members of the group agreed that it will be important to find ways of bringing together the lines of evidence that come from these different perspectives.

Following Dr. Menzie's summary of the group's deliberations, Dr. van den Berg noted that several groups had commented on the lack of transparency in the derivation of WHO consensus TEF values for mammals. He indicated that the authors of the WHO document had not realized that these values would be useful, and he said that specific references to the studies driving those TEF values would be added to the paper, at least for those TEFs that were changed by the Working Group. Adding this information for the TEFs that were adopted without modification may be difficult, since documentation as to how those values were derived is scant.

Regarding the issue of expressing the consensus TEFs as ranges rather than point estimates, Dr. van den Berg said that participants at the Stockholm meeting had decided

against this approach because many of the TEFs were derived from a variety of endpoints and so may have a range that covers several orders of magnitude. In the past, people have used this fact to wrongly claim that the TEF system doesn't work. If risk assessors wish to work with ranges instead of point estimates, Dr. van den Berg suggested that they go back to the studies from which the TEFs were derived, and develop their own TEF ranges from the ones that are most appropriate to the site they are assessing.

A member of the Planning Group noted that the 1994 Ahlborg paper does include histograms describing the studies used to derive mammalian TEFs, and that, contrary to popular belief, a large number of these values are based on *in vivo,* Tier 1-level data.

Another member of the Planning Group asked Dr. van den Berg to comment on the accessibility of the Karolinska database and on how the database would be maintained -- whether anyone had assumed responsibility for keeping it current and/or for assessing the quality of studies that are included. Dr. van den Berg said that it was his understanding that the database would be accessible to anyone who wanted to use it, and that the charge for access would be minimal. Regarding maintenance of the database, he noted that at the time of the Stockholm meeting the database was two or three months behind the calendar. Although he did not know whether the database has been similarly maintained since the meeting, he indicated that the issue of maintenance is currently being discussed. There are no plans to review the data from a quality control perspective, but informal guidelines have been established.

After this exchange, another member of the Planning Group commented on the Menzie group's discussion of detection limits as they relate to use of the TEF approach, noting that one way to address this problem is to be sure that the concentrations a lab provides are accompanied by information about the quantitative limits of the detection method.

One of the experts questioned the group's suggestion that a surrogate such as total PCBs might be useful for screening or monitoring purposes. He cautioned that this could be misleading, as it would be in the retrospective scenario, where dibenzofurans, despite

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the evaluation of point source discharges (within the framework of the Clean Water Act) and the evaluation of contaminated sites (within the framework of the Comprehensive Environmental Remediation and Compensation Liability Act). The applicability of the method is situation-specific. As with any method, appropriate caution should be exercised to avoid misuse or application of the methodology to situations where the underlying assumptions are known not to be valid. When applying the method, it should be recognized that there may be effects associated with the chemicals of concern that are unrelated to AhR and, therefore, may need to be evaluated under a separate methodology. These possibilities should be considered during the planning stage of an assessment.

2. The TEF/TEQ methodology reduces uncertainties associated with developing dose-response information for AhR agonists that exist with methods that rely on a single compound (e.g., TCDD) or on compounds evaluated as an aggregate (e.g., total PCBs). Specifically, because the method takes into account the possible effects of the suite of chemicals that act as AhR agonists, it is less likely to underestimate risks than are methods based on only one of these compounds (i.e., TCDD). Further, because total PCBs in the environment can be comprised of many compounds that vary in concentration and potency as AhR agonists, the TEF/TEQ methodology provides a means for accounting for these variables.

3. The uncertainties associated with using REPs or TEFs are not thought to be larger than other sources of uncertainty within the risk assessment process (e.g., dose-response assessment, exposure assessment, and risk characterization.) However, these uncertainties should be quantified better.

4. As is the case with any ecological risk assessment, the nature and magnitude of uncertainties should be identified and carried through the ecological risk assessment process (dose-response assessment, effects assessment and risk characterization). This could involve a number of different approaches, including qualitative analyses, assignment of ordinal rankings to sources of uncertainty, presentation of ranges, fuzzy arithmetic, and probabilistic analyses. Information on the sensitivity of the risk estimates to the uncertainties associated with the TEF approach (as well as other ERA components)

should be identified and quantified (if possible). This knowledge can be used to communicate the range of possible results to the decision maker and to identify what additional information would be the most useful for decisionmaking. Specific examples of approaches are provided in the summaries of the workshop breakout group sessions on the case studies (Appendix E).

5. Workshop participants supported the use of a hierarchical procedure for selecting REP or TEF values for use in risk assessment. In general, the most appropriate values are those that are closely related to the taxa and endpoints being evaluated. Workgroup participants agreed that uncertainties are introduced with increasing taxonomic and endpoint extrapolation. The workgroups suggested schemes for selecting REP and/or WHO TEF values, as well as schemes for considering how uncertainties associated with selecting values can be identified and tracked. These are identified in the workgroup summaries (Appendix E).

6. A database of REP and TEF values should be maintained in order to facilitate the application of the hierarchical procedure and to enable the conduct of sensitivity and uncertainty analyses. The appropriate regulatory agencies will need to consider how to insure the quality of the data in the database, document the values and the procedures used to derive them, make the database accessible, and provide guidance for its use.

7. The derivation of REP and WHO TEF values needs to be adequately documented (including specific citations) in order to support the use of these values in regulatory risk assessments. The WHO TEF document provided to workshop participants did not include documentation for the mammalian TEF values. This was viewed as a major limitation on the use of the document for risk assessment purposes.

8. The TEF/TEQ method requires analytical methods to identify and quantify the individual dioxin, furan, and PCB compounds. The accuracy and precision of available methods are considered acceptable for risk assessment purposes. The analytical measurement errors are not considered to be a large source of uncertainty within the assessment. A few of the workshop participants familiar with the analytical methods reported measurement errors in the range of 5 to 30%.

9. The costs for analyzing the suite of individual dioxin, furan, and PCB compounds are greater than those associated with analyzing an individual compound (e.g., TCDD) or for measuring "total PCBs." Workshop participants agreed that it may be possible to focus the analytical effort at different stages of the assessment, thereby reducing costs. For example, investigations may indicate that risks are due to a few of the compounds or to a particular class and these may form the basis for subsequent evaluation. Further, it may be possible to complement detailed analyses of individual compounds with simpler and cheaper analytical methods (e.g., to provide information on spatial extent of contamination).

10. Analytical detection levels for congeners need to be lower than concentrations at which important biological effects might occur. Workshop participants agreed that this can be achieved with available methods. As with any analytical program where data will be used in risk assessments, data quality objectives should be specified and care taken to insure that they are met.

11. Because physical, chemical, and biological properties vary among the individual dioxin, furan, and PCB compounds, exposure assessments that complement the TEF/TEQ methodology may require more information and resources (i.e., effort) than exposure assessments for an individual compound (e.g., TCDD) or a class of compounds (e.g., total PCBs). Fate and transport models used to support the exposure assessment will need to account for individual compounds through the various modeled components. In some cases, it may be possible to model groups of compounds with similar fate and transport properties.

12. Information on the environmental behavior of individual chemical congeners is needed to understand and use the congener-specific information in a modeling effort. With increasing use of a TEF/TEQ approach, gaps in knowledge on chemical-specific environmental behavior will become evident. Regulatory agencies will need to consider how best to acquire this information and/or develop exposure assessment tools that can complement the use of TEF/TEQ for specific regulatory applications.

13. Application of a TEF/TEQ method could be considered within the framework of a "lines of evidence" approach as described within the EPA's guidance for ecological risk assessment. As such, additional field and laboratory information could corroborate or improve the results of an assessment that is based, in part, on the application of the TEF/TEQ method. analysis. Use and integration of various lines of evidence in ecological risk assessment can often strengthen the analysis and provide a greater degree of confidence in the results than can be achieved from relying only on a single line of evidence. Each piece of information will have inherent strengths and limitations, and the amount of confidence placed on the information will also reflect the technical background of the individuals using the method and their experience with it.

14. Several workshop participants stressed the value of applying population-level assessment tools and obtaining population-level information in support of assessments (i.e., as a line of evidence). These included methods by which risks to individuals could be described in terms of potential risks to local populations. In addition, a few participants gave examples of tools that could be helpful for assessing whether population-level effects were being manifested (for retrospective assessments.) Examples included direct observations of hatching success, the condition of fledgling birds, and the age structure of populations.

15. Participants also discussed the use of bioassay tools to support the assessment. These methods could complement assessments that rely upon the TEF/TEQ approach. One participant summarized the strengths and limitations of these tools as follows. *In vitro* TEQ bioassays have the advantage of measuring the integrated effects of complex mixtures of Ah receptor agonists. In addition, such assays have the potential of identifying compounds that act via the Ah receptor which would not be identified by a chemical residue approach that measures only dioxins, furans and PCBs. *In vitro* bioassay-derived TEQ concentrations can be obtained at a lower cost than TEQ concentrations obtained by analysis of chemical residues. One potential problem with *in vitro* bioassays is that they can overestimate the toxic potency of compounds which are rapidly metabolized *in vivo* (e.g., PCB 77). However, recent research has shown that such problems can likely be circumvented. Various *in vitro* bioassays have considerable potential for predicting TEQs which are relevant to whole organisms.

16. Participants adopted the language given in the WHO document cautioning against the potential misapplication of the TEF/TEQ method to environmental media (e.g., sediments or soils). Specifically, the participants indicated that it is not appropriate to derive TEQs for these media. TEQs are relevant only with respect to specific ecological receptors. The methodology can be used to support decisions concerning the regulation of point source discharges and environmental clean ups that involve chemicals in environmental media. However, in these cases, the decision involves identifying concentrations of chemicals and/or the composition of mixtures that would yield acceptable TEQ with respect to specified ecological receptors.

Part 2: Conclusions Related to Charge Questions

I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. The WHO consensus TEF values are reported as point estimates and generally rounded off to the nearest order of magnitude. For the risk assessment case studies, additional background information used in the derivation of the TEF values is provided. Does this additional information enhance the means of evaluating uncertainties in the assessments? If so, how? If not, why?

Conclusion: Participants found this information useful. However, they indicated that additional information -- beyond that provided -- would be important for risk assessment purposes. This additional information includes better documentation of the process used to derive TEF values, references for the values employed for mammalian receptors, and access to the database.

2. Some TEFs were determined from several studies, endpoints, and exposure routes, while other TEFs were based on a single study and endpoint. Given the range of knowledge associated with specific compounds, should all TEFs be considered to have similar uncertainties? Why? Or why not?

Conclusion: All TEFs should not be considered to have similar uncertainties. Participants discussed several derivation and extrapolation issues that affect the uncertainty associated with using TEF values. They also provided an example of how these uncertainties might be tracked.

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III. EXPOSURE PROFILE

1. To what extent does the TEF approach present challenges, introduce new uncertainties, or modify old uncertainties associated with modeling the exposure of AhR agonists? To what extent does the availability and quality of congener-specific physico-chemical data limit the means of employing fate and transport or food chain models?

Conclusion: The approach will likely require additional resources to model exposure because a larger number of chemicals will need to be taken into account. Because these chemicals vary in their properties, information is needed on various physicochemical properties in order to support modeling efforts.

2. The route of administered or absorbed dose used to derive TEFs may differ from those needed to establish exposure profiles in a risk assessment. To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?

Conclusion: This was not discussed at length.

3. To what extent does the TEF approach require a more rigorous analytical design in quantifying sediments, soil, and biota AhR agonist concentrations than is apparent in other methods which aggregate stressors (e.g., total PCBs)?

Conclusion: Sampling design issues were judged to be comparable. However, as discussed in the main conclusions, there will be additional analytical costs and care must be taken to specify and meet data quality objectives.

IV. RISK CHARACTERIZATION

1. In evaluating the case studies, are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments? Do the uncertainties associated with TEFs limit the means of performing the assessments, or do the other areas of the effect and exposure characterization contribute similar or greater levels of uncertainty?

Conclusion: These uncertainties are not more problematic than other uncertainties of the risk assessment. They do not limit the means of performing assessments. However, use of the method places demands on analytical methods and on modeling of exposure.

2. Biologically-based TEQ assays on environmental samples could be employed as an alternative to the TEF-based approach. What would the strengths and weaknesses of such an approach be? To what extent could these approaches be integrated?

Conclusion: These assays should not be used as an alternative to the TEF/TEQ approach. However, they could be used to complement the analyses. They could also be used as a screening tool. These assays were thought to be most useful in retrospective assessments. There was not an agreement on how they would be used in a prospective (i.e., predictive) assessment.

Appendix A

WORKSHOP PARTICIPANTS

United States
Environmental Protection Agency
Risk Assessment Forum

Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and Wildlife

Chicago Hilton & Towers
Chicago, IL
January 20-22, 1998

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United States
Environmental Protection Agency
Risk Assessment Forum

Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and Wildlife

Chicago Hilton & Towers
Chicago, IL
January 20-22, 1998

Agenda

TUESDAY, JANUARY 20, 1998

3:00PM	**Registration**	
4:00PM	**Welcome**	*Dr. Chris Boiven, Risk Assessment Forum, U.S. Environmental Protection Agency (U.S. EPA), Washington, DC* *Mr. John Blankenship, Assistant Regional Director, U.S. Fish and Wildlife Service (FWS), Fort Snelling, MN*
4:10PM	**Scope and Charge for the Workshop**	*Dr. Charles Menzie, Workshop Chair, Menzie-Cura & Associates, Inc., Chelmsford, MA*
4:30PM	**Synopsis of the World Health Organization Workshop Held in Stockholm**	*Dr. Martin van den Berg, University of Utrecht, Utrecht, The Netherlands*
5:00PM	**Presentation of Prospective Case Study and Discussion**	*Dr. Steve Bradburg, U.S. EPA, Denver, CO*
5:30PM	**Presentation of Retrospective Case Study and Discussion**	*Dr. Donald Tillitt, U.S. Geological Survey, Columbia, MO*
6:00PM	BREAK	

Appendix B

WORKSHOP AGENDA

TUESDAY, JANUARY 20, 1998 (continued)

6:15PM **Review Structure of Workshop and Goals and Objectives of Breakout Groups** ..... *Dr. Charles Menzie*

6:45PM **Observer Comments**

8:00PM ADJOURN

WEDNESDAY, JANUARY 21, 1998

8:30AM **Expertise Group Sessions:**

Toxicity Equivalency Factors (TEFs) Experts ..... *Dr. Richard Peterson, Facilitator*
University of Wisconsin,
Madison, WI

Fate & Transport and Bioaccumulation Experts ..... *Dr. William Adams, Facilitator*
Kennecott Utah Copper Corporation,
Magna, UT

Risk Assessors and Population Modelers ..... *Dr. Charles Menzie, Facilitator*

10:30AM BREAK

10:45AM **Breakout Group Session I: Apply TEFs to Case Study 1**

Group 1 ..... *Dr. Peter deFur, Chair*
Environmental Stewardship Concepts,
Richmond, VA

Group 2 ..... *Ms. Janet Burris, Chair*
McLaren Hart/ChemRisk,
Oak Ridge, TN

Group 3 ..... *Dr. Charles Menzie, Chair*

LUNCH *(at discretion of individual groups)*

3:45PM BREAK

4:00PM **Plenary Session**
Breakout groups report on Case Study 1 and discuss commonalities and differences among their groups

5:30PM DINNER BREAK

8:00PM **Plenary Session**
Complete reports on Case Study 1 and continue plenary group discussion

9:00PM ADJOURN

THURSDAY, JANUARY 22, 1998

8:30AM **Breakout Group Session II: Apply TEFs to Case Study 2**
Same breakout groups as Wednesday

BREAK (*at discretion of individual groups*)

12:30PM LUNCH

1:30PM **Plenary Session**
Breakout groups report on Case Study 2 and discuss commonalities and differences among their groups

3:00PM BREAK

3:15PM **Overall Meeting Conclusions and Wrap-Up** ..... *Dr. Charles Menzie*

5:00PM ADJOURN

Note to Observers: We are aware that many of you did not have the opportunity to review the materials prior to the workshop. We encourage you to submit written comments to the workshop and discussion group chairs, so that your comments can be considered during the writing of the workshop summary report.

Appendix C

PREMEETING COMMENTS

Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and Wildlife

Premeeting Comments

Chicago, Illinois
January 20-22, 1998

Prepared and compiled by:
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CHARGE QUESTIONS AND PHYSICO-CHEMICAL PROPERTIES TABLE

It is reasonable to assume that the proposed WHO TEFs are appropriate for risk assessments associated with permitting discharges, attributing causality to specific compounds, and establishing remediation goals for AhR agonists. These risk assessment situations are the primary focus of the workshop. The major issue to be addressed in the workshop is the extent to which a TEF/TEQ approach can be used in risk assessments that have progressed beyond the screening stage.

The primary objective of the workshop is to identify, document, and compare uncertainties (lack of knowledge and variability) in TEF development and their impact in ecological risk assessments. To achieve this goal, two case studies that represent hypothetical situations for prospective and retrospective risk assessments have been prepared. For each case study, a series of questions and issues are raised that will help focus the panels' deliberations. The majority of issues/questions raised are directed towards effect characterization topics. However, it is recognized that assessing the exposure of PCDD, PCDF, and PCB mixtures is also a significant challenge for implementation of a TEF/TEQ approach in a risk assessment. Therefore, issues and questions concerning exposure characterizations are also provided to highlight important concepts that can not be excluded from the risk assessment process.

SPECIFIC QUESTIONS/ISSUES:

The major objective of the workshop is to address uncertainties associated with using a TEF/TEQ approach in effects characterizations for ecological risk assessments. These uncertainties need to be identified, documented, and to the extent possible, quantified. For example, there are gaps in the TEF knowledge base for mammalian wildlife, avian wildlife, and aquatic life in terms of interspecies, exposure route, and endpoint extrapolations. A challenge to the participants of this workshop is to evaluate the relative contribution of TEF-related uncertainties in relation to other effect characterization uncertainties found within an ecological risk assessment (e.g., uncertainties in identifying 2,3,7,8-TCDD dose levels of concern; extrapolating effects from the individual to the population). To place the effect characterization uncertainties associated with the use of TEFs in perspective, TEF analyses in the case studies can, for example, be compared to analyses based on total PCBs or 2,3,7,8-TCDD alone. Application of a TEF approach to an ecological risk assessment also requires additional information for parameters in the exposure characterization for the mixture. A critical need is the documentation of additional data requirements for use of a TEF approach (e.g., K_{ow}s, K_ds, BAFs, BMFs, BSAFs, biotic and abiotic degradation rates, etc.). The extent to which these exposure issues can contribute to risk assessment uncertainties needs to be estimated.

The following questions are generally organized around components of the draft U.S. EPA Ecological Risk Assessment Guidelines (U.S. EPA, 1997). It is understood that not everyone will answer every question. Please prepare responses to the questions appropriate to your area of expertise.

I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. The WHO consensus TEF values are reported as point estimates and generally rounded off to the nearest order of magnitude. For the risk assessment case studies, additional background information used in the derivation of the TEF values is provided. Does this additional information enhance the means of evaluating uncertainties in the assessments? If so, how? If not, why?

2. Some TEFs were determined from several studies, endpoints, and exposure routes, while other TEFs were based on a single study and endpoint. Given the range of knowledge associated with specific compounds, should all TEFs be considered to have similar uncertainties? Why? Or why not?

3. The TEF values provided were based on endpoints that ranged from *in vitro* biochemical responses (e.g., induction of cyp1A1) to in vivo early life stage mortality. To what extent can these endpoints be extrapolated to the measures of effects that are relevant for the assessment endpoint for each case study?

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. What are the implications, both quantitatively and conceptually, of assuming no dose-additivity or no interaction among the components of the mixtures described in the case studies? To what extent would the risk assessment conclusions differ if stressor response analyses where based on total PCBs or 2,3,7,8-TCDD alone?

2. Many TEFs are based on LC50 or EC50 values. To what extent should TEF values derived at a median response level be used in risk assessments where a no adverse effect level is being employed?

3. The TEFs values provided were typically based on a single or limited number of mammal, bird, or fish experiments. To what extent can class-specific TEFs be directly extrapolated to the species identified within each case study?

III. EXPOSURE PROFILE

1. To what extent does the TEF approach present challenges, introduce new uncertainties, or modify old uncertainties associated with modeling the exposure of AhR agonists? To what extent does the availability and quality of congener-specific physico-chemical data limit the means of employing fate and transport or food chain models?

2. The route of administered or absorbed dose used to derive TEFs may differ from those needed to establish exposure profiles in a risk assessment. To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?

3. To what extent does the TEF approach require a more rigorous analytical design in quantifying sediments, soil, and biota AhR agonist concentrations than is apparent in other methods which aggregate stressors (e.g., total PCBs)?

IV. RISK CHARACTERIZATION

1. In evaluating the case studies, are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments? Do the uncertainties associated with TEFs limit the means of performing the assessments, or do the other areas of the effect and exposure characterization contribute similar or greater levels of uncertainty?

2. Biologically-based TEQ assays on environmental samples could be employed as an alternative to the TEF-based approach. What would the strengths and weaknesses of such an approach be? To what extent could these approaches be integrated?

3. Assume that site-specific data or additional research could be gathered or performed to generate more information for the case study assessments. Provide a list of specific investigations/studies and rank them from highest to lowest priority. What is your rationale for the ranking?

Additional Questions Specific to the Prospective Case Study:

RELATIVE TO THE EXPOSURE PROFILE:

1. The state adopted BAF_w^{fd}s used by the GLWQG. What improvement in the accuracy of maximum allowable concentrations for individual congeners in water, $(MAC_w^t)_{i,j}$, can be expected through use of BAF_w^{fd}s determined from Roundtail Lake data?

2. What errors are associated with the state's application of the GLWQG TCDD water quality standards for birds and mammals without consideration of congener-specific differences in biomagnification factors from fish to tissues in wildlife relevant to the effects of concern?

RELATIVE TO THE RISK CHARACTERIZATION:

3. How should the uncertainties associated with the available fish, avian, and mammalian TEFs be incorporated into decisions about which TCDD water quality standard should be chosen for setting a TEqTMDL for regulating chemical discharges into Roundtail Lake?

Additional Questions Relative to the Retrospective Case Study:

RELATIVE TO THE RISK CHARACTERIZATION:

1. Would TEQ sediment cleanup goals be the same for each vertebrate group? If not, why would there be a difference? If the vertebrate group with the most certainty is not the group with the most restrictive sediment cleanup goal, how would you council the risk manager's concerns for the other vertebrate groups?

2. Would the TEF/TEQ-based sediment remediation goals be the same as those determined for total PCBs for the identical vertebrate class? Assume that a simple ratio of total PCB sediment concentration goal to TEQ sediment concentration goals was formulated to allow for the use of total PCBs to monitor cleanup efforts based on TEQs. What exposure and effect issues would need to be evaluated before using the less costly total PCB analysis to support the TEQ-based sediment remediation goal?

Table 1
Parameters for PCBs, PCDDs, PCDFs

	log K_{ow}			Henry's Law	Lake Trout BSAF		BAF^{fd}_t	BMF		
	(EPA GLI)[1]	(Eisler & Belisle, 1996)[2]	(MacKay, Shiu & Ma, 1992)[3]	Constant[4]	(Oliver & Niimi)[1] (g OC/g lip)	(EPA GLI)[1] (g OC/g lip)	(EPA GLI)[5] (L/kg)	BMFbe,w[6] (g fish/g egg)	BMF be, l[7] (g lip/ g lip)	BMFdl,l[8] (g lip/ g lip)
Total PCB					1.85	NA	1.17E+08	32	12	2.9
PCB-1248	NA	NA	5.8 - 6.3	NA	NA	NA	NA	NA	NA	NA
PCB-1254	NA	NA	6.1 - 6.8	NA	NA	NA	NA	NA	NA	NA
PCB-1260	NA	NA	6.3 - 7.5	NA	NA	NA	NA	NA	NA	NA
PCBs										
77	6.36	6.52	6.5	1.72	NA	0.29	9.68E+06	1.8	0.7	0.15
81	6.36	6.37	NA	NA	NA	0.67	2.24E+07	NA	NA	1.0
105	6.65	6.66	6.0	NA	2.70	4.49	2.18E+08	20	7.3	5.0
114	NA	6.66	NA	NA	NA	NA	NA	NA	NA	4.0
118	6.74	7.12	NA	NA	4.09	1.72	2.04E+08	31	11	4.3
123	NA	6.75	NA	NA	NA	NA	NA	NA	NA	0.8
126	6.89	6.90	NA	NA	NA	3.21	3.63E+08	29	11	12.6
153	6.92	7.75	6.9	42.9	4.22	1.91	3.31E+08	48	17	NA
156	7.18	7.19	NA	NA	3.97	NA	8.12E+08	NA	NA	8.6
157	NA	7.19	NA	NA	NA	NA	NA	NA	NA	11.0
167	7.27	7.28	NA	NA	NA	0.69	1.87E+08	NA	NA	5.7
169	NA	7.43	NA	NA	NA	NA	NA	46	17	13.6
189	7.71	7.72	NA	NA	NA	0.71	5.30E+08	NA	NA	9.1
PCDD										
2378-TCDD	7.02	NA	6.8	3.331	NA	0.059	9.00E+06	20.75	7.5	11.0
12378-PCDD	7.50	NA	NA	NA	NA	0.054	2.49E+07	9.7	3.5	6.3
12478-PCDD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
123478-HxCDD	7.80	NA	NA	1.084	NA	0.018	1.65E+07	NA	NA	9.3*
123678-HxCDD	7.80	NA	NA	NA	NA	0.0073	6.71E+05	16	5.8	33.5
123789-HxCDD	7.80	NA	NA	NA	NA	0.0081	7.44E+05	NA	NA	15.5*
1234678-HpCDD	8.20	NA	8.0	1.273	NA	0.0031	7.16E+06	NA	NA	45.2
OCDD	8.60	NA	8.2	0.684	NA	0.00074	4.29E+06	NA	NA	62.3
PCDF										
2378-TCDF	6.50	NA	6.1	1.461	NA	0.047	2.16E+06	NA	NA	0.4*
12378-PCDF	7.00	NA	NA	NA	NA	0.013	1.89E+06	NA	NA	NA
23478-PCDF	7.00	NA	6.5	NA	NA	0.095	1.38E+08	4.45	1.6	54.1
123478-HxCDF	7.50	NA	7.0	1.454	NA	0.0045	2.07E+06	NA	NA	64.4
123678-HxCDF	7.50	NA	NA	0.741	NA	0.011	5.07E+06	NA	NA	NA
123679-HxCDF	NA	NA	NA	NA	NA	NA	NA	NA	NA	54.9
123789-HxCDF	7.50	NA	NA	NA	NA	0.037	1.70E+07	NA	NA	NA
234678-HxCDF	7.50	NA	NA	NA	NA	0.04	1.84E+07	NA	NA	75.8
1234678-HpCDF	8.00	NA	7.4	1.425	NA	0.00065	9.47E+05	NA	NA	27.5
1234789-HpCDF	8.00	NA	NA	NA	NA	0.023	3.35E+07	NA	NA	NA
OCDF	8.8	NA	8.0	0.191	NA	0.00099	9.10E+06	NA	NA	43.3*

References:

1. US EPA, 1995 (EPA-820-B-95-005).
2. Eisler, R., and A.A. Belisle. 1996. Planar PCB Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. National Biological Service Biological Report 31. 75 pp.
3. Mackay, Shiu & Ma. 1992. *Illustrated Handbook of Physical and Chemical Properties for Organic Chemicals.* Boca Raton, FL: Lewis Publishers.
4 Values from Eisler & Belisle (1996) or MacKay, Shiu & Ma (1992).
5. Mean BAF^{fd}_t for salmonids from Table 10 of US EPA (1995), with the exception of total PCBs which are from Appendix F.
6. BMFbe,w is the BMF from forage fish to bird eggs on a wet weight basis from Braune, B.M., and R.J. Norstrom. 1989. Dynamics of Organochlorine Compounds in Herring Gulls: III. Tissue Distribution and Bioaccumulation in Lake Ontario Gulls. Environ. Toxicol. Chem. 8:957-968.
   BMFs for PCB congeners 77, 126, and 169 are from the same samples, but reported in Hoffman et al.(1996).
7. BMF be,l is the BMF from forage fish to bird eggs on a lipid basis calculated from the % lipid in the fish and bird eggs from Braune and Norstrom (1989).
   BMFs for PCB congeners 77,126, and 169 are from the same samples but reported in Hoffman et al., (1996).
8. BMFdl,l is the BMF from diet to mink liver on a lipid basis from Tillitt et al. (1996). The BMFs were normalized to feed consumption which differed among treatment groups. The BMFs in this column are the means, among treatment groups, of the BMFs for which both values (diet and liver concentrations) were above the limit of quantitation unless noted by an *.

NA = Not available
BMF = Biomagnification factor
BAF = Bioaccumulation factor
EPA = Environmental Protection Agency
K_{ow} = Octanol water partition coeffecient
GLI = Great Lakes Water Quality Initiative
OC = Organic carbon
lip = lipid
g = grams

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I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. It was not clear to me that the additional information provided reduced the uncertainty associated with the TEFs. Perhaps this will become clearer at the meeting. The rounding of the TEFs to the nearest order of magnitude introduces uncertainty in the final calculation of risk and it reflects the uncertainty associated with the individual values. Additional discussion on how this uncertainty should be dealt with in a risk assessment context is needed at the workshop.

2. Intuitively, I would say that all TEFs should not be considered to have similar uncertainties. This is based on both weight of evidence and lines of evidence for those chemicals which have been studied the most. However, it is fair to ask the question, can we quantify the uncertainty through rigorous statistical assessment of the available data on TEFs? The question posed is somewhat similar to asking the question, would a single acute toxicity test with *Daphnia magna* have the same uncertainty in deriving a water quality criterion as a genus mean acute value based on the average of several *Daphnia magna* studies as well as several other daphnid species. The answer is, of course, that we would have less uncertainty with a genus mean acute value than with a single acute toxicity test.

3. This question gets to the heart of the entire risk assessment approach for TCDD and other HOHs and deserves in depth review at the workshop. The TEF approach is one that has found favor because it provides a way forward for numerous chemicals with a similar mode of action. The complexity of assessing all PCB, Furan and Dioxin isomers is monumental and is somewhat simplified by this approach. However, care has to be taken in the use of the "model" results as measurement endpoints for the purpose of evaluating key assessment endpoints (i.e., the valued resource) in risk assessments. The data seem to indicate that the use of *in vitro* measurements and QSARs introduce additional uncertainty into the measures of effects that are ultimately used to estimate risk. A measurement of selenium in the egg of a black-necked stilt, for example, provides a reasonable

estimate of the potential for reproductive effects at the individual level. A measurement of selenium in the diet of the birds can be used to estimate egg concentrations and reproductive effects, but the uncertainty becomes greater. Measuring the selenium in the sediments where the dietary species lives as an indicator of potential for reproductive effects introduces even more error. The same analogy applies here. As a general rule, the further away you get from the a direct measure of the assessment endpoint the uncertainty becomes greater. I would add, that this does not necessarily imply that as the uncertainty increase there is a need for use of additional safety factors. The inappropriate use of safety factors has been shown to increase the conservatism and decreases the accuracy of the risk estimate. Ultimately, what has to be answered is, can TEFs be used to accurately predict population effects in aquatic ecosystems? This can only be answered by the careful use of both laboratory and field data.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ

1. When assessing chemicals with the same mode of action and the same receptor, the literature strongly supports the use of an additive model. According to Konemann, when there is no interaction between the chemicals they can be assumed to be additive. If you do not consider dioxin isomer effects to be additive then one must assume they are either antagonistic or synergistic or unpredictable. The consequence is that you have to assess each isomer independently and determine its potential to cause effects (calculate separate hazard quotients). Adding individual hazard quotients to assess the overall potential for risk has serious limitations. The use of total PCBs, which has limitations unique to itself due to the environmental degradation of the constituents, or the use of just 2,3,7,8-TCDD provides a single point estimate of the potential for risk, but does not consider the cumulative potential for risk from similar compounds co-located in the environment. The fundamental basis for using an additive model exists, what hasn't been determined accurately is when does it over predict the potential for effects? The potential for antagonism appears to be somewhat greater than for synergism.

2. The use of TEFs based on central tendency values such as LC50 or EC50 values can be justified even though most in-depth assessment typically use chronic no-effect concentrations or threshold values. The selection of a very sensitive EC50 value can in many cases be more sensitive than some chronic threshold values. Risk assessments with atrazine, diazinon, copper and cadmium have shown where there were lots of acute and chronic data that the water concentrations selected as protective of aquatic species (95%) using sensitive acute endpoints were nearly the same as the values selected using chronic no-effect levels.

3. My response to this question comes not from extensive experience with TEFs, but with having performed risk assessments where laboratory to field relationships have been examined. As a general rule, when one has to extrapolate within a class the best approach is to use the same value for the species of interest as obtained from the toxicity test. The use of safety factors in this situation provides protection, but sacrifices accuracy and predictability.

III. EXPOSURE PROFILE

1. I don't see the use of the TEF approach introducing significant new uncertainties into the exposure assessment. However, in regards to the second part of this question, the lack of congener-specific physico-chemical data provides considerable issues relative to accurately modeling or predicting the fate and transport of these materials both within the physical environment and the biota. Transport estimates within the food chain can be developed without these data if one chooses to rely only upon sediment to biota and biota to biota accumulation factors.

2. The route of administration is always important in an overall risk assessment. The key is to match the route of "exposure" in the effects characterization with that which actually occurs in natural systems. TEFs derived from *in vitro* biochemical measurements will have greater uncertainty associated with them because the

potential for metabolism to occur in the body is removed. This ultimately translates to an increase in the uncertainty associated with the final risk estimate.

3. Interesting question. Is more error introduced via the analytical techniques used when multiple chemicals are measured and quantified than when a class of chemical are measured as a group. I would think so. I'll leave this question to the chemists.

IV. RISK CHARACTERIZATION

1. Regarding uncertainty introduced into the risk assessment by the use of TEFs, I don't think the use of TEFs necessarily introduces additional uncertainty into the risk assessments that other approaches would not. However, not all TEFs are equal (i.e., some are based on QSARs, *in vitro* biochemical measures, *in vivo* effects measurements etc.), therefore, depending on what is actually used as the set of TEFs for the risk assessment may have more or less uncertainty. Clearly, extrapolating across species and perhaps classes introduces uncertainty. Additionally, estimating exposure from sediment using BSAFs has considerable uncertainty when one considers all the compounds of interest. So, does the use of TEFs introduce more uncertainty than already exists? Who knows? The question which should be asked is, can we measure and quantify the uncertainty in each part of the risk assessment? If so, this would be a useful research endeavor.

2. The use of residue based approaches for deriving water quality criteria and performing risk assessments is gaining favor for both organics and metals. I favor the approach and believe the uncertainty associated with the risk estimate would be reduced. This approach can be applied in several ways including evaluation of TEQs within a given tissue (liver) for a valued species (otters) or alternatively by comparing the TEQs in the diet of a given species against a known dietary effect concentration. I prefer the latter because it gets directly at the issue of exposure for HOHs.

The process of risk characterization typically looks at several lines of evidence to help assess the uncertainty, therefore I don't see the various approaches as mutually exclusive. Why not perform the assessment using both TEFs and TEQs for comparative purposes (cost aside)? Especially if you are locating a new industry as proposed in the prospective case study.

3. A. Gather additional data on the species or resource to be protected. I would not assume, for example, that Bull trout are as sensitive as lake trout and then divide by a factor of 10 to account for species to species extrapolation. Perform the necessary early life stage test or egg exposure study to obtain the information. At each stage of the assessment I would gather as much site-specific data as possible on the species of interest. This will reduce uncertainty.

   B. Collect additional field data at the population level at each of the sites used in the risk assessment cases. Risk assessments performed at the species level and extrapolated to the population or community level tend to be overly conservative. They typically assume constant dietary and water exposure, for example, and this is rarely true. Individual level assessments rarely consider the behavioral aspects of populations, e.g. migration, feeding behavior, habitat selection, etc. all of which effect the exposure regime. Additional on-site evaluation of the populations of interest (in the retrospective case) will provide additional information on whether or not actual effects occurring at the site.

Additional Questions Specific to the Prospective Case:

RELATIVE TO THE EXPOSURE PROFILE

1. No comment at this time.

2. No comment at this time.

RELATIVE TO THE RISK CHARACTERIZATION

3. Relative to the question as to how uncertainty should be handled in setting water quality standards - this is an area where the state-of-the-science is improving. Probabilistic techniques are emerging using Bayesian theory and Monte Carlo calculations to account for uncertainty and to predict a range of values that might be protective. The advantage of this approach is that it also provides an estimate of the confidence along the range of values identified such that one can select a value with a given level of confidence (say 90%). The approach can be used to include site-specific parameters and can be used at the population level if sufficient data are available. We recently completed such an approach for selenium to assess levels in water that are protective of bird egg concentrations to prevent teratogenic effects.

Additional Questions Specific to the Retrospective Case:

RELATIVE TO THE RISK CHARACTERIZATION

1. I would think that the TEQ sediment cleanup goals would not be the same for each vertebrate group. There are differences in sensitivity of different vertebrate species to TCDD and similar compounds (consider the variability that exists just for trout species to TCDD) and this should be evaluated and discussed as part of the effects characterization.

   Providing the risk manager with an assessment of the uncertainty associated with each of the risk estimates in the overall risk assessment is the job of the risk assessor. Hence the statements identifying the uncertainty with a given risk estimate become very important. A decision, in fact, could be made by the risk manager to set a level of protection based on a less sensitive species when the data are well characterized as opposed to using a more sensitive species, but with an uncertainty level so large that the confidence in the estimate is very low. Risk management is not a quantitative science and often involves personal judgement

and personal/societal values. The risk assessor must provide sufficient information so the manager can make an informed decision. In short, if you do not have much confidence in your risk estimate the selection of a level of protection is very difficult and often tends to be on the conservative side.

2. Relative to the second part of this question - what exposure and effects issues would have to be evaluated before using the less costly PCB analysis as an alternative to TEQ-based sediment remediation goal- I suggest the following might be important:

   1. Exposure - the quantitation of the total PCBs has to be matched to the congeners used to perform the TEF/TEQ assessment other wise there will not be a match between the exposure and effects estimates.

   2. There needs to be *in vivo* laboratory evaluation between effects observed using the TEF/TEQ approach and that obtained using the total PCB approach. This approach tests the additivity model, reproducibility and provides the first level of field verification.

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Björn Brunström

Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors (TEFs) to Fish and Wildlife

Premeeting comments

I.1. When carrying out a risk assessment based on a TEF/TEQ approach, it is important to be aware of the limitations of such an approach and the uncertainties associated with the fish and wildlife TEFs for a certain congener. The uncertainties in the TEF values add to the other uncertainties in the assessment and the background information used in the derivation of the TEFs is important in the evaluation of these uncertainties. The extent of the TEF value uncertainties in relation to the other assessment uncertainties partly depends on which congeners that are of concern.

The TEF values provided are order of magnitude estimates based on the presently available information and future research data will result in revaluation of these values. TEF values for certain compounds have been estimated from a single study and relative potencies have generally been determined only in a few species. However, it should be remembered that a conservative approach was used when deriving the TEF values. The currently available data used as a basis for TEF development and the major uncertainties in this development are discussed in the recent WHO report on proposed TEFs for mammals, birds, and fish.

The very large interspecific differences in sensitivity to Ah receptor agonists that exist within animal classes contribute significantly to the total uncertainty in a risk assessment. The WHO document only deals with the relative potencies of various Ah receptor agonists and LOAEL and NOAEL values for different species are not discussed. Other background documents give information about LOAEL and NOAEL values and the uncertainties in these values for mammalian and avian wildlife.

I.2. Since a tiered approach was used when setting the TEFs, it is obvious that some TEFs should be considered more uncertain than others. Some of the TEF values were estimated by

using a QSAR model based on enzyme induction data and these values of course are less reliable than those based on data from a carefully conducted reproduction study. Uncertainties appear to be largest for the least potent compounds since their TEFs are frequently based on biochemical effects observed in *in vitro* systems or on estimates from QSAR studies. Values also tend to be more uncertain for easily metabolized compounds, such as PCB 77, since these compounds show different relative potencies in acute and subchronic studies.

Concerning the exposure routes, it should be kept in mind that mammalian TEFs are mainly based on studies where the compounds were administered via the food and the effects were related to concentrations in the diet. In contrast, the fish and bird TEFs are based on egg injection studies in which the effects were related to egg concentrations.

I.3. Any Ah receptor-mediated response may, principally, be used when determining relative potency values. The rationale for using a tiered approach when developing TEF values is nevertheless that certain endpoints are considered more useful than others. It should also be kept in mind that metabolism is largely overlooked in *in vitro* assays and in acute studies. Also, the shapes of dose-response curves in enzyme assays may differ between congeners which leads to difficulties in the interpretation.

The most relevant compounds in the case studies all were designated fish TEF values that are based on early life stage mortality in rainbow trout. For protection of bull trout and lake trout these values should be relevant. Several of the bird TEFs are based on EROD induction studies in chicken embryos. For these values there are uncertainties associated with the interpretation of differently shaped dose-response curves and also with the extrapolation from the chicken to the bald eagle and the Caspian tern.

II.1. When 2,3,7,8-TCDD is not the major contributor to total TEQs, a response analysis based on TCDD alone would significantly underestimate the impact of the chemical stressors

present. In the prospective study, TCDD is one of the major compounds of concern and an assessment based on a TEF/TEQ approach would only decrease the permitted TCDD toxicity equivalent load from the effluent a few times.

The impact of a PCB mixture depends on the relative concentrations of the congeners in the mixture. Only if the relative concentrations of different congeners were determined in some samples, and could be predicted to be similar across the lake, would total PCB determinations be sufficient. The relative concentration of PCB 126 seems to be crucial in the retrospective case study.

II.2. The problem with using LC50 or EC50 values for determination of relative potencies is that the shapes of the dose-response curves may differ for different congeners. This primarily seems to be a problem involving the least active congeners.

II.3. Only few comparative studies addressing the relative potencies of various Ah receptor agonists across species have been carried out. Whether class-specific TEFs are valid for different wildlife species is a matter of concern. Most data suggest similarities but some studies indicate that there may be relative potency differences across species within an animal class. For the species identified in the case studies, the use of the new class-specific TEFs should give better estimations than the .old. TEFs but extrapolations between species, e.g., from the chicken to the bald eagle and the Caspian tern, are rather uncertain. However, the contribution from interspecific differences in sensitivity to TCDD to total uncertainties may be as large or larger as the contribution from differences in relative potencies across species.

III.2. The mammalian TEFs are mainly based on food intake whereas fish and bird TEFs are based on egg concentrations. When the models used predict levels in eggs of fish and birds there is no contradiction. Uncertainties are introduced when models describe the relationship

between the concentrations in sediment and those in avian diet or mammalian tissue. For instance, the high metabolic transformation of PCB 77 is accounted for in the TEF value for mammals and this means that the contribution by this compound to total TEQs will be underestimated if its TEF value is applied for a tissue concentration. However, the concentration of PCB 77 appears to be low in the retrospective case study.

IV.1. I think that uncertainties other than those associated with the TEF values contribute to a similar or even greater level to the total uncertainty. Major problems are uncertainties in the sensitivities of the wildlife species to TCDD and uncertainties in exposure characterization. Also, it should be remembered that the TEF values assigned are conservative estimates.

IV.2. In biologically-based TEQ assays, the total effects of Ah receptor agonists and antagonists in a sample are measured. Preparing extracts from fish eggs and bird eggs and injecting these extracts into eggs of laboratory species gives an opportunity to study chemical interactions and relevant end-points.

In certain *in vitro* systems, the relevant species may be studied. Disadvantages with using *in vitro* systems include that they do not accurately model all the interactions that occur *in vivo*, and that the biochemical end-points usually measured are more or less connected to adverse effects.

By combining bioassays with chemical analysis and a TEF approach, the contribution from the analyzed congeners and from non-analyzed compounds to total effects can be estimated.

IV.3. The highest priority should be given to clarifying the extent of species differences in sensitivity to TCDD for the relevant species and the basis for such differences. Are any piscivorous bird species as sensitive as the gallinaceous birds? Sensitivities are difficult to

determine for relevant species but the use of *in vitro* assays and receptor studies may give some information about those species not available for *in vivo* studies. Second, studies of the relative potencies of the congeners of concern in terms of various end-points should be carried out in relevant species. For instance, the relative potency of 1,2,3,4,7,8-HxCDF in bull trout should be examined since the mill effluent was predicted to contain high concentrations of this compound.

Both the relative potency value of 2,3,7,8-TCDF in bull trout and the BAF of this congener in Roundtail lake would be important information for the prospective case study assessment.

Additional Questions Specific to the Prospective Case Study:

2. The tentative water quality standards (WQSs) for TCDD can not be used as WQSs for TCDD equivalents. 2,3,7,8-TCDF would be the major contributor to the water TEQ concentration when using the TEFs for mammals or birds without consideration of the low biomagnification factor for this congener.

3. An uncertainty factor including uncertainties in the BAFs, BMFs, and TEFs for the different congeners should be considered.

Additional Questions Relative to the Retrospective Case Study:

1/2. The relatively low potency of PCB 126 in fish (TEF value of 0.005) means that the Caspian tern and the otter are more likely to be affected than the lake trout. A PCB sediment concentration goal to protect the Caspian tern and the otter should be related to PCB 126 as the major contributor to total TEQs. If the concentration of PCB 126 in relation to total PCB concentrations would be similar in sediments across the lake, then cleanup efforts may be monitored by total PCB analysis.

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I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

Some TEFs were determined from several studies, endpoints, and exposure routes, while other TEFs were based on a single study and endpoint. Given the range of knowledge associated with specific compounds, should all TEFs be considered to have similar uncertainties? Why? Or why not?

Toxicity Equivalency Factors (TEFs) for mammals, fish and birds should not be considered to have similar uncertainties. There is greater uncertainty in the derivation of some TEFs versus others and these uncertainties should be understood in the application of the TEFs as part of an ecological risk assessment (ERA). The uncertainties are expressed in part in the tiered approach used to derive the World Health Organization (WHO) TEFs for fish and birds. The tiered approach provides for preferential use of the more "certain" data, if available. For example, several of the WHO TEFs for fish for furans and mono-ortho polychlorinated biphenyls (PCBs) are based on no testing data and are estimated based on structural similarity assumptions and/or Quantitative Structure activity relationships (QSARs). There is obviously less certainty in these TEFs compared to TEFs derived from LD50 data on overt toxicity in developing embryos (*in vivo)* studies.

Uncertainties in the TEF value directly results in associated uncertainty in the ERA. The amount of uncertainty should be assessed qualitatively or quantitatively in order to understand the influence of the uncertainty on risk assessment results. The stakeholders in the ERA should have an accurate understanding of the confidence in the risk estimates. The greater the confidence the greater the certainty that actions will result in actual reduction of risks and attainment of the assessment goals.

Probabilistic techniques could be used to examine quantitatively the uncertainties associated with the TEFs. Probability density functions could be used to represent TEF values (as well as TEQs) in place of the existing point estimates. The stakeholder would then have a quantitative understanding of the uncertainty. The current presentation of TEF values as point estimates provides the illusion that all of derived values are "equal" in their predictive ability of dioxin-like toxicity.

The TEF values provided were based on endpoints that ranged from in vitro biochemical responses (e.g., induction of cyp1A1) to in vivo early life stage mortality. To what extent can these endpoints be extrapolated to the measures of effects that are relevant for the assessment endpoint for each case study?

Certain endpoints used in the derivation of the WHO consensus TEFs may not be relevant to the selected assessment endpoints for the case studies and ecological risk in general. For example, maximum enzyme induction levels, tumor promotion, and increased organ weight are used as endpoints in the derivation of TEFs. However, these toxic effects may not have consequences on the survival, growth, development, and reproduction of individuals, and the sustainability of populations and communities (typical assessment endpoints for an ERA). Some of the toxic endpoints used to derive

TEFs are not toxic responses but instead represent biochemical effects (binding affinity or induction of cytochrome P4501A) that may be in some way associated with subsequent toxic responses (WHO, 1997). Other toxic effects used to derive TEFs (aryl hydrocarbon hydroxylase (AHH) or ethoxyresorufin o-deethylase (EROD) activity) have been reported to not directly correlate with toxic injury (Stegeman et al., 1992). Without a clear association between the toxicity endpoint used to derive the TEF and the assessment endpoint for a specific ERA extrapolation may either impossible or extremely uncertain.

One of the primary questions that should addressed in reviewing the application of the TEF values to the ERA process concerns **endpoints**. As with the case studies, each ERA will have specific assessment endpoints that reflect site-specific risk management goals. The WHO consensus TEF values, however, represent "fixed" toxicity endpoints. Are these TEFs appropriate for use in effects characterization for all ERAs? Are the toxic effects used to derive these TEFs reliable indicators of the toxic effects of concern (those relevant to the assessment endpoint)? Should there be some site-specific flexibility in the selection of TEFs for use in an ERA? Should TEFs be derived that are species-specific and/or endpoint-specific?

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

What are the implications, both quantitatively and conceptually, of assuming no dose-additivity or no interaction among the components of the mixtures described in the case studies? To what extent would the risk assessment conclusions differ if stressor response analyses were based on total PCBs or 2,3,7,8-TCDD alone?

The TEF approach inherently assumes dose additivity and this is considered in the case studies. Possible interactions among mixtures of congeners, however are not addressed. The assumptions of additivity and no interaction could result in overestimation of risks. Non-dioxin like PCBs and metabolites may be antagonistic to TCDD-like response (Zhao et al., 1997; Biegel et al., 1989; Haake et al., 1987). PCB 153, a reported TCDD antagonist, is the predominant congener in the tissue and eggs of a number of avian species (Focardi et al., 1988; Elliott et al., 1989; Borlakogul et al., 1990; Ormerod and Tyler, 1994; Van den Berg et al., 1994; and Mora, 1996).

In other cases the assumptions of additivity may underestimate risks. Non-dioxin like PCBs and metabolites may induce toxic effects not addressed in the TEF (Safe, 1990 and McFarland and Clarke, 1989). Aryl hydrocarbon receptor (AhR) TEFs may be poor predictors of PCB reproductive toxicity (Battershill, 1994).

Many TEFs are based on LC50 or EC50 values. To what extent should TEF values derived at a median response level be used in risk assessments where a no adverse effect level is being employed?

For screening level ERAs, a no adverse effect level is preferable to a median response value, as the goal is to identify potential risks under conservative conditions. Application of a toxicity equivalency approach, however, requires the use of response

data to calculate relative potencies. TEFs derived based on median responses can still be used in risk assessments employing no-adverse effect levels, if the uncertainties are addressed quantitatively or qualitatively. The use of probabilistic methods to derive distributions of TEFs and/or TEQs in the ERA (in place of point estimates) could be used to address this uncertainty in quantitative identification of margins of "safety".

The TEF values provided were typically based on a single or limited number of mammal, bird or fish experiments. To what extent can class-specific TEFs be directly extrapolated to the species identified within each case study?

The TEF values provided represent the selection of the most sensitive test species and endpoint. As such the TEFs may over represent risks for less sensitive ecological receptors. The WHO fish TEFs are based on testing of one fish species, the rainbow trout. Use of these TEFs to characterize potential toxicity for the fish species of concern in the case studies (cold water fisheries including lake trout and rainbow trout) is entirely appropriate due to similarity in the specific species and sensitivity. However, in other applications outside of the case studies for warm water fisheries, these fish TEFs may not be directly applicable. Available data indicate that the relative risk of TCDD to early life stage survival for seven freshwater fish species are from 16 to 180 fold less than that for lake trout (Spehar, 1998?). Existing information on relative toxicity could be used to derive interspecies extrapolation factors to predict species-specific TEFs for non cold-water fish species.

The possible problems in extrapolation between WHO TEFs for mammals and the specific species of interest in the case studies is difficult to discern. More information on the specific derivation of the WHO TEFs for mammailian species is required above that provided in the distributed materials.

III. EXPOSURE PROFILE

The route of administered or absorbed dose used to derive TEFs may differ from those needed to establish exposure profiles in a risk assessment. To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?

Exposures for risk assessment for mammals are typically expressed as oral exposures (dietary, water and/or sediment). These exposure routes are often not equal to the exposure route used to establish potency of congeners (interperitoneal injections and *in-vitro* exposures). As the exposure routes are not directly comparable between exposure estimate (in the risk assessment) and the TEF, resulting TEQs are not accurate and introduce uncertainties into the risk analyses. The potency of congeners can vary by exposure route (intake orally with transfer and absorption through the gastrointestinal tract versus direct injection into peritoneum.

To what extent does the TEF approach require a more rigorous analytical design in quantifying sediments, soil and biota AhR agonist concentrations than is apparent in other methods which aggregate stressors (e.g., total PCBs)?

The TEF approach requires a more rigorous and expensive analytical program compared to the traditional analyses of aggregate stressors (total PCBs). In a practical sense this is

one of the more important questions in the general application of the TEF approach. The data that exists for most contaminated sites is in the form of total PCB measurements. NPDES permit and other regulatory monitoring requirements may not traditionally require congener specific analyses?

IV RISK CHARACTERIZATION

In evaluating the case studies, are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments? Do the uncertainties associated with TEFs limit the means of performing the assessments or do the other areas of the effect and exposure characterization contribute similar or greater levels of uncertainty?

The uncertainties associated with the TEFs are primarily related to the relevancy of the toxicity endpoints used to establish potency [see previous endpoint discussion]. Without the ability to complete an effect assessment specific to the unique assessment endpoints that is directly comparable to the exposure assessment data, the TEFs are more problematic than other uncertainties. Attaining the smallest difference in the laboratory (or field) measurements and the assessment endpoints (species and exposure route) minimizes minimizes uncertainties in the effect and exposure characterization (extrapolation error). Use of the TEFs limit the means and scope of assessments in setting forth the measurement endpoint (the toxic effect) and specifying the measurements of exposure that need to be performed (egg tissue concentrations in birds and fish).

Use of the TEFs also introduces uncertainty as it requires evaluation of risks for fish and birds based on egg tissue exposures. Prediction of egg tissue concentrations based on maternal exposures will often be necessary (due to analytical data constraints). This process is probably less certain than other established procedures to estimate oral doses for avian receptors.

Additional Questions Specific to the Prospective Case Study

The state adopted BAF_w^{fd}s used by the GLWQG. What improvement in the accuracy of maximum allowable concentrations for individual congeners in water, (MACt,w)ij, can be expected through use of BAFfdws determined from Roundtail Lake data?

What errors are associated with the state's application of the GLWQG TCDD water quality standards for birds and mammals without consideration of congener-specific differences in biomagnification factors from fish to tissues in wildlife relevant to the effects of concern?

How should the uncertainties associated with the available fish, avian, and mammalian TEFs be incorporated into decisions about which TCDD water quality standard would be chosen for setting a TEQTMDL for regulating chemical discharges into Roundtail Lake?

Additional Questions Relative to the Retrospective Case Study

Would TEQ sediment cleanup goals be the same for each vertebrate group? If not, why would there be a difference? If the vertebrate group with the most certainty is not the group with the most restrictive sediment cleanup goal, how would you council the risk manager's concerns for the other vertebrate groups?

The TEQ sediment cleanup goals would not be the same for each vertebrate group as the TEFs represent different sensitivities across the general classes (mammals, birds and fish). The TEQs for each vertebrate are also based on different exposures (oral for mammals and egg tissue of birds and fish) which whould result in different cleanup goals.

My general advice to the risk manager's concern would be somewhat practical. The vertebrate group with the most certainty in the risk results represents the most certain clean up option with the greatest chance of attaining the management goals. Specifically I would substantiate recommendations with quantitative information on the uncertainties in the assessment including the effect of the uncertainties on risk results and clean up concentrations. Clean up options for the protection of the different vertebrate classes would be represented geographically. A cost-benefit analysis would also be completed to identify for the various clean up concentration the amount of risk reduction per unit cost. Uncertainties would be considered in the cost-benefit analyses. The primary goal of risk assessment in most regulatory applications is to identify how to reduce the most risk for least amount of cost. This type of quantitative analyses would be used to demonstrate the most effective and protective options.

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Steve Bursian

I. STRESS RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. The additional background information which was provided enhances the process of evaluating uncertainties. The supplementary material provides details related to experimental design which can account for differences between studies using the same species for determination of Lethal Dose 50 (LD50), lowest observable adverse effect level (LOAEL), or no observable adverse effect level (NOAEL). These differences may be due to the type of compound(s) administered to a particular species [eg. commercial polychlorinated biphenyl (PCB) mixture as compared to weathered PCBs/dioxins/furans provided through fish collected from a contaminated site]; the method by which the compound is administered to the animal (eg. injection into air cell vs. yolk; injection on day 0 vs. day 4); the endpoint(s) which are chosen to assess LOAELs or NOAELs; the time at which endpoints are assessed (eg. 18 days of incubation vs. hatch); whether the NOAEL is actually determined from the dose-response curve or if it is estimated by dividing the LOAEL by 10; and differences in doses used between studies which could result in differences in LOAELs and NOAELs. The additional material is also helpful in terms of assessing differences between species in terms of LD50 values for specific chemicals (eg. PCB 126) that could influence toxic equivalency factor (TEF) values.

2. In the process of deriving a TEF based on several studies, a number of variables would be taken into consideration and the resulting TEF could be more accurate than one derived from a single study. For example, if one considers the data derived from studies in which PCB 126 and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been injected into eggs of different species, a consensus TEF for PCB 126 could be established which would reflect considerations made for differences in methodology and species. A TEF for a particular PCB congener that has been derived using mortality data from a cormorant egg injection study (air cell on day 4) and the chicken LD50 value for TCDD (yolk on day 0) could be very different from a TEF in which the same species and methodology was used for both TCDD and the PCB congener. It was apparent from the background material that depending on the

data chosen, TEFs for a specific congener could be different by an order of magnitude, thus, a TEF derived from a single study introduces more uncertainty than one which is based on a number of different studies.

3. TEF values based on egg injection studies utilizing embryo mortality as an endpoint would be the most relevant in terms of the avian species to be protected, particularly if the chicken was used as the animal model. In those situations in which a TEF for a particular chemical has been developed using in vitro induction of cyp1A1, for example, it could be applied to the present case studies with the awareness that in vitro induction of cyp1A1 may occur at a different concentration than an increase in embryo mortality in bald eagles. However, the variability in TEFs derived in different studies for a particular chemical and species using similar endpoints appears to be just as great in many cases as the variability in TEFs for the same chemical based on different endpoints such as in vitro enzyme induction and embryo mortality. Thus, it would be preferable to utilize a TEF based on the relevant endpoint, if available. If not, a TEF based on another endpoint could be applied if it was thought that the value was conservative and would protect the species in question.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. If there is no dose-additivity or interaction among the components of the mixtures described in the case studies, then each chemical would have to be assessed individually. The risk assessment decision would have to be based on the chemical judged to have the potential of causing the most harm to each targeted species based on its potency and its environmental concentration. If stressor response analyses were based on total PCBs or 2,3,7,8-TCDD alone in the retrospective study, one could come to different conclusions concerning the risk. The data presented in the table are from the retrospective case study and relate to the concentration of total PCBs, TCDD, and toxic equivalents (TEQ) detected in Caspian tern eggs (Table 2) and otter livers (Table 3) as well as the NOAEL

thresholds for avian eggs and mink livers (Table 4).

	Bird Egg		Mammalian Liver	
	Concentration Detected	NOAEL Threshold	Concentration Detected	NOAEL Threshold
PCBs	5667 ng/gm	5000 ng/gm	1001 ng/gm	2000 ng/gm
TCDD	4.5 pg/gm	100 pg/gm	1.43 pg/gm	60 pg/gm
TEQs	445 pg/gm	100 ng/gm	144 pg/gm	60 pg/gm

In the case of the avian species, the concentration of total PCBs detected in the egg is slightly higher than the NOAEL threshold, whereas the TCDD concentration in the egg is 22 times lower than the NOAEL threshold. The TEQs present in the egg are 4.5 times greater than the NOAEL threshold. Thus, one could conclude that an analysis based on TCDD only would suggest little risk, an analysis based on total PCBs would suggest a risk, and an analysis based on TEQs would strongly support the notion that a risk exists. In the case of the otter, an analysis based on total PCBs might indicate concern, since the concentration detected in the liver is half of the NOAEL threshold based on mink studies. The TCDD NOAEL threshold is 42 times higher than the concentration of TCDD detected in the liver suggesting that there is little risk while the TEQ concentration in the liver is twice the NOAEL threshold. For both species, an analysis based on TEQs would suggest that the contaminants present in the environment are posing a risk.

In the prospective study, the use of total PCBs would provide little protection since the proposed paper mill is expected to generate dioxins and furans only. The use of TCDD would certainly be an improvement but it would not provide the protection afforded by the TEQ approach. If the relative mass concentration ratios of each of the 7 dioxins and furans expected to be in the mill effluent are multiplied by their respective TEF values, then TCDD contributes 26% of the total TEQs while 2,3,7,8-TCDF contributes 51%.

2. The NOAEL is dependent upon the doses employed in a particular study. For example, two studies are designed to assess the effect of PCB 126 on chick bursa weights. One study employs doses of 0, 0.625, 1.25, 2.5, 5.0, and 10 μg/kg egg while a second study uses doses of 0, 1.0, and 10 μg/kg egg. In both studies, the bursa weight is reduced at 10 μg/kg egg but not at the next lowest dose. Thus, in the first study the NOAEL is determined to be 6.4 μg/kg egg while in the second study the NOAEL is 1.0 μg/kg egg. While the difference between the two values is relatively small, it would seem that considerations of the entire dose-response curve in determining an LD50 or ED50 value is more accurate than designating the dose at which no effect is observed in that particular study as the true no effect level.

3. The avian TEF for PCB 126 is 0.1 as indicated in the 1997 World Health Organization (WHO) report. Egg injection studies in our laboratory which have involved assessing the effects of PCB 126 and 2,3,7,8-TCDD in the chicken and double-crested cormorant suggest that the PCB 126 TEF values for both species are reasonably close to the 0.1 value. In the chicken, the LD50 value for PCB 126 was 2.3 μg/kg egg and the LD50 value for TCDD was 0.15 μg/kg egg. Thus, the TEF value derived in this study was 0.07. In the cormorant, the PCB 126 LD50 value was 177 μg/kg egg while the TCDD LD50 value was 4.2 μg/kg egg. Based on these data, the TEF for PCB 126 in the double-crested cormorant is 0.02. The consensus avian TEF for PCB 126 and the two values established in our laboratory are within the same order of magnitude despite the marked difference in sensitivity between the chicken and cormorant to PCB 126 and TCDD. Thus, it would seem acceptable to apply class-specific TEFs to the avian species identified.

III. EXPOSURE PROFILE

2. In egg injection studies, the site of injection (yolk vs. air cell) and the time of injection (day 0 vs. day 4 of incubation) influence the concentration of the chemical at which effects occur. Typically, yolk injections yield a lower LD50 value than air cell injections and injection on day 4 of incubation precludes exposure of the embryo during its first 96 hours of development. However, differences in LD50 values

because of injection site are relatively small and should not prevent the use of egg injection-derived TEFs for environmental risk assessments. For avian species, the use of egg injections is easier and probably more accurate than feeding the contaminant in question to laying hens and then assessing the effects of the compound on egg production and hatchability. Feeding contaminants to non-domesticated avian species such as cormorants or terns would be considerably more difficult if not impossible, while the injection of eggs collected from relevant species is feasible.

IV. RISK CHARACTERIZATION

2. Giesy et al. (1994) summarized the advantages and disadvantages of using the H4IIE assay for determination of TCDD-EQ as compared to the chemical analysis/TEF approach. The bioassay is rapid and considerably less expensive than congener-specific analysis. Since the bioassay is a mechanistically-based determination of an integrated biochemical response (induction of ethoxyresorufin O-deethylase activity), it is more biologically relevant. The bioassay accounts for interactions between the polychlorinated hydrocarbons and other types of compounds that may be present in the mixture. In a comparison study to determine the TEQs by instrumental and H4IIE bioassay analysis, the bioassay determined a higher number of TEQs in an environmental mixture when compared to the chemical analysis/TEF approach. It was possible that components of the mixture were acting synergistically or there were components in the mixture which were not quantified. If feasible, both approaches could be used and in those cases where one method offers greater protection than the other, the risk assessment would be based on the most conservative approach.

3. Perform egg injection studies with the chicken as the experimental animal (more sensitive than species of interest) to determine TEF values for each relevant chemical. If such studies could be done utilizing consistent techniques throughout, some of the uncertainty associated with the derivation of the TEFs would be eliminated.

Conduct a mink reproduction trial in which animals would be exposed to relevant concentrations of TCDD from 3 months prior to mating through weaning of the young. Mink are extremely sensitive to PCBs and TCDD. While reproductive trials utilizing commerical PCB mixtures, PCB congeners, and environmentally derived PCBs have been run, no such trial has been conducted with TCDD.

Conduct a feeding study with otter in which they would be fed diets containing TCDD, specific PCB congeners, or environmentally derived PCBs. While otter are more difficult to work with than mink, it could be done.

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Comments of Peter L. deFur on the Application of 2,3,7,8 TCDD TEF's to fish and Wildlife
November 17, 1997

General comments on the use of TEF's for wildlife and the two case studies

TEF's: The concept of TEF's and the application of TEF's is neither new nor is it entirely novel in biological sciences. Fundamentally, there is an abundant literature from the fields of endocrinology, toxicology, pharmacology, neurobiology and other areas of physiology supporting the concept of equivalencies in cellular/molecular biology. The specific development of TEF's for TCDD is also well established, although the literature may not be as old. I strongly support the effort to extend the use of TEF's from rodents and humans to fish and wildlife. This should prove fruitful in applying basic information to environmental control and clean-up and should provide important insight into the comparative aspects of environmental biology.

That said, the one point that I see limiting TEF's in this context is the metabolic differences among animals, a point also made in the present papers. The present work notes that some Ah active compounds are not metabolized in marine mammals as in other species (e.g. rodents) with subsequent different accumulations. If we accept the fact that the molecular events of Ah receptor binding are common to all Ah active compounds, then there are two major steps where substantial interspecific differences are likely to occur. The first is in the cellular events following Ah binding; the second is the process by which Ah active compounds or products of Ah activity are metabolized (either the upstream or downstream metabolic pathways). So far, most of the vertebrates examined for Ah activity, excepting marine mammals, are temperate to northern boreal animals. Few, if any show extremes in life history (lungfish), evolutionary development (platypus) or environmental adaptation (e.g. desert reptiles). I would expect to see the most remarkable differences in metabolic processing among these types of animals, as observed in marine mammals.

The use of TEF's in risk assessment or any other regulatory program or plan should pose no more or less problematic than any other analytical tool. TEF's seem to apply to fish and mammalian wildlife, but have not been attempted or well demonstrated in amphibians and reptiles.

Both cases are based on well studied situations with rich databases and numerous examples. Data on Great Lakes fish and on pulp and paper mills discharging TCDD are abundant in the

EPA files and the literature. The advantage of using such familiar types of cases will be an easier application of the method. After this exercise is completed and any modifications included, I urge EPA to consider a follow-up case that draws on a poorly studied type of situation.

Specific Review Questions:

I. Stress-response

1. Point estimates of TEF's still should include reference to the background information from which the points were calculated. As the number and variety of applications of TEF's increases, so shall the need to consider additional species less similar to the ones for which the original data were developed. The background information should enable the users to determine the extent, if any, to which new applications to other species require further modification.
2. It is clear from the literature that not all TEF's have the same or even similar levels of experimental data in the development. Yet, there seems to be no apparent reason why one compound should behave fundamentally different from those compounds for which there is a substantial database.
3. The TEF's for biochemical and cellular effects should be usable for whole organism effects. The mechanism whereby enzyme induction (or other molecular event) is related to whole organism effects, e.g. reproductive impairment, has not been elucidated in full. This information should be usable in the future, but should not prevent the application of TEF's now.

II. Stress- response and Application of TEQ's

1. I will have to give this more consideration. Part of the answer to this is found in the answer to V.1.
2. The TEF's derived at some dose to achieve median effect (response) are clearly useful in the range in which they were developed, and for the effect or mechanism for which they were developed. But the usefulness has not been challenged or tested at low doses or perhaps not at high doses for wildlife and aquatic life (presumably the high dose exposures in mammals and some rodents may confirm the applicability in this end of the range). The low dose research so far has focused on enzyme induction and similar biochemical events. Has

anyone confirmed or refuted the applicability of TEF's in very low doses in these groups of animals?

3. Class-specific TEF's should be more applicable than are more general TEF's (e.g. vertebrates). Thus far, the experimental evidence supports the class basis, even for the exceptions (marine mammals).

III. Exposure Profile

1. To what extent does the TEF approach limit exposure analysis? The challenges associated with the TEF approach are those of increasing the complexity of exposure modeling, including fate and transport. The congeners should not be collapsed together in exposure models, but should be treated individually so that congeners with dramatically different TEF's can be accounted for in the exposure, rather than assume that all congeners are similar. The physico-chemical data suggest (or more) that congeners will act differently in the physical environment. Because these congeners would likely (or certainly) have quite different TEF's and hence toxic effects, their exposures should be treated separately. Models that do not now treat the congeners separately will not suffice for use in TEF specific risk assessments.
2. Exposure route differences used in deriving TEF's may alter the final outcome if (and only if?) the route of exposure alters the absorbed and tissue dose, and if this alteration is not accounted for in the final calculation. Efficiency of uptake is high in digestive tracts of most, if not all animals; this is the primary route of exposure and of administration in laboratory work. I cannot see that this would be a problem in the derivation and use of TEF's.
3. The TEF approach will prompt or require a more detailed analysis of TCDD (and PCB) sources such as sediments, water and soils than is the case in which the congeners are aggregated. The aggregation approach simply assumes that all congeners in the total are the same, or that the aggregate can be treated in a simple, single model approach. The different toxicity of each congener will affect the final toxicity of the mixture; hence it will be much more accurate to know the real mixture toxicity based on the sum of the TEF's.

IV. Risk Characterization

1. Sources of uncertainty in an entire risk assessment are not so quantitatively predictable as to make any conclusion as to the relative contribution *a priori.*

Uncertainties in TEF's are largely from the variation in the results of experimental outcomes, whereas the uncertainties in a "field" risk assessment include fate and transport, exposures, endpoint sensitivities and population dynamics. I doubt this can be determined on a generic basis.

2. Comparing the TEF approach with a site-specific TEQ analysis, there are a few ways to approach this question. I think the first question is why would one do both? Or one or the other? I imagine that most scientists would want the congener specific TEF analysis, based on a congener analysis of the source. Given the acceptance of TEF's, and the confidence in them, as well as an exposure analysis that incorporated individual congeners, a simple analysis of the source of contamination (air, water, sediment, etc) would provide a straightforward method for determining the dose to the target species. In the cases where the TEF's are most likely to apply tot he target (endpoint) species, the TEF approach would likely provide the most accurate approach. But, in cases where the TEF's are not as likely applicable to the target species, then the total sample TEQ approach would circumvent the lack of applicability. The TEQ approach is likely to be more difficult.
3.

V. Prospective Case

1. This question about improvement in BAF $^{fd}_{w}$'s makes a comparison and it is not clear to what the new BAF's are compared or if it is to any alternative. The improvement or increased accuracy is in targeting for lower MAL's those congeners that are more accumulative. I do not see the safety in permitting the relaxation or raising of MAL's for congeners with lower BAF's in a single species or in a class. In this latter case, the permitting of more discharge of any congeners of TCDD assumes that the congeners will not ever pose a toxicity problem in the lake. If at some later time the congener with the higher MAL does pose a problem, then the loading over time will present future contamination problems. The temporal lag between discharge and effect, control and response is problematic for TCDD and congeners that have such a long half-life in the environment.
2. Without using congener specific data, the state may have to treat all congeners in an approximately similar fashion, erring in exposure, dosimetry and in toxicity. Alternatively, the state may chose to ignore all congeners for which

there are not site-specific data, as has been done in the past when only TCDD was analyzed. Both approaches have the potential for underestimating toxicity; the latter approach having been carried out by many states for years (and likely still practiced). The former error could treat all lower toxicity congeners as more toxic, thereby overestimating the effect. Unless, of course the state chooses to "average" the toxicity along with lumping the dosimetry, thereby taking some sort of average toxicity to use with a total dose of TCDD's, TCDF's and PCB's.

VI. Retrospective Case

1. I have not calculated the numeric clean-up goals for each vertebrate species. Seldom are the clean-up goals the same in such cases. Frequently one endpoint drives the clean-up because of greater BAF, greater sensitivity (more toxic in one species than the others), or because of a different target level in the clean-up goals, as is the case with some endangered species, or one for which there is a specific population recovery plan.

2.

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Joseph V. DePinto

Pre-meeting Comments in Response to Charge Questions for

Workshop on the Application of 2,3,7,8,-TCDD Toxicity Equivalency Factors to Aquatic Life and Wildlife

Since my expertise is limited to modeling the transport, fate and bioaccumulation of contaminants in aquatic systems, my comments in response to the pre-meeting questions will be confined to those questions relating to exposure and risk characterization. I will leave comments on toxicity questions to those with much more expertise in that area.

III. EXPOSURE PROFILE

1. *To what extent does the TEF approach present challenges, introduce new uncertainties, or modify old uncertainties associated with modeling the exposure of AhR agonoists? To what extent does the availability and quality of congener-specific phyisco-chemical data limit the means of employing fate and transport or food chain models?*

Until the Green Bay Mass Balance Study and the modeling work conducted in that study, fate and transport models of hydrophobic organic chemicals (HOCs) were not applied to specific congeners. Having been one of the modeling team working on that project, I can state that one of the significant outcomes of that study was that "once an accurate model for the dynamics of sorbents (solids and non-settleable organic material) in a specific system has been developed, we have enough knowledge of and appropriate formulations for the transport and fate of HOCs in surface waters that we can accurately model the concentrations in water and sediments of specific congeners of these compounds merely by having good congener-specific physico-chemical data (*e.g.*, K_{ow}, H_e, biotic and abiotic degradation rates)." This result was demonstrated for PCBs in the Green Bay study by successfully modeling PCB congeners spanning a wide range of hydrophobicity and volatility using the same sorbent dynamics and only changing the respective physico-chemical properties. This development has made it possible to use the TEQ approach as proposed in the **Prospective Case Study**. However, as the statement above suggests, we will be limited in this approach by the availability of accurate congener-specific physico-chemical parameters. As indicated in Table 1 of the Charge Questions, there are gaps in these data and potentially order of magnitude or more uncertainties in some of the properties estimated for the more hydrophobic congeners. In my opinion, considerably more work needs to be done in measuring or calculating (based on structure-activity models) these chemical properties.

The status of bioaccumulation model is slightly more problematic because of the uncertainties of in model formulation and parameterization of food chain bioenergetics and predator-prey dynamics and because of the large system-to-system variability of these ecosystem dynamics. Measurements of BAFs and BSAFs on a congener-specific basis can obviate the need for the more mechanistic food chain bioaccumulation models, but extrapolation of site-specific measurements carries with it a significant uncertainty in terms of two things: 1) a different system with a different food web will exhibit a different BAF or BSAF; and 2) the measurement in a given system is representative of a specific point in time and there may be a lag between a change in the concentration in the water column or sediments and the response of the concentration up the food chain (*i.e.*, the measurement may not have been at bioaccumulation equilibrium).

2. *To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?*

This could be problematic because of the fact that we tend to see a decrease in BAF as a function of log K_{ow} for the super-lipophilic congeners (log $K_{ow} \geq 6.5$). It is not known whether this is because the congener is so insoluble that it cannot transport as effectively across the gut wall or whether the kinetics of the bioaccumulation process is so slow that the organism cannot respond to a given exposure level in a reasonable length of time. In any event, there is likely to be a big difference between the BAF for one of these compounds if the exposure is via food intake versus direct injection.

3. *To what extent does the TEF approach require a more rigorous analytical design in quantifying sediments, soil, and biota AhR agonist concentrations than is apparent in other methods which aggregate stressors?*

If I understand the approach, projecting a AhR-based toxicity in fish or wildlife based on will require a chemical measurement of the concentration of each congener in each stressor source. In other words, if contaminated sediments are the source of toxicity, then the initial concentration of each relevant congener will have to be quantified. Of course, with current analytical methods, measurements like total PCBs are actually made by appropriately summing individual congener concentrations. This may be somewhat problematic using EPA's accepted standard method because detection limits for individual congeners are often too high to give an accurate TEQ for comparison against a effects standard.

IV. RISK CHARACTERIZATION

1. *In evaluating the case studies, do uncertainties in the TEFs limit the assessment or are other aspects of effect and exposure characterization contributing similar or greater levels of uncertainty?*

It is clear to me that uncertainties TEFs will increase the overall uncertainty of a risk assessment, simply because we are propagating more error through the calculation as we increase the number of parameters to specify. However, one must weigh uncertainty against the information obtained — or utility — in a given calculation. In my opinion, there is a potential to gain much more information using the TEF approach; therefore, it is worth using even though the error might be somewhat higher. I feel confident that, over time, experience with the approach and more empirical data will reduce the uncertainty.

2. *Biologically-based TEQ assays on environmental samples could be employed as an alternative to the TEF-based approach. What would the strengths and weaknesses of such an approach be?*

With regard to exposure modeling, if the biologically-based TEQ assays approach were used, we would have to develop models for the fate and transport of whatever it was that the TEQ assay was measuring. If we did this we would not only find it virtually impossible to parameterize such a model, but we would have no way of guaranteeing that a TEQ assay level at a source would be transported and transformed through the aquatic system in such a way that made it directly comparable to the same assay conducted on the receptor (some fish, bird or mammal). In other words, TEQ for multiple sources (including background sources) would <u>not</u> necessarily be additive at the receptor. Put another way, modeling the fate and transport of "toxicity" as a single constituent is fraught with problems and uncertainties that may indeed exceed the errors introduced by making the analysis more complex by using the TEF approach.

3. *Provide a list of specific research or site-specific data that would improve the analyses in the case studies.*

In my opinion, there are three primary areas of uncertainty associated with the type of regulatory analysis described in both case studies:

1. Quantifying the exposure distribution in water and sediments of the system of interest as a function of the various sources.
2. Confirming that BAF or BSAF measurements in one system are applicable to another; in

other words, understanding the ecosystem factors that control bioaccumulation and hence these measurements.

3. Reducing uncertainty in TEF values by building an empirical database over time.

All three of these research/data acquisition areas are very important in my opinion. Suffice it to say that application of the TEF-based for risk management requires continued research associated with its application in order to build a better experience and knowledge base.

Additional Questions Specific to the Prospective Case Study

1. *The state adopted BAF^{fd}_{w} used by the GLWQG. What improvement in the accuracy of maximum allowable concentrations for individual congeners in water can be expected through use of BAF^{fd}_{w} determined from Roundtail Lake data?*

The GLWQG BAFs were determined largely using Lake Ontario measurements using lake trout. Measurement error and time-variability aside, these BAFs could easily vary by 1-2 orders of magnitude between Lake Ontario and another system with a different food web (*e.g.*, more benthic versus pelagic or having a different number of trophic levels). To the extent that the food webs of Lake Ontario and Roundtail Lake are similar, I would not expect to gain much accuracy in measuring site-specific BAFs in Roundtail Lake. However, if the food webs are significantly different or if there had been a significant perturbation (*e.g.*, one or more very bad recruiting years) in one of the trophic levels (such the prey fish), then site-specific measurements would certainly be advisable.

4. *What errors are associated with application of TCDD water quality standards for birds and mammals without consideration of congener-specific differences in biomagnification factors from fish tissues?*

If I understand this question correctly, there is potentially significant error involved in not accounting for BMF from fish to wildlife. The BMF could be significant on a congener-specific basis, which in turn might have a significant effect on the standard. Good data on the fraction of the wildlife diet coming from fish and the age and size of fish in their diet is crucial and perhaps an even greater source of error in many cases.

5. *How should uncertainties associated with the available fish and wildlife TEFs be incorporated into decisions for setting TeqTMDL?*

We must attempt to quantify the uncertainty in TEFs for each target group and then

propagate that error through the calculation of TeqTMDL for each. Then the actual TMDL allocated to the discharger should be based on the target group yielding the allowable loading that is statistically lowest without making a Type I error.

Additional Questions Relative to the Retrospective Case Study

1. Would the TEQ sediment cleanup goals be the same for each vertebrate group? If not, why would there be a difference? How would you handle a situation in which the group with the most certainty is not the group with the most restrictive sediment cleanup goal?

I would think that sediment cleanup goals would vary from group to group; because, even though the source may be the same and may have the same congener distribution for each group, the pathway to each group is very likely to be different and each trophic level in those pathways may be subject to different exposure distributions and may bioaccumulate and metabolize that exposure differently. Therefore, I would not be surprised at all that computing a cleanup goal based on a TEQ would yield different values.

Refer to my response to the last question for my opinion on how to handle a situation where unequal certainty exists among groups.

2. *Would the TEF/TEQ-based sediment remediation goal be the same as those determined for total PCBs for the identical vertebrate class? Assume that a simple ratio of total PCB sediment concentration goal to TEQ sediment concentration goal was formulated to allow for the use of total PCBs to monitor cleanup. What exposure and effect issues would need to be evaluated before using the less costly total PCB analysis to support the TEQ-based sediment remediation goal?*

This is an excellent question, but it is difficult to answer without going through significant calculation and modeling. But most importantly, the concentration of PCBs (and other chemicals of interest) in the sediments of Yuckymuck River have not been measured (or at least not specified); therefore, it is impossible to know if the two goals will differ. But given the total PCBs in the lake surface sediments are 110 ppb, I would venture an educated guess that the PCBs in the river sediments are still well over 1 ppm. Therefore, a goal based simply on getting total PCBs down below 1 ppm would probably require removal of more sediment than the TEQ-based remediation goal.

Using a simple ratio of goals to permit measuring total PCBs as a means of monitoring cleanup progress is fraught with error. I can think of no *in-place* or removal-treat-and-replace sediment remediation process that would not be congener-specific in its removal efficiency.

Even using a simple dredging and disposal approach would not necessarily work. This is because the congener distributions of PCBs and PCDFs would no doubt change with depth in the sediments; therefore, a goal and ratio based on surface sediments would not necessarily be constant through the full treatment depth of the sediments. If the spill occurred 30 years ago and loss is by burial, the spill chemicals may have penetrated quite deep into the sediments.

Conclusion

I am strongly in favor of applying the TEF/TEQ approach for risk management of fish and wildlife; however, we must move forward by maintaining a concurrent research and data acquisition program that will allow us to continue to reduce uncertainty in decision-making.

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0. General and preliminary comments

The scope of the workshop's discussion is confined by the organizers to considerations of direct effects only. While perhaps useful in limiting the scope of the discourse for the sake of manageability, this seems dangerously restrictive in the context of ecological risk assessments. After all, **indirect effects** such as trophic cascades certainly do play a very important role in the ecotoxicology of TCDD and its congeners. Perhaps this restriction should be relaxed somewhat.

Like many, I have serious and strong reservations about the use of the "hypothesis-testing" approach in environmental risk assessment and management, including use of the hazard quotient, no observed effects levels, and their ilk. The conceptual difficulties with EPA's approach are many and have been widely discussed (e.g., Barnthouse et al. 1986; Landis and Yu 1995; inter alia). Whether or not to regulate or remediate should be framed as a **decision problem**, not a hypothesis testing problem. Much of the use of TEFs (toxicity equivalency factors) has heretofore been embedded in hypothesis-testing approaches which I find barely intelligible. It is heartening, however, that the TEFs should be of use beyond the rarefied context of hazard quotients. I think it will be important for the workshop discussion to consider how TEFs will continue to be useful when hazard quotients are replaced by probabilistic methods of decision analysis.

II.3. Extrapolating class-specific information to particular species

Although one might hope that TEFs will provide a means of freely translating toxicity information within the big matrix of chemical congeners and biological species, there appears to be a considerable amount of interspecific variability in toxicity of TCDD itself (and presumably this cannot be erased in the TEF method). It might be very interesting to explore the available information about TEFs for the existence of **allometries** in which this residual variation might be at least partially explained by a species' body size, typical egg size, or other easily measured species-level variable. If even crude allometric relationships exist, they may be very useful in making the TEF method more accurate with little additional effort.

IV.1. Are uncertainties of TEFs more problematic?

I doubt that the uncertainty about TEFs is any more problematic than that of the other sundry inputs to a quantitative risk characterization. The magnitudes of these uncertainties may be

fairly similar to those we've seen in other inputs, and even if they're considerably bigger, it shouldn't necessarily lead to any fundamental incompatibility. It is important to understand, however, that the uncertainty in TEFs will likely primarily be lack of knowledge (i.e., incertitude or ignorance), rather than variability. We have argued that it may be necessary to use different uncertainty propagation techniques to handle this kind of uncertainty (Ferson and Ginzburg 1996). In particular, the indiscriminant application of Monte Carlo techniques in this case can lead to erroneous conclusions that underestimate the risks involved.

The task of identifying, and quantifying, the uncertainties associated with TEFs belongs primarily to the empiricists who collect the original toxicity data and the synthesizers who collate this information and compute the TEF values. The former must report their measurement errors in full detail; the latter must propagate these uncertainties using appropriate techniques. Reviewers can help by checking that the results seem reasonable and by guessing at what possible mistakes or omissions may occur, but they cannot be expected to develop characterizations of uncertainty if the requisite underlying details are missing.

IV.3. Further empirical investigations for the case studies

Most of the documents focus on effects on juvenile survivorship. Are there known to be no effects from common environmental concentrations of TCDD etc. on other demographically important variables? Possibilities include time to reproductive maturity, onset of adult senescence, growth rate, reproductive investment, among others. Since the toxicological effects are believed to be additive, I would supposed they are likely to also be cumulative in time with iterated exposures. Thus one might expect to see effects in later life stages. Unless it's clear that no effects on such variables are possible (via the Ah receptor mechanism or otherwise), I think it would be very important that further specific empirical and synthetic studies be conducted to extend the TEF method to such variables. It seems doubtful that a TEF value for one life stage is really general for all life stages.

Often a biochemical response (e.g., induction of cyp1A1) is observed in lieu of measuring effects on juvenile survivorship. It is harder and harder to justify regulations based merely on measurable biochemical effects in non-human species. Unless this biochemical effect has an obvious and direct consequence on some population-level vital rate (reproduction, mortality,

growth rates), or perhaps on some organismal-level variable related to individual health of humans or a listed endangered species, we should expect to encounter "so what?" questions from the regulated communities and the public.

Extra Note:

The word 'congener', like 'species', has both a meaning in chemistry and another meaning in biology. The documents are consequently rather confusing.

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Responses to General Questions

Because I often had difficulty understanding exactly what was being asked in some of these questions, my responses below contain my paraphrase of the question prior to the answer.

I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES.

1. I was very perplexed by this question Was there any information in the descriptions of the case studies which was useful for reducing the uncertainties in the derivation of the WHO TEFs and their application to each particular assessment?

No. I believe the greatest uncertainties in the application of the WHO TEF values are in the reliability of the extrapolations to other untested species, and extrapolations to endpoints biologically or biochemically distant from the endpoints used to derive the TEFs.

2. Should all TEFs be considered to have similar uncertainties? Obviously, no. The uncertainties associated with each of the TEFs have multiple sources. As referred to above, these are most importantly the cross species extrapolations, and the cross-endpoint extrapolations. As pointed in several of the documents provided, the uncertainties in the application of any given point estimate of a TEF increase the more distant the endpoint on which the TEF is based is from the endpoint of interest in the assessment. I believe this is nothing more than common sense and toxicology 101. There are several examples which exemplify these uncertainties; as eloquently pointed out in document 6F by Cook, et al.. In Table 2 of that document TEFs are listed across endpoints. By looking at the TEFs for a given chemical (across endpoints), it is apparent that the relative difference in the TEFs is not the same across chemicals (see below).

Table 1.

	TEF Ratio	
	In vivo RBT liver EROD/ RBT ELS Egg Mortality	*In vitro* RBT liver EROD/ RBT/ELS Egg Mortality
Congener		
2,3,7,8-TCDD	1.0	1.0
1,2,3,7,8-PeCDD	2.7	3.9
1,2,3,4,7,8-HxCDD	1.5	4.2
1,2,3,6,7,8-HxCDD	20	10
1,2,3,4,6,7,8-HpCdd	33	133
2,3,4,7,8-PeCDF	5.9	5.6
1,2,3,4,7,8-HxCDF	1.7	4.6
1,2,3,7,8-PeCDF	12.5	6.3
2,3,7,8-TCDF	16.7	6.7
PCB 126	0.4	41
PCB 81	6.5	4.8
PCB 77	28	17

Looking down the row of each ratio, it is notable that relative difference in the TEFs derived from the biochemical endpoint of EROD induction to the more ecologically relevant endpoint of ELS egg mortality is clearly non constant across the chemicals and spans a range of nearly 200 fold. Also of interest is the lack of concordance in the relative ratio for any given chemical (i.e. looking across the rows). As pointed out by Cook et. al., the difference, hence the best example of uncertainty, is for PCB congener 126.

Since uncertainty is also a general function of the information richness of the data set, the trout data serves as the basis for another point. In general, the rainbow trout and lake trout data sets are the most information rich data sets we have for formulating and evaluating TEFs. And this data reveals the uncertainties above. It is very difficult to say whether these same observations about uncertainty would hold if the data set were on another species entirely, for example with a bird species. In other words, it is difficult to say if the differences would be greater or less?

In addition to this source of uncertainty, there is also uncertainty derived from cross species extrapolations. For example, the LC50/NOEC values derived from the data of Spehar et al. are as follows.

Species	LC50/NOEC Ratio
Fathead Minnow	2.3
Channel Catfish	1.7
Lake Herring	5.2
Medaka	2.4
White Sucker	2.2
Northern Pike	2.1
Zebrafish	6.2
Avg	~ 3

Note that the NOEC values included non-lethality (non-acute) measures such as growth. Also the range (estimated) of egg LC50 values is 35 fold (lake trout to zebrafish).

Clearly, there are significant uncertainties in the risk benchmark values for any given species. Would this same pattern hold true for the other PCDDs and PCDFs of interest? Would the magnifude of the differences among species be more or less?

3. To what extent can the TEFs be extrapolated to the measures of effect that are relevant to the assessment endpoints? The best case, i.e. the one with the least uncertainty, is the case where the extrapolation is biologically proximate—as is the case when the risk endpoint is focused on early life stage mortality and the TEFs are based on the same endpoint. So, in both cases, the fish assessments are on the firmest footing. The bird assessments, because the TEFs are based primarily on biochemical endpoints, are the least certain, and potentially the most conservative—if the lessons from the fish data set are ultimately applicable.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. What if we didn't do a total TEQ approach? Quite simply, you would miss important information in the estimation of the overall risk. It is clear from all of the information provided, and the other literature in this area, that a total TEQ approach to the risk assessment is a rationale one. I agree that additivity at the cellular level is a reasonable assumption for these chemicals.

2. Are we erring in our assessments by using TEFs based on median response levels? Probably not. The toxicology 101 answer to this question is "Not as long as the slopes of the dose response curves and the magnitudes for the range of the responses are similar across the chemicals of interest. From the data I've seen so far, it appears as though, at least for TCDD, the slopes of the various dose-response curves are generally similar (cf. The Spehar et. al. data). It also turns out that for this particular class of chemicals the dose response curves are quite steep, with ratios of EC50 values to NOEC values on the order of 2-3X. The downside to this type of dose response curve is that *in the effective range* small changes in exposures can result in large changes in levels of effect. The positive aspect is that below this narrow range, no measurable effects are likely.

3. To what extent can class-specific TEFs be extrapolated within each case study? As per the data in my answer to question 2 of Section I above, there are indeed significant uncertainties associated with applications across biological endpoints or species. For the cases provided, these uncertainties are probably less problematic because it is evident from the datasets that we are likely to have TEF values and toxicologic benchmark values for species that are the most sensitive. For example, because the TEFs for fish are derived from the lake trout and rainbow trout datasets, and because all of the other data on toxicity of TCDD suggests that these are the most sensitive species, one can have confidence that extrapolating across all other species of fish is not under conservative (i.e under protective).

I would be willing to make similar conclusions for estimates of risk to wildife which are derived from TEFs based on mink. I think the extrapolation with the least certainty is that with birds.

III. EXPOSURE PROFILE

1. Does the TEF/TEQ approach make it more difficult to assess "exposure" than if the assessments were focused on one chemical alone? Clearly yes, if only for the simple reason that this approach deals with the aggregate uncertainty associated with dealing with a larger number of chemicals. While we have built a fairly reasonable data set on which to describe (or model) the likely fate profile of TCDD and some of the PCBs, it is clear that we do not have the same level of understanding for all of the compounds which are included in these TEQ calculations. We have what I would consider "adequate" physico-chemical data on which to estimate fate and trophic level exposure *if* we are willing to assume little or no biodegradation through metabolism at any (every) trophic level. However, we clearly know this is not the case for many of these chemicals. The conservative modeling approach is to assume no appreciable losses at any given level of the food chain. The application of BSAFs and BAFs derived from the literature are an improvement in realism, but are still subject to uncertainty depending on the difference between the trophic structure of the system on which the BSAFs/BAFs are derived versus the system in which these factors are being applied. As pointed out in the GLI, the strongest application is where site specific BSAFs and BAFs are available.

In addition to the complexity of estimating general exposure to a large suite of chemicals, there is also the uncertainty introduced by assuming that the internal kinetics and dynamics of these chemicals are the same among species (and across the chemicals). For example, there is data to suggest that the ratio of the whole fish or muscle tissue concentration to the estimated gonadal tissue concentration of TCDD in trout is approximately 3. Do we really know how valid this assumption is for all the other Ahr agonists?

2. How much uncertainty is introduced because TEFs are often derived from exposure routes that do not simulate realistic exposure and tissue deposition? Given all the other areas of uncertainty and other data gaps, I don't believe this is an area of great concern. While I have often wondered about whether slow accumulation over long periods of time (for example over the lifespan of a lake trout) leads to significant tissue pools (or "compartments") which are not bioavailable and effectively "sequestered", I haven't seen any data which address this.

3. Does the TEF approach require that the analytical data be more rigorous than with aggregated measures like total PCBs? Clearly the TEF approach requires very specific data, and the concentrations of many of the analytes are often close to the limits of detection. In addition, in most cases relative amounts of the various analytes varies over several decades of concentration. And finally, the TEFs themselves vary over several orders of magnitude. The net effect is that the very low concentration, high potency, analytes generally contribute most to the TEQ calculation. Because these low concentration analytes are often the least certain from a general analytical standpoint, a more rigorous analytical design is generally necessary. All of the standard QA/QC rigor associated with stable isotope spiking, blanks, etc. etc. become paramount.

IV. RISK CHARACTERIZATION

1. Are the TEFs the dominant source of uncertainty in these assessments? I'm not expert in uncertainty analyses, but would guess that the uncertainties in the TEFs are of similar magnitude to the other uncertainties.

2. Should cellular assays of TEQ content in extracts be used to make these assessments? These assays can provide valuable data, particularly for screening purposes. They have high throughput, are standardizable, and are relatively simple. However, because they are subject to potential interferences (depending on how "refined" the extract is), they are best utilized as an exploratory tool. When a full and specific assessment is required, the specific analytical data should be used.

ADDITIONAL QUESTIONS RELATIVE TO THE EXPOSURE PROFILE OF THE PROSPECTIVE CASE STUDY.

1. Will site specific $BAF_{w,fd}$s from Roundtail Lake improve the accuracy of the allowable loadings over that of the $BAF_{w,fd}$s used from the GLWQG? Because they take into acount site specific differences in trophic structure (and hence trophic transfer) and bioavailability, the allowable concentrations derived from site specific data would certainly be more "accurate" for that system—accuracy relative to the models being used. From a risk management perspective this implies that the calculations would be more certain in preventing adverse impacts. However, based on the information provided about Roundtail Lake, and the nature of the Lake Ontario data on which the GLWQG is derived, I would not anticipate that the differences between the two would be large.

2. In estimating the WQS for TCDD based on estimated exposure and effects risks to bald eagles and river otters, the state appears to have used only the BAFs/BSAFs etc. relevant to TCDD (i.e. the calculation assumes that the practical bioaccumulation potential for all the Ah_r agonists of relevance in the assessment will be the same for all compartments of the Roundtail Lake system). According to the data in the GLWCG document, the Bioaccumulation Equivalency Factors for most of the other PCDDs and PCDFs are signifantly less than one—i.e. much less "practically bioaccumulative" than TCDD. Therefore, assuming that all materials will have bioavailabilities and bioaccumulative properties similar to TCDD appears to be significantly conservative. The assessment of the potential current risks from PCBs is an indication of the potentially overly conservative nature of this calculation.

3. I believe the assessment makes a prudent choice in selecting TE_qTMDL estimated from the fish data. As stated in my response to an earlier question, the best data set we have for relative TEFs is the fish data set. The bird data set is not sufficiently robust yet. Since we know very little about the River Otter, and it's clear that there is a very large degree of difference among the mammals in sensitivity to dioxins, it is difficult to say exactly just how conservative (or overly conservative) the numbers might be. While I do not dispute the logic and the scientific underpinnings to the calculations that have been used to derive the wildlife values of approximately 3 fg/l, I remain eager to see an example where this calculation has been supported by field data. It is difficult to imagine that it is not significantly overly conservative.

One additional comment on the Prospective study. I believe it is appropriate to consider the form in which the allowable TEqTMDL enters Roundtail Lake via the discharge. If we assume that much of the measured mass loading that is contained in an effluent (particularly a pulp mill effluent) enters in a form that is largely already associated with organic material, have these assessments adequately attempted to account for the possibility that most of the mass of the material will never become freely dissolved or otherwise bioavailable. The models that are used generally assume that whatever is discharged is all discharged in a freely dissolved form, is instantaneously well mixed throughout the system, and then partitions to equilibrium (again instantaneously) based on affinities for organic carbon (living or dead). I contend that while this approach is in many ways "necessary", it is likely a very conservative one from an exposure standpoint. Since there are so many other uncertainties, should this source of conservatism (i.e uncertainty) also be articulated and dealt with? Is it reasonable to propose that a significant amount of the mass flux of material that would be associated with a pulp mill never partitions to a bioavailable form?

I think the 503 regulations for sludge application to land are a good example of an attempt to deal with estimating allowable loadings taking into account the form and availability of chemicals, in this case metals, as they enter the environment. The analyses that did not take this into account, i.e. the ones that tried to establish acceptable loadings based on total metal concentrations, ended up producing estimates of acceptable loading that were low and impractical. My point here is that the form of entry of the chemical, particularly ones that are poorly soluble and carbon reactive, can potentially be an important factor to take into account when trying to estimate "acceptable" loadings.

ADDITIONAL QUESTIONS ON THE RETROSPECTIVE CASE STUDY.

1. Pardon my sarcasm, but is this a rhetorical question? The answer has direct parallels with the calculated WQS' in the prospective study. In that example, the values were different for the three vertebrates groups, the fish, the birds and the bald eagles. This is expected because of the input values that go into the derivation; the variable TEFs and the variable BAFs, BSAFs and FCMs for each group. The common convention for risk managers is to use the lowest, ostensibly most restrictive, value. However, this is generally judged against the amount and quality of the input data relative to the estimated risk and protection goals. In the case of the WQS derived to protect the otter in the prospective study, I assume from the information given that it was considered prudent, given the very low number, to get additional data to better understand the exposure of otters in that system.

2. No, because the TEF/TEQ based sediment goals are based on chemicals which have a potency/unit mass (or mole) which is much higher than for total PCBs. I interpret the rest of this question to be "Can total PCBs, or any other co-occuring contaminant with similar properties, be used as a proxy measure for TEQs for determining the progress of a remediation attempt? Unless you believe that the PCDDs, PCDFs and coplanar PCBs will behave differentially to the bulk PCBs during the clean-up process, such that the ratio of these compounds to the bulk total will change, then I can't think of a reason why you couldn't use totals to monitor. I would presume that a confirmatory analysis would be done to confirm that the TEQ clean-up goal had been met.

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Workshop on the Application of
2,3,7,8-TCDD Toxicity Equivalency Factors (TEFs)
to Fish and Wildlife

Pre-meeting comments

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I. **Derivation of specific TEF values.**

1. *Does the additional information provided enhance the evaluation of uncertainties in the assessments?*

Documents such as the draft WHO report identify some of the data gaps, sources of variability and uncertainty, and possible shortcomings of the TEFs used and thus are helpful in evaluating uncertainties in the assessments. Rather than the many papers and reports provided, a single document summarizing all the TEF values in the literature might be more useful. Such a document could also review the major sources of uncertainty and perhaps even provide estimates of the magnitude of each.

2. *Should all TEFs be considered to have similar uncertainties?*

Theoretically, the degree of uncertainty associated with each "consensus TEF" should be compound-specific. This is because certain compounds may be more strongly affected by the variables that lead to uncertainty. For example, a compound that is broadly resistant to metabolism may show less variability (uncertainty) in TEF values obtained in different systems than a compound that exhibits differential metabolism among systems. Similarly, some compounds (e.g. 2,3,7,8-PCDDs) will have high affinity for the AHR in most species, while for other compounds (e.g. mono-ortho-PCBs) there may be substantial species-specific variation in their ability to bind the AHR.

Whether this is true can be evaluated by looking at the range of TEF values for each compound in a variety of systems. As an example, Figure 1 shows a comparison of all published relative

potency values for HAH in fish. This graph appears to illustrate different degrees of variability in the estimates, indicating different degrees of uncertainty depending on the compound chosen.

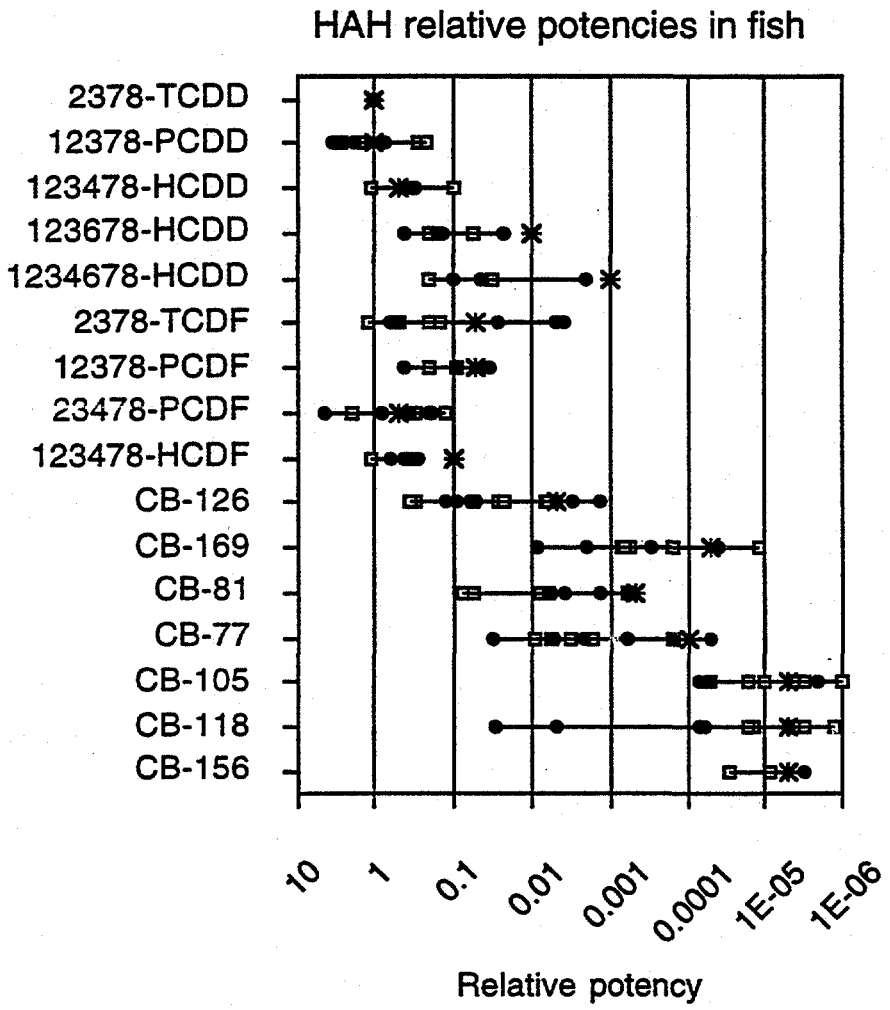

Figure 1. A comparison of all published relative potency values for HAH in fish. Data were obtained from *in vivo* studies (filled circles, references (1-8)) and *in vitro* studies (open squares, references (9-14)). The values for mono-ortho-PCBs (105, 118, 156) include "upper bound" estimates; in general responses with these congeners are minimal or absent. Asterisks indicate the "consensus Fish TEFs" used in the risk assessment scenarios.

3. *To what extent can the endpoints used for TEF determination be extrapolated to endpoint(s) of concern ("Measures of effect" or "Assessment endpoints")?*

Obviously, the goal should be to determine TEF values using the endpoints (and species) of concern—for example larval mortality or reproductive success in lake trout or Caspian terns in the retrospective scenario. If this is not possible, then one should choose the endpoints that are as closely related mechanistically as possible to the endpoint of concern. One might then look at the

relationship between the chosen TEF endpoint and the "endpoint of concern" in other systems where it is known, and attempt to quantify or predict the degree of uncertainty introduced by using the surrogate endpoint.

With regard to the use of CYP1A induction, there are often some misconceptions about the relationship between this biochemical response and toxicity. Although the mechanism of toxicity of dioxin-like compounds is not completely known, available evidence suggests that it involves changes in the expression of genes involved in the regulation of cell growth and differentiation. CYP1A induction is relevant as an endpoint for TEF determination for two reasons.

a) In a general sense, induction of CYP1A occurs in parallel with the changes in gene expression that are responsible for dioxin toxicity. CYP1A induction signals activation of the Ah receptor (AHR), which is the common initial step in toxicity. In this way, CYP1A induction is a surrogate for toxicity.

b) In addition to acting as a surrogate for AHR-dependent toxicity, induction of CYP1A can also be directly responsible for some forms of toxicity. This may occur through the generation of reactive oxygen species, for example. Such a mechanism could be important for some endpoints of concern, such as cardiovascular toxicity involved in early-life stage mortality in fish (15-17).

The correlation between potency to induce CYP1A and toxic potency is often strong (e.g. 18, 19), but is not perfect. CYP1A induction is usually measured as an acute effect, whereas effects of concern may occur only after chronic or subchronic exposure. Thus, some compounds may induce CYP1A acutely but—because of rapid metabolism, for example (e.g. PAH)—may not produce the sustained activation of the AHR that appears to be important for toxicity (20, 21).

Another endpoint that is sometimes considered for TEF determination is the accumulation of highly carboxylated porphyrins. This effect is AHR-dependent and also appears to be linked mechanistically to induction of CYP1A. However, it also appears to involve two additional steps—induction of aminolevulinic acid synthase and binding of HAH to the induced CYP1A (12, 22, 23)—that complicate the determination or interpretation of relative porphyrogenic potencies.

II. Application of the TEQ Approach

1. *What are the implications of assuming no dose-additivity (??) or no interaction among the components of mixtures? How would risk assessment conclusions differ if analyses were based on total PCBs or TCDD alone?*

?

2. *Should TEF values derived using median response levels (LC50 or EC50) be used in risk assessments where a "no adverse effect" level is being employed?*

According to receptor theory, the relative potencies for full agonists should be independent of the location on the dose-response curve where effects measurements are made. In the real world, parallel dose-response curves are not always seen because of a) antagonism and partial agonism, and, b) artifacts introduced by additional phenomena such as enzyme inhibition.

(a) **Partial agonism** occurs in situations where there are differences in the *intrinsic efficacy* of compounds and where other factors (such as receptor number) are such that compounds with lower intrinsic efficacies are incapable of producing the same maximal tissue response as compounds with higher intrinsic efficacies (e.g. see reference 24). (Intrinsic efficacy refers to the inherent property of a chemical that determines the activity of the chemical-receptor complex (24, 25). Intrinsic efficacy is distinct from affinity, which is the probability of a chemical binding to the receptor.) There is evidence for partial agonism of some PCB congeners in some systems (26). Because of their lower intrinsic efficacy, partial agonists will antagonize full agonists under certain conditions (25).

(b) Compounds may *appear* to be partial agonists or have non-parallel dose-response curves as a result of secondary effects on the endpoint measured. For example, in some systems compounds that induce CYP1A protein can also bind to and inhibit the activity of the enzyme (27). This inhibition will result in reduced levels of maximally-induced CYP1A activity (EROD) and an underestimate of the EC50 for CYP1A induction. This will lead to an overestimate of relative potency (TEF) values (11, 13, 19).

For risk assessments in which a "no adverse effect level" is being employed, it may make sense to use TEFs derived from lower level responses to avoid the potential problems discussed above. Such lower response levels might include "threshold responses" (6), initial slopes ("slope-ratio" methods) (28), or EC values based on 25% (29) or 10% (19) of the maximal response caused by TCDD.

3. *To what extent can class-specific TEFs be used?*

Whether "class-specific TEFs" exist is an important question. There seem to be some differences that are characteristic of a vertebrate class (e.g. low activity of mono-*ortho* PCBs in fish) but there has not yet been a systematic attempt to compare within-class and among-class variability in relative potencies. It might be possible to address this question by determining relative potencies of several HAHs in several species in each of several vertebrate classes, and then using multivariate statistical techniques to evaluate the within-class and among-class patterns.

IV. Risk Characterization

1. *Are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments?*

It would be useful to quantify the degree of uncertainty associated with each step of the risk assessment process, and then to focus on the steps for which the uncertainties are greatest.

2. *Strengths and weaknesses of biologically based TEQ assays? Integration?*

The strengths of bioassays for determining TEQs include: a) relatively low cost, b) the response integrates additive effects plus any non-additive interactions that may occur between components of mixtures, c) the responses reflect the presence of all AHR agonists, including compounds that may not have been identified by chemical analysis. For acute bioassays, and depending on the source of the extract (e.g. sediment vs. tissue), a possible disadvantage is that rapidly metabolized compounds such as PAH may contribute more significantly to the bioassay response than they would likely contribute to toxicity in the target species. Because of the advantages inherent in each approach—i.e. bioassay-derived TEQs and TEQs calculated from chemical data and TEF values—a combined approach is desirable.

3. *Additional data or research for use in the risk assessments?*

Species-specific TEF values and relative sensitivities for the species of concern, i.e. bull trout, river otter, bald eagle, Caspian terns would be helpful. It is important to characterize both the relative potencies of HAHs (TEFs) as well as the absolute "dioxin sensitivity" of the target species relative to species used to determine the levels of concern (e.g. no-effect thresholds). For example, in the retrospective scenario , risk assessment for Caspian terns uses TEFs (and no-effect thresholds?) based on chicken data. Common terns are approximately 80-fold less sensitive to TCDD than chickens (based on EROD induction in embryo hepatocyte cultures (30)) and exhibit different TEF values (e.g. for PCB-126, which drives the TEQ in this assessment).

In both scenarios, it would be useful to have long-term data on population structure, productivity, etc. for the species of concern so that possible population-level effects of current chemical burdens (in the retrospective study) or future increases (in the prospective study) could be evaluated. For example, in the retrospective scenario, TEQ levels in Caspian tern eggs are well above the level of concern established using data from other species of birds. What is the reproductive success of Caspian terns at this site now, in comparison to past success at this site and current success at less contaminated sites?

Retrospective Case Study

2. *Use of total PCB analyses to monitor cleanup efforts?*

Total PCB levels could provide a useful surrogate for TEQs in monitoring cleanup. However, to the extent that (a) PCB congener composition or (b) concentrations of PCBs relative to other HAH classes change with time or depth in the sediment, the ratio of total PCB to total TEQ could change. Why not use bioassay-derived TEQs to monitor cleanup?

Miscellaneous comments on WHO (1997) Draft Report on Derivation of TEFs for humans and wildlife

1. An important question is raised in this document (p. 9-10): To what extent do relative potencies for lethality mirror relative potencies for sublethal effects? Direct comparisons of lethal and sublethal endpoints are scarce. In mammals, the huge difference in TCDD LD50 values (guinea pig 1 ug/kg to hamster 5000 ug/kg) is not necessarily reflected to the same extent in potencies for sublethal effects (e.g. see ref. 31). In birds, the correlation may be stronger (19).

2. With regard to the molecular basis for TEFs across species, it is noted that homologs of the AHR and ARNT exist in the nematode *C. elegans*. These homologs have not yet been isolated, but are predicted based on computer-predicted coding regions (exon structure) of genomic DNA sequences (32). However, in the putative *C. elegans* AHR, the "PAS-B domain", which has been associated with ligand-binding in the mammalian AHR, is not well conserved (32). Based on this, it has been hypothesized (32) that the ligand-binding characteristics of the *C. elegans* AHR homolog may be different than those of vertebrate AHRs.

An additional complication in understanding the molecular basis of dioxin action is the identification of a second AHR in fish (32) and mammals (33). The functions of the second AHR, including its ligand-binding properties, are not yet known.

In the discussion of the species differences in the AHR (p. 28), it should be noted that an AHR gene has been identified in lamprey as well as in cartilaginous and bony fish (32). Interestingly, however, adult lamprey appear to be non-responsive to AHR agonists, as CYP1A is not inducible in lamprey treated with 3,3',4,4'-tetrachlorobiphenyl (34). The comparative biochemistry and molecular biology of the AHR has been reviewed recently (35).

3. Antagonistic effects (pp. 36-37). According to receptor theory, antagonistic properties do not result from differences in receptor-binding *affinity*, but rather from differences in *intrinsic efficacy* (see discussion above). This is an important distinction because it means that low-affinity compounds will not necessarily act as AHR antagonists. But compounds with lower intrinsic efficacies may act as partial agonists, and partial agonists will antagonize full agonists under certain conditions (25). In addition, because receptor number influences whether

compounds with lower intrinsic efficacy will act as full or partial agonists, there will be tissue- and species- differences in antagonistic properties of a given chemical.

4. Hexachlorobenzene (p. 41) is a low-affinity AHR agonist in rat (36). It has a relative potency (in rat) of approximately 0.0001 based on receptor-binding affinity and approximately 0.0005 based on porphyrogenicity (36). HCB has a similar relative potency of approximately 0.0001 for EROD induction and uroporphyrin accumulation in chicken embryo hepatocytes (23).

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I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. Does additional information on TEFs enhance the means of evaluating uncertainties in the assessements?

To answer this question, I examined Tables 1 and 2 in the Retrospective Scenerio. While there are some changes (e.g. fish TEQ1 for PCB 77 is 0.031 and fish TEQ2 is 0.62), such differences have very little effect on the relative contributions of total PCBs, total PCDDs or total PCDFs to total TEQ concentrations. Therefore, I do not think that the additional information is valuable, particularly when one considers all of the other uncertainties which go into a risk assessment.

2. Should all TEFs be considered to have similar uncertainties?

I do not think that all TEFs should be considered to have similar uncertainties. The WHO meeting in Stockholm established the use of a tiered approach for ranking studies from which TEFs could be derived. I think this approach is reasonable, and TEFs obtained from *in vivo* studies should be (and were, at the WHO meeting) ranked higher than other types of studies. For example, in fish, TEF values that are based on mortality following egg injections are more likely to be "accurate" (I use the word "accurate" to mean that they are more likely to be predictive of the relative potency *in ovo* than are values obtained from *in vitro* studies or from methods that use QSAR. This statement is not meant to indicate that *in vitro* and/or QSAR derived TEFs are of no value - they certainly are. They can be particularly useful for helping one decide what *in vivo* studies are required. For example, several studies with avian hepatocytes have shown TCDF to be either equipotent or more potent than TCDD at inducing EROD activity. For the time-being, a TEF for TCDF in birds of 1.0 seems reasonable, but *in vivo* studies are warranted to test this prediction.

TEFs that were derived from several studies (rare for fish and birds, common for mammals) should be considered to have less uncertainty than TEF values obtained from single studies.

3. To what extent can different types of endpoints that were used to derive TEFs be extrapolated to effects that are relevant for the assessment endpoint for each case study?

Retrospective Scenario:

In general, one should be cautious when using TEFs that have only been derived from *in vitro* biochemical responses. However, it should be noted that compounds which contribute the most to total TEQ concentrations (see below) have been tested for overt toxicity *in ovo* both in fish and birds (albeit in a limited number of species).

Fish Approximately 93% of the total TEQ concentrations in both shiners and lake trout was obtained from the following compounds: PCB 126, 1278-TCDD, 12378-PCDD, 2378-TCDF, 1,2,3,7,8-PCDF and 23478-PCDF. The TEFs for all of the these compounds were obtained from studies which determined mortality in rainbow trout following injection of compound into the egg (ie. a Tier 1 study). In my opinion, the total TEQ is highly relevant to the assessment endpoint of interest, despite the fact that lethality-based TEFs have, to date, only been reported in one species of fish.

Birds Approximately 85% of the total TEQ concentration in Caspian tern eggs was obtained from the following compounds: PCB 77, PCB 126 and PCB 105. TEFs for all of these compounds were derived from egg injection studies which measured lethality. In my opinion, the total TEQ is highly relevant to the assessment endpoints of interest for birds. Despite the fact that TEFs for many other compounds were only obtained from studies that measured either EROD induction *in ovo* or in cultured hepatocytes ,or were from QSAR estimates, these compounds contribute, in total, only approximately 15% to the total TEQ.

Mammals For mammals, the TEFs for most of the compounds were derived from several studies, and TEQ estimates are likely to be relevant.

It should also be noted, that there are generally quite good correlations between relative potencies of compounds as EROD inducers and their respective toxic potencies (as long as one considers some of the "problems" with *in vitro* assays - such as differences in efficacy and

metabolism - see Bastien and Kennedy, Organohalogen Compounds (1997) **34**, 215 - 220. *In vitro* derived REPs can be very useful for predicting *in vivo* TEFs.

Prospective Scenario:

Above comments for the Retrospective Scenario are of relevance for this case.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. What are the implications of assuming no dose-additivity or no interaction among the compounds? To what extent would the risk assessment conclusions differ if the stressor response analyses were based on total PCBs or 2,3,7,8-TCDD alone?

In general, is dose-additivity is not assumed, then the risk assessments need to be based solely on TCDD and total PCBs. In the following, differences between using TCDD and total PCBs vs. the TEQ approach is examined. No-effect thresholds indicated in Table 5 of the Retrospective Scenario were used in all cases.

Retrospective Scenerio:

Shiners and Lake Trout

The concentration of TCDD in shiners and lake trout is much lower (230-fold and 55-fold, respectively) than the no-effect threshold for fish (30 pg/g). PCB concentrations in these species of fish are 14-fold and 5-fold lower than the no effect level (5 ug/g), shiners and lake trout, respectively. However total TEQ concentrations of 1.3 pg/g and 4.2 pg/g in shiners and lake trout, respectively are at a level which approaches levels which might be expected to have some effect in sensitive species (eg. lake trout). I say this because the lowest value for no-effect threshold in fish indicated in table 5 is 3 pg/g. PCB 126 and two PCDFs are major contributors to total TEQ concentration. It should be noted, however, that 4.2 pg TEQ/egg is much lower than the reported LD50 for TCDD in lake trout (74 pg/g).

Caspian Tern

The concentration of total PCBs in Caspian tern eggs is 5.7 ug/g, which is higher than the no-effect threshold for birds indicated in table 5 (1-20 ug/g). Thus, sensitive species might be expected to have some effects using a risk assessment that is based on total PCBs alone. A risk assessment that used TCDD alone would conclude that levels of TCDD were much below the no-effect level (concentration of TCDD is 4.5 pg/g and the the no-effect threshold is 100 pg/g). In contrast, if one were to use the TEQ approach, the total TEQ concentration (426 pg/g) exceed the no-effect theshold of 100 pg/g by approximately 4-fold. Thus, the TEQ approach certainly indicates more reason for concern than does a risk assessment that is based on TCDD alone. In addition, the TEQ approach might indicate more reason for concern than would an assessment that is based on total PCBs.

Otter

The concentration of TCDD in otter liver of 1.4 pg/g is much lower than the no-effect level indicated in table 5 for mammals (60 pg/g). The concentration of total PCBs of 1 ug/g is approximately ½ of the no-effect level (2 ug/g). However when the TEQ approach is used, a much different conclusion is reached. The total TEQ concentration is 144 pg/g, which is higher than the no-effect threshold of 60 pg/g. PCB 126 contributes the most to the total TEQ.

Prospective Scenario:

I did not to make the type of detailed analysis for this that I did above for the Retrospective Scenario because residue levels in eggs were not given - my reasoning would be the same, however.

2. To what extent should TEF values derived at median response levels be used in risk assessments where no adverse effect level is being employed?

I not see a serious problem at all with using LC50 or EC50 values (assuming one carefully considers, and accounts for situations where compounds are not full agonists at eliciting a particular effect; eg. EROD induction, in some cases).

3. To what extent can class-specific TEFs be directly extrapolated to the species identified in each case study?

Given that only a limited number of species have been tested, one cannot be absolutely certain. However, in my opinion, large errors are not likely to be made. For example, PCB 126 has been assigned a TEF of 0.1 in birds, based on Tier 1 studies with chickens. REPs for PCB 126 as an EROD inducer have been determined to be very close to this TEF in hepatocyte cultures prepared from a large number of avian species. My conclusion, is that the TEF of 0.1 for PCB 126 is likely to be reasonable across avian species, including those of interest in the present scenarios. Further studies are required to determine relative potencies of compounds in different species of fish, but the values derived from egg injection studies in rainbow trout are likely to be relevant to fish in general (based on studies we and others are seeing in hepatocyte cultures) and, almost certainly, to be relevant to bull trout (Prospective Scenario).

III. EXPOSURE PROFILE

1. To what extent does the TEF approach present challenges, introduce new uncertainties, or modify old uncertainties with modeling exposure to AhR agonists?

The modeling of exposure to contaminants, including AhR agonists is beyond my area of expertise. However, based on the data provided in Table 1 in the document entitled, "Charge Questions and Physico-Chemical Properties Table", it would seem that BAFs have come from a very limited number of studies, and I would question how reliable these are across species. In addition, one needs to have a lot of information on feeding patterns of the species being studied.

2. To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?

For fish and birds, the exposure route used to derive TEFs for the most important contributors to total TEQs (see above) was from egg injections. It is my opinion that these values of definitely of relevance to the species of interest in the case studies. Values for mammals are also relevant.

3. To what extent does the TEF approach require a more rigorous analytical design?

The TEF approach requires the measurement of dibenzo-*p*-dioxins, dibenzo furans and non-ortho PCBs by GC-MS. This increases the analytical costs over the costs of total PCBs.

IV. RISK CHARACTERIZATION

1. Are the uncertainties associated with TEFs more problematic than other uncertainties of the risk assessments?

In my opinion, the uncertainties associated with TEFs are no more problematic than other uncertainties which are associated with the risk assessment for the tow scenarios.

2. What are the strengths and weakness of using biologically-based TEQ assays, and to what extent could these approaches be integrated?

Biologically-based TEQ assays (I refer her to *in vitro* assays) have the advantage of measuring the integrated effects of complex mixtures of Ah receptor agonists. In addition, such assays have the potential of identifying compounds that act via the Ah receptor, which would not be identified by a chemical residue approach measuring only dioxins, furans and PCBs. Some of these assays are considerably less expensive than chemical residue analysis (particularly where measurement of dioxins, furans and no-ortho substituted PCBs is required).

One potential problem with such *in vitro* assays is that they can over estimate the toxic potency of compounds which are rapidly metabolized *in vivo* (eg. PCB 77) but recent research has shown that such problems can likely be circumvented. For example, Bastien and Kennedy (Organohalogen Compounds (1997) **34**, 215-220) and others have reported that the REPs of rapidly metabolized compounds are dependent on the length of time between the addition of the compounds to the cells and analysis. Thus, various bioassays under development have considerable potential for predicting TEQs which are relevant to whole organisms. For the two case scenarios, I would recommend the incorporation of *in vitro* bioassays.

compounds to the cells and analysis. Thus, various bioassays under development have considerable potential for predicting TEQs which are relevant to whole organisms. For the two case scenarios, I would recommend the incorporation of *in vitro* bioassays.

I would recommend incorporation of at least one *in vitro* bioassay into the risk assessments for both scenarios. This might either be the H4IIE bioassay or an assay which uses a reporter gene. In addition, I would consider using primary hepatocyte cultures for species of interest (eg. Caspian tern and bull trout). Such methods can be very useful in predicting the sensitivity of species of concern to complex mixtures of compounds that elicit effects which are mediated by the Ah receptor.

3. What additional research do you recommend?

I would recommend incorporation of a study that would include the addition of extracts from soil and tissues to hepatocytes cultures prepared from species of concern. For example, this could be done for the bull trout in the Prospective Scenario and for Caspian terns for the Retrospective Scenario. Such methods are now routine in my laboratory and others, and show considerable promise for risk assessment purposes. If bull trout cannot be obtained from any location (due to their endangered status) , then rainbow and/or Lake trout could be used. A small number (approximately 10 eggs) of Caspian tern eggs could be obtained from another location, incubated, and primary hepatocyte cultures could be prepared. The advantage of doing such studies is that one can obtain important information regarding species sensitivity to complex mixtures of compounds that elicit effects which are mediated by the Ah receptor which would not be identified by the chemical-based TEQ approach.

I would also recommend inclusion of other biologically-based TEQ (eg. H4IIE, CALUX) assays into the assessments.

Additional Questions

Prospective Case Study:

The questions asked here go beyond my area of expertise.

Retrospective Case Study

Sediment cleanup goals would be the same for birds and mammals since PCBs are, by far, the most important contributors to total TEQ concentrations in these taxa. However, in fish, PCDFs are major contributors. Total PCBs could be used to monitor the results of clean-up efforts providing a good correlations were found between major PCB congeners and the following: PCDFs, TCDD and PCB 126.

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Dr. Landis's expertise is in environmental toxicology, ecological risk assessment, and population biology. He holds a bachelor's degree in biology from Wake Forest University, a master's in biology from Indiana University, and a doctorate in zoology from Indiana University. He is currently director and professor at the Institute of Environmental Toxicology and Chemistry at Huxley College, Western Washington University. He belongs to six professional societies, including SETAC, ASTM, Sigma Xi, and the Genetics Society of America. Dr. Landis is currently the principal investigator in a project entitled "Novel Models for the Evaluation and Interpretation of Ecological Datasets Applied to the Ecological Risk Assessment of Biotechnological Products" funded by EPA. His current research includes regional risk assessment, the application of metapopulation dynamics in estimating the impacts of toxicants, and microcosm/mesocosm research. Dr. Landis also is co-developer of the Community Conditioning hypothesis, a non-equilibrium description of the impacts of toxicants to populations and communities. He serves on a variety of advisory committees.

Workshop on the Application of 2,3,7,8 -TCDD toxicity Equivalency Factors (TEFs) to Fish and Wildlife

Answers to Charge Questions

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General comments:

The assessment of compounds that have modes of action similar to that of TCDD and yet also have estrogenic type interactions is challenging. Many of my comments that are specific to the charge questions are based on two factors. First, the risk assessment process stated here is based on the derivations of LC50s or NOELs (no observed effects levels), methods that misrepresent the toxicity of the compounds. Second, in both case examples, the risk assessments are purely toxicological not ecological.

In my reading of the material supplied to us only one paper, the draft by Elonen et al, used the dose-response curves in order to judge the relative toxicity of TCDD to a group of organisms. The other papers used a median lethal dose, a no-effects level or a lowest observed level to compare toxicity. The failings of the NOEC and LOEC (lowest observed effect level) approaches have been discussed (Stephan and Rodgers 1985, Chapman et al. 1996, Chapman and Chapman 1997) although debate continues (Dhaliwal et al 1997). A summary of the problems of NOECs and LOECs can be also found in Landis and Yu (1995).

Basically, NOECs and LOECs are artifacts of the hypothesis testing process and the concentrations selected by the researcher. While they may be of some interest within a set of experiments conducted under identical conditions with similar experimental variance, replication and statistical power, they can not be compared in a strict sense between laboratories or species because the statistical power of the experiments change. As the statistical power changes so does the results of the NOEC and LOEC. As the statistical power decreases, the NOEC and LOEC will increase even without a real change in the concentration-response curve.

An alternative approach using regression techniques and curve fitting have been proposed (Stephan and Rodgers 1985, Moore and Caux 1997). Specific points along this curve can then be compared (an EC_x) in order to determine relative potencies at concentrations that correspond with acceptable effects. In this instance we can compare numbers with similar units. The uncertainty in the comparisons can also be quantified since the error in the estimates will also be available. This is a much better situation than comparing statistical artifacts.

The second failing of the ecological risk assessments provided to us as examples is that they are still toxicological assessments. Only direct toxicity is considered, as is appropriate for determining effects upon a particular receptor. However, the goals of these example assessments is to attain the same fish populations as before. Oneofakind lake has depressed fish and tern populations. It is claimed to have healthy pelagic and benthic invertebrate communities, but since health is undefinable ecologically I have no idea what this means. Roundtail lake has seen the introduction of mysids that have drastically altered the food web and the bull trout populations. The introduction of paper mill effluent would constitute another stressor, with the impacts partially controlled by the other introductions. Observed alterations in the fish dynamics could be due to historical impacts, the rates of migration due to landscape structure, or the toxicity of the effluent.

Reference:

Caux, P-Y and D. R. J. Moore. 1997. A spreadsheet program for estimating low toxic effects. *Environ. Toxicol. Chem.* **16**:802-806.

Chapman, P. M., R. S. Caldwell and P. F. Chapman. 1996. A warning: NOECs are inappropriate for regulatory use. *Environ. Toxicol. Chem.* **15**:77-79.

Chapman, P. F. and P. M. Chapman. 1997. Author's reply: *Environ. Toxicol. Chem.* **16**:125-126.

Dhaliwal, B. S., R. J. Dolan, C. W. Batts, J. M. Kelly, R. W. Smith, S. Johnson. 1997. Warning: Replacing NOECs with point estimates may not solve regulatory contradictions. *Environ. Toxicol. Chem.* **16**:124-125.

Landis, W. G. and M.- H. Yu. 1995. *An Introduction to Environmental Toxicology: Impacts of Chemicals on Ecological Systems.* Lewis Publishing, Boca Raton, FL.

Moore, D. R. J. and P-Y Caux. 1997. Estimating low toxic effects. *Environ. Toxicol. Chem.* **16**:764-801.

Stephan, C. E. and J. R. Rodgers. 1985. Advantages of using regression analysis to calculate results of chronic toxicity tests. In *Aquatic Toxicology and Hazard Assessment: Eighth Symposium.* R.C. Bahner and D.J.H. Hansen, eds., American Society for Testing and Materials, Philadelphia, pp. 328-339.

I. Stress-Response profile relative to the derivation of specific TEF values.

3, The TEF values provided were based on endpoints that ranged from in vitro biochemical responses to in vivo early life stage mortality. To what extent can these endpoints be extrapolated to the measures of the effects that are relevant for the assessment endpoint for each case study?

The more the test is run under conditions similar to the exposure in the field, the easier and more confident the extrapolation. Biochemical responses observed from in vitro tests are more like bioassays for exposure to specific concentrations than indications of toxicity. Early life stage mortality tests are more useful, but rarely does the dosing correspond to situations typical of the field. Each test allows more confidence in the prediction, and the greater the number of endpoints measured the better the characterization of concentration effects. However, laboratory tests can not take the place of properly designed field studies or taking advantage of natural experiments (spills, prior contamination etc.).

II. Stress-Response profile relative to the application of the TEQ approach.

2. Many TEFs are based on LC50 or EC50 values. To what extent should TEF values derived at a median response level be used in risk assessments where no adverse effect level is being employed?

In keeping with my introductory comments, the use of LC50 and EC50 values is inappropriate, but no more than the use of a no adverse effect level for the risk assessment. The use of the LC50 and EC50 for TEFs uses a part of the concentration-response curve that is of relatively little interest for the protection of ecological endpoints. No adverse effect level is a statistical artifact at best, at worst it is trying to prove a negative and that can not be accomplished scientifically. A more appropriate alternative would be to settle on an acceptable effect, even one as small as an EC10. Then use the EC10 values from the LC50 or EC50 data to calculate the TEFs. Once the risk assessment goal is quantified, then the appropriate endpoints for the computation of the TEFs is trivial.

The concentration-response curves illustrated in the Elonen et al. manuscript demonstrate the variability of the slopes. Given the same EC50, the compound with the shallowest slope will have greater effects at lower concentrations.

3. The TEFs values provided were typically based on a single or limited number of mammal, bird, or fish experiments. To what extent can class-specific TEFs be directly extrapolated to the species identified within each case study?

In the Elonen et al. manuscript (Table 5), the range of $LC_{egg}10$ and $LC_{egg}50$ both have a five-fold range in toxicity for seven teleost fish. The data presented in Figure 3 show a twenty-five fold range from lowest to highest $LC_{egg}50$ values among the fish. Without comparable data for other Ah receptor compounds it is not possible to tell if the ratios between TCDD and other compounds shows a comparable interspecific variability. Do comparable data exist for the ratios and can that be used to examine the range of TEFs? Getting more data would answer that specific question, otherwise it simply is speculation.

III. Exposure profile

IV. Risk Characterization

1. In evaluating the case studies, are the uncertainties associated with the TEFs more problematic than other uncertainties of the risk assessments? Do the uncertainties associated with TEFs limit the means of performing the assessments, or do the other areas of the effect and exposure characterization contribute similar or greater levels of uncertainty?

Given the current methods of estimating the TEFs, reliance on NOECs and LC50 values, the uncertainty in the estimates of these values at realistic levels of impacts is high. Without the basic biological effects data, the basic yardstick by which to judge impact is uneven and bent. It is like measuring a centimeter with only a meter stick marked in meters. It does not matter than there is uncertainty is the other factors as much because they are not the yardstick by which impacts are measured.

2. Biologically-based TEQ assays on environmental samples could be employed as an alternative to the TEF-based approach. What would the strengths and weaknesses of such an approach be? To what extent could these approaches be integrated?

Data from well designed experiments from environmental samples is always a preferred approach for several reasons. 1) It provides data for sediments and water conditions that will be found at the site of interest. 2) Field work can provide a measure of the temporal and spatial heterogeneity of the environment and the fate and bioavailability of the contaminants. 3) Data from field samples can provide a measure of uncertainty provided by the laboratory studies and the TEF approach. 4) Site specific data forces the investigators to pay close attention to the site and reality instead of laboratory tests and models.

3. Assume that site-specific data or additional research could be gathered or performed to generated more information for the case study assessments. Provide a list of specific investigations/studies and rank them from highest to lowest priority. What is your rationale for the ranking?

Highest to lowest ranking; assuming that this is a prospective risk assessment.
1) Obtain as much data as possible on the spatial and temporal distributions of the species of interest, their supporting food web, and the organisms that alter the physical structure of the habitat. This information will eliminate a lot of the guesswork about exposure and population effects. Particularly important are data about other stressors, patch distribution and landscape form that may confound predicted impacts.

2) Simulate the dosing of the system using a model multispecies system that includes fish as a receptor. Have specific questions and predictions in mind to guide the experimental design. If the models and toxicity data can not effectively predict the risk to a model system there is little hope that it can predict risk to the ecological system of interest. It should also be possible to obtain correlations between biomarkers, reproductive success and population and community alterations that should allow the answering of so what type questions.

3) Get reliable concentration-response data that actually includes accurate estimates of effective levels of concern, not NOELs (not real) and LC50s (too high). For bioavailablity studies use sediments and water from the site of interest in order to gain site-specific data. These studies should allow the elimination of a great deal of the uncertainty in the toxicological and exposure aspects of the risk assessment.

Additional questions specific to the prospective case study:

1. The sate adopted BAFs used by the GLWOG. What improvement I the accuracy of the maximum allowable concentrations for individual congeners in water (MAC) can be expected through the use of BAFs determined from Roundtail lake data?

This is a crystal ball, not a scientific question. The accuracy in indeterminable without doing the experiment. The important fact is that it is the BAFs from the Roundtail lake data that should be the most relevant to a risk estimation since they can provide a range of values assisting in the quantification of the variance, and data on spatial and temporal variability. This type of data will not be available using model results. After all, models produce output, not data.

3. How should the uncertainties associated with the available fish, avian and mammalian TEFs be incorporated into decisions about which TCDD water quality standards should be chosen for setting a TEqTMDL for regulating chemical discharges into Roundtail Lake?

Tell me how much uncertainty the decision maker can live with. The uncertainties need to reported fairly and as accurately as possible. How the decision is made is more a political issue when such unspecified and indeterminable criteria such as no adverse effect are used.

Additional questions specific retrospective Case Study:

1. Would TEQ sediment cleanup goals be the same for each vertebrate group? If not, why would there be a difference? I the vertebrate group with the most certainty is not the group with the most restrictive sediment cleanup goal, how would you council the risk manager's concerns for the other vertebrate groups?

Of course the clean up goals will be different for each vertebrate group depending upon the route of exposure. Terrestrial mammals will be exposed in a very different fashion compared to sediment dwelling fish. Seed eating birds are likely to have little concern about sediment concentrations compared to fish eating birds. Reptiles and amphibians that burrow in the mud during parts of the year will have a direct exposure to the sediment for prolonged periods. Amphibians have to breed in the water, mammals and birds do not and so have different exposure routes and sensitive stages.

The second part of the question is amusing. For the most vertebrate groups are not represented by any toxicity data and when they are for only a few species. Given the lack of representation of the different vertebrates the level of uncertainty is going to relatively high no matter what. Considering the problems with estimates of exposure, lack of tissue data for most species, and the lack of truly comparative toxicology, I do not hold out much hope for reducing uncertainty for vertebrate groups, only the few well studied species.

How about uncertainty factors for extrapolation across vertebrate types? Considering the reported 25 fold difference in TCDD toxicity in teleost fish, how much more uncertainty is there between vertebrate groups. I suspect the answer is species specific given the precise mode of action of the TCDD and similar compounds. Very subtle alterations in biochemistry may give rise to big differences in realized toxicity in a largely stochastic fashion.

2. Would the TEF/TEQ-based sediment remediation goals be the same as those determined for total PCBs for the identical vertebrate class? Assume that a simple ratio of total PCB sediment concentration goal to TEQ sediment concentration goals was formulated to allow for the use of total PCBs to monitor cleanup efforts based on TEQs. What exposure and effect issues would need to be evaluated before using the less costly total PCB analysis to support the TEQ-based sediment remediation goal?

No, total PCBs are comprised of many compounds that work with very different modes of action compared to the TCDD like PCBs. The proportion of the various PCB types will be important in estimating the likely toxicity resulting from the mixture. Why not a TEF for estradiol mimics as well as TCDD mimics?

I am generally against clean up goals set on chemical concentration alone. Chemistry does not estimate toxicity very well, and when have been so caught up in numerical analytical goals that toxicity prevention can get lost.

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MEMORANDUM

TO: U.S. EPA TCDD TEF Workshop

FROM: L.S. McCarty

DATE: November 3, 1997

TOPIC: Answers to Questions/Issues for the Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors (TEFs) to Fish and Wildlife

CHARGE QUESTIONS AND PHYSICO-CHEMICAL PROPERTIES TABLE

I have an objection with a statement in the opening paragraph: "It is reasonable to assume that the proposed WHO TEFs are appropriate for risk assessments ... " I agree that this is the basis for the subsequent questions on refinements of the TEF approach to assessments beyond the screening stage, but do not believe that it is a universally agreed upon assumption for either the initial application or the refinements being considered by the workshop. In fact, it should be made clear that such an assumption clearly establishes this workshop as a policy-based exercise. The workshop is a means of obtaining the best professional judgement of scientific experts on how, in their opinion, to most suitably apply available but incomplete scientific facts to serve policy objectives. Without explicit clarification there is a danger that such deliberations may be perceived by many as being a purely scientific discussion when it is not. I both understand and support the general need for some degree of precautionary activity, but strongly object to dressing it up as science. Good, reasonable policy incorporates input from a variety of sources and does not need a scientific aura for respectability.

Rather than stating that it is reasonable, I believe that a list of the assumptions required to enable the TEF process to be used in risk assessment be presented, both for the screening and advanced cases. Any reader can then judge the degree of reasonableness for themselves. This is consistent with the concerns which prompted the Levin-Thompson bill currently under debate in the U.S. Senate. This bill illuminates the need for identification and clarification of both the scientific and policy basis of assumptions used in risk assessment. Such a separation of science and policy in should make the risk assessment process more transparent and understandable. The recent Presidential/Congressional Commission on Risk Assessment and Risk Management

(1997) has made a call for improved risk communication and a clear identification of science and policy aspects of the TEF approach would also contribute to achieving such a goal. I have also commented on the confusion of policy for science in risk assessment (Power and McCarty, 1997).

It is my opinion that the TEF approach as currently constituted is not sufficiently rigorous or comprehensive to be employed in other than screening level risk assessments for aquatic, avian, and mammalian wildlife. The approach represents a reasonably founded policy for screening that also serves as a useful guide for directing additional scientific research. However, the limitations and restrictions specified in the meeting description and charge questions represent little more than a detailed list summarizing why, at this time, it should not be used beyond an initial screening risk assessment.

The method addresses only Ah-receptor mediated effects. This provides only an illusion of full protection since non-Ah-receptor-mediated effects associated with the dioxin-like chemicals may still cause adverse effects by other modes of action. Thus, the overall goal of environmental protection may not be achieved using a TEF risk analysis alone. The method assumes strict additivity and, although a reasonable assumption, cases of over- and under-protection are possible for a variety of reasons. The TEF approach is strictly a toxicological approach dealing only with direct effects and ignoring indirect and nondirect (induced) effects. Nondirect or induced effects are the result of changes in physical/ecological conditions which are not either a direct or indirect biological response of an organism to a chemical stressor, but may be a sequela. Examples of this would be changes in benthic communities associated with changes in sediment texture or quality resulting from biological or physical/chemical events associated with the contaminant of concern, or loss of habitat associated with ecological or anthropogenic events related to the chemical contamination of concern. In the traditional toxicological sense, no pharmacological dose of a chemical can be described to model the situation, but such effects may combine with or dominate the direct toxicological effect (Munkittrick and McCarty, 1995).

Ecological dynamics in the field are not considered. Population (both intra-species and interspecies) and community level compensating factors can have substantial influences on the nature and degree of response in natural field populations are ignored. This issue is particularly problematic as empirical information questions the validity, or at least the accuracy, of the

extrapolation method: "However harmful effects (e.g. effects on survival, growth and reproduction) of dioxin-like chemicals are often difficult to detect at the population level. Therefore, methods to assess and predict effects on individuals are required" (WHO, 1997). Also, the method does not address interactions, both positive and negative, with other stressors and factors in the real environment that is being assessed.

Despite the problems noted above and my views of them, I will attempt to include answers to the supplied questions which are in the context of the question asked.

SPECIFIC QUESTIONS/ISSUES

I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. The additional background information available for some TEFs provides an attractive, but illusionary means of evaluating uncertainties. I am not aware of a comprehensive list of possible sources of uncertainties, with a quantitative ranking of the possible contribution of each. Thus, it is not possible to quantitatively evaluate the data that is available and assign valid, comparable uncertainty rankings. Although some qualitative assessment may be carried out, it too is prone to being misleading since it is a evaluation of only the uncertainty information known. It is be quite possible that influencing factors for which there is currently no information could dramatically alter any uncertainty evaluation made with incomplete knowledge. As well, since the overall uncertainty level is not quantified, the relative magnitude and significance of any uncertainty reduction cannot be determined.

By my estimate about 25% of the proposed WHO TEFs (Table 5) are rounded to the nearest 1/2 order of magnitude (significant digit is 5 rather than 1). I think that the statement in this question that TEFs are generally rounded to the nearest order of magnitude is overstating the case. With 25% rounded to the nearest 1/2 order of magnitude I think that is more representative statement of the actual rounding practice. I note that this is stated correctly in Tables 1-3 in the retrospective case study.

2. All TEFs should not be considered to have similar uncertainties. As noted, a variety of studies, endpoints, and exposure routes have been employed. Until such time as either there is a common experimental basis for the TEF scheme or there is quantitative knowledge of the toxicokinetic

and toxicodynamic relationships between various tests, endpoints, and exposure routes, the uncertainty associated with derivation remains problematic. Although there are greater amounts of background information for some congeners, the information base for all is insufficient or incomplete. Therefore, all uncertainties associated with each TEF are not quantifiable and the similarities in the uncertainties associated with each TEF are unknown. Although TEF estimate uncertainties may lie in a similar range, or be of modest influence compared to other uncertainties, partial quantification at this time would impart a false sense of accuracy.

3. There is a question as to whether any of the TEFs derived from *in vitro* and *in vivo* laboratory testing can be reliably extrapolated to the effects relevant to the chosen assessment endpoints. Assessment endpoints are usually clear goals related to the maintenance of populations of certain valued or threatened/endangered species or, more specifically, the maintenance of reproduction and protection of sensitive life stages in these species. Protection of the community is assumed to be accomplished when the sensitive or sentinel species are protected. This is a very broad, unfounded assumption.

Success in protecting a community/ecosystem is closely related to population modellers knowledge of the system being examined and their ability to employ the toxicological data in their models to address the assessment endpoints selected. Currently toxicological data that are or can be quantitatively related to growth, reproduction, and survival (mortality) are most likely to be of use, since these are the effects that current models have been developed for. Any other endpoints are likely to be of little use for extrapolation modelling and of little use for risk assessment purposes.

The following has been noted on page 3 of the WHO Draft Report (WHO, 1997) "However harmful effects (e.g. effects on survival, growth and reproduction) of dioxin-like chemicals are often difficult to detect at the population level. Therefore, methods to assess and predict effects on individuals are required." It appears that most TEFs based on laboratory tests are likely to be unreliable or at least unvalidated for prediction of populations/communities of organisms in field situations at the current state of the knowledge. If relatively dramatic effects of dioxin-like chemicals, such as survival, growth, and reproduction, are difficult to detect at the population level it suggests that compensating mechanisms are active. Without a good knowledge of the number, types, and effectiveness of such compensating mechanisms it will not be possible to

reliably extrapolate laboratory data. Since a variety of effects observed in laboratory testing are more subtle or less clearly linked to survival, growth, and reproduction, they will be of even lesser utility in predicting effects in the field.

In summary, since many TEFs are based on effects that are poorly linked to survival, growth, and reproduction, and since it appears that compensating mechanisms in field populations/communities are poorly understood for the effects of dioxin-like chemicals, accurate extrapolation using current TEFs to protect selected populations in the field is unlikely.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. Conceptually, no additivity or mixture interaction would result in a lower estimation of risk. Risk would be based on the extrapolated effect of only the most toxic congener, that is to say the chemical present with the an expected or observed ambient concentration closest to or most in excess of an estimated or regulated adverse effect level. This is the opposite situation to that where simple, non-potency adjusted mixture additivity is employed and a higher estimation of the risk would result. The degree of underprotection or overprotection of these different approaches to mixture toxicity compared to the TEF approach cannot currently be assessed quantitatively since considerably more toxicology and ecology knowledge and data would be required. Furthermore, there are insufficient data to perform a qualitative evaluation. The TEF approach, although clearly based on current scientific understanding and principles, is best viewed as a policy based on good judgement, and should not be presented as having strong empirical support for risk assessment extrapolation.

The risk assessment conclusions for the retrospective case study would not be completely different if based on total PCBs or 2,3,7,8-TCDD alone. For this the TEF approach is assumed to be used to adjust potency but only the single most potent congener is used to assess risk relative to the proposed guideline. Based on Table 1 it can be seen that neither the total TEQs nor the PCB TEQs exceed the provisional fish guidelines of 30 µg/g. Similarly, the TEQs from PCDDs and PCDFs as groups or any individual congener alone does not exceed the guideline. Also, the total PCB concentrations do not exceed the provisional guideline of 5,000,000 µg/g.

The original TEQ analysis for birds (Table 2) finds exceedences of the provisional guidelines for

total TEQ (100 μg/g) by PCBs, but not for the TEQs from PCDD or PCDF. Using a non-mixture approach the total TEQ guideline is exceeded by PCB-126 . No other individual congener exceeds the it. The total PCB concentration in Caspian tern eggs exceeds the 5,000,000 μg/g limit by a relatively small amount. Thus, the conclusion of a modest adverse effect on birds can be obtained from either the detailed TEQ analysis or the total PCB analysis. The analysis for mammals (Table 3) is different. The original TEQ analysis finds exceedences of the provisional guidelines for total TEQ (60μg/g) by both the TEQ total and the PCBs, but not for the TEQs from PCDD or PCDF. Using a non-mixture approach the total TEQ guideline is exceeded only by PCB-126. The total PCB concentrations do not exceed the provisional guideline of 2,000,000 μg/g. In this case the exceedence estimated by the TEQ analysis is not confirmed by the total PCB analysis.

Although the results of the risk assessment do change somewhat, the general conclusion drawn from them would not change substantially with an alteration from mixture additivity to consideration of only the most significant single congener or to consideration of total PCB concentration alone. The conclusion is that, in this watershed, there are levels of certain organochlorine chemicals present in organisms above the proposed effect levels and the dominant source is PCBs, in particular PCB-126. Of course, the conclusion depends on the nature of the residue levels present in the study and the above conclusion would not be universal for all cases. However, the multiple receptor approach with foodchain considerations does appear to be robust and appears to provide more certainty than a less diverse examination would provide.

2. Given the uncertainties and variability in the data on which TEFs are based, any differences caused by the use of median response level versus no adverse effect level data is likely within the considerable noise associated with the TEF estimation process. However, an estimate of the contribution can be made. There are empirical data to suggest that differences between acute and chronic responses in conventional aquatic toxicity data is usually of the order of a factor of 10 or less (see Rand *et al.*, 1995). Also, some fish TCDD TEFs calculated at the threshold of EROD induction were about four to five times larger than international TEFs (I-TEFs), while being similar to I-TEFs when conventional ED50 data were employed (Parrott *et al.* 1995). This suggests that at low concentrations typical of environmental exposures, fish TEFs may be different from mammalian-based TEFs and/or there may be a difference between TEFs

calculated at median response levels versus those calculated from information closer to no effect levels. If the latter is the primary source of the difference, then it supports the contention that TCDD TEF toxicity estimates are affected by differences in endpoint response proportion, that such differences may be as great as the order of a factor of 5, and that such differences represent nonconservative errors in the risk assessment process using TEFs since congeners appear to be more toxic compared to TCDD than when compared a median response levels. It should be noted that the opposite appears to be true for TEFs for PCBs since they are often smaller that I-TEFs when estimated away from median response levels.

3. Extrapolation of class-specific TEFs (e.g., primarily based on single or limited mammal, bird, or fish data) to species identified in the case studies is currently a matter of policy rather than science. It is not uncertainty, but rather ignorance, that is the main controlling factor. In addition to the general laboratory-to-field extrapolation problems discussed in the response to question I.3, there is now the differences between the species used in class TEF development and the species selected in a given risk assessment. There are exposure and toxicokinetic differences. These include differences composition and timing in exposure routes (e.g., water, diet (sediment, foodchain)), lifestage and other seasonal factors, and metabolic handling differences. Toxicodynamic factors such as differences in Ah-receptor density in target tissues, as well as possible differences in receptor character, also complicate extrapolation.

In addition, the choice of assessment-specific species is not based on a rigorous scientifically-based method, and it is clearly not optimized for toxicological extrapolation. For example, in the prospective study bull trout "... as a potentially very sensitive species (probably as sensitive as or more sensitive than lake trout), was chosen because of its status as a threatened species." while bald eagle and the river otter were chosen as "representative bird and mammal species" without any detailed technical justification being supplied. Knowledge concerning TEF extrapolation is largely qualitative, semi-quantitative at best, and if TEFs are to be used it should be clear that such use is based on professional judgement and is a policy-based assumption rather than a scientific fact. At the moment TEF extrapolation should be considered as good policy but inadequate, incomplete science.

III. EXPOSURE PROFILE

1. The exposure modelling uncertainties associated with TEFs are those common to modelling

the fate of any chemical contaminant or contaminant mixture. The TEF approach has an advantage that, unlike the case where a mixture of chemicals may contain a diverse group of chemicals with differences in mode of toxic action, dose additivity is an integral part of the approach. The ranking of the potency of various congeners does provide an advantage since the degree of accuracy on the ambient level estimation can be adjusted relative to potency. For congeners not on the TEF list, chemical analysis can be avoided. For low potency congeners, analysis can be less rigorous as their contribution is likely modest anyway. Analytical efforts can then focus on for high potency congeners, since these have the greatest contribution and should be determined most accurately. A similar logic applies to fate/transport and foodchain models, since the level of effort and degree of accuracy can be tailored to the potency of the congener.

Although there are some differences in the availability and quality of congener-specific physico-chemical data I believe that any deficiencies here are less significant than in the knowledge of physical, chemical, biological, and ecological processes and relationships used in fate/transport and foodchain models.

2. Exposure route differences between the data used to derive the TEFs and the exposure profile(s) in a particular case study can be of great importance and effort are required to address this issue. The closer or more representative the dose surrogate is to the dose at the site of toxic action, the more useful and more readily interpretable it is likely to be from a toxicological point of view. Parrott *et al.* (1995) provide a useful example. Liver concentrations of PCDD/F congeners were better predictors of EROD activity than oral doses. There were some differences in the ranking of potencies of the PCDD/Fs between fish and mammalian data As well, fish TEFs calculated at the threshold of EROD induction were about four to five times larger than international TEFs, suggesting that at low concentrations typical of environmental exposures, TEFs may be different from mammalian-based TEFs which are often based on median response levels. This suggests another twist related to different exposure routes. Since an estimate of the received dose is not usually obtained in exposure-based dosing, some of the differences in TEF estimates reported in different species or endpoint testing may be simply related to differences in the amount of the received dose.

In summary, estimates of received doses are more readily interpreted from a toxicological point of view. However, if only received dose data are available information on bioavailability,

partitioning, and metabolic breakdown differences may be missing. This is the very data needed to facilitate risk assessment which is commonly focused on concentrations of dioxin-like chemicals in environmental media. Thus, unless bioavailability, partitioning, and metabolic breakdown differences between organisms, congeners, and test endpoints are available, along with either an exposure or received dose estimate, application of TEFs in risk assessments will be difficult and potentially misleading.

3. In all regulatory approaches based on comparison with a critical effect or no-effect level it is important to minimize measurement and manipulation errors and uncertainties to the extent reasonably possible. The simple total PCB approach relies on summing PCB data and comparing the result to a guideline level. In the TEQ approach congener-specific measurements are manipulated by equations containing several parameters and the errors/variability increases as a result. The greater the uncertainty in the parameters the greater the uncertainty in the product which is the basis of the comparison. Thus, in the interests of keeping uncertainty down, and perhaps comparable to the simple total PCB approach, chemical analysis of AhR agonists should be more rigorous and thereby produce less uncertain estimates that will allow the TEQ product to exhibit a similar uncertainty.

The above comments are based largely on mathematical considerations. On the other hand both methods have substantial, but unquantified errors and uncertainties associated with toxicological and ecological aspects. Thus, the overall extent to which any additional analytical efforts would substantially reduce TEF methodological uncertainty is unknown.

IV. RISK CHARACTERIZATION

1. The uncertainties associated with TEFs are not more problematic than other uncertainties associated with case study risk assessments. In fact, given their relatively narrow focus and comparatively detailed examination, they are likely less uncertain than some of the other aspects of the risk assessment process. With the TEF approach at least some attempt has been made to quantify the differences in toxic potency. On the other hand, as noted elsewhere, assumptions required to project populations, communities, and ecosystem effects from controlled toxicity testing results are rather broad and, for the moment, little quantification of the influence of current practice has been attempted. Also, bioavailability directly from the environment, as well as at various stages in the foodchain (direct bioavailability from dissolved water phase, dietary

absorption efficiency from ingested sediment and prey organisms), is a major source of variability. Although addressed in some degree in the current BSAF, BAF, BMF, and FCM approaches, detailed consideration would allow for better understanding and quantification of this likely important source of variability. I expect that it would be at least a significant a source of variability as the TEF toxicity scheme.

2. At this time I do not believe that biologically-based TEQ assays with environmental samples represent a useful or viable extension to the current TEQ screening approach to regulation. Certainly such activities would be useful in the examination of the validity and accuracy of TEQ screening, and should provide useful insights helpful to further refinement of the scheme. However, it is premature and unwise to use research tools in a regulatory process.

3. For regulatory purposes I would not desire any further site-specific data. As I noted earlier I do not believe that the TEF approach should be used for anything other than a screening risk assessment. Although there can be debate about what constitutes a screening risk assessment and a detailed site specific risk assessment, the case studies provided certainly tend more towards the latter. I do not believe that there is enough understanding of the toxicology and, especially, ecology to further refine such regulatory approaches at this time. Even the current status is providing a false sense of scientific validity and I would not wish to have it go any further. Additional work in basic research is needed to better understand the toxicology and ecology in a field situation to aid in better understanding extrapolation. Only then would additional site-specific data be of substantially greater utility.

Additional Questions Specific to the Prospective Case Study

RELATIVE TO THE EXPOSURE PROFILE

1. I trust the question refers to BAF^{fd}_{l} rather than BAF^{fd}_{w} since the latter does not appear in the GLWQG, Table 1 in the Charge Questions, or Figure 5 of the prospective study. Improvements in the accuracy of congener-specific MACs using site-specific data for BAF^{fd}_{l} determination will be a function of how different the site-specific values would be compared to those values used in the GLWQG determination process. It will also depend on whether the first or second most preferred method of deriving baseline BAFs is followed (see GLWQG, 1995, page 2). Since the values used in the GLWQG consider all routes of exposure and all aspects of environmental fate, including metabolism, a very thorough extensive sampling and analysis program on Roundtail

Lake would be required to improve the estimates. However, even given that, the low to nondetectable levels of Ah-receptor stressors currently in the system make it unlikely that improvements could be made in a prospective study since non-detect data points would confound the analysis, especially for PCDD/F.

2. Not answered.

RELATIVE TO THE RISK CHARACTERIZATION

3. As presented in the prospective case study the water quality standard estimates of 0.032, 0.028, and 0.021 pg TCDD/L have too many significant digits. The equations used (e.g., 1 or 2) employ parameters with various significant digits. However, the TEF estimates which are used in the equations are declared to be a single significant digit which is rounded to the nearest order or 1/2 order of magnitude, depending on the source of the statement. Thus, values with 2 significant digits, such as are presented, represent a serious distortion of the actual precision of the output of the formulas. Conventionally, the output of such an equation is presented with a level of significance no greater than that of the least precise parameter. In this case it is the TEF. Thus, rather than a choice of 3 values the choice should be between either 0.01 or 0.05 pg TCDD/L if 1/2 order of magnitude precision is used. If the precision is at the order of magnitude level there is only one estimate: 0.01 pg TCDD/L. Given the uncertainty and lack of precision in the other input parameters of these equations I am inclined to go with the order of magnitude estimate. This represents a more realistic consideration of the uncertainties in the estimation process.

Additional Questions Relative to the Retrospective Case Study

RELATIVE TO THE RISK CHARACTERIZATION

1. It is very unlikely that the sediment cleanup goals would be the same for each vertebrate group, although I cannot confirm this without doing the detailed calculations. The reason for the expected difference is that the three formulas used to estimate fish, bird egg, and mink TEQ relationships to sediment use differing BASF/BMF and TEF values, as can be seen from the information in the included tables. These differences are appropriate and expected since the target organisms occupy different locations in the food chain. Given the variety of data sources and limitations, and numerous assumptions required I feel it will be difficult to quantify meaningful differences in certainty of clean-up goals. If there are substantial differences in the

sediment cleanup goals from the various methods, the scientists should offer a best professional judgement ranking the values and the manager should consider additional non-scientific (i.e., economic, technological etc.) factors in the choice of a final project cleanup value.

2. Not answered.

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Dr. Menzie specializes in assessing environmental risks of toxics in aquatic and marine systems. He has many years of experience working with EPA on projects related to the development of the ecological risk assessments, case studies, and guidelines. Dr. Menzie has a good working knowledge of EPA's risk assessment guidance and has used this in risk assessments for several aquatic and marine environments. He has also used a risk or hazard assessment framework to identify research needs related to the fate and effects of toxics in estuaries. He has performed cross-media risk assessments for ocean-dumped wastes. He investigates marine and estuarine environmental problems on all coastal areas of the United States, including Alaska and Hawaii, and provides multi-media assessments of various remedial action alternatives.

Dr. Menzie has chaired multiple workshops and colloquia related to the development of the EPA ecological risk assessment guidelines. Additionally, he has prepared case studies and process diagrams, performed peer reviews, and other tasks for EPA related to ecological risk assessment. Dr. Menzie will serve as the chair of the EPA workshop on shrimp virus issues related to ecological risk assessment. Dr. Menzie acted as the facilitator for a series of public stakeholder meetings on shrimp virus issues in July of 1997. He was chosen as the chair based on his extensive experience with ecological risk assessment, environmental risk in marine and estuarine systems, and his skill as a facilitator and chair.

To: Eastern Research Group

From: Charles Menzie

Topic: Pre-meeting comments on TEF Charge Questions

I. STRESS-RESPONSE PROFILE RELATIVE TO DERIVATION OF SPECIFIC TEF VALUES

1. Does the additional information enhance the means of evaluating uncertainties in the assessments? If so, how? If not, why?

   The additional background information is useful for evaluating the uncertainties in the assessments primarily because these give insight into the methodology used to derive the estimates. The "uncertainties" probably have more to do with the methodology than to rounding issues.

2. Should all TEFs be considered to have similar uncertainties?

   No. Because TEFs are "models" based on empirical data, the amount and quality of data affects the level of confidence that can be given to each value. The derivation of TEFs is commonly based ona weight-of-evidence approach. Therefore, as the weight of evidence increases, there is greater certainty about the TEFs as well as the variability of these values.

3. To what extent can endpoints be extrapolated to the measurers of effects that are relevant for the assessment endpoint for each case study?

   The different measured endpoints are related to the endpoint of interest. As long as the same type of related endpoint is used to develop relative measures of effects, extrapolation is possible. There is greater uncertainty associated with using endpoints that are surrogate measures of the effects of interest than endpoints that are more directly related. This source of uncertainty is difficult to quantify. However, where data sets exist for several endpoints, it may be possible to quantify the extent to which relative measures diverge from one another.

I. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. What are the implications of assuming no dose additivity or no interactions among the components of the mixtures?

Most environmental exposures of consequence occur at relatively low doses. Most available information suggests that an additivity (i.e., non-synergistic and non-antagonistic) model is appropriate under such circumstances. The use of such a model is consistent with our knowledge of effects under low dose exposures. Alternatively, it is unlikely that sufficient information would be obtained in the near future to support an alternative model. Assuming additivity is probably the most appropriate approach and is more likely to overestimate than to underestimate effects.

2. To what extent should TEFs derived at a median response level be used in risk assessments where a no adverse effect level is being employed?

The question suggests that there is a potential "apples and oranges" problem associated with mixing these different types of information. This is not the case. Median response data are selected because they provide useful – and more stable values – of relative measures than do data at the tails of dose-response curves (e.g., NOAEL values). However, these relative measures can still be combined with absolute toxicity data at the tails of a distribution for the purpose of estimating risks. In such cases, there would be uncertainty associated with the selected toxicity data but the relative measures would still be appropriate.

3. To what extent can class-specific TEFs be directly extrapolated to the species identified within each case study?

It would be useful to have measures of variability among species within a class for both toxicity and relative measures of toxicity. Without such information, it is difficult to comment on the uncertainties associated with extrapolation.

I. EXPOSURE PROFILE

1a. To what extent does the TEF approach present challenges ....?

The approach reduces uncertainties associated with estimating risks associated with mixtures because it makes greater use of the information available on the relative toxicities of the compounds within the mixture. Because the mixture is variable in composition, a method that accounts for such variability is likely to provide a better estimate of effects than a method that assumes a specific composition.

1b. How does the approach affect fate and transport modeling considerations?

The approach does require more detail to be included in fate and transport models. For simple models, the impact will be small. However, for large models with extensive computations, the additional effort (models runs and times) can become demanding. Modeling these complex mixtures will require the same types of considerations that have been given to models of petroleum hydrocarbons. The recent work of the Total Petroleum Hydrocarbon Workgroup (TPHCWG) is a good example. This group has divided the complex mixture of petroleum hydrocarbons into manageable fractions for the purpose of modeling and for risk assessment.

2. To what extent do exposure route differences used in deriving the TEFs affect their application in the case studies?

TEFs are relative measures of effects. However, it is possible that the relationships between administered, absorbed, and effective doses could vary depending on route of exposure and that these do not vary consistently among compounds. Thus, there is greater uncertainty with using TEFs that are based on routes of exposure different from those being evaluated in the risk assessment.

To what extent does the TEF approach require a more rigorous design...?

The TEF approach will require greater analytical costs. Based on experience, the analytical cost may be higher by a factor of two to ten as compared to total PCB measurements. The TEF approach will also require greater efforts to perform QA/QC, data validation, and data management.

I. RISK CHARACTERIZATION

1. Are the uncertainties associated with TEFs more problematic than other uncertainties?

No. Use of TEFs does not introduce greater uncertainties into the analysis in cases where toxicity data are based on literature values (as compared to direct measures of toxicity.) These uncertainties do not limit the analysis.

2. What would be the strengths and limitations of a biologically-based TEQ approach?

The major strength is that such an approach provides a better measure of the effects of the mixture and avoids having to rely upon a reconstruction of the effects from an estimated "sum of the parts." The major disadvantage has to do with having an acceptable approach and the analytical costs associated with implementing that approach.

3. Provide a list of investigations and rank them.

I would rely upon a weight-of-evidence approach. This would consist of three components: a) field observations of effects using an ecoepidemiological approach, b) laboratory exposures using extracts of sediment, water, or fish, and c) an assessment of effects based on chemical measurements. All of these contribute to an overall understanding of effects. I place greater reliance on field observations for retrospective studies and on laboratory toxicity tests for prospective analyses.

Additional Questions for Prospective Case Study

RELATIVE TO EXPOSURE PROFILE

1. The Roundtail Lake data are more relevant for site-specific evaluation. Therefore, MAC based on these data should be more appropriate than GLWQG.
2. The approach should be internally consistent. If a TEF approach is being applied to assess toxicity, then it should also be used to evaluate exposure. Otherwise, the improvements gained on effects may be offset by uncertainties and errors associated with modeling exposure.
3. I suggest that a Monte Carlo approach be used. The approach should adhere to recent EPA policy concerning the use of probabilistic methods. A policy decision will need to be made concerning level of protection. Typically, this is selected as a value at the tail of the distribution (e.g., 95th percentile.) In lieu of Monte Carlo analyses, other probabilistic methods may be helpful.

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Dr. Metcalfe is currently chair of Environmental and Resource Studies at Trent University in Peterborough, Canada. He received a B.Sc. degree at University of Manitoba in zoology/chemistry, a M.Sc. in biology from the University of New Brunswick, and a Ph.D. in biochemistry from McMaster University. He is a recent winner of the "Excellence in Research and Technology" award from the Ontario Ministry of the Environment. Dr. Metcalfe has a range of experience in international projects concerning aquatic contaminants. Particular research interests are in determining fate and toxic effects of halogenated aromatic hydrocarbons, polynuclear aromatic hydrocarbons, and alkylphenol ethoxylate surfactants in the aquatic environment. Dr. Metcalfe has been involved in international projects in Belize, Mexico, Ecuador, Argentina, and Indonesia, as well as conducting several research projects on toxicity in the Great Lakes. He has published more than 70 journal articles and other refereed publications.

C.D. Metcalfe

Response to Charge Questions

from Chris Metcalfe
Trent University, Peterborough, Ontario, Canada

November 14, 1997

I. Stress-response profile related to the derivation of TEF values:

Question 1. The additional information for derivation of TEFs provided in the case studies informs the reviewers that the toxic endpoints of interest in these case studies are reproductive success and recruitment within the populations of exposed organisms. This informed me to place greater emphasis on TEFs that have been derived using toxic endpoints that affect recruitment, such as early life stage mortalities.

Questions 2 and 3. TEFs will vary in level of certainty. There is a good toxicity data base with *in vivo* and *in vitro* mammalian models from which TEFs for wild mammals can be derived. For fish, there is a comprehensive data base for TEFs that are based upon early life stage mortalities in salmonids, but data for other *in vivo* endpoints are incomplete. I am particularily concerned about TEFs derived for birds, which are mainly based on *in vitro* assays using endpoints that are only peripherally related to effects that are relevent to the assessment endpoints in the case studies (i.e. recruitment).

II. Stress-response profile relative to the application of the TEQ approach:

Question 1. The implications of assuming no dose-additivity or no interactions in the case studies are a major leap of faith for the risk assessment process. The

limited information on this subject indicates that other non-toxic halogenated aromatic hydrocarbons (HAHs) exert a modulating effect upon the toxicity of planar HAHs; hence a TEQ-based risk assessment based upon an assumption of no interactions will over-estimate the toxic risk to fish and wildlife. However, having said this, risk assessments based upon concentrations of TCDD or total PCBs would offer no major advantages over the TEQ approach. Basing toxicity assessments upon TCDD concentrations would be problematic in the retrospective case study where planar PCBs contribute to a large percentage of the total TEQ, and in the prospective study where chlorinated dibenzofurans are major contributors to the total TEQ. Estimates based upon total PCBs do not take into account the changes in congener proportions that take place through a process of partitioning in the environment and biomagnification through food-webs.

Question 2. For me, estimates based upon EC50 or LC50 values are not a problem for calculating NOAELs. As stated in the documentation for this exercise, the dose-response curves for planar HAHs tend to be so steep that there are not likely to be large differences in EC50s and NOAELs. The use of median response levels for risk assessment based on NOAELs will add a safety factor that partially compensates for the uncertainties that are inherent in the TEF estimates.

Question 3. There are some problems in extrapolating TEFs based upon tests with a limited number of test species to an entire taxonomic group. In the case of fish, TEFs based upon early life stage mortalities with salmonids are particularly appropriate for assessing risk to salmonid species of esthetic or economic value; a situation that is common for assessing risk in temperate lakes. However, these TEFs may be of limited value for risk assessment in warm-water environments with species such as bass and channel catfish. The mustelids appear to be particularly sensitive to the toxic effects of planar HAHs, so risk may be underestimated for

these mammals when using TEFs based upon rodent models. The limited amount of data available on TEFs for birds indicates that interspecies differences in sensitivity are large, so applications of TEFs to the avian species identified in the case studies may be inaccurate; either under- or overestimating the toxic risk.

III. Exposure Profile:

Question 1: The TEF approach presents challenges for modeling the environmental distribution and exposure dynamics of planar HAHs. I am not particularily concerned with the quality of the physico-chemical data for these compounds. In most cases, there are adequate data for Kow, Koc, H, etc. for each of the toxic compounds, and where there is not, estimates can be made from empirical relationships or structure-activity relationships. However, I am concerned that there are few data on the relative rates of biodegradation of these compounds. There may be a tendency to model bioaccumulation and biomagnification of planar HAHs solely on the basis of ability to partition into lipids (or fugacity); forgetting the effect of biotransformations and excretion on this process. We particularily do not understand the relative biotransformation capabilities of various taxa, since it appears that different groups of organisms (e.g. fish-eating birds; marine mammals) may have different mertabolic capabilities for PCB congeners, PCDDs and PCDFs.

Question 2: It is difficult to assess the effect of differences in exposure routes on estimates of TEFs. For instance, injections of eggs in studies with fish and birds may not reflect the normal toxicokinetics and partitioning of contaminants that occur in eggs as a result of parental transfer of contaminants. More work is needed to assess this problem.

Question 3: The methods required for analysis of specific congeners of PCDDs, PCDFs and PCBs, (in particular, coplanar PCBs) are definately more rigorous, time-consuming and expensive than methods for aggregate stressors. This means that only a small number of analytical labs with appropriate technical expertise and analytical instrumentation (e.g. high resolution GC-MS) will be able to provide the data that is appropriate for risk assessment, and tight research budgets will limit the number of samples that can be analyzed. In addition, some of the analytes identified in these risk assessment scenarios are often not routinely analyzed (e.g. PCB congener 81).

IV. Risk Characterization:

Question 1: In my opinion, uncertainties in modeling the bioaccumulation and biomagnification of planar HAHs are a limitation of the risk assessment process that may exceed the uncertainties associated with calculating the TEFs.

Question 2: *In vitro* or *in vivo* biological assays to determine TEQs may be a useful approach. However, protocols must be developed to define the degree of sample fractionation prior to the assays. For instance, typical *in vitro* assays for EROD induction with H4IIE cell lines have been conducted with environmental samples that have undergone considerable fractionation to isolate planar HAHs. Use of these samples may result in overestimates of biological responses. Use of a more crude fraction also containing non-toxic PCBs, for instance, may significantly modulate the degree of EROD induction.

Question 3: Studies are needed to address:

i) The appropriateness of an additive approach for estimating total TEQs.

ii) The relative rates of transformation and elimination of planar HAHs in

different taxa, and the effects upon bioaccumulation and biomagnification.

iii) The influence of exposure route on estimates of TEFs.

Additional Questions:

Questions Specific to Prospective Case Study:

Questions 1 and 2: No comments until I can further examine the basis of the BAFs used by the GLWQG.

Questions Specific to Restrospective Case Study:

Question 1: I would council a risk manager to develop TEQ sediment cleanup goals that ensure protection of the vertebrate group with the most certainty in TEQ estimates. In my opinion, the lack of certainty in TEQ estimates (e.g. 10-20 fold?) would probably exceed the differences in sediment cleanup goals calculated for the various vertebrate groups.

Question 2: I do not consider a ratio of total TEQs to total PCBs to be an effective method for setting TEQ-based sediment remediation goals. The reason for this opinion is illustrated in the attached figure (from Metcalfe and Metcalfe, 1997, Sci. Total Environment) that shows variations in total TEQs for coplanar PCBs relative to total PCBs in different components of the Lake Ontario food web. The ratio varies among environmental compartments and groups of biota; probably as a result of differences in metabolism and bioaccumulation of coplanar PCBs relative to other PCB congeners. This is especially noticeable when comparing TEQ/PCB ratios in biotic and abiotic compartments.

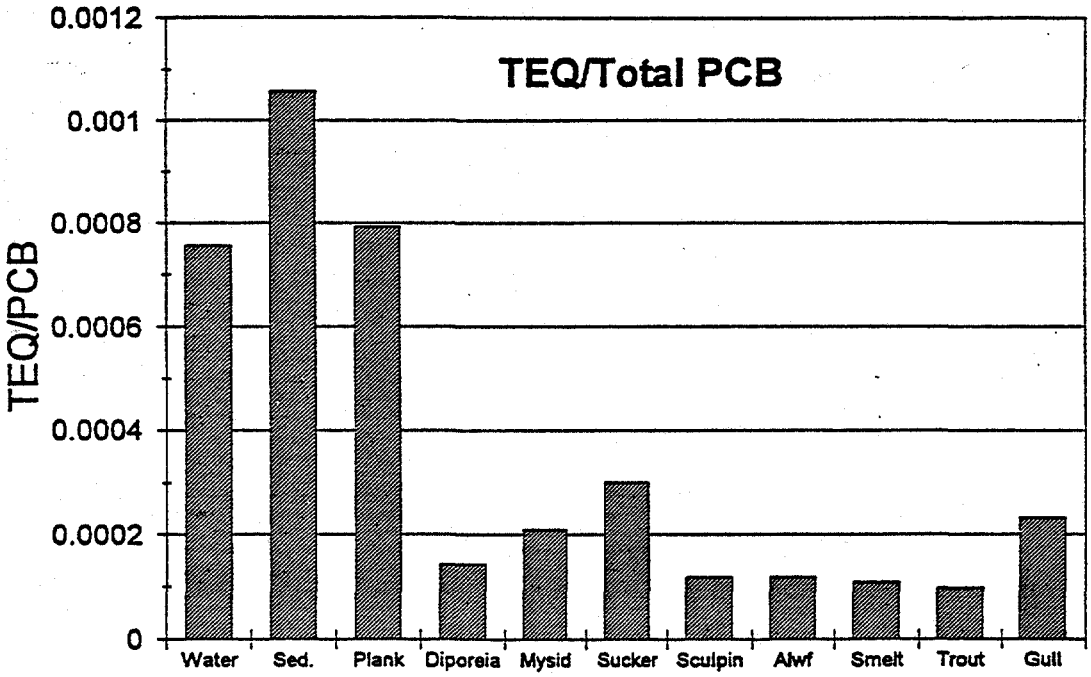

Fig. 11. Ratio of Toxic Equivalent Quantities (TEQs) calculated for total mono-*ortho* and non-*ortho* PCB congeners relative to total PCB concentrations in water, sediment and biota from the Lake Ontario food-web.

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Michael W. Meyer

Response to Charge Questions for Workshop on the Application of TEFs to Fish and Wildlife

Section I.

1. Because of the variety of species and endpoints used in developing TEFs, additional information describing TEF derivation is required for uncertainty analysis. Ideally, standard protocols would be established for congener specific TEF derivation (same species, same endpoint) and a TEF profile be established for each class (mammal, fish, bird) Unless or until this is established, additional information should be provided for all TEFs which comprise >10% of a calculated TEQ, including endpoint, species, and study citation. The effect of TEF rounding on the risk assessment process should be investigated via model sensitivity analysis.

2. If the TEF is derived from an enzyme induction endpoint, from QSAR studies, or if multiple TEFs have been calculated for the same congener in different studies, an uncertainty value should be assigned to the TEF. Perhaps a "sliding scale" of uncertainty could be assigned to all TEFs comprising >10% of a calculated TEQ (i.e. zero uncertainty assigned to TEFs that are derived from embryo toxicity studies using the "target" species, with more uncertainty added incrementally as quantitative rigor diminishes).

3. TEF values developed using *in vivo* early life stage endpoints for relevant species can be directly used to predict a stress response in a risk assessment. However I am skeptical of using biochemical responses unless they have been closely correlated to a toxic endpoint in a relevant species.

Section II.

1. The implications are that no antagonistic or synergistic effects are occurring between a complex mix of congeners as they compete to bind with the AhR receptor - if synergistic effects do occur the risk assessment would be too permissive, if antagonistic effects occur it would be too conservative. If one where

to use the total PCB and 2,3,7,8-TCDD no-effect thresholds presented in the Retrospective Case Study (i.e. 5 ug PCB, 100 ppt TCDD/g bird egg), the Caspian tern eggs collected from Oneofakind Lake would be close to the no-effect threshold. However, the calculated egg total TEQ for the same Caspian tern eggs exceeds a reported total TEQ avian egg no-effect level (calculated by Giesy et al.1995 *Arch. Env. Cont.* 29:309-321) by a factor of nearly 60. This reflects the author's establishment of a 2,3,7,8-TCDD no effect level of 7 ppt vs. 100 ppt in the Retrospective Study.

2. Calculating a NOAEL from the slope of the LC50 or EC50 dose-response curve may not protect the most sensitive individuals in a population. This could be permissive if the risk assessment targets an endangered or declining species.

3. The existing data for birds and mammals indicates that use of TEFs derived from chicken or mink studies will provide highly protective, conservative calculated TEQs. Chicken and mink are nearly an order of magnitude more sensitive to TCDD TEQs than other species within their respective classes, and wild mink may consume a limited amount of contaminated fish in their natural diet. Establishment of conservative TEQ standards is desirable from the perspective of the risk assessor and the resource, but will predictably result in controversy amongst the regulated community. If a permitting process uses the most conservative calculated TEQ to establish effluent discharge, and achieving that new discharge goal requires substantial capital investment by the regulated parties, you can expect litigation and delay in implementation of the new rule. The cost-benefit of this trade off should be addressed from a policy perspective.

Section III.

2. One will need to assume that assimilation efficiency and detoxification/ metabolism routes are similar when one pools TEFs derived from various dosing (injection, oral gavage, dietary) experiments. One also then needs to assume that wildlife contaminant exposure in the natural environment will result in similar

assimilation, metabolism, and effects patterns. These assumptions should be kept in mind when establishing a TEF.

3. Sediment, soil, and biota will likely have differing congener patterns within the same environmental system due to differential metabolism/degradation of the various PCB/TCDD congeners present in the parent contaminant. An understanding of these differences may be necessary to predict risk within the various biotic and abiotic compartments of an ecosystem, requiring additional sampling and analysis costs. A biologically based TEQ assay may be the preferred route to travel (see Section IV #2).

Section IV.

1. To be quite frank, it is difficult to answer this question without simulating the risk assessment for the various contaminants and species of concern. In most risk assessments there is a great deal of uncertainty in describing exposure (limited diet studies for few species, few prey items characterized to congener content, etc.) and effect (species sensitivity, endpoint characterization, etc.). How that uncertainty compares to that generated by extrapolation of TEFs between species and endpoints is beyond the capability of my hand calculator.

2. Biologically-based TEQ assays are by far the best conceptual approach and most economical means of using TCDD TEQs in the regulatory process. The cost associated with collecting the data required to conduct a calculated TEQ risk assessment may prohibit a meaningful characterization of exposure in most scenarios. Therefore a bioassay would be cost effective. Furthermore, the bio-based TEQ would theoretically account for antagonism/synergism between congeners in complex mixtures. Unfortunately, none of the existing bioassays appears ready to go on line for routine screening in risk assessment exercises. For instance, though bioassay TEQ values (using rat liver hepatoma cell line H4IIE) and congener specific calculated TEQs were very similar in an experiment where mink were fed diets containing Saginaw Bay carp, suggesting the additive assumption to

be correct (Tillet et al. 1996. *Env. Science Tech.* 30: 283-291), large discrepancies exist between bioassay TEQ values and congener specific calculated TEQs values in birds (Tillet et al. 1991. *Arch. Env. Tox. Chem.* 21:91-101). An understanding of these inter-class differences and receptor binding mechanisms is necessary before a bioassay can be implemented.

3. Suggested research/site specific data

a. Establish a standard protocol for deriving TEFs (early stage mortality endpoint) and determine the TEFs for the most relevant congeners for all 3 classes.

b Establish standard bio-based TEQ assay and conduct research to understand mechanisms responsible for different results between classes.

c. Establish protocol for quantifying site-specific biomagnification/bioaccumulation factors to quantify TEQ exposure including dietary habit studies, prey base contaminant characterization, magnitude of trophic level biomagnification, etc.

d. Conduct additional research to provide scientifically defensible TEQ effect levels for mammals, birds, and fish if current data is insufficient. Investigations of potential TCDD TEQ interactions with residual DDE in Great Lakes systems is also desirable.

Prospective Case Study

2. It has been shown that non-ortho PCB congeners are more readily bioaccumulated and are more resistant to metabolism when compared to ortho-substituted PCB congeners. It follows that wildlife tissues may contain a larger proportion of dioxin-like PCB congeners/g total PCB, enriching the toxic potency of the total PCBs measured in their tissues. While not firmly established, it is also likely that species differ in their ability to assimilate/metabolize the various PCB and TCDD congeners.

3. A risk assessment model should be developed which simulates exposure and

effect thresholds under a ranges of values which reflect the uncertainty inherent in the model and its parameters. This model output should then produce a range of possible TeqTMDL with associated risk attached (zero risk for the lowest value, "x"risk for the greatest value). Once this range has been established, a final rule can be developed which is most protective of the ecological concerns while utilizing the best available technology.

Retrospective Case Study

1. It seems obvious that the variability in BSAFs, as well as thresholds of effect between vertebrate groups, will result in different sediment clean up goals. In addition, it does not seem possible to provide a scientifically defensible TCDD TEQ threshold of effect for Caspian terns and river otters as their sensitivity to these compounds has not been experimentally established. Indeed, it seems that Caspian terns are insensitive to the embryo toxic effects of PCBs (hatching success was not depressed despite eggs PCB levels of 19-40 ug total PCB/g wet weight; Struger and Weseloh, 1985, *Colonial Waterbirds* 8:142-149). No data is currently available on the relative sensitivity of otters to TCDD TEQs as compared to mink though rumor has it such work is underway. I would therefore council the risk manager to go with the sediment TCDD TEQ value that protects lake trout, a species whose TCDD TEQ for early life stage mortality is well characterized. I would then request that the responsible party support a dose-response study for river otters/TCDD-TEQs, or, at a minimum, a study which compares river otter TCDD sensitivity to that of the mink. I'd exclude the Caspian tern from the risk assessment because of their insensitivity to the toxic effects of PCBs.

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USEPA Workshop on the Application of TEFs to Aquatic Life and Wildlife
January, 20-22, 1998, Chicago, Illinois

Answers to Premeeting Questions

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III. EXPOSURE PROFILE

1. In addition to uncertainties in the TEFs themselves, there are numerous challenges and uncertainties associated with the application of TEFs to environmental risk situations. In the prospective study the consultant choose to calculate permitted concentrations in water ($TEqC^t_w$ values) based on the initial premise that each compound contributed alone to the TCDD toxic equivalence. In the final step it appears that the mass loadings of each compound were then distributed on the basis of their relative mass distribution in the effluent. This is a complicated process and since I did not have access to the modeling program I was only able to carry out the initial calculation for allowable water concentrations for fish as shown:

$$(TEqC^t_w)_{pecdd} = \frac{(TEqC^t_w)_{tcdd}(BAF_l^d)_{tcdd}(f_d)_{tcdd}}{(BAF_l^d)_{pecdd}(f_d)_{pecdd}(TEF_{pecdd})} = \frac{0.021x9.0E+06x0.2}{2.49E+07x0.08x1} = 0.019 pg/L$$

The f_d values were determined using DOC , POC and K_{ow} values as described in EPA-820-B-95-005. The complete set of $TEqC^t_w$ values are shown below (pg/L):

	Fish	Avian	Wildlife
1,2,3,7,8 PeCDD	0.019	0.021	0.0029
1,2,3,4,7,8 HxCDD	0.115	0.106	0.018

2,3,7,8 TCDF	1.2	1.29	0..183
1,2,3,7,8 PeCDF	1.9	2.05	0.289
2,3,4,7,8 HxCDF	0.026	0.028	0.004
1,2,3,4,7,8 HxCDF	2.28	2.45	0.348

It is apparent that with the DOC and POC values quoted in the prospective study there would be considerable errors if the BAF^d_i values from the background literature were applied without converting them to BAF^t_i values using f_d values. For instance the $TEqC^t_w$ values for PeCDD would be increased by nearly a factor of 3.

When the WASP4 model is applied to the calculation of maximum allowable loads ($MAL_{i,j}$) for each congener, several other parameters are required in addition to maximum allowable water concentrations (MAC^t_w). These are sediment related parmaters (settling flux, respusension flux, log K_{OC} etc.) and two important physico-chemical parameters, the Henry's Law constant for vapor/water partitioning and the photolysis rate constant. In the Lake Ontario TCDD study it was determined under steady-state conditions, that for a given annual load to the lake from the Niagra River, 6% would be transported out of the lake via the St. Lawrence River, 25% would be incorporated into the bottom sediments, 31% would volatilize and 38% would undergo photolysis. The Henry's Law constants have been determined by a number of investigators and are known with reasonable accuracy for several PCBs and PCDDs/PCDFs, although only a very small number of PCB values are shown in the physico-chemical parameters table (Table 1). Since PCBs have absorption maxima at wavlelenghts below the lowest sunlight wavlelenght (less than 300 nm) photolysis may not be as important a removal process for these compounds as it is for PCDDs/PCDFs which absorb light in the UV-B region (300-340 nm). Based on studies carried out in the laboratory using 50/50 acetonitrile:water solutions a value of

0.002 was selected for the quantum yield (ϕ) for TCDD in the Lake Ontario study. Our studies confirmed this value for both pure water and acetonitrile/water solutions photolyzed at 300 nm in the laboratory. However for reasons that are not completely clear quantum yields for PCDDs and PCDFs are an order of magnitude higher in sunlight than at 300 nm, a finding corroborated by work carried out in the laboratories of Derek Muir and Barrie Webster. Furthermore Dung and O'Keefe (Environ. Sci. Technol. 28: 549-554, 1994) and Friesen et al (Environ. Sci. Technol. 24: 1739-1744, 1990) have shown that dissolved organics potentiate the photodegradation of PCDDs/PCDFs. Taken together these studies show that photodegradation should be given serious consideration in any studies modeling the transport and fate of PCDDs/PCDFs in the aquatic environment. A major uncertainty at the present time is the lack of knowledge on the extent to which PCDDs/PCDFs photodegrade when they are bound to suspended sediment particles.

2. In mammalian and avian species the extent of absorption and the tissue distribution of toxic compounds do not appear to differ significantly between i.p. and oral routes of administration. However if toxicity to fish eggs is used as an endpoint for risk characterization and the water quality standard is based on tissue residue measurements in whole fish, then it must be noted that concentrations in fish eggs are 2 to 3 times lower than maternal tissue concentrations.

3. Since measurements of individual congeners are required for the TEF approach analytical methods must be much more rigorous than those used in the determination of total compound type concentrations. In the case of PCBs, the non-ortho (coplanar) congeners are the most toxic but they may only constitute 1% of the total PCBs. However PCBs are generally analyzed using GC/EC instrumentation, a relatively nonspecific analytical technique. Under these circumstances the trace signals from the coplanar congeners may be obscured by coeluting diortho congeners. Therefore relatively complex cleanup methodologies based on carbon chromatography must be used to separate the coplanars from the noncoplanars prior to GC/EC analysis. In PCDD/PCDF

analysis individual congener identity is accomplished more readily since analyses are carried out by GC/MS and isomer identity within a given chlorine group is simplified by the fact that most biota accumulate predominantly 2,3,7,8-substituted isomers. Rigorous cleanup methodologies are still required since PCBs are generally present in environmental samples at higher concentrations than PCDDs/PCDFs (ng/g - μg/g vs. pg/g) and certain PCB congeners can interfere with the analysis of PCDDs/PCDFs by low resolution GC/MS.

IV. RISK CHARACTERIZATION

1. As pointed out above in answer to Question 1 on Exposure Profiles, there are some major uncertainties associated with some of the physico-chemical parameters used for modeling the fate and transport of PCBs and PCDDs/PCDFs. On the other hand there are uncertainties associated with the TEFs determined for avian and mammalian species. In the case of both avian and mammalian species an uncertainty factor of 10 was used to adjust NOAEL levels from subchronic exposure studies conducted in the laboratory to chronic exposures which biota would experience in the field. Is this appropriate ? Another uncertainty relates to the nature of the diets consumed by predatory avian and mammalian species. If a diet contains a high proportion of fish-eating biota then food chain biomagnification can be significant and the Great Lakes Water Quality Criteria may severely overestimate MAC^t_w values. On the other hand MAC^t_w values can be underestimated if plant-eating terrestrial biota are consumed.

2. If the TEFs are indeed additive then biologically-based TEQ assays may not provide any additional information from a risk assessment viewpoint. However there are several literature citations in the Interim 1993 Report on TCDD Risks in Aquatic Life and Wildlife (p 4-4) suggesting that an additive model may not always be appropriate. Recent studies conducted at the Wadsworth Center, NYSDOH have shown that PCBs 126 and 169 inhibit TCDD-induced estradiol (E_2) metabolism by hydroxylation at both the 2 (CYP1A1 activity) and 4 (CYP1B1

activity) positions in certain human cancer cell lines (Shaokun Pang, Ph.D. Thesis, 1997). In the absence of TCDD the two PCB congeners induced E_2 metabolism. Under these circumstances a biologically-based assay would provide more definitive information on the risks associated with a defined mixture of compounds. On the other hand a nonadditive model would make it extremely difficult to regulate the compounds on an individual basis. Perhaps the most useful approach would be use a bioassay to adjust TEF values. As pointed out in the Interim Report the bioassay would also need to be calibrated against a biological endpoint of environmental significance.

3. Since the total PCB concentrations in gull eggs from Roundtail Lake approach 3 μg/g a major concern would relate to the coplanar and mono-ortho PCB concentrations in the gull eggs and also in sensitive mammals such as mink and otter. In the retrospective study a total PCB concentration of 5.7 μg/g in Caspian tern eggs results in a TEQ value of 400 ρg/g, which is x10 higher than the no-effect threshold. In conjunction with these monitoring studies the state should determine the status of the river otter population in the area. Results from the population survey may indicate that the more stringent WQS for river otters should be adopted in the risk assessment. The next research priority would be the determination of BAF^t_l values for those Ah active PCBs which are present in high enough concentration to be measured in water samples. Since the fish currently in Roundtail Lake have no detectable concentrations of PCDDs or PCDFs additional monitoring of biota or sediments for these compounds is probably not warranted. However it would be appropriate to determine PCDD/PCDF residue levels in biota from other lakes where there are discharges of known magnitude from pulp and paper mills.

In the retrospective study, the fact that the TEQ concentrations in the Caspian tern eggs and the otter livers exceed the NOAELs for these species is a major concern. Three research questions arise from this concern: (1) Could the low success rate in Caspian terns be explained by interactions between the DDE residues in the tern eggs and the PCBs/doxins ? Field observations coupled with residue measurements might useful information, (2) The manager of

the area should determine if the anecdotal accounts of low numbers of mink and otter are valid, and (3) the proportions of fish and fish-eating biota in the diet of mink and otter should be assessed. Consumption of fish-eating biota could have a considerable impact on the biomagnification of PCBs and dioxins by these mammals.

Additional Questions on the Prospective Study

1.Since there is no information on either dissolved or total aqueous concentrations of the chemicals, field derived BAFs cannot be derived. However field-derived BSAFs can be determined for PCB 77 and PCB 126 lake trout and sediment data and it is possible to use this information to obtain a ratio of the BAF^d_l using the following equation:

$$\frac{(BAF_l^d)_i}{(BAF_l^d)_r} = \frac{(BSAF)_i(K_{OW})_i}{(BSAF)_r(K_{OW})_r}$$

This value can then be compared to the ratio determined from the GLWQG document. With i = PCB 126 and r = PCB 77 the ratio was determined to be 5.7 compared to a ratio of 37.5 determined from Table 1. Therefore we might expect some errors if the GLWQG values are used.

2. The bald eagle data are most suitable for this type of comparison since the data can also be analyzed by the BMF approach described on pages 33 through 44 of the USFWS Critique of the GLWQG Document. Basically the established water standard for bald eagles needs to be divided by 21, the BMF for forage fish to bird eggs. This will adjust all the dietary components for

biomagnification from forage fish, using the equation on page 3-13 of the GLWQI Criteria Document for TCDD (the BMFs for forage fish to piscivorus fish and piscivorus fish to bird eggs have already been taken into consideration by using two BAFs and the factor of 30, respectively). The BMF-adjusted WQS then becomes 0.0013 pg/L instead of 0.028 pg/L. Using the chicken embryo NOAEL of 100 pg/g for bald eagles together with a safety factor of 10 the same WQS can be determined by the USFWS approach as shown below:

$$\frac{NOAEL}{totalBMF} = \frac{100x10^3}{0.736x21 + 0.184x10 + 0.056x659} = TargetDietaryConcentration$$

$$\frac{TargetDietaryConcentration}{BAF} = \frac{1840pg/kgforagefish}{172{,}100kgforagefish/L} = 0.0011pg/L$$

It is more difficult to determine a BMF-adjusted WQS for mink since the diet-to-mink BMF is presented on a lipid basis in Table 1. If we assume that the lipid concentration in the mink is 4% compared to a lipid forage fish concentration of 8% then the wet weight BMF for TCDD would be 5.5 and the WQS value should be divided by this number to give a BMF-adjusted WQS of 0.0005 pg/L rather than the value of 0.00292 pg/L. However if the USFWS approach is used assuming a NOAEL of 60 pg/g, as per the retrospective study, and a diet composed exclusively of forage fish then the WQS would be 0.0063 using the x10 safety factor. The discrepancy between the two approaches is partly related to the fact that the EPA method uses a daily toxic dose (TD) whereas the USFWS uses the NOAEL body burden. The

NOAEL/TD ratio is 7 for the bald eagle compared to 60 for the mink.

Additional Questions Relating to the Retrospective Study:

1. The cleanup goals would not be the same for each vertebrate group since the order of sensitivity of the groups is mammals>birds>fish. As shown in the retrospective study document TEQs can be directly linked to sediment concentrations of the chemicals via BASFs. Therefore the most restrictive sediment cleanup standard would be based on the otter TEQs and the extent of cleanup required would depend on the extent to which the TEQs exceed the NOAELs.

2. This question can be addressed by considering the equation for calculating TEQs in birds and mammals on page 8 of the retrospective study. In addition to the organic carbon-normalized congener concentration in the sediment (C_{oc}) and the appropriate TEF, this equation involves the use of two partition coefficients, a BSAF and a BMF. When the shiner BSAFs were determined from the field data and the BMFs from Table 1 were used TEQs were obtained which can be compared with the TEQs listed in Tables 2 and 3:

Caspian Tern Eggs

	TEQ Calc.	TEQ Table 2
PCB 77	29	54
PCB 126	232	275
2,3,7,8-TCDD	2.3	4.5
2,3,4,7,8-TCDF	4.1	9.58

Otters

	TEQ Calc.	TEQ Table 3
PCB 105	2.5	2.6
PCB 118	4.8	4.8
PCB 126	98	99.8
2,3,4,7,8-PeCDF	25.96	25.92

It is apparent that there is considerable agreement between the calculated TEQs and the TEQs derived from tissue concentrations. However these data were obtained using field-derived spottail shiner BSAFs. As shown in the table below the agreement would have been much lower if the EPA lake trout BSAFs had been used:

	BSAF shiners Oneofakind Lake	BSAF lake trout Oneofakind Lake	BSAF EPA Table 1 Questions
PCB 77	0.91	1.82	0.29
PCB 105	10	14	4.49
PCB 118	7.3	23.5	1.72
PCB 126	5.76	5.1	3.21
2,3,7,8-TCDF	0.006	0.069	0.047
2,3,7,8-TCDD	0.14	0.35	0.059
2,3,4,7,8-PeCDF	0.017	0.035	0.095

Consequently if generic BSAFs cannot be used, field-derived BSAFs must be determined for individual congeners using state-of-the art analytical methods. If this were the case there would be no savings in analytical costs by analyzing sediments for total PCBs and then determining individual C_{OC} values by a ratio calculation.

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Specific Questions/Issues

I. STRESS-RESPONSE PROFILE RELATIVE TO THE DERIVATION OF SPECIFIC TEF VALUES

1. Inclusion of the WHO draft report (July 30, 1997) on derivation of toxic equivalency factors (TEFs) for polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs) for humans and wildlife is useful in evaluating uncertainties in the fish-, bird-, and mammal-specific TEFs as they relate to the retrospective and prospective case studies.

In the case studies the fish receptors of concern are lake trout and bull trout. By consulting Table 3 of the WHO report one finds that fish-specific TEFs for PCBs, PCDDs, and PCDFs were based on early life stage mortality in rainbow trout. This is useful information because the fish receptors of concern are species of trout. So there is a relatively low level of uncertainty in extrapolating the fish-specific TEFs to lake trout and bull trout because they are a closely related fish species to rainbow trout. In addition, the endpoint upon which the fish-specific TEFs were based, early life stage mortality, is relevant to recruitment which is the assessment endpoint proposed for fish in the case studies. Thus, in assessing the risk to recruitment of lake trout and bull trout caused by exposure to PCBs, PCDDs, and PCDFs there is a relatively low level of uncertainty in using the internationally agreed upon TEFs for fish.

In the retrospective and prospective risk assessments the wild bird receptors of concern are the Caspian tern and bald eagle, respectively, and the assessment endpoint is also recruitment as it was for fish. In consulting Table 4 of the WHO report for bird-specific TEFs one observes that the TEFs for PCDDs and PCDFs are based on induction of ethoxyresorufin-O-deethylase (EROD) activity in the chicken embryo. This is important, because there is greater uncertainty in these EROD induction-based TEFs for PCDDs and PCDFs, with respect to the assessment endpoint of recruitment, than there would be if they had been based on embryo mortality. In this context, it is useful to find in the WHO report that the TEFs for essentially all of the environmentally relevant PCBs were based on the LD50 for embryo mortality in the chicken. Thus, the bird-specific TEFs for PCDDs and PCDFs are more uncertain than those for the PCBs when assessing the risk to recruitment of Caspian terns and bald eagles caused by the presence of complex mixtures of PCDDs, PCDFs, and PCBs in the eggs.. In addition, it is important to note

that all of these TEFs whether they were based on EROD induction or embryo mortality tended to be based on results obtained in chicken which is the most sensitive of all bird species to aryl hydrocarbon receptor (AhR) agonist toxicity. Yet the wild bird receptors of concern in the case studies, the Caspian tern and bald eagle, are not closely related to the chicken. This may be significant because it is uncertain to what extent TEFs determined in a highly sensitive species like the chicken can be extrapolated to more TCDD insensitive and distantly related bird species like the Caspian tern and bald eagle. It is concluded, in assessing the risk to recruitment in lake trout and bull trout versus Caspian terns and bald eagles, due to exposure of the fish or bird embryo to TCDD and related compounds, that there is more uncertainty in using the internationally agreed upon bird-specific TEFs than the fish-specific TEFs. Another point is that of the two case studies, there is more uncertainty in estimating TCDD equivalents (TEQs) from bird-specific TEFs in the prospective study. This is because the mill effluent of concern in this case study is predicted to contain only PCDDs and PCDFs and bird-specific TEFs for these classes of AhR agonists are the most uncertain for birds.

In the two case studies the mammal receptor of concern is the river otter and the assessment endpoint, recruitment, is the same for both case studies. In consulting Table 2 of the WHO report no information is provided on which species were used in the derivation of the mammal-specific TEFs, however, from the text of the report it is clearly stated that the majority of these TEFs were based on studies in laboratory rodent species. Furthermore, it is stated in the report that relative potencies of the PCBs, PCDDs, and PCDFs toward mink reproductive toxicity are not different from those of the rodent models from which most of the data to derive the TEFs were obtained. This interpretation is useful, because in the case studies the mammal-specific TEFs will be used to determine TEQs in river otter liver and there is uncertainty in the extent to which TEFs can be extrapolated across species as well as across endpoints. Another way in which the WHO report was useful is that it demonstrates that the most rigorously determined TEFs among the three vertebrate classes are those for mammals. In fish and birds a TEF might be based on one study whereas in mammals the results of several studies using different routes and durations of exposure are available for consideration in the derivation of TEFs. This leads to the conclusion that the mammal-specific TEFs probably have less uncertainty than those for fish and birds.

2. Within and between the three vertebrate classes (fish, birds, and mammals) there is a range of uncertainty in the TEFs determined for individual PCDD, PCDF, and PCB congeners. However, if TEFs are acknowledged to be order of magnitude estimates of actual relative potencies for AhR-mediated responses in a particular species it might not be necessary to apply an additional uncertainty factor to the TEQs that are generated by the TEF approach in order to acknowledge the uncertainty that exists in these estimates. In general the mammal-specific TEFs would appear to have the least uncertainty because they are derived from a larger number of studies than is the case for fish and birds. In the latter two vertebrate classes, it would seem that the degree of uncertainty associated with the TEFs for PCBs is probably similar because the studies that were relied upon to derive the TEFs for PCBs used an egg injection route of exposure and an LD50 for embryo mortality as the basis for deriving the TEFs. However, for PCDDs and PCDFs there is a significant difference between the endpoint used to derive the TEFs for fish and birds. In the case of fish the TEFs were based on embryo mortality whereas for birds they were based on EROD induction which is more uncertain because it is an adaptive rather than a toxic response.. Thus, the degree of uncertainty in the TEFs varies across vertebrate classes and would appear to be less in mammals than fish and birds. The uncertainty in the TEF for a particular PCDD, PCDF, or PCB congener is influenced by a number of factors including whether it was based on an in vivo or in vitro study, species, route and duration of exposure, endpoint assessed, and reproducibility of the results in similarly designed studies.

3. The measures of effect in the case studies pertain to reproductive success as measured by effects on egg production and viability and/or larval and offspring survival. Until evidence is presented to the contrary for each vertebrate class (fish, birds, and mammals) it would seem to be more uncertain to extrapolate TEFs based on cytochrome P4501A1 induction (determined in vivo or in vitro) to these measures of effect, than to extrapolate from TEFs based on clearly adverse developmental and reproductive toxicity endpoints such as early life stage mortality in fish, embryo mortality in birds, or a reduction in litter size in mammals.

If one is going to rely on EROD induction based TEFs for PCDDs and PCDFs in birds, which is currently the situation for bird-specific TEFs, then it would be prudent to show that

relative potencies (REPs) for a few of the most environmentally relevant PCDD and PCDF congeners following injection of graded concentrations into bird eggs give rise to REPs for EROD induction and embryo mortality that are within an order of magnitude of one another. Also to the extent such information is available for PCBs in bird embryos it would be helpful to include the findings in the WHO report. If REPs for these two endpoints are similar for PCBs in birds it would suggest this will probably also be the case for the PCDDs and PCDFs.

II. STRESS-RESPONSE PROFILE RELATIVE TO THE APPLICATION OF THE TEQ APPROACH

1. Assuming no additivity of the PCDD, PCDF, and PCB congeners that are AhR agonists would underestimate the risk which exposure to this mixture of chemicals poses to recruitment of the fish and wildlife receptors of concern in the two case studies. Furthermore, if an alternative method of ecological risk assessment, based on total PCBs relative to an Aroclor standard or TCDD alone, were applied to the prospective and retrospective scenarios they would both probably underestimate the risk to the fish, bird and mammal receptors of concern.

In the prospective scenario, mill effluent will contain, in addition to TCDD, three PCDDs and four PCDFs which are AhR agonists, but no PCBs. Since the mill is not a source of PCB contamination, the measurement of total PCBs would be inappropriate. Relying on the concentration of TCDD alone in fish and wildlife tissues as the exposure metric has the problem of neglecting the potential contribution to AhR-mediated toxicity of the other PCDD and PCDF co-contaminants in the effluent which have the potential to bioaccumulate in the fish and wildlife receptors of concern. For example, if TCDF which is present in the mill effluent at a 20 times higher concentration than TCDD was found to bioaccumulate in bald eagle eggs to a higher concentration than TCDD, then TCDF would contribute more TEQs to the eggs than TCDD (bird-specific TEF for TCDF = 1.0). However, this potentially greater contribution to egg TEQs by TCDF would be missed if the exposure analysis were based solely on TCDD. Thus, the risk assessment conclusions reached from relying on total PCBs or TCDD alone would underestimate the actual ecological risk posed by the discharge from this particular mill and would result in higher concentrations of PCDDs and PCDFs being permitted in the effluent than would be justified from an ecological risk perspective.

For the retrospective study, involving the PCB spill it would not be appropriate to assess exposure of fish and wildlife to these halogenated aromatic hydrocarbons by measuring TCDD alone because it was not a contaminant of the used hydraulic fluid. The major source of PCBs in the used fluid was Aroclor 1248. However, it would also be inappropriate to monitor the impact of this spill on fish and wildlife by measuring total PCBs in the tissues of such animals. This is because Aroclor 1248 is contaminated with PCDFs which are not detected by measuring total PCBs. Also the used hydraulic fluid might be actually enriched in PCDFs when compared to Aroclor 1248 if it was used at high temperatures that could result in PCDF formation. Thus, the main point is that certain PCDFs that would be expected to be present in significant concentrations in the used hydraulic fluid that was spilled into the Tuckymuck River would not be detected by measuring total PCBs in fish and wildlife inhabiting the river.

Another point is that weathering of the PCBs that were spilled into the river would result in PCB concentration profiles in the fish and wildlife receptors of concern (lake trout, Caspian terns, and otter) that are different from both that of the PCBs spilled and an Aroclor 1248 standard that might be used to quantify total PCBs. In this regard it is possible that lake trout and Caspian tern eggs and otter liver will have greater concentrations of PCB 126 than are present in Aroclor 1248. This enrichment of these tissues in PCB 126, which is a major contributor to TEQs in this particular case study, might be missed if exposure to AhR agonists is based on total PCBs. Thus, if TCDD alone or total PCBs were used to assess exposure in the retrospective case study the results obtained would underestimate, retrospectively, the risk to recruitment of fish and wildlife caused by the spill.

2. There is less variability in the LC50 and EC50 on a dose response curve than there is in the LC1 or EC1 which are closer to the NOAEL. Therefore, REPs based on the LC50 and EC50 should be more accurate than those based on a certain percent response at the lower end of the dose response curve near the NOAEL in deriving TEFs. In my judgement TEFs derived in this manner can be used in risk assessments where a NOAEL is being employed. The only exception to this generalization is if TEFs are based on REPs for EROD induction in cell culture systems where full dose response curves are unable to be generated for certain congeners. In those cases it has been recommended that REPs for this particular response be based on ED10 values.

3. The assumption in deriving vertebrate class-specific TEFs (WHO, 1997) was that they could be used to determine TEQs in fish, bird, and mammalian species, respectively, with less uncertainty than if a single set of TEFs was used. However, significant uncertainty still remains in extrapolating these new TEFs across species. This is reflected in the new TEFs still being referred to as "order of magnitude estimates". This certainly applies in directly extrapolating the fish-specific TEFs (determined in rainbow trout) to lake trout and bull trout, the bird-specific TEFs (based on studies in chickens) to Caspian terns and bald eagles, and the mammal-specific TEFs (determined in laboratory rodents) to the river otter.

Those TEFs that are the most uncertain are the ones derived solely from either QSAR or AhR binding affinity studies followed by TEFs that are based on CYP1A1 induction in vitro and in vivo. Bird-specific TEFs are the most problematic in this latter regard because all PCDD and PCDF TEFs for birds are based solely on EROD induction in the chicken embryo. Thus, for the Caspian tern and bald eagle there is more uncertainty in directly extrapolating the bird-specific TEFs for PCDDs and PCDFs.

Nevertheless there is such a paucity of studies, particularly in fish and birds, on the magnitude of species differences in REPs for the same endpoint that it seems prudent to assume for each vertebrate class that the TEFs can be extrapolated across species with a one order of magnitude uncertainty until there is evidence to the contrary. In support of this assumption sets of TEFs for PCDDs, PCDFs, and PCBs, determined by various authors/agencies, were recently used to determine TEQ concentrations in lake trout eggs from the Great Lakes. It was found that the TEQs so determined varied by less than one order of magnitude in spite of the different sets of TEFs that were used (Cook et al., 1997). In light of these findings, and recognizing that TEFs are one order of magnitude estimates, it would appear that class-specific TEFs can be directly extrapolated to the fish and wildlife receptors of concern in the case studies.

The REP determined for PCB126 in rainbow trout eggs, based on the endpoint of early life stage mortality, the Peterson laboratory has shown to be accurate in predicting the egg dose of PCB 126 that caused early life stage mortality in lake trout eggs. Thus, between these two closely related fish species, rainbow trout and lake trout, the REPs for PCB 126 were quite similar. Whether this will be the case for fish species that are not as closely related is not known, but is an

important area for future research.

III. EXPOSURE PROFILE

1. The TEF approach, in and of itself, does not present new uncertainties or modify old uncertainties associated with modeling the exposure of AhR agonists, because it is not applied until after the concentration of an AhR agonist has been estimated in a particular tissue for the receptors of concern (i.e., fish egg, bird egg, or mammal liver).

2. The TEF of a particular congener is based on its potency for producing a particular response, relative to that of TCDD, when both compounds are administered by the same route.
For fish and birds, the majority of TEFs were based on the egg injection route of exposure. This is significant because the concentrations of PCDDs, PCDFs, and PCBs in eggs when multiplied by such TEFs should give rise to a TEqC that has a greater level of certainty associated with it than if a different route of exposure had been used. Also in lake trout it has been shown that the potency of TCDD in causing early life stage mortality is essentially identical irrespective of whether TCDD is transferred naturally from the female to the oocytes prior to spawning, is directly injected into the egg, or is taken up by the egg following waterborne exposure to TCDD.

3. No comment.

IV. RISK CHARACTERIZATION

1. Uncertainties associated with the TEFs are not more problematic than the other sources of uncertainty in the ecologic risk assessment nor do they limit the means of performing the assessment. In my judgement uncertainties associated with estimating exposure to the various PCDD, PCDF, and PCB congeners, retrospectively and prospectively, are greater than those associated with the TEFs.

2. The H4IIE bioassay, has the advantage over the TEF-based approach of assessing interactive effects of AhR agonists. The endpoint of such a bioassay is that a "net" AhR-mediated response in cell culture, such as induction of cytochrome P4501A1 activity, is determined. TEQ

concentrations are then estimated by comparison to a TCDD standard curve after appropriate corrections for dilution of the tissue extract are made. Another strength of the TEQ bioassay is that it is relatively inexpensive when compared to congener-specific GC/MS, and can be used, therefore, to screen a large number of samples for high concentrations of TEQs in a more cost effective manner. The weakness of the method is that it will detect other AhR agonists that are not PCDDs, PCDFs, and PCBs, such as polyaromatic hydrocarbons and several other classes of compounds, and can lead to false positives.

The two approaches could be integrated if the H4IIE bioassay, the recently developed CALUX bioassay, or an equivalent, validated, TEQ bioassay were used to screen large numbers of environmental samples for TEQs. Congener-specific GC/MS which is more cost prohibitive could then be reserved for confirming PCDD, PCDF, and PCB congener related AhR agonist activity in only the most highly contaminated samples and for confirming reduced AhR agonist activity in designated "cleaned up" media such as lake or river sediments in the case studies.

3. List of specific studies that would reduce uncertainty in the case study assessments (ranked from highest to lowest priority)

1. There is a need for determining a NOAEL and LOAEL for effects of TCDD in reptiles (snakes and turtles) and amphibians as well as TEFs that can be used to determine TEQs for these species.
2. There is a need for a laboratory-conducted, dose response, developmental and reproductive toxicity study in mink exposed in utero and via lactation to TCDD or PCB 126 alone. Such a study does not exist causing uncertainty in the NOAEL and LOAEL used for TCDD in piscivorous mammals.
3. There is a need to determine the NOAEL and LOAEL for TCDD and PCB126 in bull trout. The bull trout is related to the lake trout, the most sensitive fish species to TCDD-induced early life stage mortality. Given its threatened status, it might be significantly more sensitive than lake trout to TCDD-induced early life stage mortality. If so, determination of the NOAEL and LOAEL for bull trout might change the conclusion of an ecological risk assessment which otherwise would have relied on the higher NOAEL and LOAEL for TCDD in lake trout eggs.

4. For fish and birds there is a need to conduct cross-species comparisons of REPs based on a population relevant endpoint such as embryo mortality. This should be done for those PCDD, PCDF, and PCB congeners that are generally considered to be the major contributors to the TEQ concentrations in fish and bird eggs in North America. The question to be addressed is: for each individual congener tested, in fish and bird species of widely differing sensitivity to TCDD-induced embryo mortality, will the REPs vary by more than one order of magnitude?
5. There is a need to determine, for a wide variety of environmentally relevant egg or body burden mixtures of AhR agonists in fish and wildlife, if in ovo exposure (fish and birds) and in utero and lactational exposure (mammals) causes population relevant signs of toxicity (i.e., developmental and/or reproductive) by an additive interaction.
6. TCDD embryotoxicity studies need to be conducted in long-lived aquatic species that live in close contact with contaminated lake and river sediments such as snapping turtles and sturgeon.

Additional Questions Specific to the Prospective Case Study

RELATIVE TO THE EXPOSURE PROFILE:

1. No comment.

2. No comment.

RELATIVE TO THE RISK CHARACTERIZATION:

3. A source of uncertainty in applying TEFs across species of the same vertebrate class is not knowing to what extent TEFs vary between species. Until the scientific literature clearly demonstrates (within the same vertebrate class for those PCDD, PCDF, and PCB congeners that are generally considered to be the major contributors to TEQs) that the TEFs determined for one species are consistently more than one order of magnitude different from TEFs determined for the same endpoint in a different species - it is recommended that an uncertainty factor not be applied to the TEFs.

Richard E. Peterson

Additional Questions Relative to the Retrospective Case Study

RELATIVE TO THE RISK CHARACTERIZATION:

1. The TCDD equivalents sediment clean up goal would be determined by which wildlife receptor of concern would have its recruitment adversely affected at the lowest concentration of TCDD equivalents in eggs (lake trout or Caspian tern) or liver (otter). This sediment clean up goal, because it is the most restrictive, would also be protective of recruitment in the other two species.

If the vertebrate group with the most certainty is not the group with the most restrictive sediment clean up goal it might still play a useful role in directing the sediment clean up. That is, the sediment clean up goal for the "less sensitive but more certain group" could represent an "upper bound" for clean up whereas the sediment clean up goal for the "more sensitive but less certain group" would represent the "lower bound" for clean up. By bracketing the sediment clean up goal in this way would set limits on what is acceptable.

2. No comment.

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Mark Servos

TEF Workshop: Responses to questions

Stress response profile

1. There is very little gained by using the exact values for TEFs. The values are derived from information for which there is a lot of variability with the experimental data, approaches, species, etc. Using the actual values would in many ways be misleading because it gives more credibility to the number than is justified.

2. No. There is certainly more data available for some chemicals. There should be more credibility give to studies that use whole organism responses as an endpoint or that have been validated in field studies. Only a few congeners generally contribute to the REQ and these are the ones for which the uncertainty is most critical. Emphasis should be on estimating the uncertainty of these.

3. The farther you move from a whole organism response the less faith we can have on the predictive ability of the measure. The whole organism responses integrate the many complex responses in the fish. Many factors can alter, inhibit or modify biochemical responses at the cellular level dramatically alter the interpretation of the relative toxicity. Early life stage mortality is an endpoint which we can apply with some certainty. However, there are numerous mechanism by which these chemicals can interact with organisms and cause adverse effects. The early life stage mortality is a very well studied and sensitive endpoint for fish. In birds most of the studies are at lower levels of organization. Of particular interest in birds would be the validation of the TCDF TEF of <1.

Stress-response profile relative to the application of the TEQ Approach

1. A single chemical approach would seriously underestimate the potential Ah-receptor mediated toxicity. The TCDD alone would not be considered a major problem at any of the trophic levels in the northern lake scemario. Looking at t-PCBs alone would also be very misleading as the t-PCBs are less than would be expected to cause any responses in the trout eggs or otter liver. In contrast the t-PCBs are in the range that would raise concern in the terns; 5.657 compared to 5 (1-20) ug/g no effect threshold. If we assume additivity, the conclusions are very different. The total-TEQs in trout eggs is below the threshold for effects (30) but approaching the range of reported values. The total-TEQs in the terns is 4 times higher than the threshold values. This driven by the high levels of PCB 126, 77 and 81 with a relatively small amount of 2,3,4,7,8-PCDF. Mink liver also have high values (although less than terns) but the total-TEQs are driven by PCB 126 and 2,3,4,7,8-PCDF. By assuming additivity we have ignored potential antagonistic and synergistic effects which could alter the expression of toxicity.

2. The threshold values may differ from the EC50s leading to a misinterpretation of the relative toxicity of congeners. The assumption that the dose-response slope are parallel is often valid and is testable although it may differ according to species and response measured. Caution should be used when making conclusion based on this type of data.

3. In the spill case study, it would seem reasonable to apply the TEFs to all of the species within each class of biota with some caution. There will be considerable variability within each class but if caution is used and the limitations recognized this approach will be very useful. The closer the phylogenetic relationship the higher the level of confidence in extrapolating the results. The lake trout TEF can be applied with considerable confidence to the lake trout and salmon but less so to the carp and sturgeon. Many fish species such as fathead minnows show a much reduced response (EROD) to exposure to various chemicals which may affect the application of the TEF developed from trout, etc.

III. Exposure profile

1. The need to model numerous chemical presents a challenge. The weakness of the physical/chemical and biaccumulation data for specific congeners introduces considerable uncertainty. This leads to many assumptions or simplified approaches being employed. The particularly important weakness is knowledge as to the extent of bioaccumulation and the changes in the relative composition of congeners at different trophic levels resulting from differential metabolism and/or biomagnification. This can lead to very different relative importance of each congener in different organisms. The chemical focused for remediation may differ depending on the trophic level that is at risk. For example, in the spill senario, the PCDFs are the most important (more than half) the lake trout while the terns are driven by the PCBs especially PCB 126. PCB 126 is the dominant congener of concern for otter but PCB 77 and 81 are important for terns.

2. For the persistent slowly metabolized or excreted compounds this would not be a significant problem. For other compounds it could be if the actual dose is not considered. There is some concerns about continuous exposure to congeners that do not bioaccumulate (this could be happening in a pulp mill discharge). A tri-substituted dioxin found in the pesticide TFM caused induction of MFO enzymes in fish even though it was relatively water soluble and easily degraded. A constant exposure to low levels may result in responses which would not be seen in experiments where a single dose is administered and the chemical is quickly metabolized.

3. Congener specific analysis is difficult and expensive. It require additional steps in the clean-up and high resolution GC-MS detection.

IV. Risk characterization

2. The biologically based TEQs could be employed as an alternative to address some specific questions. Biologically derived TEQs could be used as a surrogate for more expensive chemical analysis to monitor the success of remediation or to detail the distribution of the contamination. However, the concentrations would have to be validated and it would have to be demonstrated that the chemicals of concern were causing the biological response in the environmental samples being monitored. The biologically derived TEQs would differ based on the type of cell line used (fish vs mammals) so the appropriated procedure would be important. Biologically derived TEQs have been used to demonstrate that chemicals other than PCDD/Fs at pulp and paper mills were present and contributing to the MFO induction response. In a case such as this the biologically derived TEQ would respond to the other chemicals and fail to demonstrate a reduction in the chemicals of concern (e.g. PCB 126, PCDF).

3. site specific studies, etc.

- Relative contribution of items in the diet of the birds and otter. Are the PCBs associated with bioaccumulation through the aquatic system in the lake?
- The role of other sources to the diets. To determine the relative role of the lake and determine if remediation will reduce levels. The calculation of the concentration of chemicals in the birds and mink did not consider that only part of the diet comes from the lake.
- The seasonal contribution of the lake to loadings and reproductive success in terns.
- Reproductive success of the terns and hatchability of lake trout. To determine if there is a predicted effect.

Risk Characterization

The clean-up goals would not be the same for each vertebrate group. The fish are just approaching the threshold values so there would be no apparent need for remediation. The birds have the highest TEQs but also a higher thresholds than the otters. The birds and the mammals have about the same BMF for PC 126 but the mamamals have a BMF of 54 compared to only 1.6 for 2,3,4,7,8-PCDFin birds. The otter metabolized some of the PCBs, most notably the PCB 77 and 81 which changes the relative composition of the congeners at this trophic level. PCDF contributed the most to the PCDD/PCDF TEQ value in terns and otters. However, when considered alone or with the PCDD/PCDF totals they are predicted to not be high enough to cause the effects. There is a huge difference between the TEFs of PCB 77 and 81 in birds and mammals but the value for PCB 126 which is the major contributor in both is the same. The focus should be on the PCBs in the higher trophic levels but these goals would have to be translated into sediment

concentrations goals. The sediment cleanup goals should be set for the group with the most restrictive values. However, if the uncertaintity is high the values may be too low and unnecessary and expensive cleanup goals may be set. If the terns are getting only a small dose from the lake when we have assumed it all comes from the lake then remediation may not result in the desired goal. On the other hand the poor prediction of the BMFs or other factors may set a value to low which is not adequate to protect the most sensitive species. If possible the various scenarios should be presented with some level of confidence (or lack of) for the risk manager to use as needed. To use the t-PCBs to guide remediation we would need to ensure that the key toxic congeners are changing in proportion to the total-PCBs and the relative bioavailability of the congeners is not changing with the remediation.

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Comments on the questions related to the Workshop on the Application of TEFs to Fish and Wildlife.

I.1

With respect to additional information provided, I do not think that it will provide the workshop with more information regarding the use of TEF values for wildlife, as it mostly concerns guidelines for TCDD only. During the WHO Stockholm meeting all available material has been evaluated for "Eco-TEFs". Therefore it does not enhance the means of evaluating the uncertainties more.

I.2

The uncertainties in "Eco-TEFs" are much larger than those obtained from mammalian studies due to the limited information available. Nevertheless, by using a tiered approach (see WHO document) and the rounded off procedure the most protective way was chosen which was possible.

I.3

At the TEF Stockholm meeting a tiered approach was followed for "Eco-TEFs" in which priority was given to more classical toxic parameters, e.g. ELS mortality, above biochemical effects or QSARs.

II.1.

If it is assumed that no additivity or no interactions exist, each compound involved should be evaluated seperately and the basic information for this process is than lacking, because it was simply not available. Actually, because we acknowledge the Ah-receptor mechanism and derived TEF concept we are worrying about these compounds. In addition, there are sufficient in vivo and in vitro studies which support this TEQ/TEF approach. In general non-additive interactions which have been reported are general within one of a magnitude or even much lower. For ecotoxicology the largest uncertainties do not seem to be these non additive interactions but large differences in species sensitivity.

II.2.

From a practical point of view there is not much choice as at best usually the EC50 or LC50 values were reported. As the efficacy of a dose respons curve varies, especially for PCBs, another value maybe better. An EC10 or or even lower might be better. However I feel that the amount of information now available in literature does not permit such an approach. With respect to differences in slope of the dose respons curve I am not convinced that the statements which have been made by the critics that this phenomenon makes the TEF concept impossible to work with are that valid. Opponents using this "difference in slope" argument have to my knowledge this argument not solidly supported by statistical analysis.

II.3.

I think that the differences observed in species specific TEFs are less of a problem than the value which is actually used as a LOAEL or NOAEL for the species of concern. In the environment we have a huge amount of species variation and we know from lab studies with different taxa that sensitivities towards these compounds, usually TCDD, can vary more than three orders of a magnitude. Therefore the right choice of LOAEL/NOAEL seems to much more essential to the process.

III.1.

One of the challenges within the present approach of the TEF concept comes from the fact that the efficacy (Ymax) of the response varies a lot, especially with PCBs. This difference in efficacy strongly influences the TEF values we work with. I would like to know how this problem should be approached in future and what the fundamental reasons are behind this phenomenon. At my lab we have developed some ideas, but sofar have not come up with a good solution or suggestion (Maybe this topic belongs to another question).

I think food chain models will work pretty nicely as long as the compounds are highly resistant against biotransformation. As soon as compounds are more effectively metabolized the modeling becomes more difficult due to species differences in metabolic capacities. Luckily for most of the PCDDs, PCDFs and PCBs which accumulate in the food chain this seems not to play a dramatic role. I think other fate and transport models will also be a good approach for these compounds as long as you stay with the more hydrophobic compounds. In addition, I think that physico-chemical data can be much

easier obtained for these compounds than biological data, while further more physico-chemical data could be easier estimated using e.g. log Kow.

III.2

The TEF concept could certainly be strongly improved if values are derived in the future from e.g. tissue levels instead of administered dose. Using PB-PK modeling in combination with the right toxicodynamic models would improve the risk assessment for these compounds significantly and even bridge the differences between species. The present TEF concept has always been presented as an "interim" method for risk assessent during the last 10-15 years, but in fact nobody ever came up with a better method. In addition, governmental agencies have not put much effort in improving the TEF-concept either. So for the time being we are just stuck with this interim method if we want to do (eco)risk assessment for these compounds.

III.3

I do not think that the present TEF concept requires a more rigid design of analysis as most labs which are involved already analyze the non and mono ortho PCBS in addition to the 2378-PCDDs and PCDFs already measured. Measuring total PCBs is rather risky for sediments etc. as you might miss geographical and temporal changes in the most toxic PCBs in the matrix easily. As these are the congeners you are interested in, this information should not be ignored. However, it should be noted that TEQ values from e.g. sediments have more a comparative meaning than an actual toxicological one. As is illustrated nicely with both risk assessment exercises much more information is necessary before e.g. a risk assessment can be done for a top predator. In other words when ecological risk assessment is done on these compounds models should work as long as possible with the congener specific approach. The combination between TEQs and toxicity should be done in the final step of final food. In general the use of TEFs and TEQs should not exceed that of a single trophic level.

IV.1.

I think that the problems with the TEFs and associated TEQs are in general greater than those for a number of other groups of environmental contaminants eg. OP-esters, because:

a) It involves an exceptionally high number of compounds with a specific mode of action.

b) These compounds show a large variability in physico-chemical properties.

However, if one would develop an appropriate TEF model for the polyaromatic hydrocarbons and genotoxicity, I am sure similar problems as with the dioxin TEF model would be encountered.

See also earlier comments I made regarding the uncertainties of the present TEF concept.

IV.2.

I think that biological based assays, e.g. Caluc Ah-receptor, could serve very well as a prescreening method for selecting abiotic environmental samples for further chemical analysis. In fact they could also serve as a way of measuring TEQ tissue levels from target species. However, these bioassays could never be used in biological samples as long as the species specific sensitivity is unknown. Alternatively, a large general safety factor for eco risk assessment in combination with these bioassays might do it also. I do believe that these bioassays can save us a lot of money on expensive chemical analysis as long there limitations are acknowledged.

IV.3.

I think that the present amount of information is adequate for the two case studies presented. From my expertise (TEFs, toxicity and pharmacokinetics) the recent WHO evaluation tried to incorporate as much as possible all available scientific information. More information was simply not available. It might be desirable to have the WHO TEF database available at the meeting for consultation, If necessary I can bring it with me.

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Dr. van Hattum received a Ph.D. in biology from Vrije University in Amsterdam and a master's degree in chemistry at the State University of Utrecht. At Utrecht his major areas of study were analytical chemistry and chemical oceanography. Most recently he has researched food chain transfer and effects of planar PCBs in top predators, analysis and bioaccumulation of organic compounds, risk assessment of water discharges from offshore installations, design of biomonitoring programs, and sustainability and environmental quality in river basins. Dr. van Hattum has participated in several advisory committees for the Dutch government on secondary poisoning in mammals and birds and on the development of environmental quality criteria for PCBs. He is currently a member of the board of the Environmental Toxicology Section of the Dutch Society of Toxicology, a member of IAWQ, SETAC, and the editorial board of *Environmental Pollution* journal. He has published more than 40 articles and reports, 4 books or chapters, and he has made presentations at a number of conferences in Europe and Canada.

Premeeting Comments

With much pleasure I have read the extensive set of documentation. I was impressed by the profound and critical approach laid down in the different documents. Below I have tried to answer some of the charge questions in my field of expertise. Some of the citations include recent technical reports on Dutch and Danish otter studies, which can be made available on request.

I. Stress-response profile relative to the derivations of specific TEF-values.

Q1,2,3 The material provided for the workshop and the references cited in the case studies, provide further experimental corroboration of the validity of the approach laid down in the WHO-document for the evaluation of the hazards of AhR agonists, such as PCBs, diobenzodioxins and dibenzofurans. Although many questions remain unanswered (WHO-1997), it helps to identify critical compounds, pathways, species at risk, and to focus emission reduction programmes. Especially the material from recent experimental or review studies on mink (Tillitt *et al.* , 1996; Leonards *et al.* , 1995) provides substantial evidence for the extreme sensitivity of this species, the cause-effect linkage between contaminants in the diet and reproductional effects, and the soundness of the TEF-approach as a framework to account for the joint toxicity of mixtures of contaminants. The uncertainty of the proposed TEQ-based no-effect concentrations (NOEC) for mink probably is much lower than for other chemicals, for which NOECs usually are being extrapolated using safety-factors from experimental studies with 'surrogate' laboratory species (Luttik *et al.* ,1993).

II. Stress-repons profile relative to the application of the TEQ-approach

Q1. Rejecting the additive dose-interaction model of the TEQ-approach, would imply separate risk-assessments for all potentially active individual congeners. In that case the focus should be directed towards the compounds that are most likely to induce effects. Depending on the target organism, a significant proportion of the toxic potency may be left out of the evaluation. Based on the TEQ-values calculated for the Oneofakind Lake case study, it can be hypothesised that individual congeners may

contribute represent at most up to 30-40% (23478-PCDF) of the total dioxin-equivalent conentration for fish, and 60-70% (PCB-126) for birds and mammals. Reasoning further along this line, an evaluation based solely on total PCBs would probably underestimate fish early life stage mortality, and an evaluation based solely on 2378-TCDD would underestimate potential reproductional effects in mammals or birds.

Q2. I am not familiar with the discussions held during the preparation of the WHO-TEF document. Based on analogy of risk assessments conducted for other compounds, chronic NOEC (no observed effect concentration) or NOAEL-based values (no observed adverse effect level) if available, should be preferred. Relationships between NOECs and other endpoints values have been studied systematically for several compounds and test-species (Slooff *et al.* 1986), and have resulted in specific extrapolation factors, which are used in the Dutch risk-assessment protocols (Luttik *et al.* , 1993). I have no knowledge if similar surveys have been made for AhR-agonists.

Q3. If available, data from eco-epidemiological studies for potentially affected species should be used to evaluate the feasibility of the lethal body-burden concept and within-class extrapolations of toxicity endpoints for AhR-agonists. In Leonards (1997) a comparison was made of no-effect and critical levels for otter, mink and seal, expressed as concentrations in the liver of the target-species. Hepatic TEQ-based NOECs for mink (0.4 -9 ng/g lipid wt.) and otter (1 - 2 ng/g lipid wt.) were comparable. A lower NOEC was found for seals (0.1 ng/kg lipid wt.). Due to the large differences in toxicokinetics and biotransformation between the species, fish diet-based NOECs for TEQs (ng/kg wet wt.) exhibited a much different ranking, ranging from 0.7 ng/kg wet wt for otters, and 1-50 ng/kg for mink and 8 ng/kg wet wt for seals. This demonstrates that within-class extrapolations of toxicity endpoints should be dealt with only with great care.

III. Exposure Profile

Q1. The proposed risk-modelling framework requires the input of high-quality data for calibration of currently used parameter estimates (BAFs, BSAFs, BMFs, rate constants) in chemical fate and bioaccumulation modelling. Special attention should be given to habitat- or target species-specificity of parameter estimates, in order to

judge the validity of the application of generic parameter-estimates. In the studies conducted in food chains of European river otters (*Lutra lutra*) in Danish and Dutch habitats (Smit *et al.* , 1996; Leonards *et al.* , 1997), we observed extremely high otter-fish BMF values (indicated in Table 1) especially for some non-ortho substituted PCBs (126 and 169), which are much higher than the mink-diet based BMFs reported by Tilliitt *et al.* , (1996) and applied in the case-studies. As concentrations in otters and fish are influenced by factors such as age, sex, reproductional activity, and other species-specific factors, BMF estimates may be highly sensitive to sampling design and experimental methodological choices made in the various studies.

Especially with respect to the role of non- and mono-ortho substituted PCBs, the availability of high-quality analytical exposure data is a limiting factor. The analytical procedure (pre-separation followed by HRGC-MS) is costly, and a rigorous analytical quality control is required in order to produce accurate and precise exposure data. Most of the currently involved laboratories in OECD countries have participated in round robin exercises or proficiency testing-programmes. There is a need for development of low-cost analytical techniques for AhR agonists, which e.g. can be used in combination with AhR-responsive bioassays.

Most of the old exposure assessment data for PCBs are expressed as equivalent technical-mixture concentrations, as concentrations of selected dominant di-ortho substituted congeners, e.g. PCB-153, or as total concentrations of individual congeners. In most European monitoring programmes and regulatory practices attention is focused mainly on di-ortho PCBs. Therefore, there also is a need to develop and evaluate the feasibility of generic or habitat-specific extrapolation algorithms to derive TEQ-exposure profiles from e.g. PCB-153 concentration data.

Q2. Exposure route differences may have a profound influence on the actual dosage at receptor sites, due to variations in e.g. bioavailability, feeding-preferences, toxicokinetics and biotransformation of contaminants in the target-species and in species from lower trophic levels. The differences in susceptibility to dietary PCBs between mink and otter, as discussed previous section, seem to be related to variation in toxicokinetics and lipid-metabolism between both species.

IV. Risk Characterization

Q1. Many methodological, habitat-related and biological factors contribute to the uncertainty of parameter estimates used in risk assessment models. Complementing the angle taken in the case studies, with evaluations based on trials with probabilistic models would provide insight in the effect of the uncertainties on the extent to which target species are protected.

Q2. The added value of some of the recently developed bioassays and biomarkers, is that they provide insight in the total quantities of AhR-responsive compounds. In studies conducted by Murk *et al.* (1996) and (1997) good correlations were found between CALUX-based TEQs and values derived with TEF-values from measured concentrations. Care should be taken to account for confounding response of other potentially active compounds, such as e.g. PAHs in sediments. In the study of Smit *et al.* (1996) a combined approach was applied, in which the low-cost and sensitive CALUX-assay was used for screening purposes and selection of samples for further extensive chemical analysis.

Q3. With respect to gathering of site-specific data my recommendation would be:

- exposure concentrations in sediments, fish and tissues of predatory species species to examine if generic BSAF and BMF values can be used, or that site-specific values should be applied. For the prognostic case-study the predicted water column partitioning (dissolved, DOC-bound and POC-bound) should be corroborated with experimental data.
- ecological assessment of status of targeted species, assessment of influence of other natural or anthropogenic stress-factors
- confirmation of predicted risks with relevant laboratory-bioassays
- uncertainty analysis with probabilistic modelling

V Prospective case-study

Q1. Due to variable hydrodynamic conditions, large variations may be expected in the transport, distribution and bioavailability of contaminant in the water column. The applicability of generic BAF^{fd} values needs to be investigated. Additional studies could contribute to the accuracy and precision of the proposed water quality objectives.

Q2. Based on the high biomagnification of some non-ortho substituted PCBs (PCB-126, PCB 169) in the food chain of the otter, an approach which ignores congener-specific biomagnification, may result in an underestimation of risks of these congeners to sensitive predators.

VI Retrospective case-study

Q2. In the study of Smit *et al.* (1996) TEQ-based NOECs and critical levels (for Vitamin A reduction) in otter-liver, were extrapolated with congener-specific BMFs and BSAFs to equivalent critical levels and quality objectives in fish-diet and sediments. Significant double-logarithmic correlations were observed - for sediments and biota - between concentrations of Σ 7PCBs (summation of 7 selected congeners, which usually account for 50% of total PCBs : 28, 52, 101, 118, 138, 153, 180) or indicator congeners (PCB-153) and TEQ levels. Although the relative contribution of individual congeners to the total TEQ-based concentration appeared to be species-specific, extrapolation factors could be derived to express the proposed critical levels on the basis of Σ 7PCBs and of PCB-153. As a rule of thumb, a one order of magnitude range of uncertainty may be introduced due to this extrapolation. Nonetheless, as most of the European fish and sediment-monitoring data are based on these standard congeners, this provided a framework to evaluate the quality of potential otter habitats in the Netherlands. Similar relationships also have been observed in recent (unpublished) studies planar PCBs in sediments and cormorant food chains in the Rhine-Meuse estuary.

As correlations between total PCBs and TEQ-based concentrations may be site-and species-specific, my recommendation would be to validate such extrapolations with measurement data. Some of the low-cost biomarker techniques also could have potential for screening purposes in this context.

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Table 1. Average fish diet-based biomagnification factors (lipid weight basis) of PCBs for otters in Danish (Lymfjord) and Dutch (Lakes Oude Venen) habitats.

PCB No.	BMF* Oude Venen (NL)	BMF** Lymfjord (Denmark)
28	0.044	0.47 (0.002 - 8.4)
31	0.049	
44	0.014	
49	0.022	
52	0.016	0.54 (0.2 - 2.3)
101	0.066	2.1 (0.03 - 36)
105	12	7.9 (0.7 - 50)
118	15	35 (2 - 251)
128	9	
138	26	31 (4 - 297)
149	0.16	
153	15	28 (2 - 172)
156	30	37 (3 - 505)
157	19	84 (2 - 2086)
158	4	
166	2	
167	6	13 (2 - 83)
170	15	
180	123	63 (5 - 442)
187	23	
189	50	144 (12 - 1073)
194	21	
77	1.4	2.5 (3 - 7.9
126	70	130 (4.2 - 900)
169	348	108 (3 - 1700)
Σ PCBs	14	36 (2.9 - 209)
Σ TEQs***	41	95 (3.5 - 640)

* Geometic mean BMFs for 5 otters from Leonards *et al.* (1997); ** From Smit *et al.* (1996), geometric mean values (di-ortho PCBs n=20; non/mono-ortho PCBs n=9) and minimu to maximum ranges of BMFs between brackets; *** calculated with TEFs from Ahlborg *et al.* (1994). BMFs are expressed on a lipid normalized basid and calculated for an average diet composition.

Appendix D

DETAILED SUMMARIES OF EXPERTISE GROUP DISCUSSIONS

FATE AND TRANSPORT EXPERTISE GROUP

Facilitator: William Adams

Group Members: Joseph DePinto, Lynn McCarty, Christopher Metcalfe, Patrick O'Keefe, Mark Servos, Phil Cook, Cynthia Nolt, and Lisa Williams (notetaker)

Discussion started with the questions distributed.

Question 1: How well do we know the uncertainties associated with accuracy and precision of analytical chemistry data, including measurement of BAFs, BSAFs, and BMFs?

BAFs may vary from one aquatic system to another. Modeling of these aquatic systems may allow estimates of BAFs to be made. Modeling BAFs requires parameters like K_{ow}s and Henry's Law constants for individual congeners. K_{ow}s are known reasonably well, but Henry's Law constants are usually calculated themselves and may be uncertain to within a couple of orders of magnitude.

BAFs can be determined empirically by measuring concentrations of congeners in biological tissues and in water. Concentrations of some compounds, including TCDD itself, may be near the detection limit in water, especially in the dissolved phase. Water concentrations may be calculated from other partitioning coefficients.

Precision and accuracy of the compound-specific measurements needed to determine BAFs and BSAFs vary among matrices. The sources of uncertainty in these measurements include analytical variability, the extent to which sampling protocols represent the real heterogeneity in the system, and magnitude of the real heterogeneity. Analytical variability increases as concentrations approach the limits of quantification (LOQ) and detection (LOD). In sediments and tissues, concentrations of individual PCBs, PCDDs, and PCDFs can currently be determined to within ± 30% for most samples. As concentrations approach the LOD, concentrations can be determined to within a factor of 5 to 10. Concentrations in ambient water samples are near LOQ and LODs in most samples, so determinations are generally accurate to within a factor of 10. Sampling protocols need to be designed using power analyses. The real heterogeneity of concentrations of these compounds may be huge for sediments within a given aquatic system because of the heterogeneity of sediment types. Concentrations vary spatially and with organic carbon type and amount, particle size distribution, and other sediment characteristics. Concentrations of these compounds in individual fish within a population may vary by an order of magnitude. Heterogeneity in water samples within a system is not well studied, but varies with solids dynamics in the system.

Analytical techniques are available for all of these compounds. Many commercial laboratories are currently analyzing 2,3,7,8-substituted PCDDs and PCDFs. Fewer are regularly analyzing non-ortho-substituted PCBs, but more would likely add these to their available analysis if regulations began requiring quantification of these

compounds. No capital expenditures would be required as the methods currently being used for analysis of PCDDs/PCDFs are very similar to those used for the non-ortho-substituted PCBs. The most likely method to be used for these analyses is a carbon column separation step followed by gas chromatography/mass spectrometry with isotope dilution internal standards.

The uncertainty in sampling and analysis of the individual congeners is similar to that introduced by sampling and analyzing total PCBs or 2,3,7,8-TCDD alone. The samples required would be the same for any of these analyses. Measurements of total PCB concentrations may have greater uncertainty than those for individual congeners. Analysis of total PCBs is generally done by analyzing individual congeners and then summing their concentrations. Laboratories may use a different number of congeners to quantify total PCBs and may use Aroclor mixtures or individual congeners as standards. Analysis of total PCBs is less expensive than for congener-specific analysis which includes the non-ortho-substituted PCB congeners. The difference in the cost of analyzing 2,3,7,8-TCDD and analyzing all of the 2,3,7,8-substituted PCDDs and PCDFs is negligible.

Question 2: Are chemical fate and transport properties (hydrophobicity, volatility, photolysis, biodegradability, etc.) well characterized for all chemicals with TEFs? If not, what uncertainties are introduced in exposure predictions?

Hydrophobicity (K_{ow}) and volatility are better known and generally more important in determining fate and exposure than photolysis and biodegradability. K_{ow}s are known reasonably well. Using K_{ow}s to predict K_{oc}s introduces an uncertainty of about an order of magnitude (based on a 95% confidence interval) because of inherent differences in the nature of organic matter. Henry's Law constants for some congeners may only be accurate to within a couple of orders of magnitude, so in systems in which volatilization is a significant fate pathway, this uncertainty could have a significant impact on predicting water concentrations. Biodegradation is system-, congener-, and concentration-specific, so generalizability of this process is very poor. The time scale for this process is very long, so this is a less significant process in determining overall fate than partitioning, sorption, volatilization, and many other processes. Photolysis is also generally of minor importance to an overall mass balance for these compounds, but sensitization could result in this process being important for some compounds in some systems. In mass balance exercises in major rivers and bays, approximately 80% of the accuracy of the model was determined by the accuracy of the modeling for the solids dynamics of the system.

Question 3: What degree of uncertainty is associated with biotransformation/ metabolism in the food chain?

Biotransformation, metabolism, and differential absorption patterns alter congener patterns more significantly in birds and mammals than they do in fish and other biota. Congener patterns among fish species and fish tissues are relatively homogeneous. This is not true for birds and mammals that eat those fish. Relative to the pattern in fish, concentrations of PCB 77 decrease and those of PCB 126 increase as a percentage of total PCBs (see Figure D-1).

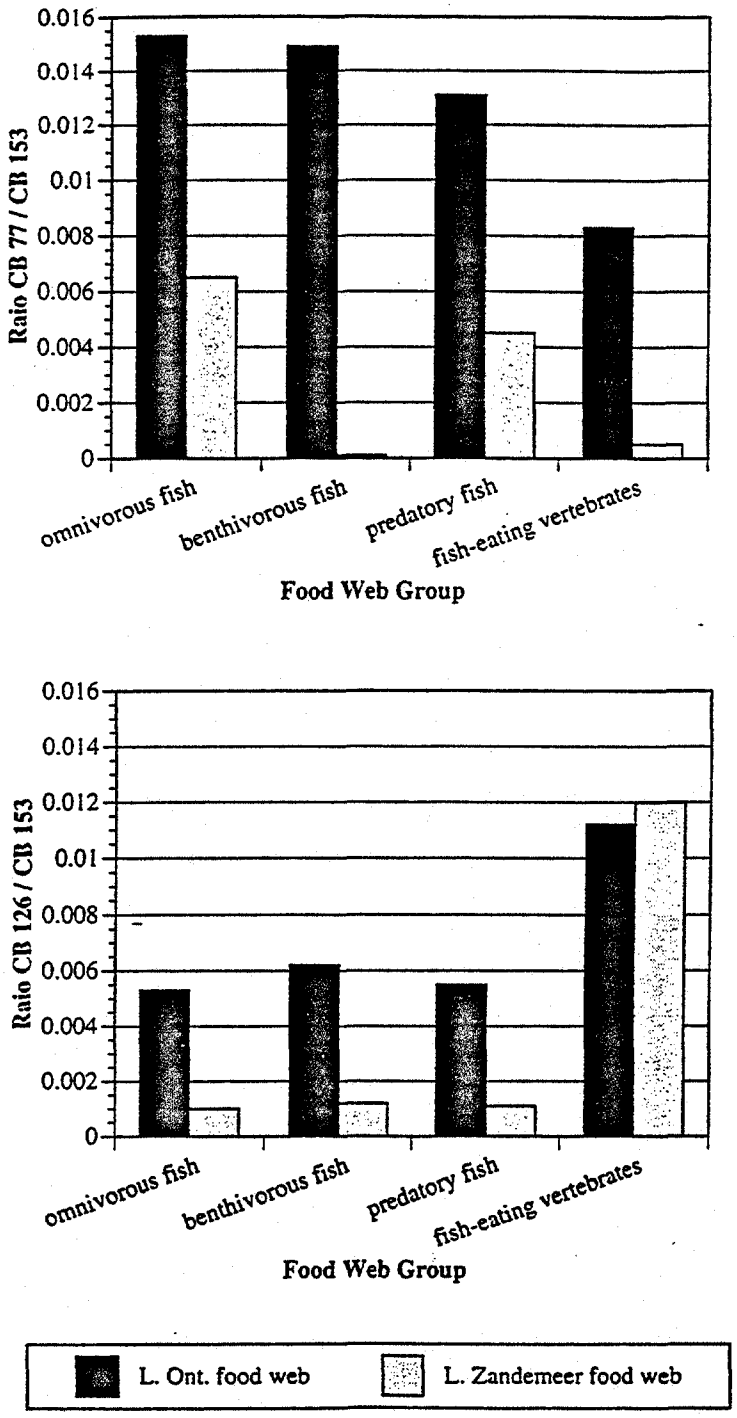

Figure D-1.

These shifts, especially for PCB 77, may be quite species-specific. Changes in patterns among tissues within a bird or mammal are not well characterized. Most environmental data on birds is for eggs and most mammalian data is for livers.

Biomagnification factors (BMFs; ratio of concentration of a given compound in the tissue of the predator to the concentration in the tissue of its prey) are needed for the species of concern, especially for fish-eating birds, for a given assessment. BMFs for a given species in one system could be applied in another system if the dietary composition is known. The basic ecology (food web structure and dietary composition) of a system is usually less certain than the BMFs themselves.

The TEF approach is critical in estimating risk to birds and mammals in particular because of the change in congener patterns from source Aroclors and through the food chain. For birds and mammals at the top of the food chain, these changes in pattern appear to be even more species-specific or class-specific than they are source-specific (in chronic exposures). In lakes with only atmospheric sources of PCBs and compared to those with local sources with varying patterns of PCBs, the patterns of congeners in the top of the food chain are similar although significant differences in patterns are observed low in the food chain. In the Great Lakes, analysis of archived samples of fish and bird eggs have shown little or no change in congener patterns within species over a time period, while absolute concentrations dropped by an order of magnitude.

Question 4: For these classes of chemicals (PCDDs, PCDFs, and PCBs), what are the greatest sources of inter-ecosystem variability in bioavailability and bioaccumulation? Are there any unique considerations for exposures in marine ecosystems?

The greatest sources of inter-system variability in bioavailability and bioaccumulation are solids dynamics and food web structure. This variability is most important in predicting absolute concentrations of PCBs, PCDDs, and PCDFs rather than in predicting the relative proportions among them. The solids dynamics is perhaps the most difficult to determine, especially in a marine system. Systems can be compared by knowing the distribution of contaminants between sediment organic carbon and the freely dissolved phase (p_{socw}; Cook and Burkhard, National Sediment Bioaccumulation Conference, September 1996). This relationship can be measured for one congener and generalized to the others. Differences in benthos structure may have significant influences on absolute concentrations at the top of the food chain for compounds with K_{ow} greater than 6. At the top of the food chain, the source of the contaminants to critical tissues is important because birds and mammals are mobile. For example, a migratory bird may arrive on a breeding ground and begin feeding locally and then transfer lipid from the bloodstream to the developing egg. In that case, the contaminants in the eggs would reflect local sources and diet. If the eggs are developing during migration or if the bird arrives on the breeding ground and must rely on stored lipid to produce eggs, then the contaminants in the eggs would reflect other sources of contamination.

Question 5: From the standpoint of TEF applications, what are alternatives to, or improvements for, the waste load allocation process model described in Figure 5 of the prospective scenario?

The waste load allocation process model needs to be validated, but it is expected that this process is more likely to change the values of the parameters than the tool itself. Overall, this model appears to be a good approach for dealing with this complex mixture of compounds with additive toxicity and which are bioaccumulated and exhibit chronic toxicity. The model would need to be more complex for an acutely toxic substance or condition. The assumption in this model of the existence of an assimilative capacity for these persistent compounds can be questioned on philosophical grounds. Loss processes from a given system are dominated by physical movement within or from the specific system rather than by chemical destruction.

A critical component of this waste load allocation process model is the system level mass balance model. A mass balance model can be very complex, so a hierarchy of mass balance models may need to be developed. Different mass balance models could be developed for different types of systems and with varying degrees of complexity and number of input parameters required. There is nothing unique about mass balance modeling for this application. If one can model TCDD for a given system, then one can model other PCBs, PCDFs, and PCDDs. The modeling will become more accurate as more parameters are measured in more systems.

The overall uncertainty in the waste load allocation model is unknown, but is largely related to knowledge of the system, not knowledge of congener-specific information. For example, ratios of BAFs among congeners across various systems are fairly constant (varying by less than a factor of 2 or 3), whereas absolute BAFs among systems are less certain. The uncertainty can be reduced with more measurements and can be explored using Monte Carlo simulations. Regulators may be given guidance on an amount of the waste load to be allocated to the uncertainty. Overall uncertainty in calculated final MALs may currently be a couple of orders of magnitude.

TEF EXPERTISE GROUP

Facilitator: Richard Peterson

Group Members: Bjorn Brunstrom, Steve Bursian, Jay Gooch, Mark Hahn, Bert van Hattum, Sean Kennedy, Martin van den Berg, Steve Bradbury, Mike DeVito, Don Tillitt, and Tim Kubiak (notetaker)

Dr. Richard Peterson opened the session by describing basic terminology for the discussion. Two terms, Toxicity Equivalency Factors (TEFs) and Relative Potency (REP), were defined to provide clarity. In the group discussion, Toxicity Equivalency Factors (TEFs) were defined as consensus values derived from multiple REPs. A REP pertains to the relative potency of a dioxin-like congener to TCDD in a single study. Additionally, it was discussed that a receptor could be defined as both a species under assessment and for pharmacological use of Ah receptor interactions. It was recommended that "target species" be used for the species and "receptor" be reserved for pharmacological use.

Dr. Peterson reviewed and handed out copies of the nine questions to be considered by the workgroup.

An issue was raised pertaining to the specific purpose of the World Health Organization TEFs. Group members questioned how broadly they are to be applied, and whether these values are for screening only. These questions were subsequently addressed in the case studies and are addressed elsewhere.

The following discussions pertain to the numbered questions prepared for the expertise group. It should be noted that six of the twelve individuals in this group also attended the WHO TEF meeting in Stockholm, where the WHO TEFs were developed for non-human mammals, birds, and fish.

Question 1. Are taxa-specific TEFs a reasonable approach given current scientific understanding of relative differences between fish, birds, and non-human mammals?

Discussion centered on the use of TEFs for the various classes and their use for site-specific analysis to be protective. There was considerable discussion about the meaning of the term "protective." It was generally agreed that the term was misused in the sense that there was no numerical uncertainty factor included to provide a any margin of safety due to uncertainty. Derivation was by a tiered approach (WHO 1998). Site-specific use of the TEFs was thought to provide predictive value in the interpretation of dioxin-like exposure, risk, and effects. While the WHO TEFs were rounded up or down to the nearest half or whole order of magnitude, the differences were not large. Fish and bird TEFs were considered predictive because the documentation was available for specific REP to TEF conversion and these values represented a toxicological endpoint for the most important congeners. There was a clear indication that a statistical validity assessment of the different TEFs was not performed.

Question 2. What are the sensitivity analysis arguments for using the WHO TEFs that have been rounded to provide maximally harmonized TEFs across taxa versus the unrounded TEFs that are more taxa-specific?

The discussion resulted in a finding that, despite rounding, the TEFs are not less but more predictive. The relative contribution of PCDFs, PCDDs, and PCBs to total TEQs does not change. Differences in species sensitivity is a larger source of uncertainty.

Question 3. How would you distribute uncertainty in the methodology among the following categories:

A. High dose to low dose extrapolation

Data indicate that using REPs from EC_{50} versus EC_{10} does not result in much difference. *In vitro* CYP1A protein induction is less consistent and there are problems associated with *in vitro* tests. The greatest confidence in the data occurs at the EC_{50} level of relative potency. Therefore, greater uncertainty involves the extreme ends of the dose-response curves, such as in the EC_{1-10} range. Inflection points vary in dose-response.

B. Species differences in relative potency

There was general agreement that there is a large degree of uncertainty associated with the comparative data across species when looking at REP data sets. This includes all tiers of the WHO database. The uncertainty is not associated with major variation but with a lack of interspecies comparative data for the same endpoint. For example, the chicken response to embryo toxicity is 70X more than double-crested cormorant. For PCB 126 the difference is 40X. REPs between the two are 0.07 and 0.02 for PCB 126. The question is what are minor differences for REPs? There should be some evolutionary differences. CYP1A1 has little difference in REPs and EC_{50}s are not statistically different. As long as dose-response curves are parallel, it works. There was some concern that there may be an overestimation of relative potency. While there is a lack of toxicity data for many REPs, those based on mortality data show some coherence.

C. Species differences in the sensitivity to TCDD

For birds, the data are limited. There is a need to be able to explain the mechanism of action at the receptor level relative to different species' sensitivity. It was mentioned that receptor occupancy determines toxicity, but receptor populations in tissues and organs are not routinely measured. There are some limited data on four species that supposedly can explain this but they were not identified. There is still high uncertainty in this area due to lack of information, that is equal to B above and greater than A.

D. Experimental versus environmental exposures

There is not a high degree of uncertainty. Target tissue doses are important in the environment. Routes of exposure in laboratory are different from environmental measurements in some cases, but for bird eggs there appears to be consistency between lab egg injected doses and maternally deposited doses. Egg injection can underestimate responses based on methodology.

E. Differences in the relative potency across endpoints

There was general agreement that there is some uncertainty, as with items B and C, above. For fish and birds, REP/TEF data rely on ecologically relevant population assessment endpoints through a reproductive endpoint (LD_{50}) for many congeners. Some other TEFs in mammals may have greater uncertainty.

F. Use of the assumption of additivity

TCDD & 153 are a limited data set showing antagonism at certain relative concentrations. Generally, data for dioxin-like congeners are consistent with additivity. Others present problems, because there is not much information. There appears to be low uncertainty relative to other sources. Mink data clearly support the additive model. Also low uncertainty based on chickens, lake trout, and brook trout. Environmental mixtures have a factor of 2 or 3 for variation. For fish, the departure factor from additivity is small (2-5). Methods to verify additivity have not yet been established.

Question 4. Are there different uncertainties due to chemical classification of the chemicals (i.e., dioxins versus dibenzofurans versus biphenyls)? If so, is there a biological explanation for this difference?

Mono-ortho PCBs produce mixed effects and induce more than CYP1A1. This is problematic in fish, since fish are not responsive to mono-ortho-substituted PCBs. Among vertebrates, there are class-specific differences. For humans, PCBs 118 & 105 give high tissue TEQs. The respective class differences are four orders of magnitude. PCB 126 drives TEQ calculation across classes and results in reduced uncertainty. Uncertainty is generally lower than for items B and C, above.

Question 5. If we decide that a given species is the most sensitive species in the ecosystem of study and relative potency values are available for that species for all of the congeners present, should we use the relative potencies specific for that species or should we use the WHO TEF values for all chemicals?

The purpose of the TEF is for risk assessment. WHO TEFs were rounded to half/whole order of magnitude. While there are great data gaps across species, there is also uncertainty that the assessed species is the most sensitive. Species-specific data could be used.

Question 6. If we chose to use relative potencies specific for the species of interest instead of the WHO TEF values, will we decrease the uncertainty in the TEF methodology?

Use of species-specific values will reduce uncertainty.

Question 7. How would a surrogate species be identified given that relative sensitivities among species cannot be easily predicted?

It is common sense to use similar species. There may not be a need for surrogates, if a species of interest can be used. Uncertainty associated with extrapolations across species is greater than that associated with extrapolations across endpoints.

Question 8. Given the toxic endpoints used to establish each set of TEFs, how far-ranging should the assessment endpoint diverge before the TEF predictive uncertainty is high? How would uncertainty be valued to account for these other endpoints when communicating risk?

The answer to this involves seeing data on species-related differences in REPs. Having not seen this sort of data makes the question difficult to address. At a minimum, tier consistency for a congener reduces uncertainty.

Question 9. What are the advantages and disadvantages of using the TEF methodology over that for total PCBs? Based on the type of AhR agonists that are present at a contaminated site is one approach preferable to the other?

Pharmacodynamic differences between species result in a need for better exposure assessment, which is important for ecosystems assessment. TEFs should be applied only to biotic matrices, not to abiotic. Different commercial mixtures vary in congener composition, resulting in further uncertainty associated with total PCB assessment. It may be possible to use indicator congeners to track and extrapolate from known-composition mixtures.

An observer addressed the need for feedback on the global background of contamination. For fish and wildlife, other Ah-active compounds, such as azobenzenes, hexachlorobenzene, some chloronapthalenes, should be receiving attention.

RISK ASSESSMENT/POPULATION MODELING EXPERTISE GROUP

Facilitator: Charles Menzie

Group Members: Janet Burris, Peter deFur, Lev Ginzburg, Wayne Landis, Mike Meyer, Pat Cirone, Robert Pepin, and Steve Wharton (notetaker)

CM: provided an overview of goals of the workshop and reviewed key issues:

- we should ask ourselves how we as a group will help the other groups understand the risk asssessment process
- risk assessment terminology is important and should be clarified (three handouts provided)
- screening level vs. baseline ERA; we should have a common working definition

WL: For screening, the CCME has defined a Tier 1 assessment (not probabilistic, uses an array of risk quotients, done quickly, some lab/field data).
JB: In Superfund, screening level ecological risk assessments (SLERAs) are used to identify whether there is a problem, and the results are then used to design or scope a more in-depth ecological risk assessment (ERA), which is likely probabilistic.
CM: Typical characteristics might include identification of possible receptors, use of literature values, limited additional investigation, benchmark comparisons ("quick and easy" or "off-the-shelf" comparisons).
MM: They are often used as a measure of exposure to identify the need for additional studies.
SW: The number of versions of SLERAs is increasing, with states and non-governmental professional organizations generating their own approaches (e.g., ASTM, Soil Screening Levels).
LG: It is important that we not restrict our definition to only non-probabilistic risk assessments; this is not necessarily the case for all SLERAs.

CM: How do we characterize SLERAs in the context of TEFs?
WL: All TEFs are already a screening approach; they are an expression of a relative value (i.e., high-med-low); this is Tier I.
PdF: TEFs go beyond screening by virtue of the congener-specific nature of their analysis; TEFs shift the boundary between screening and full-blown risk assessment (toward more detailed).
CM: We have the potential to move to more complicated evaluations using the TEF approach. What are the next couple of steps in this process, and do the TEFs lend themselves to taking these steps? In taking these steps, do we move toward more or less uncertainty?
JB: ERAs will typically address specific receptors, pathways, and endpoints beyond the limits used to derive the TEFs. In some ways TEFs may restrict your approach, because some assumptions are inherent in the TEF approach. The decisions are not transparent in the WHO document and these may affect their applicability. One

should track the toxicity, assumptions, and uncertainties used in the TEF approach.
CM: Is there a way to expose these? Would we need to collect additional data to complete the SLERA and move beyond it to the ERA? Yes; examples include:

- site-specific data
- more complex data
- field data
- relate TEFs to field conditions (chicken versus eagle)
- try to reduce uncertainty of lab-derived TEFs

MM: Screening is setting up plausibility of effects, baseline allows conversion of TEF to actual pg/g values.

Uncertainty Terminology

LG: There is another choice between deterministic and probabilistic. There is a problem with the probabilistic description of uncertainty. The mode associated with each distribution assumes independence (e.g., triangular distribution results from algebraic extension of a normal distribution), but not all environmental data are actually independent; rather, they are sometimes dependent. A "tight" mode may underestimate extremes owing to too much central tendency. If variables A and B are dependent or correlated, we often don't know their actual relationships due to lack of adequate data.
WL: Correlation matrices developed in the field lead us to focus on dependent variables.
LG: Probability bonds allow expression of uncertainties through generalized application of full algebra. Alternatively, "fuzzy arithmetic," where you do not assign a specific uncertainty to distributions, allows description relationships. Ignorance versus variability should be identified so that we apply the correct tools to reduce uncertainty when possible. Fuzzy arithmetic does not consider dependent/independent terms. We are more in the ignorance mode rather than the variability mode when extrapolating between species.
CM: Variability is measurable, but not reducible; lack of knowledge (ignorance) is reducible.
PdF: It is important that everyone agrees with these concepts.
CM: Which of these are we tracking? There are two different techniques to propagate these forms of uncertainty. How do the risk assessments document elements of uncertainties? Should the details of each uncertainty be carried forward throughout the risk assessment all the way to the decisionmaker (risk manager)?

[At this point, the group discussion turned to the issues raised in the charge questions raised by the Planning Group; the comments/conclusions are listed under specific questions where possible, otherwise, they are grouped at the end of this section.]

Question 1. How does one characterize a risk assessment with total PCBs and congener-specific PCBs?
PC: Do you lump the endpoints, or do you carry through the individual endpoints? Congener endpoint may be behavior whereas the endpoint for total PCBs may be

lethality. Is this a piece of information that should be provided to the risk manager?
WL: It depends on the nature of the site.
SW: It also depends on the nature of the risk management decision to be made and the level of uncertainty deemed acceptable in the decisionmaking process.

Question 2. How does one treat uncertainties associated with variable detection limits for individual PCB, dioxin, and furan congeners?
PdF: Do we agree that where mixtures of compounds are at fairly high levels, those ERAs are more certain than those where the levels are near the detection limits? Generally, yes.
WL: It may depend on the nature of the risk management decisions that need to be made.
PdF: This also raises the multiple stressor issue.

Question 3. How does one characterize a risk assessment where the risks are primarily due to the PCB or dioxin congener that has the lowest toxicity limit but the highest concentration, or the highest toxicity but the lowest concentration?

Question 4. How does one distinguish the risk characterization of a "screening assessment" from a "final risk assessment" where both rely on TEFs?
CM: The differences in uncertainties between screening and final risk assessments may be based on policy decisions related to screening level assessments. For a SLERA (minimum case), what information do you carry on through the assessment—a simple narrative providing a qualitative uncertainty analysis?
LG: If you use any numbers, you should fully disclose the associated uncertainties. One should have some idea of the spread.
WL: Be honest about your uncertainties, and if you need to, go out and take some samples.
MM: The site-specific ERA can build on the SLERA.

Question 5. Describe the lines of evidence that should be included in all TEF risk assessments.

Question 6. Is a quantitative uncertainty analysis appropriate for the TEF toxicities? (Toxicological uncertainty)
WL: There is some quantification available, although exposure models (fate and transport) are problematic in that they are not necessarily empirically derived (e.g., K_{ow}) and may create the perception of "falsely precise" values..
PdF: They also may be based on lab as opposed to field data.
LG: TEFs are not the only component of uncertainty in the risk analysis.
PdF: We are willing to use available data to do screening, including TEFs.
MM: We should ask ourselves does your risk estimate bound the realistic value?

GROUP CONSENSUS: There was a general desire to provide the basis for a numeric estimate even in the SLERA.

Question 7. Do you need to present all toxicity data when you do the risk assessment or is a reference to an EPA or WHO summary document on toxicity sufficient?

Question 8. How do you describe the severity of effect? By individual congener or by TEQ?

Miscellaneous Issues

LG: We should carry forward some form of uncertainty analysis.
PC: Do congener-specific ERAs leave out information that total PCBs ERAs capture or presents more realistically?
CM: This issue should be dealt with in Problem Formulation - up front endpoints.

Exercise in Cataloging Uncertainties

CM: Derivation of TEFs by WHO included multiple uncertainties: lab studies, rounding up, limited information, lack of knowledge (including technical limitations in measurement).
LG: Uncertainty is associated with the level of aggregation (i.e., only three classes of animals are used in the ERAs and many chemicals versus using many species and only a few chemicals).
CM: Only some information is available; confidence is given qualitatively. There may be a need to access the original data to quantify uncertainties.
PdF: Each TEF should come with a set of information regarding the uncertainties associated with the derivation of these values.
CM: For the compounds that drive the risk, they should be based on higher quality data/less uncertainty.
MM: For the regulators to adopt the risk assessment, they should be given the information regarding uncertainty so that they may use it with the responsible parties to reduce the risk.
WL: EROD versus toxicity relationship is not expressed in the TEFs. Are there data for a higher-tiered TEF, or does the toxicity data have the lowest uncertainty?
CM: If this is important, we don't have the information at hand.
WL: There has not been a plot of the correlation of the tiered approach and the associated reduction in uncertainties. This would be helpful.

RECOMMENDATION: Justify the tiered approach qualitatively. There may be knowledge not presently available in the WHO report.

Application of TEFs

CM: What thoughts do you have on other uncertainties associated with application of TEFs?
PdF: There are uncertainties related to sources: Is the list of chemicals correct? What form are they in? Are there similarities or differences in the chemicals in the environment brought about through biological, chemical, and physical transfers?
WL and PdF: Biotic transformations prior to entering aquatic systems are important.

CM: Compare the uncertainties of this approach with modeling total PCBs.
WL: We should ask ourselves several questions: How well do these models work?

Are they predictive? Are they applicable to all media/endpoints? Are model uncertainties constant across all compounds?
WL: TEFs have been described as order of magnitude estimates. When modeling populations, order of magnitude changes are very important.
LG: It is foreseeable that we may likely have to apply standard food-chain models in the future (to reduce modeling uncertainties).
WL: Detection limits are a source of uncertainty
PdF: Whether the risk assessment is based on a few congeners as opposed to many congeners is important in determining heterogeneity in exposure.
WL: There are spatial and temporal issues associated with sampling; aggregation of samples destroys heterogeneity of environmental data.
CM: Would you expect greater variability in tissue with congeners than an aggregate (total PCBs)?
WL: At lower concentrations, variability would likely be greater with congeners.

RECOMMENDATION: Variability in tissue concentrations for congeners vs. aggregate Ah receptor agonists may present significant uncertainty. Data addressing this issue should be reviewed/compiled.

Effects-Related Issues
CM: What are the sources of uncertainty?
PdF: The greater the phylogenetic extrapolation, the greater the uncertainty. Also, uncertainty associated with life stage sensitivities may be as great (due to metabolic changes related to age/life stage).
LG: Simplistic description of populations using extreme endpoints (e.g., death) is meaningless. We should use more meaningful endpoints. Resilience should also be considered.
PdF: There may be genetic determinants of population effects (e.g., *Fundulus*).
WL: Patch dynamics present uncertainties (breeding occurring in one area versus adults living in another).
LG: Endpoints derived for one life stage applied to another life stage (e.g., survival of adults versus larva)
MM: When you get down to dietary habits, you need precise data for TEF development. Preferential exposure by congener may be different than total PCBs.
WL: Compounds are distributed preferentially into food items, and BAF for exposure may be determined by log P.
MM: You can differentiate dietary habits by age.
PdF: Gender, life stage, and overall condition will contribute uncertainties.
WL: The shape and slope of the dose-response curve (TCDD) may add to uncertainty with respect to measured values in environmental media.
CM: Consider other dose-response interspecies differences (Spehar's paper). There are significant interspecies differences and there may be a broad range within a species by compound.
WL: Source(s) of population regulation are important.
MM: When you look at the overall population versus the impacted (or studied) population, the level of risk may be different.
CM: Social considerations (values) come into play when determining level of

protectiveness (extinction of protected species versus prevention of population declines).
WL: The shape of the dose-response curve will impact what happens on a meta-population level.

CONSENSUS: Congener-specific information is important for exposure and effect characterization, predicting severity of adverse effects, magnitude (number of individuals affected), and subtle effects. The details of uncertainties associated with congener-specific ERAs should be retained throughout the risk assessment and interpreted for the decision maker. Congener-specific risk assessments are actually a form of multiple stressor risk assessment. These points should be discussed during problem formulation and in the selection of appropriate assessment endpoints.

Charge Questions Related to Prospective Case Study
CM: Are the TEF uncertainties greater than or less than other approaches?

CONSENSUS: They are no worse than other approaches (ignorance exists in both areas), they are not the only source of uncertainties, and TEFs may present advantages.

CM: Biologically based TEF assays, what would be their strengths?
SW: Generally, assays are cheap, and they provide an aggregate response.
WL: They allow direct testing (validation) of risk hypotheses (risk estimates).
WL: If TEF data contain bioassay (toxicity) results, then you may make direct extrapolations to risk management decisions.

CM: What would be their disadvantages?
LG: The metabolic processes are undefined.
MM: We are extrapolating from assays on a limited number of organisms to populations.
SW: There is an inability to know which compound to regulate.

CONSENSUS: Bioassays provide another line of evidence. Biocriteria versus chemical criteria present difficulties in implementation. Testing methods need improvement.

Responses to Charge Question IV-3 . (discussion also relevant to Question 5, above)
LG: Additional demographics on species are necessary, both historical and present conditions.
MM: Precise dietary habits are needed, including ecological implications.
WL: Abiotic characterization of ecosystems is needed (e.g., physical, chemical, meteorological)
LG: Additional information on patch dynamics is needed for interpreting population effects, especially for statutorily protected species.
PC: We should exercise caution discounting risk when laboratory assays or modeling fails to demonstrate predicted risks. They represent one line of evidence.

Appendix E

DETAILED SUMMARIES OF CASE STUDY DISCUSSIONS

Breakout Group Assignments

Wednesday, January 21, 1998—10:45AM-3:45PM
Thursday, January 22, 1998—8:30AM-12:30PM

Group 1	Group 2	Group 3
Chair: Peter DeFur	Chair: Janet Burris	Chair: Charles Menzie
TEFs Experts		
Jay Gooch (fish)	Mark Hahn (fish)	Richard Peterson (fish)
Martin van den Berg (mammals)	*(See EPA/DOI Planning Group)	Bert van Hattum (mammals)
Sean Kennedy (birds)	Björn Brunström (birds)	Steven Bursian (birds)
Fate & Transport/BAF Experts		
William Adams	Patrick O'Keefe	Mark Servos
Joseph DePinto	Christopher Metcalfe	Lynn McCarty
Population Modeler		
Lev Ginzburg	Mike Meyer	Wayne Landis
Risk Assessor		
Peter deFur	Janet Burris	Charles Menzie
EPA/DOI Planning Group		
Gerry Henningsen	*Mike Devito	Pat Cirone
Lisa Williams	Tim Kubiak	Cynthia Nolt
Robert Pepin	Steve Wharton	Don Tillitt
	Steve Bradbury	
	Phil Cook	

WORKGROUP #1

Facilitator: Peter deFur

Prospective Case Study

The discussion focused on the prospective case study, which involved permitting a new pulp/paper mill on a generic lake in the midwestern United States. The point of the discussion was to review the uncertainties and issues raised by using TEFs to evaluate permit application and discharge conditions for the hypothetical facility. The facilitator briefly reviewed the case, going over the basic facts and issues. The group discussed the elements of the case: the complex effluent, the multiple chemicals with dioxin-like activity, the ambient atmospheric input of dioxin-like materials, and the level of scientific knowledge and uncertainty surrounding the case. The group agreed that several issues were critical: bioaccumulation, ambient inputs, and species variability.

The group opened with a discussion of the bioaccumulation factors used in this case and the issues associated with application of BAFs to TEFs. The charge questions included several for this case that the group felt were a good starting point, specifically: "What errors are associated with the use of BAFs, given the uncertainties?"

The group discussed the scientific nature and derivation of the BAFs , and compared these with the TEFs for wildlife. The BAF issues are largely ones of application, and do not so much directly affect the TEFs as they influence the final outcome of the technical analysis in which the toxicity of a "mixture"—in this case, a complex effluent—is assessed. The TEF approach requires the use of BAFs because of the nature of dioxin-like compounds.

The group agreed that there is significant uncertainty in "generic" BAFs determined for one site with the intent of using the BAFs elsewhere. However, the

species used in deriving the BAFs and the basic similarity of the systems (both northern freshwater systems) provides greater usability in the application to the system in the case study. Such is not always the case for other applications. The group agreed that the greatest certainty in BAFs exists in systems where the species and the aquatic conditions are the same. The group recommended that research efforts to improve the understanding and use of TEFs focus on applicability to the same system under different conditions rather than on expanding to new systems. The group felt that there was less variability in the system than in the variation among systems, and that the need for site-specific factors was greatest when moving across widely different systems. The interspecies variation largely came up when addressing the species that had no counterparts in other systems. One great source of variability among ecosystems derived from the differences in trophic structure, especially the number of trophic levels and the nature of the top level.

The use of a Monte Carlo or other probabilistic approach generated some discussion, but most agreed that the Monte Carlo approach could give greater insight into the variations in the system, providing that the Monte Carlo was not misapplied. Additional information on this issue is provided at the end of this summary of the workgroup discussion and in the summary of discussions in the Risk Assessment Expertise Group (Appendix D).

At least one member felt that the use of probabilistic approaches was largely over-rated. He noted that everyone had a propensity for using the same independent variable approach with the same distributions, mostly normal (in statistics). This member noted that the normal functions used to predict the distributions of these things do not follow the way in which the effects actually occur in the natural world. In fact, he noted, real-world variables are often dependent and not normal in distribution, but either non-normal or stochastic. Thus, some of the conditions cannot be predicted in the Monte Carlo approach because the wrong formulas are being used. Other statistical approaches will offer a different analysis of those dependent and non-predictable events. Two important

points are that the variables are often linked, that is they are dependent, and that the distributions are not normal, in a statistical sense. Additional discussion of this topic is provided later in this summary.

In discussing this case, the group decided that it would be better to understand the behavior of a single congener (i.e., TCDD) across many different systems than to understand the behavior of all congeners in a single system and then expand to the next system. The reason for this is that the behavior of congeners in relation to one another is more constant than other variables. Thus, by knowing how one congener performs, the others can be extrapolated with greater certainty than other extrapolations. The group agreed that acquiring BAF data for a suite of chemicals would be ideal, but the utility of such data is directly related to its specificity.

The data should be coherent among data types and forms for calculated versus measured values and for field measurements versus estimates. Different approaches (e.g., TEFs for individual congeners versus TEQs for a whole effluent) should reveal concordance or lack thereof. These approaches then turn out to be data checks.

The present case offers a scenario with multiple sources and multiple chemicals and the requirement to conduct a TMDL for the water body. In this scenario, the TEF makes it possible to examine options and compare data from widely disparate sources. This is a positive feature of the approach.

Retrospective Case Study

This case, which involves contaminated sediments from a spill, was handled somewhat differently in the group discussion than the case study from the previous day. For this case, after discussion with the steering committee, the facilitator urged the group to reach a decision regarding the use of the TEF approach in applying the available data to a decision. The group was asked to

determine if a decision could be reached, and specifically if the TEF approach would or would not affect the outcome of the decision.

The facilitator summarized the case as follows. A chemical spill took place previously, resulting in contaminated sediments in an upstream segment of a river that flowed into a lake. Despite some time passing, populations of several species of wildlife seemed to remain at some level of risk or impairment. Some of the data on the populations may be more qualitative and observational than quantitative. Three species have already been identified as species of concern: lake trout, otter, and Caspian tern. Data were provided to assess the toxic load from the PCBs using two alternative approaches, either based on total PCB levels in the tissues (and sediments), or based on the TEF approach for all dioxin-like compounds summed across individual congeners. The case provided previously determined decision reference values for action for each of the three species, using either approach. These reference values were given as single values and as ranges, indicating that if the predicted exposures exceeded these reference values, some sort of action would be recommended.

The case description included the notes that atmospheric deposition is a source of TCDD, that there are indications of population effects on wildlife, that the analysis already includes a conceptual model and selection of endpoints, and that the exposure pathway has some predictions, but uncertainties as well. There is knowledge of prior eutrophication, with unknown consequences. The group agreed, reluctantly, to treat the source(s) as constant, without degradation or recalculation or loss. This assumption makes the "no-action" option less viable.

The group could not make a recommendation for the site to be cleaned up, but attempted to determine whether the data warranted some type of conclusion about the risks that might lead to an action, which might be to leave the system alone, study it more, or identify management options to reduce/control toxicity).

A brief analysis and summary of the data on exposure and reference dose revealed that the two approaches gave somewhat different conclusions. The TEF approach yielded exposure concentrations higher than the reference values, for the most part, while the total PCB-estimated exposures yielded values that were somewhat lower, and less clearly exceeding the reference values. This observation was in a general form, recognizing that the magnitude of the ranges made it difficult to determine whether or not the predicted values overlapped the ranges in Table 5 of the case study.

The group discussed how to interpret the data on exposures from the two estimation methods. The resulting discussion revealed that individual members of the group did not view the results the same, in that some members did not see such a great difference in the interpretation based on the two approaches. The group members who thought that results did not differ between the two approaches saw both as giving borderline "positive" (exceeding the reference dose) results. Everyone in the group considered the data to be at least in the borderline category for making a decision, but everyone agreed that there was room for interpretation of the meaning and significance of the impacts of the exposures. One concern expressed by several members of the group was the lack of data on ranges, or on variability of the data. The ranges of values given for the NOAEL in Table 5 was not sufficient. The group wanted data on TEF variability, BAF variability, toxicity, and so on. There was a general discussion of the need for species-specific dose-response functions for the biological processes in this ecosystem, and several members wanted to see the measures or other expressions of variability in the data, including the TEFs.

The group had a range of opinions regarding the quality and sufficiency of the data in supporting a decision on the case using the TEF approach. Basically, all thought more data would make the decision easier, but only part of the group were satisfied enough with the available data to make a decision. Others wanted to see site-specific data before concluding that a decision could be made, and still others thought that population data were needed for the three species before decisions could begin.

All in the group were satisfied that the TEF approach provided a useful way to understand the data, the case, and the situation. Also, the available data were sufficient to support use of TEF approach at least for screening level analyses.

Discussions Relevant to Both Case Studies

A number of points were raised in the course of the two days that the group agreed were important to include in the summary report of the workshop. The following paragraphs were drafted by individual members of the workgroup to reflect the nature of the discussion and the agreement or lack thereof among the members of the group.

Need for Ground-Truthing of the TEF Approach. Field verification of TEFs and the resulting TEQs is highly desirable, in the least, and was considered essential by some. The TEQ approach has been examined in the field for birds (Tillitt et al., 1992; Murk et al.; Kubiak et al., 1989; Harris et al.), fish (Cook and Peterson's retrospective work on lake trout in the Great Lakes), and mammals (otter in Europe). Effects observed in the field are generally consistent with what would be predicted from exposures expressed on a TEQ basis. Differences in TEQs among colonies of double-crested cormorants in the North American Great Lakes explained more of the differences in hatching success among colonies than did differences in total PCBs (Tillitt et al.). Among-species variability in absolute sensitivity to dioxin-like compounds is greater than among-species within-class variability in TEFs.

In the ecological risk assessment process, exposure and effects data are integrated and the potential for risk is characterized. As a general rule, when exposure levels exceed the effects level (threshold), expressed as a risk quotient greater than 1.0, excess risk is expressed. When excess risk is calculated (e.g., when the summation of TEQs exceeds a threshold effects value), it is important that the potential for effects to occur in natural environments (i.e., at the population or community level) be assessed. There is a need to ground-truth the

TEF/TEQ approach such that when this approach is used to demonstrate risk that measured effects at those exposure levels have been observed in field populations.

Status of the TEF Approach as a Screening-Level versus Decisionmaking Tool. As a general rule, screening level assessment/ranking/scoring tools should have an accuracy in the range of a factor of 5-10. For more definitive/quantitative risk assessment, the accuracy of the assessment tool should be less than a factor of 5, preferably between 2 and 3. In general, the uncertainty associated with the derivation of a TEQ based on class-specific TEFs (i.e., TEFs for fish, mammals, or birds) is on the order of a factor of 5-10. In light of this, we conclude that the risk ratios derived using the application of a TEQ value based on TEFs are best viewed as a screening tool, to provide direction in determining additional data needs. We recognize that in some cases, for example where the species and endpoints are in fact the species and endpoints on which the TEF values are derived, the TEQ portion of the risk quotient may be sufficiently accurate to justify more confidence in the estimation of risk inferred from the hazard quotient. In these cases, obtaining a better estimate of the TEQ would be less of a priority than obtaining a better estimate of exposures.

This level of certainty in the TEF-derived estimates was apparent in the interpretation of data from the two case studies. One of the reasons the group members held somewhat different interpretations was the understanding of the data that support the TEFs used in these cases. The members with greater familiarity with this data were more satisfied with the final outcomes.

Uncertainty. Throughout the two case study discussions, group members urged the incorporation of variability and/or uncertainty in the numerical expressions and elsewhere. This expression of uncertainty may take different forms, only one of which is a Monte Carlo or probabilistic analysis. The group acknowledged the dual nature of uncertainty, which includes both statistical variability and the unknown. The group was confident that the former type of uncertainty was adequately, if not always, expressed in statistical ways, often as

standard error or mean, as confidence limits, or other such numerics. However, the latter type of uncertainty, unknowns (also called ignorance), is less well expressed and is not conveyed through traditional statistics or through probabilistic approaches.

Both no-effect levels (NOAELs) and computed values, whether of risk or of variables such as BAFs, have to explicitly incorporate error. No meaningful comparison of values (e.g., benchmark versus NOAEL) is possible otherwise. In the retrospective case study, the benchmark values did come with a range, but the computations did not include explicitly propagated errors in the input values, which are significant.

Uncertainty does not have to be described in a probabilistic framework. Lack of knowledge, or ignorance, does not easily lend itself to a frequentist's interpretation. We have to clearly distinguish natural variability (heterogeneity) from ignorance-based uncertainty. The first can be described probabilistically; the second, by either range or in terms of fuzzy arithmetic (i.e., using formulas that do not recognize frequentist views but attempt to reflect subjective uncertainty). Reference to such techniques (Ferson and Ginzburg, 1996) can be found in Dr. Ginzburg's pre-meeting comments (Appendix C).

Grounding of the TEF Approach in Ecology. The idea of a so-called "no-effect" concentration certainly provides a useful first cut, but it effectively disconnects toxicologically-based decisions from ecological considerations. In practice, and in our case study, the "no-effect" concentration is exceeded, and another level of analysis (i.e., population or ecosystem dynamics) is need to make ecologically-based judgments. Specifically, we need information about:

- the slope or, preferably, the shape of the dose-response curve above the "no-effect" level; and
- demographic characteristics of the target species, including an idea of the strength of population regulation (density-dependence).

In the retrospective case study, even though a no-effect level is exceeded, population level consequences are uncertain and may not warrant immediate action but rather a closer look at population dynamics. The group was confident that many, if not most, cases would exhibit some ambiguity in the interpretation of effects of dioxin-like chemicals on animal populations. Thus, data on and evaluation of population dynamics is the next step.

Measurement Issues. The group agreed that current knowledge of dioxin, Ah receptors, the dioxin-like compounds, and the mechanism of action of the dioxin-like compounds necessitates measuring all of the relevant congeners when taking environmental samples. It makes no sense scientifically to measure only the one congener, TCDD.

WORKGROUP #2

Facilitator: Janet Burris

Prospective Case Study

The following sections summarize the discussions completed by Group 2 during its review of the prospective case study. The summary is organized according to the primary issues discussed, and indicates which members contributed to the related text.

TEF Derivation and Application in Ecological Risk Assessment (Janet Burris and Mark Hahn). The group reached an agreement that the TEFs used in ecological risk assessment should be selected using a hierarchical approach. Species-specific values should be used if available or a value for a closely (phylogenetically) related species. The WHO consensus TEFs would be used as a default. The proposed system also considered in vivo results preferable to in vitro in selection of the most appropriate endpoint . See the section entitled "*Selection of TEF values for use in a TEQ-based Ecological Risk Assessment),*" *in the* Group 2 summary of the retrospective case study, below, for a more complete description of the proposed hierarchy.

The group expressed the need to have more information on the derivation procedures used to identify the WHO TEFs, as well as the underlying data. This information is necessary to understand the uncertainties in the values and to carry those uncertainties through the risk assessment.

The group felt that the rounding procedures used in the WHO TEFs do introduce some uncertainty in the ecological risk assessment. This uncertainty is, however, quantifiable and can be evaluated in a sensitivity analysis where results are calculated using both rounded and unrounded values. A sensitivity analysis can also be performed to evaluate uncertainties associated with the use of species-specific TEFs versus the WHO TEFs and endpoint-specific TEFs versus the WHO

TEFs. Further quantification of the uncertainties in the derivation of the WHO TEFs may be limited as the TEFs were not derived in a systematic manner. The exact process used to derive the WHO TEFs is not specified in the current draft report.

Discussion of Appropriate TEF Values for Species of Interest in the Case Study (Janet Burris). The group's selection of TEFs for the species of interest was:

- Bald Eagle: The group recommended using the WHO avian TEF values. The rounded and unrounded values should both be used to perform a sensitivity analysis. The group was comfortable with extrapolating from the endpoints used to derive the TEF to the reproductive assessment endpoint, but this needs re-evaluation

- Bull Trout: The group recommended using the rainbow trout values and the endpoint of early life stage mortality.

- Otter: The group recommended using the WHO TEFs as a default, since values for the otter or more closely related species are not known to be available. Uncertainties in the extrapolation from the TEF endpoint to the assessment endpoint are not large, as these values (the TEFs) represent relative potency values.

TEF Approach Compared with TCDD or Total PCBs (Bjorn Brunstrom). The group agreed that the TEQ approach provides significantly more information compared with an assessment based on TCDD alone, total PCDDs, or total PCBs. However, use of the TEQ approach should not replace or exclude risk assessment based on total PCBs, since non dioxin-like effects may also be important. In the prospective case study, the TEF/TEQ approach provides information on the potential toxicity of the mixture of congeners in the effluent and identifies the specific congeners that may make the largest contribution to toxicity.

Using a TEQ approach in the modeling process is principally similar to modeling a single compound. The challenge is to get valid fate and transport-related parameters for a number of compounds.

The Use of Median Values to Derive TEFs (Mike DeVito and Janet Burris). The group discussed the issue of the use of median values (EC_{50} and LC_{50} data) to derive the TEFs and the implications of this on their application in risk assessments where no effect levels are used to identify risks. The group decided that median values for the derivation of TEFs is appropriate and does not effect their application in risk assessment.

Derivation of REP values typically use the ratio of ED_{50}s, EC_{50}s, LOELs or NOELs of the test chemical compared to TCDD. A question often asked about the use of the TEF methodology is whether the TEF should be based on the ratio of the lower end of the dose-response curve as opposed to the ED_{50}s. The relative potency for full agonists should be independent of the level of response where the measurements are determined. One advantage of using ED_{50}s is that the ED_{50} can be determined with greater accuracy and precision than the NOEL, LOEL, or either the ED_{01} or ED_{10}. The increased precision and accuracy are related to the greater ease of measuring a 50% response above background. In comparison, determination of LOELs, NOELs, or ED_{10}s and ED_{01}s are fraught with uncertainty. The ability to accurately detect NOELs and LOELs, ED_{10}s, or ED_{01}s is dependent upon the magnitude of the maximal response compared to the controls and the variability in the measurement of the control and lower-dose groups. Estimates of these low-dose parameters are also highly dependent upon study design and dose selection. The uncertainty of the ED_{01} or ED_{10} estimate is much greater than the uncertainty of the estimated ED_{50} (DeVito et al., 1997). The increased uncertainty of the estimate of the low-dose parameters would increase the uncertainty of the REP. While REPs may be dependent upon where on the dose-response curve they were derived, the greater accuracy and precision of the ED_{50} determination provides a significant advantage for its use in estimating the REP.

While the relative potencies of full agonists are independent of the measured response, the same is not true for partial agonists. Partial agonists do not have the same intrinsic efficacy of full agonists and may antagonize the effects

of full agonists under certain conditions. Assigning REPs or TEFs for these chemicals is problematic. Under certain conditions (predominately low-dose exposures), the interactions of full agonists and partial agonists may be additive. Under high-dose conditions, the interactions of full and partial agonists maybe antagonistic. The use of the TEF methodology for partial agonists should be viewed with caution. The interactions may be additive in the low-dose region but non-additive in the high dose region of the dose-response curves.

Use of Bioassay-derived TEQs (Christopher Metcalfe). The group discussed the potential for using bioassay-derived TEQs in the prospective risk assessment scenario. Reservations were expressed over the value of this technique for monitoring total TEQs discharged in wastewater. The potential for generating "false positive" responses in *in vitro* assays was considered high. For instance, in pulp mill effluents, there are high concentrations of potent EROD-inducing compounds (e.g. retene) which are not AhR-agonists. PAHs will also give a response in some *in vitro* assays if they are not removed from complex environmental mixtures by fractionation. The biologically-based TEQ assays are not chemical-specific and therefore do not show causality.

Discussions with Sean Kennedy indicated that bioassay-derived TEQs could be applied to estimating burdens of planar HAHs in fish and wildlife in the lake system, both before construction of the mill and for monitoring purposes after the mill is operational. This was discussed by the group and was considered a valid application for these techniques.

Challenges and New Uncertainties Associated with Modeling the Exposure of AhR Agonists (Christopher Metcalfe). The group concluded that the use of the TEF/TEQ approach in this case study introduces no uncertainties in exposure estimation that are not also common to other chemical-specific assessments. The challenge of the TEF/TEQ method is that it requires the modeling of individual congeners, as well as modeling of the fate and transport of the many congeners, in comparison to traditional modeling for individual or lesser numbers of chemicals.

Measurements of specific congeners may be problematic, considering congener-specific detection limits; and the problem will be greater for analyses of water, due to the higher volumes required than for sediment or tissue samples. When the detection limits are high, uncertainty is high, but the uncertainties are offset by the advantages of using congener-specific as opposed to aggregate (i.e., total chemical) values.

The group further discussed the fate and transport issues in two parts: physical/chemical and metabolic parameters. Regarding the former, there were concerns expressed about the quality of chemical and physical data that could drive the mass balance models involved in the risk assessment process for the prospective scenario. The quality of log K_{ow} data was considered relatively high, but there was concern over the lack of empirically-derived K_{oc} data, and over the quality of K_{oc} values derived from K_{ow}s. There was also concern over the lack of credible Henry's Law constants (H) and photolysis data for planar HAH congeners, although it was acknowledged that this may not be a problem for hydrophobic compounds that bind readily with the particulate phase. Finally, there was discussion of and general agreement with a point made by Joe DePinto (in the Wednesday morning meeting) and by Phil Cook, that the partitioning of the planar HAHs between sediment and water and the particle dynamics in the aquatic system are the most important processes that will drive the mass balance model. There was concern expressed that "getting these processes right" will require an extended and expensive research effort, a luxury that may not be feasible for the risk manager. For further discussion of this topic, see the section of this summary dealing with generic mass balance modeling, below.

Regarding metabolic parameters, concern was expressed over the lack of information on the metabolic capacity of target organisms for planar HAHs. This may have implications for estimating BAFs and BSAFs. It was pointed out that many fish-eating birds and mammals appear to "enrich" PCB congener 126 and appear to metabolize PCDFs relative to the levels in the fish they are eating. Therefore, a knowledge of the metabolic capacities of fish-eating target vertebrates

for the various planar HAHs is essential for generating BAFs in the prospective scenario.

Requirements and Considerations in Analytical Design Associated with TEF-Based as Compared to Aggregate Analyses (Patrick O'Keefe). The TEF approach requires quantitative analytical data on a large number of PCB and PCDD/PCDF congeners. Consequently, more complex and costly analytical methods must be selected compared to those used for the measurement of total PCBs. In general terms, sample extracts will need to be analyzed by gas-chromatography/mass spectrometry (GC/MS) using isotope-labeled internal standards rather than by GC with electron capture (EC) detection, the current method-of-choice for total PCBs. While many laboratories engaged in PCB analysis do not have the equipment or expertise to carry out these procedures, those laboratories currently involved in PCDD/PCDF analysis do have the appropriate equipment and expertise.

Since compound identification is more specific using GC/MS methods, the possibility of false positives is considerably reduced compared to using GC/EC. Quantitation is also improved when isotope-labeled internal standards are included rather than the surrogate compounds used in GC/EC. However, it should also be understood that, in the TEF approach, the PCB and PCDD/PCDF congeners with the largest contributions to the TEQs are usually present at very low concentrations relative to the total PCB concentration. Consequently, rigorous quality control procedures will be required to ensure accuracy and precision in the analytical data. In addition to the generally accepted internal quality control samples (blanks, duplicates and spikes), standard reference materials should be used for calibration purposes. Currently, fish tissue and soil sample standard reference materials are available for 2,3,7,8-substituted PCDDs/PCDFs and for coplanar (non-ortho) PCBs. Similar materials are not currently available for mono-ortho PCBs. Round-robin studies using selected samples from different matrices represent an alternative method for comparing the results from different laboratories.

In the prospective case study, there are five matrices of concern: avian eggs (bald eagles), fish tissue (bull trout and lake trout), mammalian tissue (otter), sediment, and water. In this scenario, a fish tissue TEQ residue level of 9 pg/g was judged to be the level of concern. Since the proposed pulp mill would release PCDDs/PCDFs but not PCBs, the discussion can be limited to the two former compound classes. Laboratories with proficiency in PCDD/PCDF analysis can achieve detection limits of 1 pg/g for individual PCDD/PCDF congeners. With this detection limit, it should be possible to obtain reliable data near the level of concern for fish, especially since the major contributor to the TEQ value (2,3,7,8-TCDF) has a TEF value of 0.05. Accuracy of ± 30% should be achievable with a signal-to-noise value of 10, but would be reduced for data near a detection limit of 3:1, the minimum detection limit used in many laboratories.

It is difficult to determine the residue levels of concern in the bald eagles and otters, since the data are discussed in the scenario using water quality considerations which are in turn related to ingestion levels leading to toxic responses. However, if it is assumed that the no-effect threshold levels in the retrospective study (100 pg/g for bird eggs and 60 pg/g for mink liver) are also appropriate for the prospective study, then laboratories that are capable of analyzing the fish tissues should have no problem meeting the higher detection limits of the avian and mammalian species. The same considerations apply to sediment samples, since the biota-sediment accumulation factors are all less than 1 and therefore the sediment concentrations will exceed the fish tissue concentrations.

When the tissue levels of concern are translated into water quality guidelines, using biota accumulation factors, the maximum allowable total water concentrations (MAC_w) will all be less than 2 pg/L, and in many cases they are below 0.1 pg/L. Currently, there are no routine laboratory procedures available that are capable of meeting these detection limits.

This discussion of the prospective scenario assumes that all of the permissible discharge will be allocated to the pulp mill. However, in the

description of the risk assessment scenario it was proposed that only 25% of the maximum allowable load (MAL) would be allocated to the pulp mill. If the mill is required to measure this increment at the levels of concern for the three species described above, the data will be very close to the 1 pg/g detection limit, and accuracy will probably not be better than a factor of 2 and possibly even a factor of 10. As a final note, the group realized that, in this scenario, use of the TEF approach will require the plant to assume responsibility for producing state-of-the art analytical data. However, by doing this the plant management will have considerable flexibility in controlling the mix of PCDDs/PCDFs in the plant effluent.

Challenges in the Modeling the Food Chain Transfer of AhR Agonists (Janet Burris and Mike DeVito). The group concluded that sound modeling could be completed if the transfer of congeners from the sediment/water interface and sediment transport within the lake are both well understood. However, the group observed several challenges in the modeling of food chain transfer.

First, there is concern that the poor understanding of biodegradation and metabolism of specific congeners may limit modeling. Biodegradation and metabolism rates are absent or incomplete. Second, composition of the diet and metabolism affect the transfer of congeners. There are large mixture composition changes of congeners from plankton to fish and from fish to fish-eating birds. Third, the food chain transfer of congeners is species-specific. To address this factor, knowledge of the composition of the diet of species within the food chain needs to be clearly understood and considered in the modeling exercise. Fourth, the data available for estimates and projection of food chain transfer are good for fish but are not adequate for wildlife. Measured biomagnification factors (BMFs) are better for fish, but are much less certain than those for fish-to-wildlife transfers.

The group observed that BMFs should be consistent with the dosimetric basis of the TEF. In ecological risk assessments of dioxin-like chemicals, BMFs

and TEFs are important parameters in exposure and toxicity assessment, respectively. The BMF is a function of the physical/chemical and pharmacokinetic parameters of the individual chemical. The TEF of a chemical is related to its binding affinity to the Ah receptor and its pharmacokinetic parameters compared to TCDD. Because BMFs and TEFs are both dependent upon pharmacokinetics, differences in pharmacokinetics of a chemical between species may alter the BMF and the TEF in the same direction. For example, if the BMF increases between two different species for a test chemical, while the BMF remains constant for TCDD, the TEF may increase across these species as well, since retention or accumulation of the chemical has increased relative to TCDD across the two species. A note of caution is that the relationship between BMF and TEF is not direct. Chemicals that have high BMFs may have low or no TEFs. However, if BMFs for a chemical change dramatically between species, the TEF may also change dramatically between species. Hence large changes in BMF between species warrant further examination of the TEF for that congener.

Bioaccumulation Factors (BAF), Biomagnification Factors (BMF), and Toxic Equivalency Factors (TEF) (Mike Meyer). Site-specific BAFs for PCB and dioxin congeners will provide greater accuracy in prediction of wildlife and fish tissue congener concentrations than will extrapolation of the BAF derived for the GLWQG. The GLWQG BAF was derived from Lake Ontario data that predicted tissue concentrations of total PCBs and 2,3,7,8-TCDD in lake trout. The trophic structure of water bodies can differ greatly, as a function of the complexity and structure of the food web. Stressors other than chemical contamination (e.g., climate, nutrient loading, introduction of exotic species, and so on) can create perturbations in the recruitment at various trophic levels (such as prey fish). Elimination, reduction, or additions of organisms to the food chain can affect the trophic transfer of chemical contaminants, thus altering BAFs from site to site. When using the TEF approach for risk assessment, calculations of BAFs become more complex, as the individual congeners will have unique BAFs, and those BAFs can vary between sites. If this level of precision and accuracy is desired, sampling, modeling, and database management efforts will become more complex and costs

will increase. However, the additional information will allow risk managers to provide congener-specific discharge allowances or remediation goals.

It has been shown that non-ortho PCB congeners are more readily bioaccumlated and are more resistant to metabolism when compared to ortho-substituted PCB congeners. It follows that wildlife tissues may contain a larger proportion of dioxin-like PCB congeners per gram of total PCBs than do fish, reflecting the increased toxic potency of the total PCBs measured in their tissues. Therefore, direct extrapolation of the TCDD BMF from the GLI will provide erroneous risk estimates. A recent study in the Netherlands demonstrated the effect of this error. In that study, a diet-specific BMF of 14 was calculated from fish to otter on a total PCB basis, however the BMF for total TEQs was 41 (Leonards et al., 1997, Env. Tox. Chem 16: 1807-1815). This was mainly due to the high BMF of PCB 126. When incorporating BMFs into the risk model, it is essential that congener-specific BMFs be used, and, when possible, species-specific BMFs should be measured at the risk assessment site.

Possible Errors in the Application of the TCDD Water Standard (Janet Burris). The group identified two possible errors in the application of the TCDD water standard to the prospective scenario. The standard does not consider the enrichment of PCB 126 from fish to wildlife or the loss of chlorinated dibenzofurans in some species of birds. Some members of the group observed that underestimation of effects may result.

Other Possible Approaches: Generic Mass-Balance Modeling (Christopher Metcalfe). The group explored the idea of alternate or improved approaches to the solution of the problem presented in the prospective case study. How could we do it better? Several group members acknowledged that a model for the system could be constructed but it was not clear whether such a model would be predictive given the uncertainties. Other existing food chain models could be used if they could handle the metabolism issues. Another idea that was put forth involved the use of a generic mass-balance model.

A potential problem with the prospective scenario for TEQ-based risk assessment is the complexity of the mass-balance modeling exercise. Discussions at the workshop indicated that knowledge of sediment-water partitioning and particle dynamics within an aquatic system are essential for accurate prediction of assimilative capacity. Our experience with mass-balance models indicates that it may take several years of research effort to obtain the necessary information to develop an accurate model. In a prospective scenario, this level of research would not be possible. Therefore, several members of Group 2 felt that another approach to this situation would be to develop "generic" mass balance models for different types of ecosystems that could be used by risk managers to make decisions. Generic models could be developed for:

- Small eutrophic and small oligotrophic lakes;
- Small embayments that connect to larger lake systems;
- Large embayments that connect to large lake systems;
- High flow and low flow rivers; and
- Marine or estuarine systems with high or low tidal flushing.

Risk managers could use these generic models to make initial decisions on the siting of industrial facilities, and so on. More complex site-specific mass-balance models could be used later, at the discretion of the risk manager or siting applicant affected by the decision. In the latter case, it may be beneficial to adopt a "polluter pays" policy, in which the applicant is responsible for paying for the more complex modeling exercise.

Overall Conclusions Concerning the Use of the TEF/TEQ Approach in the Prospective Case Study and Associated Uncertainties (Janet Burris). The group observed that uncertainty is less manageable in the prospective case study (risk assessment) than in the retrospective case. In a retrospective case, actual measurements of congeners in the environment or within the food chain could be used to decrease uncertainties. In a prospective application, such measurements are not possible.

Uncertainties in the exposure profile appear to be equal to or greater than those associated with stressor-response (effects) assessment. A sensitivity analysis would be beneficial to evaluate the various uncertainties in the risk estimates. Suggested parameters for sensitivity analyses include: TEFs, K_{bw}, K_{oc}, P_{socw}, and BMFs (location- and species-specific)

The group observed that risk managers are attempting to "titrate the system" to permit the release of the last increment of chemical into the system, based on its full assimilative capacity. Use of the TEF/TEQ approach reduces uncertainty in the assessment, as there is not a better approach to be applied, and use of a TEQ is more appropriate than the current TCDD standard. However, the uncertainty in the exposure profile may be result in a high enough uncertainty that the risks of loading the system beyond capacity are much greater than the manager is willing to face.

Measuring Uncertainty at the Population Effect Level (Mike Meyer). At present, little effort has been made to assess the impact of "threshold levels of effect" on target wildlife populations. In most cases, the assumption is that early life stage mortality measured in laboratory studies (with species such as rats and chickens) translates into population level effects in wildlife (such as otters and bald eagles). This extrapolation is not supported by correlational data from the field nor with laboratory studies using relevant wildlife species (the exception being feeding studies in mink). This 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 (see Meyer, 1998, Env. Tox. Chem. 17: 137-138). Until this uncertainty is addressed, the effectiveness of the TEF approach to establish water quality guidance is suspect.

Retrospective Case Study

The following sections summarize the discussions completed by Group 2 during its review of the retrospective case study. The summary is organized according to the primary issues discussed.

Selection of TEF Values for Use in a TEQ-Based Ecological Risk Assessment (Mark Hahn). As part of a TEQ-based ecological risk assessment (ERA) for PCBs, PCDDs, PCDFs, and other compounds that act via the Ah receptor (AhR), toxic equivalency factors (TEFs) or relative potencies (RPs or REPs) are used to convert congener-specific chemical residue data into 2,3,7,8-TCDD toxic equivalents (TEQs). Although the TEQ approach is based on the broad similarities in relative potencies that exist across different endpoints and species (Safe, 1990), specific REP values can vary between species and across endpoints within a species. In an ideal ERA situation, congener-specific relative potencies would be known for the species of concern (e.g., lake trout) and the endpoint of concern (e.g., early-life-stage mortality). Often, however, such data are not available. The use of REP values determined in a different species or for a different endpoint, or use of a "consensus TEF," represents an important source of uncertainty in a TEQ-based ecological risk assessment. This uncertainty is separate from the uncertainty occurring as a result of species differences in sensitivity to TCDD, which affects the choice of the "threshold" or action level to which the calculated TEQ is compared.

In the absence of species-specific REP values for the endpoint of concern, a decision must be made as to which REP or TEF values provide the most accurate measure of relative potency for use in calculating TEQs from congener-specific residue data. In essence, the decision involves choosing between the uncertainty introduced by species differences in relative potencies (for the same endpoint) and endpoint-dependent differences in relative potencies (in the same species). In some cases, both types of uncertainty may be present. Common sense suggests that one should select the REP or TEF value that represents the best (i.e., most

accurate) information available. However, since the uncertainty or "potential error" inherent in a given REP/TEF choice is not always known (i.e., quantifiable), the choice is often not clear.

The approach described in Figure E-1 provides a framework for thinking about the different kinds of REP or TEF values that may be available, and the types of uncertainty inherent to each. Using this matrix, selection of a REP or TEF value is based on a hierarchical approach involving use of the best available information, relative to the ideal choice—a species-specific REP for the endpoint of concern.

Framework for choosing relative potency values for use in Ecological Risk Assessment for fish, birds, and mammalian wildlife.

	Same species	Related species (e.g. same genus or family)	WHO TEFs ("Class-specific")
Tier 1 1a (endpoint of concern)	Best		
1b (other *in vivo* toxic endpoint)			
Tier 2 (*in vivo* CYP1A)			
Tier 3 (*in vitro* CYP1A)			
Tier 4 (QSAR)			Worst

Figure E-1.

In the first column, four tiers reflecting and prioritizing the various *in vivo* and *in vitro* endpoints used to determine REP values are listed. These categories are based on the tiered approach used by WHO in deriving TEFs for fish and birds (van den Berg et al., 1997). The first tier has been subdivided to differentiate *in vivo* data for the endpoint of concern (Tier 1a) from other *in vivo* toxic endpoints (Tier 1b). As with the WHO TEF approach, the highest priority is given to REPs determined for the *in vivo* endpoint of greatest concern. Lower priorities are assigned to REP values determined using endpoints more distantly related to the assessment endpoint.

The top row in the matrix indicates the phylogenetic relatedness of the species of concern to the species in which REPs were determined. It is divided into three levels, reflecting different degrees of uncertainty. If REPs are available for the species of concern, there is interspecies extrapolation and so no uncertainty associated with species extrapolation (although there could be differences between populations within a species). If REP data are available for a closely related species—a species within the same genus or family, for example—uncertainty is higher due to potential species differences, but not as high as when REP data are from a more distantly related species within the same class or when "consensus" TEF values (such as the WHO TEFs) are used.

The matrix might be used to consider and select among the types of REP data available, by comparing the relative position of each set of REP data to the ideal. An example is provided later in this discussion.

The rationale behind this hierarchical approach is a mechanistic understanding of AhR-mediated toxicity as well as empirical data that support such the extrapolation of relative potency data across endpoints and/or species. There is abundant evidence that most effects (endpoints) of dioxin-like compounds, whether biochemical effects such as induction of CYP1A1 or toxic effects such as ELS mortality, occur through the same initial step—binding to the AhR. The structure-activity relationships are similar across various endpoints,

including receptor binding, CYP1A induction, and various forms of toxicity (Safe, 1987; Safe, 1990). The basic AhR-dependent mechanism of toxicity is the same in most vertebrate species. Most vertebrate taxa express an AhR (Poland and Glover, 1987; Hahn *et al.*, 1994; Hahn *et al.*, 1997) and are sensitive to dioxin toxicity (Poland and Knutson, 1982; Cook *et al.*, 1991), although there may be exceptions (Jung and Walker, 1997). Despite the commonality of the basic mechanism, however, there may be species- or endpoint-dependent variation in specific details of the mechanism that result in different REPs.

The basis of the four-tiered approach used to derive the "class specific" WHO TEFs for fish and birds has been described (van den Berg et al., 1997). This approach involves weighting REP values based on the endpoint for which they were derived, with preference to REPs determined for *in vivo* toxicity in developing embryos.

The basis for the phylogenetic approach reflected in the top row of the matrix in Figure E-1 is both theoretical and empirical. It assumes that two species that are more closely related phylogenetically will have REP values (determined for the same endpoint) that are similar or identical. This approach is supported by data such as that showing that the REPs for CB-126 to produce ELS mortality in lake trout and rainbow trout are similar (Zabel *et al.*, 1995). However, it is clear that a more systematic effort to test this assumption will be needed. Moreover, although it is expected that closely related species will in general exhibit similar REPs, exceptions to this assumption for certain species and/or congeners may be revealed as additional data are collected.

As stated earlier, it is important to keep in mind that the issue of species- or endpoint-specific differences in REP values is separate from that of species differences in sensitivity to TCDD. In fact, there may be little or no relationship between the two issues. Two species that differ widely in their sensitivity to TCDD can have similar REP values for most congeners. For example, chickens are 119-fold more sensitive than Pekin ducks to *in vitro* effects of TCDD, yet for

TCDF and PCB congeners 126 and 81 the REPs differ less than 5-fold between these species (Kennedy *et al.*, 1996).

The matrix in Figure E-1 is intended to provide a framework for thought and discussion concerning the selection of REPs for ecological risk assessments. There are a number of practical questions that arise when considering this approach:

- Often, REP data sets are incomplete. Is it appropriate to draw REPs from multiple data sets to calculate TEQs for a given species? For example, in performing a risk assessment for lake trout, the only "Tier 1a" REP value that exists *for lake trout* is for CB-126. For other congeners, REPs exist only for rainbow trout or other fish species. A "best available information" approach would lead one to choose the lake trout REP for CB-126, and rainbow trout REPs for the other congeners.

- The phylogenetic approach assumes that closely related species will exhibit similar REP values. But how close is "close"? Can we expect species within the same family to show greater similarities in REPs than occur between families, or must the species be within the same genus before such similarities are evident? Again, more data are needed to resolve this question.

- One of the most difficult questions concerns choosing between uncertainties based on species differences versus endpoint differences, in the absence of data that would allow one to quantify the uncertainty in each. For example, suppose a risk assessor is performing an assessment for Caspian terns, using measured, congener-specific concentrations of PCBs, PCDDs, and PCDFs in tern eggs. There are no data on REPs for ELS mortality in Caspian terns, but let us suppose that there are REP values (A) for *in vitro* CYP1A induction in Caspian terns, and (B) for *in vivo* ELS mortality in domestic chickens (the latter used to establish the WHO "consensus TEFs"). Perhaps there are also data for *in vivo* CYP1A induction in embryos of common terns, a closely related species (C). Figure E-2 illustrates the positions these three types of data would have in the matrix. Which of these three sets of REP data would provide the most accurate estimate of TEQs in Caspian terns? One option when confronted with such a decision might be to perform the TEQ calculations with each set of REPs; a comparison of the resulting TEQ values might provide a measure of the uncertainty in selecting any one of the REP sets.

Matrix showing position of three choices of REP values for the scenario described above.

	Same species	Related species (e.g. same genus or family)	WHO TEFs ("Class specific")
Tier 1 1a (endpoint of concern)	?		B
1b (other *in vivo* toxic endpoint)			
Tier 2 (*in vivo* CYP1A)		C	
Tier 3 (*in vitro* CYP1A)	A		
Tier 4 (QSAR)			

Figure E-2.

An example related to the last of these practical questions can befound in the retrospective scenario discussed at the workshop. In this case, the avian species of concern is the Caspian tern. Figure E-3 shows TEQ values and STEQ determined using two different sets of REPs. The first set of values are based on the WHO TEFs which are derived largely from chicken embryo data (van den Berg et al., 1997). The second set of values are based on REPs from *in vitro* CYP1A induction in embryo hepatocytes from common terns, *Sterna hirundo* (Lorenzen *et al.*, 1997), a species closely related to the Caspian tern (*Sterna caspia*). There is a substantial difference in the STEQ calculated using each set of values; the difference is due largely to the 3-and 17-fold lower relative potencies for CB-126 and CB-77 in common terns as compared to the WHO TEFs.

TEQ values and ΣTEQ, determined using two different sets of REPs, for Caspian terns in the retrospective scenario.

	Bird TEF[1]	Caspian Tern Egg TEQ	Common Tern REP[2]	Caspian Tern Egg TEQ
PCB-77	0.05	54.17	0.003	0.32
PCB-126	0.1	275	0.03	82.5
PCB-169	0.001	0.32	0.02	6.4
TCDF	1	2.79	0.4	1.1
ΣTEQ for all congeners (including those not shown)		426		184

[1] WHO TEFs (van den Berg et al., 1997).

[2] REP values for *in vitro* CYP1A induction determined for common tern (Lorenzen *et al.*, 1997).

Figure E-3.

The WHO TEFs, based largely on chicken embryo mortality, are thought to be preferable because the endpoint used is more relevant to the effect of concern. However, the differences between WHO TEFs and common tern REPs could indicate some fundamental difference between terns and chickens in the relative potencies of these congeners. The comparison is useful in providing an indication of both the magnitude and source of the uncertainty (in this case, two PCB congeners). Thus, this type of analysis contributes to the risk assessment itself as well as identifying additional data that might help to reduce the uncertainty.

When confronted with a lack of REP data for the species and endpoint of concern, alternative REP values must be chosen. This choice involves the introduction of uncertainty based on species differences and/or endpoint

differences in relative potencies. There is currently insufficient data to determine which type of uncertainty is greater, and thus to guide the selection of particular values. A best available information approach is recommended; this may involve use of multiple REP values and sensitivity analysis of the resulting TEQs.

Selection of Appropriate TEF Values for Species of Interest in the Case Study (Janet Burris). The group recommended construction of a hierarchy for selection of TEFs for use in the retrospective risk assessment. The group could not complete the hierarchy in consideration of the time and the absence of data and derivation procedures for the TEFs in the WHO report. The group recommends expressing consensus TEF values as a range instead of point estimates, since risk management decisions are often not point estimates. Use of a range could provide the manager with and understanding of the uncertainty and confidence in the results.

In its review of the retrospective case study the group selected TEFs for the species of concern as follows:

- Lake trout: The group recommended using the rainbow trout REPs for all congeners except PCB 126, for which the lake trout REP was selected. Lake trout is the species of concern, and it was decided that a species-specific value would result in less uncertainty than a REPs for another species. Both REPs (lake trout = 0.003 and rainbow trout = 0.005) are for early life stage mortality. The group agreed that uncertainty is introduced into the assessment by extrapolation from rainbow trout to other non-salmonid species (e.g., largemouth bass). This uncertainty could not be quantified, since it represented a lack of knowledge about relative REPs between fish species.

- Caspian tern: The group recommended using the WHO TEF of 0.1 (embryo mortality) and a common tern *in vitro* EROD REP of 0.03. The common tern value represents data for a more closely related species (compared to the chicken data used for the WHO TEFs). However, the common tern REP is based on an *in vitro* endpoint, which is less useful. In this case, the group decided to run the analysis with both REPs to provide a sense of uncertainty by giving a range of reasonable risk estimates. This would be one way of avoiding the use of a single point estimate in the risk assessment.

- Otter: The group recommended using the TEFs in the WHO report, as values could not be identified for the otter or another closely related species. The group could not fully evaluate the uncertainties in the mammalian wildlife TEFs, since the derivation of these values is not fully described in the WHO report. The group observed that the values seem consistent with mink exposure studies of AhR agonist mixtures.

The group would have liked to use the same TEF selection hierarchy for mammals as was recommended for birds and fish. However, no description of the derivation procedures for the mammalian TEFs or the underlying data is included in the present WHO report. Questions raised by the group included:

- What endpoints were used? Were *in vivo* endpoints used?
- What species-specific data was available?
- Can cellular effects, tumor promotion, and other endpoints be extrapolated to reproductive effects?
- Were the endpoints used in the derivation applicable to reproductive and population level effects?
- How were consensus values selected? Were these the most conservative values?
- What rounding procedures were used?

The group agreed that the risk assessor would need this information to document the values and assumptions for the risk assessment, to examine the uncertainties, and for "transparency" requirements.

Exposure Issues (Janet Burris). The group concluded that the exposure assessment is driven by the analytical measurements used to determine concentrations of congeners in sediment and tissues. Several members of the group observed that if sediment remedial goals were needed, these could be easily identified based on the linear relationship between sediment concentration and receptor tissue concentrations, as depicted in the equations in the retrospective case study. In other words, to reduce tissue concentrations in the organisms of concern, sediment concentrations would need to be decreased proportionately.

The group concluded that use of the TEF/TEQ approach in the fate and transport modeling is not different from traditional chemical-specific methods. Use of a congener-specific approach in the retrospective case study does not create a new problem for the exposure assessment. It could, however, change the approaches engineers would use in developing remediation plans.

Analytical Considerations (Patrick O'Keefe). In the retrospective scenario, the analytical problems are not as difficult as those associated with the prospective study. In the first place, data on concentrations of chemicals in the water are not required to carry out the risk assessment. Second, the TEQ values for the avian and mammalian species of concern—the Caspian tern and river otter, respectively—are determined by relatively high concentrations of certain PCB congeners, primarily PCB 126. Using the detection limit of 1 pg/g discussed above, regulatory agencies should be able to assess the effects of an order of magnitude reduction in sediment concentrations on tissue residues in the species of concern.

Sampling programs need to be carefully designed in order to answer the questions posed in a risk assessment. For instance, fish samples should be collected in the same vicinity as sediment samples for calculation of BASFs, and sediment samples should be collected along an appropriate grid to monitor chemical input from an effluent, as in the case of the prospective scenario. In addition to analytical precision, sample heterogeneity will play a major role in determining the number of samples required to obtain data with an acceptable variance. For statistical purposes, it may be necessary to carry out a preliminary sampling program to obtain information on the variability of the chemical concentrations with respect to sampling location. This is especially true in the case of sediments where organic carbon concentrations can have a major influence on residue concentrations. For tissue samples, other variables such as sex and age are important factors in determining contaminant concentrations.

Food Chain Modeling (Janet Burris). The group concluded that a full food chain model was not necessary. Such a model would be difficult to construct due to lack of equilibrium in system, heterogeneity, and detection limit issues. It would be especially difficult to build a model from sediment to the water interface to fish. A partial model would, however, be useful. Under assumption of steady-state could use the linear relationship between sediment and biota levels to assess various sediment remediation options without having to deal with lower trophic levels. The partial model would use site-specific BSAF/BMFs. Such a partial model would allow the risk manager to examine reductions of chemicals in target

model would allow the risk manager to examine reductions of chemicals in target species under given remediation scenarios, but would be difficult to use to predict chemical movement over time.

If not all of the exposure data were available as provided in the case study, then the group recommended obtaining sediment samples in a transect of depositional zones to get a sense of gradient, obtaining measurements of congeners in prey species, and relating these to the dietary composition and forage habits of the predators.

Risk Characterization (Janet Burris). The prospective assessment is set up to provide a point estimate of exposures and risks that can be identified as a hazard quotient for an individual organism. The real question is how or whether the adverse effect to individuals is reflected in the population.

Risks are not identified for the lake trout. The risks for the Caspian tern are summarized below and were identified by the group as being "on the edge."

	Threshold	Exposure	Individual Hazard Quotient
(WHO TEF)	100 pg/g	426 pg/g	4
Common tern	100 pg/g	185 pg/g	2
Total PCBs	5 μg/g	4.5 μg/g	<1

The group discussed the likelihood of population-level effects. Based on the hazard quotients for embryo mortality, the group did not expect population effects, but acknowledged that not all possible endpoints were assessed. The potential effect of species-specific TEFs is noted here. The common tern exposure is 2 times the threshold. Exposure based on the WHO TEF is, however, 4 times higher than the threshold. Basing the assessment on TCDD or any other single compound would in most cases underestimate the potential risk in comparison to the TEF/TEQ approach.

The group also noted that Caspian terns are not year-round residents and could be getting exposures elsewhere. The group advised that further data would be required to evaluate the origin of exposure including possible reference samples and a weight-of-evidence evaluation.

Risks for the otter were also identified as being "on the edge:"

	Threshold	Exposure	**Individual** Hazard Quotient
TEF	60 pg/g	144 pg/g	2
TCDD	60 pg/g	1.4 pg/g	<1
Total PCBs	2.0 μg/g	1.0 μg/g	<1

Action Decision (Janet Burris). When asked what they would do next, the group concurred they would leave the site alone. They recommended monitoring trends in TEQs with time to illustrate declining risk probabilities to the population. No further loading of the system should be permitted. The monitoring should include further diagnostic studies to better characterize risks, including expression of risks in the context of population-level effects. Group members felt that the risk characterization should also include a description of what the system may look like in 10 years, an estimate of effects of a 100-year flood and redistribution of contaminated sediments, and an estimate of habitat destruction that could result in more risks. In order to understand if there is a population level effect, we would need to understand the level of decreased reproduction associated with a meaningful reduction in the population.

When the group voted on action versus no action in the retrospective case study, two members voted for action and eight for no action.

If the risk manager chose to proceed with remediation, the otter would be the primary species of concern. The otter is related to the mink, but is known to have greater sensitivity to AhR agonists. The group would provide scenarios to the risk manager and discuss population level effects in the context of adverse effects associated with the remedial alternatives.

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WORKGROUP #3

Facilitator: Charles Menzie

Prospective Case Study

The group initiated their discussions by outlining and reviewing the features of the case study. This provided a basis for understanding the approach that would be taken. Key aspects of the analysis included identifying the organisms of concern, the pathways of exposure (conceptual model), and the target concentrations in water that are judged "acceptable" for the various organisms.

Our subsequent discussions are organized around two categories. The first involves points that were made relative to the case study. The second involves a discussion of how to identify and track uncertainties in an ecological risk assessment process that includes application of the TEF/TEQ method.

Part 1: Points Drawn from the TMDL Case Study

With respect to the case study, the group discussed various issues. Each of these issues is described further in the paragraphs that follow.

Rounding and Significant Digits. Some participants noted that care should be taken to not overstate (via calculation) the number of significant digits. Some numbers presented in the case study appear to be presented at a level of precision that is unlikely to have been achieved. By presenting the numbers to two or more significant numbers, a false sense of precision is given.

Application of REP versus TEF Values. The participants discussed how values should be selected for use in the TEF/TEQ methodology. The group concluded that, where available, REP values should be selected over generic TEF

values. The group believed that uncertainties were reduced if taxa-specific unrounded values were utilized in lieu of the "order of magnitude" values presented in the WHO TEF report. A hierarchical approach was suggested, within which the best and most appropriate values were selected first. The REP values are not rounded and have their own level of significance. When asked if the group would usually elect to use REP values even when they needed to extrapolate to another species (e.g., trout to largemouth bass), the group favored use of REP over TEF values. In part, this view reflected concerns over the rounding done when the WHO TEF values were developed. A few members of the group did not agree with this, because of the variability that might exist around REP values within a class of animals. When asked if they felt that the results would be compromised if they were required to use TEFs, the participants said that they would not, but that there would be additional uncertainty in the estimate.

Uncertainty in TCDD Toxicity Values and Target Concentrations. The group acknowledged that there was uncertainty in the target numbers used in the analysis. Underlying this uncertainty was the toxicity data as well as the models used to derive the concentration of TCDD in water.

Selection of Chemicals and Percentage Allocation. The group noted that for this exercise, we did not have to pick the chemicals. However, we should recognize that atmospheric PCBs also add to the Waste Load Allocation. For this exercise, it had already been decided that the site would be given 25% of total allocated load. This allows us to focus on this smaller set of chemicals.

Uncertainty Analysis. There was considerable discussion concerning uncertainty analysis. A more detailed account of this discussion is provided later in this workgroup report. However, workgroup participants discussed the need to track and document uncertainties in the ecological risk assessment process. At a minimum, this includes a narrative discussion. It is also possible to talk about the sources and potential magnitudes of uncertainties. One member of the workgroup noted that we could make a semi-quantitative attempt to estimate the magnitude; for example, we can say REPs have uncertainty of about 2 or 5.

Part 2: Identifying and Tracking Uncertainties

The workgroup discussed procedures that might be used to identify and to take into account the various uncertainties in the assessment. We worked through a process that is based on the assessment of multiple stressors. A key aspect of this approach is writing down the criteria used to judge the uncertainties associated with each aspect of the analysis. This information is also useful for helping clarify the issues of concern in discussions with managers and with stakeholders.

After much discussion, the group agreed upon an ordinal ranking system for uncertainty that reflected our level of confidence regarding the relative uncertainty in the information. We chose values of 1 (most confidence) to 4 (least confidence). We selected a range of 1 to 4 in part because this is consistent with the number of categories in the WHO TEF document.

A quantitative aspect of uncertainty which the group did not include but acknowledged as important was the magnitude of the error around the values. Such information would be important for sensitivity analyses. Our group did not address this because of time and data constraints. However, we believe that this is important to consider.

The group then identified the areas of uncertainty within the ecological risk assessment process. These do not represent all the possible areas, and some areas could have been broken into smaller components. Our purpose here was to illustrate the concept rather than arrive at a definitive approach. The areas that were evaluated include:

- uncertainty criteria for TEF values;
- uncertainty in comparing TEFs to target water levels;
- uncertainty factors for BAF values;

- uncertainty factors associated with species extrapolations; and
- uncertainty factors associated with the exposure model.

Each of these areas is described in more detail below.

Uncertainty Criteria for TEF Values. Our group had two separate discussions concerning these criteria. The first was on our first day; on the second day we revisited the issue and made some modifications. The criteria we came up with on our first attempt were:

- Level 1: egg injection with mortality endpoint;
- Level 2: whole organism with other endpoints;
- Level 3: *in vitro* studies (e.g., enzyme induction); and
- Level 4: QSARs (from *in vitro* data).

Based on this set of criteria, we initially assigned the bull trout REP an uncertainty level of 1, the value for eagle an uncertainty level of 2, and the value for river otter an uncertainty level of 1. These assignments reflect the levels of confidence given in the WHO report. When we revisited these assignments, it was noted that some participants had more certainty in the fish values than in those for mammals and birds and that the initial assignments did not capture this. The following ranking procedure was subsequently proposed:

- Level 1: REP with population-relevant endpoint;
- Level 2: TEF from *in vivo* study with toxicological endpoint;
- Level 3: TEF based on biochemical response; and
- Level 4: TEF based on QSAR or enzyme induction.

Individuals familiar with the derivation of the TEF values noted that there was more uncertainty associated with the bird values than with the mammal values (which are based on a rich body of data). These individuals had greatest confidence in the fish values. Based on our reassessment of uncertainty levels, the

uncertainty for the bull trout remained at 1, but uncertainty for the bald eagle increased from 2 to 3 or 4, and for the river otter from 1 to 2 or 3.

Uncertainty in Comparing TEFs to Target Water Levels. The group assigned uncertainty factors based on taxonomic extrapolation from the available toxicity databases. It was noted that there were other sources of uncertainty in these values, including the models used to derive the water concentrations in the criteria documents. Considering only the taxonomic extrapolations, the following criteria were developed:

- Level 1: same species;
- Level 2: same genera;
- Level 3: same family; and
- Level 4: same class.

Because bull trout are in the same genus as the reference species, comparison of the TEF with the water quality criterion was assigned an uncertainty factor of 2. Because bald eagles are raptors and the bird standard is based on galliforms, this comparison was assigned an uncertainty of 4. Finally, since river otter and mink are in the same family, the comparison for mammals was given an uncertainty ranking of 3.

Uncertainty Factors for BAF Values. The following criteria were proposed:

- Level 1: site-specific measurement;
- Level 2: lab validated with field;
- Level 3: field data with no lab, or lab with no field corroboration; and
- Level 4: BAF based on K_{ow} (prediction).

The group agreed that the BAF for 2,3,7,8-TCDD would be assigned an uncertainty value of 2. We also decided that the value should be 2 for the relative BAFs of all congeners.

Uncertainty Factors Associated with Species Extrapolations. The group noted some of the differences that can occur among taxa. We decided to use the same taxonomic extrapolation uncertainty criteria:

- Level 1: same species;
- Level 2: same genera;
- Level 3: same family; and
- Level 4: same class.

The assigned uncertainty factors were both congener- and taxa-specific. For the eagle, the three dioxin congeners (Congeners 1-3) were assigned an uncertainty of 1, the first furan (Congener 4) was assigned an uncertainty of 4, the next two furans (Congeners 5 and 6) were assigned an uncertainty of 3, and Hx (Congener 7) was assigned an uncertainty of 2. For the bull trout, all congeners were assigned an uncertainty of 1. For the river otter, dioxins were assigned an uncertainty of 1 and furans were assigned an uncertainty of 2, based on metabolic considerations.

Uncertainty Factors Associated with the Exposure Model. We noted that this was a simplified TMDL model that incorporated K_{ow} and Henry's Law constants, and that there are chemical- and environment-specific factors added into these models. We noted that the model assumes equilibrium or at least steady state; in the real world, however, non-equilibrium conditions are likely to be present. Further, we noted that there were uncertainties associated with the various physicochemical parameters used to predict the behavior of the chemicals.

For fish, we assigned the exposure model an uncertainty of 2 or 3, while for eagle and otter we assigned an uncertainty of 3 or 4. The higher uncertainty associated with the eagle and otter models reflect the anticipated increase in uncertainties associated with relating exposures at these higher trophic levels to sources within the lake. The workgroup noted that any exposure model is expected to have uncertainty associated with it, given all the simplifying assumptions that need to be made.

Representation of Uncertainties

The different sources and "levels" of uncertainty can be displayed in a table and are amenable to mathematical representation and analysis. As an example, the results of our analysis are illustrated in Figure E-4. The table indicates which areas of uncertainty are potentially most important. It also provides an indication of the levels of uncertainty that accompany results of the risk analysis for specific organisms of interest.

Relative Uncertainties in the Ecological Risk Assessment Including Us of TEF Values

Ranks for uncertainty									
Species/Congener									
	TEFs	BAFs	Species Sens./Extrapola	Exposure Mod	Threshold concentration			Total	
Bull trout	1	2	2	2	2		Species specific	9	30 Bull Trout
1		2	1				Congener specific	21	
2		2	1						
3		2	1						
4		2	1						
5		2	1						
6		2	1						
7		2	1						
Bald Eagle	4	3	4	4	4			19	55 Bald Eagle
1		3	1					36	
2		3	1						
3		3	1						
4		3	4						
5		3	3						
6		3	3						
7		3	2						
River Otter	3	3	3	4	3			16	48 River Otter
1		3	1					32	
2		3	1						
3		3	1						
4		3	2						
5		3	2						
6		3	2						
7		3	2						
Criteria are described in the text. This approach and these values are presnted for illustration only.									

Figure E-4.

Retrospective Case Study

TEF/TEQ Issues Related to Measurements

The retrospective case study is based on measurements of individual PCDDs, PCDFs, and PCBs in tissues and environmental media. The group considered several issues related to using such information in ecological risk assessments that rely, in part, on a TEF/TEQ approach.

Accuracy and Precision of the Measurements. Because the TEF/TEQ approach relies on information related to individual compounds, we discussed the ability of available methods to provide accurate and precise results. Individuals familiar with the methodologies used to identify and quantify individual PCDDs, PCDFs, and PCBs in tissues and environmental media believed that the accuracy and precision of the measurements was good. Reanalysis of samples gives similar results. There are certified reference standards. For the concentrations provided in the case study, one individual noted that the measurement error was probably about 30%. Another reported that he observed coefficients of variation on the order of 100%. Measurement error tends to increase with decreasing concentration. Overall, the group concluded that analytical error was not a large source of uncertainty in the overall analysis.

Variability in data can arise from biological variability. In particular, it was noted that different size or sex of fish would be a source of variability. Further, analytical variability in lipid measurements could be a source of variability in data that are reported on a per lipid basis. Variability in analysis of environmental media can also result from variability in the physical and chemical characteristics of these media (e.g., grain size and organic carbon content of sediments.)

Detection levels. The group discussed detection levels with respect to the use of concentration data for risk assessment purposes. The group concluded that available analytical methods could achieve detection levels low enough to support

the TEF/TEQ approach as it is applied to ecological risk assessment. However, it was noted that detection levels should be stated as part of the Data Quality Objectives and that the laboratory should be informed of the detection levels they will need to meet. When considering the detection levels that need to be met, one participant noted that it was important to be aware of the dose response curve. It is also helpful to consider which compounds contribute most to the toxicity of the mixture and to be sure that detection levels are adequate to quantify ecologically significant concentrations of these compounds.

Design issues. The workgroup concluded that sampling design issues were comparable between the TEF/TEQ method and other methods used to evaluate risks associated with PCDDs, PCDFs, and PCBs. The case study included sample sizes of 12. Participants noted that they would want to know values for eggs from 12 different terns or 2 nests. Such information would be important to understanding uncertainty.

Costs. Analytical costs associated with congener analyses are higher than for total PCBs or analysis of an individual compound.

Other Effects not Captured in TEF/TEQ. Participants noted that some PCB compounds could affect the species of concern via toxic mechanisms other than binding to the AhR receptor. Care must be taken to identify effects that may be important during planning stages of the analysis.

Working with Partial Data Sets. The TEF/TEQ methodology involves an assessment of PCDDs, PCDFs, and PCBs. However, for some situations, only one of these groups may be important. The workgroup concluded that partial data sets for one of these three groups would be adequate for evaluation if available information indicated that this was the only group of importance at the site or for the application. If available information indicates that background concentrations of other groups contribute significantly to the TEQ estimate, then those groups would have to be included in the analysis because the TEF/TEQ approach involves

comparisons to TEQ benchmarks or dose-response curves. In such cases, it would be inappropriate to consider the effects of any one group alone.

Utilizing Surrogate Methods in Concert with the TEF/TEQ Methodology

The workgroup concluded that there were opportunities to complement the TEF/TEQ methodology with surrogate analytical approaches. It was noted that once you have this information, and have calibration between individual congeners and total PCBs, you could use total PCBs as well. This would involve validating and calibrating as you go. Surrogate methods could be employed during investigations or in helping guide remedial measures. Workgroup participants noted that surrogate approaches work for the Great Lakes. It is not known how well these methods might work in other systems.

Comparison of TEQ vs. Traditional Total PCB Approach

The workgroup concluded that the traditional PCB approach would have missed important aspects of the problem. The traditional total PCB method would have underestimated risks as compared to the TEF/TEQ method.

Adequacy of Available Information for Decisionmaking Purposes

The workgroup identified several pieces of information that would be desirable for supplementing the information already at hand. These are discussed below.

Background Conditions. The case study did not provide information on background conditions. Therefore, even though body burdens of PCBs could be explained in terms of exposure to PCBs in the lake, there is the possibility that PCBs in the lake and in the tissues is comparable to those found in other lake systems. This could be evaluated by examining other lake systems or by evaluating conditions upstream of the spill. The group felt that a better

understanding of this issue would be needed before proceeding with a recommendation concerning management options.

Reasonableness of Association Among Concentrations in the Species of Interest. The workgroup concluded that it was important to examine the data with respect to the underlying conceptual model and relationships among media and receptors from other systems. Body burdens may vary among clutches of eggs. Typically, there are higher concentrations in the second clutch.

System and Food Web Issues. Workgroup participants concluded that additional information on food web relationships would be valuable. For example, it was noted that relationships within the case study were being inferred by assuming simple food chains. However, food chains could be much more complex and quite different from those assumed or inferred from the available data.

Vertical Distribution of Compounds in Sediment Cores. Information on concentrations in sediment cores would provide insight into the history of deposition, including pre-spill conditions. Such information could also be used to judge the rate of recovery.

Deriving "Acceptable" Target Levels for Environmental Media

Mechanics of Back Calculating Target Levels. The workgroup concluded that this would involve working the exposure equations backward. This would involve beginning with "acceptable" TEQ levels in ecological receptors and deriving "acceptable" target levels in sediments or water. The major challenge here is that the TEF/TEQ methodology involves tracking a number of compounds. This is primarily a logistical challenge. However, back-calculating will require information on the environmental behavior of the individual compounds. However, it may be possible to limit back-calculation to those compounds that contribute most to the TEQ levels in ecological receptors. Back-calculation would involve applying appropriate mass-loading models as well as biological uptake

models. These could be simple or complex. One participant noted that the analysis should extend beyond simple calculations of average concentrations. Individual animals do not experience the average, but rather the overall distribution.

Regression analyses. Apart from bioassay-based approaches (e.g., cell lines), use can be made of regression relationships between individual congeners, which can easily be measured with low cost GC-ECD techniques, such as PCB 153 and concentrations of toxic congeners (e.g., PCB 126) or total TEQ concentrations. In several ecosystem studies, relationships have been observed for PCB 153 and total TEQ concentrations in various fish species, otter, invertebrates, and cormorant eggs covering several orders of magnitude in PCB TEQ concentration. As a rule of thumb, a 0.5 to 1.0 order of magnitude range of uncertainty may be involved in extrapolations based on this relationship. As the regression relationships may be species- or site-specific, a preliminary validation may be required. Members of the workgroup recommend further exploration of this regression approach, using available data from monitoring studies, and further assessment of the feasibility of this potentially cost-effective approach.

Body burdens in some animals are size- or age-dependent. In the case of otters, for example, a recommendation was made to sample young carcasses.

Risk Management Options

The workgroup concluded that the decision "to clean up or not clean up" was one of several possibilities. The workgroup discussed several possible risk management options that could be explored using technical information.

Evaluating the Future Potential and Time Course for "Recovery". This is the "no action" or "limited action" option. Essentially, this option would involve providing the risk manager with information concerning how the system may change in the future. With respect to the TEF/TEQ approach, this will involve

understanding how concentrations of individual compounds in media and tissues will change in the future. Processes that could be involved include burial in sediments, degradation rates, metabolism, dissolution, and loss rates via evaporation and advection from the system.

Developing Additional Lines of Evidence. Workgroup participants discussed additional lines of evidence that could support decisionmaking. These included direct observations of effects on populations and bioassays. An example of observations that could be made on birds is to look for scelma (Great Lakes edema/mortality syndrome) in tern eggs.

Identifying Alternative Remedial Strategies. Remedial options could vary in type and magnitude. The efficacy of these alternatives could be judged by applying "what if" scenarios utilizing the TEF/TEQ methodology.

Integrating Lines of Evidence from Different Levels of Ecological Organization

The workgroup discussed the strengths and limitations of different lines of evidence that could be used to complement the TEF/TEQ approach. These discussions underscored differences in perspective related to "bottom up" approaches represented by applying the TEF/TEQ concentration-based methodology and "top down" approaches represented by making direct observations on populations. Bioassay methods fall in between. The group acknowledged that these different approaches had various strengths and limitations. The group concluded that it would be useful to explore how these different lines of evidence could be brought together to provide an overall assessment. With respect to organisms and population biology, it would be beneficial to foster exchanges between scientists working with the various bioassays, conservation biologists (working with populations), and ecological risk assessors. It would be helpful, for example, to have these groups work on a model for the first year of life for salmonids.

For this case study, participants noted the importance of having information for several trophic levels. In the present case, the fish populations do not appear to be at risk. However, species at even higher trophic levels—the tern and the otter—exhibit levels that indicate potential risks.

Appendix F

WRITTEN COMMENTS FROM OBSERVERS

DEPARTMENT OF HEALTH AND HUMAN SERVICES

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National Institute of
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Date: January 16, 1998

From: Angélique P.J.M. van Birgelen, Ph.D.

Subject: 3,3',4,4'-Tetrachloroazobenzene, hexachlorobenzene, 1,2,3,4,6,7-hexachloronaphthalene, 1,2,3,5,6,7-hexachloronaphthalene, and 1,2,3,4,5,6,7,-heptachloronaphthalene as additional dioxin-like compounds for inclusion in TEF concept

To: Chair of the Workshop on the Application of 2,3,7,8-TCDD Toxic Equivalency Factors to Fish and Wildlife

Cc: Drs. K. Abdo, J. Bucher, and G. Lucier

Inclusion in TEF concept

A dioxin-like compound is a compound that binds to the aryl hydrocarbon receptor, results in dioxin-like effects, and bioaccumulates. These are the three factors for inclusion of dioxin-like chemicals in the TEF scheme (Ahlborg *et al.*, 1992, 1994). 3,3',4,4'-Tetrachloroazobenzene (TCAB), hexachlorobenzene (HCB), 1,2,3,4,6,7-hexachloronaphthalene (PCN 66), 1,2,3,5,6,7-hexachloronaphthalene (PCN 67), and 1,2,3,4,5,6,7-heptachloronaphthalene (PCN 73) are compounds which bind to the Ah-receptor, result in dioxin-like effects, and have been shown to bioaccumulate and should therefore be included in the TEF concept.

Sources of TCAB, HCB, and PCNs

TCAB

TCAB is present as a contaminant of 3,4-dichloroaniline (DCA) and its herbicidal derivatives Propanil, Linuron, and Diuron (Poland *et al.*, 1976; Sunström *et al.*, 1978; Bunce *et al.*, 1979; Hill *et al.*, 1981). In addition, environmental contamination by TCAB occurs from the degradation of chloranilide herbicides (acylanilides, phenylcarbamates, and phenylureas) in soil by peroxide-producing microorganisms (Bartha *et al.*, 1968; Bartha and Pramer, 1969; Lay and Ilnicki, 1974). It is also formed by the photolysis and biolysis of 3,4-dichloroaniline (Mansour *et al.*, 1975; Miller *et al.*, 1980).

HCB

HCB was used as a fungicide for crops such as wheat, barley, oats, and rye to prevent growth of fungi. In the mid seventies the application of HCB as a fungicide was discontinued due to concerns about adverse health effects. In Tunisia however, HCB was still used as a fungicide, seed-dressing, and scabicide in sheep in 1986 (IPCS, 1997).

In industry, HCB has been used in the manufacture of pyrotechnics, tracer bullets, and as a fluxing agent in the manufacture of aluminum. HCB has been used as a wood preserving agent, a porosity control agent in the manufacture of graphite anodes, and as a peptizing agent in the production of nitroso and styrene rubber for tires (IPCS, 1997).

HCB is generated as a by-product in various chemical processes such as thermal chlorination, oxychlorination, and pyrolysis operations in the manufacture of chlorinated solvents such as carbon

tetrachloride, trichloroethylene, and tetrachloroethylene (IPCS, 1997). HCB is a by-product during the manufacture of pesticides, such as pentachloronitrobenzene, chlorothalonil, dacthal, pentachlorophenol, atrazine, simazine, propazine, and maleic hydrazide (IPCS, 1997). In the herbicide Propanil it has been found in concentrations up to 10-14% (IPCS, 1997). Furthermore, HCB is released into the environment by waste incineration. The release of HCB from all municipal incinerators in the U.S. was estimated by the EPA to be between 57 and 454 kg per year as documented in 1986 (IPCS, 1997).

PCNs

Polychlorinated naphthalene formulations have been used in industry as dielectric fluids in capacitors, transformers, and cables. The products containing technical PCNs are still in use or disposed in landfills. PCNs are also formed during production of technical mixtures of chlorobiphenyls and can be found in concentrations up to 1% in various polychlorinated biphenyl formulations (Falandysz *et al.*, 1996).

Binding to the Ah-receptor

TCAB and HCB have an affinity for the Ah-receptor 5- and 10,000-fold lower that TCDD, respectively (Hahn *et al.*, 1976; Poland *et al.*, 1976; Schneider *et al.*, 1995). Preliminary results from an Ah receptor binding assay indicate a relatively high binding activity of the hexa- and heptachlorinated naphthalenes (Hanberg *et al.*, 1990).

Dioxin-like effects

TCAB

TCAB exposure results in typical dioxin-like effects in rodents which include chloracne and dermal lesions, body weight loss, thymic atrophy, hepatotoxicity, developmental toxicity, induction of cytochrome P4501A1, anemia, and an increase of porphyrins in chick embryo liver cell cultures (Hsia *et al.*, 1980, 1981, 1982; Hill *et al.*, 1981; Mensink and Strik, 1982; Schrankel *et al.*, 1982; Hsia and Kreamer, 1985; McMillan *et al.*, 1990).

HCB

HCB results in dioxin-like effects, such as induction of hepatic CYP1A1 and CYP1A2 activities, hepatic porphyrin accumulation and excretion, alterations in thyroid hormone levels and metabolism, alterations in retinoid levels, liver damage (hepatocellular enlargement, bile duct proliferation, necrosis), reduction in reproduction, splenomegaly, increase in mortality, neurological alterations (such as tremors, paralysis, weakness, hyperexcitability), teratologic effects, and immunotoxic effects (IPCS, 1997). HCB is a carcinogen in rodents (IPCS, 1997). HCB exposure also results in phenobarbital-like effects, such as induction of hepatic CYP2B activity (IPCS, 1997).

PCNs

PCN 73, and a mixture of PCN 66 and PCN 67 induced EROD and AHH activities in a rat hepatoma H-4-II cell line (Hanberg *et al.*, 1990, 1991).

Bioaccumulation

TCAB

TCAB has a log octanol/water partition coefficient of 5.53 to 6.69 (US-EPA, 1985; Hashimoto *et al.*, 1994). The solubility in water is calculated to be 1 µg/l (US-EPA, 1985). In male Sprague Dawley rats administered radiolabeled TCAB by gavage, 66% of the dose was excreted in urine and

feces after 24 hours (Burant and Hsia, 1984). The pattern indicated a biphasic elimination, consisting of an early rapid phase with a half-life of 18 hours and a slow terminal phase with a half-life greater than 20 days.

HCB

HCB has a log/octanol water partition coefficient of 5.5 (IPCS, 1997). The solubility in water has been reported to range from 0.005 (mg/L at 25°C) to 0.035 ppm (IPCS, 1997; Kenaga, 1980). The (whole body) half-life of HCB in male Wistar rats has been reported to be 20 days (Yamaguchi *et al.*, 1986). In male Sprague Dawley rats and male white rabbits, the half-life was calculated to be 24 days and 32 days, respectively (Scheufler and Rozman, 1984). The major route of excretion was via the feces in both rats and rabbits. In rhesus monkeys, the half-life has been estimated to be 2.5 to 3 years (Rozman *et al.*, 1975).

PCNs

PCN 66 and PCN 67 were selectively retained in the liver of rats exposed to Halowax 1014, a commercial mixture of PCNs (Asplund *et al.*, 1986, 1994; Jacobsson *et al.*, 1992). In marine mammals such as harbour porpoise a BMF value greater than 1 was observed only for the pair of PCN 66/PCN 67 (Falandysz, 1997). PCN 66/PCN 67 and PCN 73 have been found at high concentrations in cod liver samples from Southern Norway (Schlabach *et al.*, 1995).

Relative potency estimates for TCAB, HCB, and PCNs

Table 1. Relative potency estimates for 3,3',4,4'-tetrachloroazobenzene (TCAB).

Effect	TCDD	TCAB	Relative potency for TCAB	Reference
Binding affinity to the Ah receptor (nM)	0.27	1.1	0.2	Poland *et al.*, 1976.
EC50 for binding to the mouse hepatic Ah receptor (nM)	1.22	6.03	0.2	Schneider *et al.*, 1995.
ED50 (nmol/kg) for induction of aryl hydrocarbon hydroxylase in chicken embryos	0.31	2.0	0.2	Poland *et al.*, 1976.
LD50 (ng/egg) in chicken embryos.	0.2	44	0.005	Higginbotham *et al.*, 1968; Schrankel *et al.*, 1982.
Cytochrome P4501A1 induction in the skin in a 90-day oral gavage study in female B6C3F1 mice with TCDD and TCAB.			0.000003-0.00001	Hébert *et al.*, 1993.

Table 2. Relative potency estimates for hexachlorobenzene (HCB).

Effect	TCDD	HCB	Relative potency for HCB	Reference
Binding affinity to the Ah receptor (nM)	0.18	2100	0.00009	Hahn *et al.*, 1976.
EC50 for EROD induction in chicken hepatocytes (nM)	0.014-0.020	130-150	0.00009-0.0002	Sinclair *et al.*, 1997.
EC50 for accumulation of uroporphyrin in chicken hepatocytes (nM)	0.002-0.004	25-35	0.00006-0.0002	Sinclair *et al.*, 1997.
Hepatic porphyrin accumulation in female rats			0.0007	Cantoni *et al.*, 1981.

Table 3. Relative potency estimates for polychlorinated naphthalenes (PCNs).

Effect	Relative potency for PCN 66/PCN 67	Relative potency for PCN 73	Reference
AHH activity in a rat hepatoma H-4-II cell line	0.003	0.003	Hanberg *et al.*, 1990.
CYP1A1 activity in a rat hepatoma H-4-II cell line	0.002	0.003	Hanberg *et al.*, 1990, 1991.

Impact on TEQ

TCAB

Based on an annual production volume of 10 million pounds of Propanil in the US and the concentration of TCAB in Propanil ranging from 1,000 to 2,700 µg/g, this could lead to an annual release of 12,000 kg of TCAB into the environment due to Propanil alone (Sunström *et al.*, 1978; Bunce *et al.*, 1979; Hill *et al.*, 1981; US-EPA, 1987 as cited in McMillan *et al.*, 1991). With an annual production volume of 0.1 to 1 million pounds of DCA and a concentration of TCAB in DCA ranging from 9 to 8,600 µg/g, this could lead to a production of 3,900 kg of TCAB per year in the US (Sunström *et al.*, 1978; Bunce *et al.*, 1979; Hill *et al.*, 1981; US-EPA, 1985). Analyses of a rice plot treated with 6.7 kg Propanil/hectare indicated a TCAB concentration of 0.09 ppm (Kearney *et al.*, 1970). Six of 99 soil samples from the rice-growing states of Arkansas, California, Louisiana, Mississippi, and Texas contained 0.01 to 0.05 ppm TCAB, whereas no residual concentration of Propanil was detected (Carey *et al.*, 1972). Assuming TCAB is three orders of magnitude less potent than TCDD (to pick a number), this indicates that the concentration of TCAB in the mentioned soil samples, calculated as TEQ could be as high as 90 ng TEQ/kg soil. For comparison, the mean level of dioxin-like compounds (PCDDs and PCDFs only) has been estimated to be 8 ng TEQ/kg soil (US-EPA, 1994). Using the same calculation for the production of TCAB due to Propanil and DCA, this could lead to an annual release of 16 kg TEQ in the environment.

HCB

Levels of HCB measured in bald eagle eggs from the British Columbia coast from 1990 to 1992 ranged from 0.012 to 0.025 mg/kg wet weight (Elliott *et al.*, 1996). Assuming HCB has a relative potency of 0.0001 (to pick a number), this could be as high as 25 ng TEQ/kg wet weight. For comparison, the concentration of PCDDs, PCDFs, and PCBs (planar and mono-ortho substituted) ranged from 120 to about 320 ng TEQ/kg in bald eagle eggs from the same areas (Elliott *et al.*, 1996).

PCNs

The concentration of PCN 66/PCN 67 in cod liver samples from Southern Norway ranged from 927 to 123,000 pg/g wet weight (Schlabach *et al.*, 1995). Using a relative potency value of 0.003 this equals to 2.8 to 369 pg TEQ/g wet weight. In the same study, the TEQ based on PCDDs, PCDFs, and non-ortho PCBs was calculated to range from 175 to 2000 pg TEQ/g wet weight. In the samples, up to 37% of the total TEQ was derived from PCN 66/PCN 67 and 1,2,3,4,5,6,7-heptachloronaphthalene (PCN 73), with 25% derived from PCN 66/PCN 67.

Awareness of limited data

I am very well aware of the limited data sets available to derive a TEF value. The ones available include chicken embryos and *in vitro* systems in chicken hepatocytes, binding assays to the Ah receptor, *in vitro* studies with a rat hepatoma cell line, and *in vivo* studies in rodents. However, TEF values are interim values which will change until more data become available. By setting TEF values for the mentioned congeners and using these preliminary values for the calculation of the total TEQ in selected samples, scientists and regulatory agencies can be made aware of the need for designing robust studies to derive relative potency values and continue - or even start - measuring the mentioned compounds in biota.

Conclusion

In summary, TCAB, HCB, and PCNs should be included in the TEF concept based on binding to the Ah-receptor, their dioxin-like effects, and their bioaccumulation. The limited data available on environmental levels of TCAB, HCB, and PCNs suggest that these compounds could considerably add to the total TEQ in environmental samples.

Acknowledgements

I would like to thank Dr. John Bucher for giving me the challenge in the National Toxicology Program to be involved in the technical report on the toxicity of 3,3',4,4'-tetrachloroazobenzene, Dr. George Becking for the opportunity to participate in the IPCS task group meeting on hexachlorobenzene, and Prof. Bo Jansson and Dr. Jerzy Falandysz for their efforts to keep me updated on chlorinated naphthalenes.

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22 January, 1998

The Comments of an Observer to the Expert Reviewers and EPA at the Chicago Hilton Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and Wildlife

Thank you for allowing me the opportunity to be a part of this workshop as an observer. It has been an educational event for me and something that I will build upon, especially the acronyms.

Thank you also for all your hard work and persistence in your pursuit of truth. I will feel safer knowing you are all working so hard on the subject of this workshop. I have had conversations with many of you. Some of my comments may have seemed vague and generally out of place. But they are not so out of place, for the questions I raise must be asked at each step that we take in our work. We must all develop a long range outlook on our home the earth because it is the only environment that most will have for a very long time. To look ahead 3 generations is definitely not enough. Even 30 generations is not enough. I am not certain what is the appropriate amount of foresight when it comes to the degradation of our environment.

The minimum time span that should be included is the useful life span of the chemical in question. How long does it take for this chemical to break down into something that all scientists are certain is safe? Whether the chemical in question is a PCB, dioxin or one of the latest supposedly safe chemical compounds such as glyphosate. Even that is looking to have the characteristics of an endocrine disruptor. As long as I have your attention I'd like to throw nuclear waste into the arena, as well, because there are agencies hard at work presently attempting to set limits on the amount of nuclear waste that can be recycled into consumer products.

The reason I believe such prudence is due is to ensure that we are not leaving our descendants an intolerable environmental mess. It is important that industry include the full costs of a product in its price to the consumer. Almost never included are the costs of production waste, environmental pollution, habitat loss, health problems for factory and field workers, health problems for the consumers and long term storage of toxic wastes.

We must stop passing these problems and costs on to future generations of unsuspecting people, many of whom will never have any use for the products or byproducts that will degrade their bodies and environment. When we increase a pollutant into our environment at any level it has some effect, whether it is presently measurable or not. When a permit is granted to an industry to add additional pollutants because our legal system has deemed it safe, the effects are felt around the world, especially when it comes to such things as dioxin and PCBs. You may think of these effects as social or economic problems, and therefore not in your field. It is then safe to ignore the obvious problems that we have saddled other neighborhoods, states or countries with. Maybe we cannot prove that a particular PCB or dioxin was made by a particular factory, but we did add to the whole ecosystem by granting that permit to increase pollutants production. As such we are just as liable as the next polluter or discharger, as the legal people would say.

In conclusion, I must urge you all to do as much interdisciplinary communication as possible. Get really wild and speak to a psychologist or psychiatrist about their patient that has been chemically injured and has behavioral problems. It is with these inventive communications that your new ideas will form. Remain open at all times to new ideas. Most of all, think in terms of many hundreds of generations in an attempt to slow down the constant degradation.

Sincerely,
Paul Goettlich

Date: February 25, 1998

To: Susan Brager Murphy, TEF Workshop Coordinator

cc: Charles Menzie, Linda Birnbaum

From: Brent Finley, Workshop Observer

Subject: Expanding the TEF Approach to include Hexachlorobenzene (HCB)

Introduction

As part of the effort to address potential human health risks posed by chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) in the environment, the U.S. EPA adopted an interim procedure in 1987 based on dioxin "toxicity equivalence" factors (TEFs) for estimating the hazard and dose-response of complex mixtures containing CDDs and CDFs in addition to 2,3,7,8-TCDD (USEPA, 1989). The adoption of TEFs for CDD/CDF congeners was explicitly stated and recognized by researchers to be an interim science policy measure. The technical subcommittee that was gathered to derive and periodically update the TEF scheme noted that a general (order of magnitude) approach was needed to characterize potential risks posed by the 209 CDD/CDF congeners other than 2,3,7,8-TCDD because of the lack of detailed toxicity data on almost all of these congeners. With the development of updated TEFs in 1989 (i.e., I-TEFs), it was again noted by the subcommittee that the TEF approach was an "interim" approach and should be replaced as soon as practicable with a bioassay method. Over the past several years, several efforts have been made to expand the TEF approach to include mixtures of coplanar polychlorinated biphenyls (PCBs) (Ahlborg et al. 1994; Safe et al. 1994), but a consistent set of TEF values has yet to be adopted by USEPA for PCBs.

In the draft 1997 W.H.O. document, it was suggested that the health risks associated with exposure to HCB (and other chemicals) could be assessed using the 2,3,7,8-TCDD-based TEF scheme. This suggestion was based on an apparent concern that: 1) HCB might possess "dioxin-like" properties and, 2) it is important to understand the "total TEQ body burden" of humans and ecological species. This suggestion was further detailed by Angelique van Birgelen at the workshop in Chicago. During her presentation, she elaborated on the information she had written for the W.H.O. document. It was not clear to me whether any of the other workshop participants supported or disagreed with her position. In general, I have some serious reservations about the practicality and applicability of a "TEF" for a chemical which exists not as a mixture but as a single compound (such as HCB), and I specifically have some misgivings about applying a TEF to HCB, a compound whose toxicity is already well characterized. I have detailed some of my observations and concerns below.

1. A TEF for HCB is not Warranted Because There are no HCB "Mixtures"

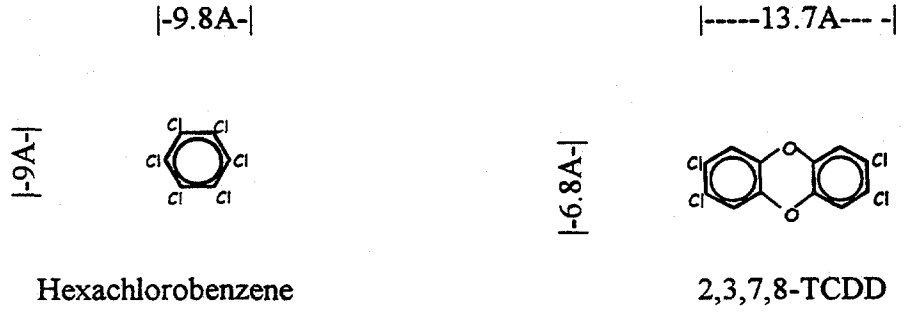
The TEF approach was originally developed as an interim approach for complex mixtures of CDD/Fs. Because it is obviously impractical to conduct long-term bioassays on each and every one of the CDD/F congeners, the TEF approach was developed as a "short-cut" that would allow for assessments of complex mixtures using existing congener data (in vitro studies, etc.). As discussed in the W.H.O. report (1997), there are a large number of compounds which could contribute to the total concentration of TCDD TEQs. These chemicals include polycyclic aromatic compounds such as biphenyls, 2- and 3-ring polycyclic aromatic hydrocarbons and other heterocyclic compounds. Depending on the degree of substitution, many congeners of polycyclic compounds can exist. For most of these compounds, little or no toxicity data exists to characterize a dose-response relationship. If in fact it can be demonstrated that some or all of the various congeners in a chemical class are likely to possess significant "dioxin-like" activity, then perhaps development of TEFs for this chemical class is appropriate.

On the contrary, dose-response relationships have been developed for HCB using the results of several bioassays to characterize effects from subacute to chronic exposures. Attempting to incorporate HCB into a TEF scheme is therefore inconsistent and contrary to the TEF concept because it ignores the more accurate assessment techniques that have already been applied to HCB toxicity data for characterizing adverse effects. More importantly, because there are no congeners or isomers of HCB, its inclusion in the TEF scheme seems counterintuitive.

2. There is Insufficient Evidence to Conclude HCB Meets W.H.O.'s Definition of "Dioxin-Like"

As defined by the W.H.O.(1997) in their draft workshop proceedings, there are four criteria that W.H.O. has proposed to determine whether a chemical might be evaluated using the TEF scheme. Each of these criteria, as they apply to HCB, is discussed below.

- *Structural Similarity to TCDD* – As is shown in the figure below, HCB is a monocyclic (single-ringed) aromatic compound with full chlorine substitution, whereas TCDD is a coplanar, polycyclic compound with chlorine substituted at four locations. As such, HCB lacks the structural dimensions (a 6.8x13.7 Angstrom box of chlorine substitutions) required for TCDD-like toxicity (Hahn et al. 1989; McKinney and Singh, 1981).



Hexachlorobenzene 2,3,7,8-TCDD

Extensive evidence exists on CDDs/CDFs to show that small configurational changes such as chlorine substitution on a specific carbon atom drastically affects the potency of a compound. For example, 2,3,5,7-TCDD is not considered as a candidate for a TEF because of the subtle difference in the placement of a single chlorine atom. The significant structural and configurational differences between the polycyclic CDDs/CDFs and monocyclic HCB is compelling evidence that support the conclusion it does not meet the first criterion for a dioxin-like compounds.

- *Binds to the Ah Receptor* – The evidence supporting HCB binding to the Ah receptor has been described as "equivocal" and "at best a very weak competitor" (Goldstein et al. 1986; Linko et al. 1986). The binding affinity of HCB is far less than that of every 2,3,7,8-substituted CDD/F, and in fact, is much less than that observed for naturally occurring aromatic compounds such as polycyclic aromatic hydrocarbons and indole carbazoles (Ames et al. 1990; Kleman and Gustafsson, 1996). Therefore, it would seem that HCB fails on this criterion.

- *Dioxin-Like Toxic/Biochemical Responses* – HCB can induce several responses that can also be induced by TCDD, including CYPIA1/IA2 induction, thyroid hormone alterations, hepatic retinol depletion, porphyrin accumulation, hepatic hypertrophy, and immunotoxicity. However, the HCB doses required to elicit these effects are orders of magnitude higher than TCDD doses required to elicit the same degree of effect. In addition, the mechanism by which HCB produces toxic effects may be quite different than that for TCDD. For instance, oxidative metabolites of HCB (pentachlorophenol, tetrachlorohydroquinone) have been implicated in the manifestation of hepatic porphyria and other effects (Rietjens et al. 1995; Schielen et al. 1995; Van Ommen et al. 1989). Conversely, the toxicity of TCDD is generally attributed to the interaction of the parent compound with the Ah receptor.

- *Persistence* – In humans, the half-life of HCB has been estimated to be approximately 215 days (Freeman et al. 1989), which is less than a tenth of the half-life reported for TCDD of approximately 7.5 years (Needham et al. 1994). Clearly, the pharmacokinetics of HCB is vastly different from that of TCDD. Indeed, one of the deficiencies of the TEF approach, particularly with TEFs based on *in vitro* or acute *in vivo* responses, is that it does not account for differences in kinetics, an important determinant of toxicity. In summary, it is questionable whether HCB can be considered "persistent" relative to TCDD.

3. Numerous Chemicals Meet W.H.O.'s Criteria of "Dioxin-Like"

As noted above, there are several chemical classes which could be interpreted to meet the somewhat arbitrary criteria of "dioxin-like," even as defined by the use of W.H.O criteria. However, it is unreasonable to suggest that TCDD-based TEFs will be derived for each isomer of each of these chemical classes. Even if sufficient resources existed to establish such TEFs over the next 5-10 years, the result would be an unwieldy collection of hundreds or thousands of TEF values. This is unlikely to occur, and therefore I believe that simply because a chemical meets a definition of "dioxin-like" is insufficient reason to attempt to establish a TEF for that compound. This is particularly true for HCB which, as noted above, does not satisfy W.H.O.'s criteria.

4. A TEF is Not Warranted Because Sufficient Toxicological Criteria Exist for HCB

The TEF approach was originally developed for PCDD/F congeners since data to characterize toxicity was limited or not available. This is not the case for HCB. Rather, the toxicity of HCB has been extensively studied, and the dose-response relationships for various health effects have been well characterized. Toxicity values for HCB and TCDD used for risk assessment are compared in the table below.

Endpoints	Toxicity Value	HCB	TCDD
Human Health	Cancer Slope Factor	Based on liver tumors in female rats (Eturk, 1986)	Based on liver tumors in female rats (Kociba et al. 1978)
	Oral Reference Dose	Based on liver effects in rats (Arnold et al. 1985)	Not available
Ecological	Mammals	Based on survival and reproductive effects in mink (Rush et al. 1983; Bleavins et al. 1984)	Based on reproductive effects in rats (Murray et al. 1979)
	Fish	Based on survival in several aquatic species (USEPA, 1988)	Based on survival in rainbow trout and northern pike (USEPA, 1993)
	Birds	Based on survival and reproductive effects in several species of birds (Vos et al. 1971)	Based on survival, reproductive & developmental effects in several species of birds (Nosek et al. 1992; Hudson et al. 1984)

Currently, the cancer slope factor and reference dose for HCB are derived without prejudice to the mechanism by which adverse effects were produced. As such, any "dioxin-like" activity imparted by HCB in the critical toxicological studies is already accounted for in the existing slope factor and reference dose.

For ecological risk assessment endpoints, there are no promulgated toxicity values as there are for human health. However, the quantity and quality of available toxicological studies for ecological receptors of potential concern for HCB is as good or better as that for TCDD. Controlled studies of subchronic and chronic HCB exposures have been conducted with 10 species of mammals and five species of birds. These include wild species such as the mink and the kestrel which are frequently identified as receptors of interest for ecological risk assessment. These studies have assessed the dose-related effects of HCB on the survival, growth, reproduction and development of these species; all endpoints clearly related to risks to exposed populations. The studies with

Japanese quail indicate that hatchability of eggs and survival of chicks are sensitive endpoints for birds and provide a sound basis of a toxicity reference value for this taxonomic group (Vos et al., 1971; Schwetz et al., 1974). Similarly, the studies with mink show that reductions in litter size and survival of kits are sensitive endpoints and provide a documented basis for developing a toxicity reference value for mammals (Bleavins et al., 1984; Rush et al., 1983). Because these studies are generally of good quality and have assessed the dose-related effects of HCB exposure on relevant endpoints in a relatively large number of species, there are substantial data from direct tests to evaluate HCB toxicity and little justification, if any, for abandoning these data for this individual compound for "an order of magnitude" TEF.

The dose-related health effects of HCB observed in long-term animal feeding studies are a far more accurate and direct measure of HCB toxicity than an indirect "TEF-estimate" which is based on the potency of an entirely different chemical. Chemical-specific information, based on chronic bioassays for all endpoints of concern, is clearly a more preferable basis for risk assessment.

5. The Relative Potency (REP) of 0.0001 is Overestimated

Although an REP of 0.0001 can be calculated for HCB based on relative binding affinity to the Ah receptor *in vitro* (Hahn et al. 1989), this is likely to overestimate the carcinogenic potency. Using the relative carcinogenic potencies of HCB [$(1.6\ (mg/kg\text{-}day)^{-1}$] and TCDD [$(156{,}000\ (mg/kg\text{-}day)^{-1}$] in female Sprague-Dawley rats following lifetime exposures (Eturk, 1986; Kociba et al. 1978), an REP of 0.00001 may be viewed as a conservative upper bound (under the unlikely assumption that all HCB-induced liver tumors are attributable to a "dioxin-like" mechanism of action). Because HCB-induced tumors are primarily attributable to a non-dioxin-like mechanism of action, the REP for carcinogenic effects is likely to be much less than 0.00001, and in all likelihood is closer to (if not equal to) zero.

Conclusions

The TEF approach was developed and adopted specifically to address the potential risks posed by related constituents (*i.e.*, congeners) with similar structural features that might elicit a response or toxic effect under an identical mechanism of action. HCB is a well-studied chemical for which current risk assessment methods are superior to the TEF approach. Given that there is no discernible benefit in adding HCB to the TEF scheme, I strongly recommend that the TEF approach not be applied to HCB for characterizing potential risks.

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