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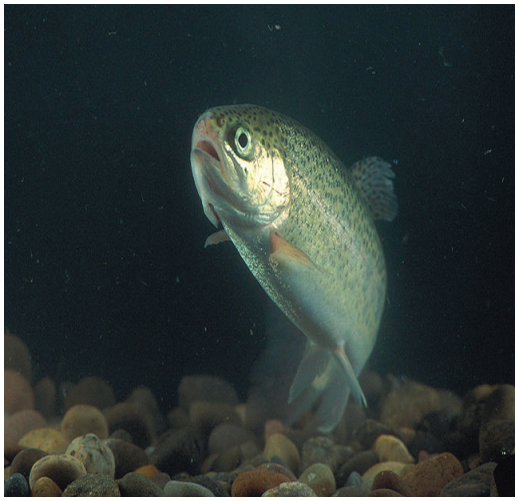
Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment



Office of the Science Advisor
Risk Assessment Forum

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Office of the Science Advisor
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, D.C. 20460

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PREFACE

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are commonly found as contaminants in complex mixtures in the environment, including in animal tissues. For more than a decade, the U.S. Environmental Protection Agency (EPA) and other organizations have estimated the combined risks that such mixtures pose to human health using a method known as the toxicity equivalence methodology. Application of this methodology in ecological risk assessments has proceeded more slowly, in part because of the variety of species from different taxonomic classes (e.g., fish, birds, and mammals) that need to be considered.

As both data and experience with the methodology have accumulated experts have come to the consensus that the toxicity equivalence methodology can strengthen assessments of ecological risks (Van den Berg *et al*., 1998, 2006; U.S. EPA, 2001a; NRC, 2006). Consultations between EPA and the Department of Interior (DOI) on water quality criteria, based on 2,3,7,8-TCDD alone, for protecting endangered species in the Great Lakes led these agencies to more intensively explore the application of the toxicity equivalence methodology in ecological risk assessment. In 1998, EPA and DOI sponsored a workshop that recommended the development of further guidance on application of the toxicity equivalence methodology (U.S. EPA, 2001a). This framework has been developed in direct response to that workshop recommendation. In July 2003, EPA released a draft framework for a 90-day public comment period. In addition, an external peer review was conducted over several months from October 2003 to February 2004, culminating in a final report on February 9, 2004. Links to these documents can be found at http://www.epa.gov/osa/raf/tefframework/.

Organized in accordance with EPA's *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998), this framework is intended to assist EPA scientists in using the toxicity equivalence methodology in ecological risk assessments that involve dioxins and dioxin-like chemicals, as well as to inform EPA decision makers, other agencies, and the public about this methodology. While this framework touches on many aspects of ecological risk assessment, it is not intended to be a comprehensive guide to risk assessment involving dioxin-like chemicals. Rather, the framework provides an introduction to the toxicity equivalence methodology, offers considerations for how and when to apply it, and presents practical examples of its use. Readers are referred elsewhere for details on topics such as chemical analysis, environmental fate and transport modeling, and development of stressor-response profiles for dioxin-like chemicals.

The Ecological Toxicity Equivalence Factor (Eco-TEF) framework is intended for guidance only. It does not establish any substantive "rules" under the Administrative Procedure Act or any other law and will have no binding effect on EPA or any regulated entity. Rather, it represents a nonbinding statement of policy. EPA believes that this framework provides a sound, up-to-date presentation of a method for use in conducting risk assessments involving dioxins and dioxin-like chemicals, and serves to enhance the application of the best available science. However, EPA and others may conduct risk assessments for dioxins and dioxin-like chemicals using approaches and methods that differ from those described in this document for many reasons, including, but not limited to, new information, new scientific understandings, and new science policy judgments. The science surrounding hazard and risk analysis for dioxins and dioxin-like chemicals continues to be intensively studied and thus is rapidly evolving. Specific guidance presented in the framework may become outdated or may otherwise require modification to reflect the best available science. Application of this framework in future risk assessments will depend on EPA decisions that its approaches are suitable and appropriate.

These judgments will be tested and examined through peer review, and any risk analysis will be modified as deemed appropriate.

This framework was prepared by a Technical Panel under the auspices of EPA's Risk Assessment Forum. The Risk Assessment Forum was established to promote scientific consensus on risk assessment issues and to ensure that this consensus is incorporated into appropriate risk assessment guidance. To accomplish this, the Risk Assessment Forum assembles experts from throughout EPA in a formal process to study and report on these issues from an Agency-wide perspective.

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This framework was prepared by a technical panel under the auspices of EPA's Risk Assessment Forum and reflects the contributions of participants at a 1998 workshop on the application of 2,3,7,8-TCDD TEFs in fish and wildlife.

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LIST OF ABBREVIATIONS AND ACRONYMS

2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
AHR	aryl hydrocarbon receptor
BAF	bioaccumulation factor
BCF	bioconcentration factor
BSAF	biota-sediment accumulation factor
DOI	U.S. Department of Interior
EC	effective concentration
ECO-TEF	ecological toxicity equivalence factor
ED	effective dose
EPA	U.S. Environmental Protection Agency
EROD	ethoxyresorufin-O-deethylase
HpCB	heptachlorinated biphenyl
HpCDD	heptachlorinated dibenzo-*p*-dioxin
HpCDF	heptachlorinated dibenzofuran
HxCB	hexachlorinated biphenyl
HxCDD	hexachlorinated dibenzo-*p*-dioxin
HxCDF	hexachlorinated dibenzofuran
IPCS	International Programme on Chemical Safety
LD	lethal dose
LOAEL	lowest observed adverse effect level
NATO/CCMS	North Atlantic Treaty Organization/Committee on the Challenges of Modern Society
NOAEL	no-observed adverse effect level
NRC	National Research Council of the National Academies
OCB	octachlorinated biphenyl
OCDD	octachlorinated dibenzo-*p*-dioxin
OCDF	octachlorinated dibenzofuran
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDFs	polychlorinated dibenzofurans
PeCB	pentachlorinated biphenyl
PeCDD	pentachlorinated dibenzo-*p*-dioxin
PeCDF	pentachlorinated dibenzofuran
QSAR	quantitative structure-activity relationship
ReP	relative potency
RPF	relative potency factor
TCB	tetrachlorinated biphenyl
TCDD	tetrachlorinated dibenzo-*p*-dioxin
TCDF	tetrachlorinated dibenzofuran
TEC	toxicity equivalence concentration
TEF	toxicity equivalence factor
TEFs-$NATO_{89}$	TEFs (sometimes also referred to as I-TEFs) adopted by the NATO/CCMS in 1989
TEFs-WHO_{94}	TEFs adopted by the WHO in 1994

TEFs-$WHO_{98/05}$	TEFs adopted by the WHO in 1998 and 2006; developed at a WHO-ECEH and WHO-IPCS expert meetings in 1997 (mammalian, avian and fish TEFs) and 2005 (mammalian TEFs only).
TMDL	total maximum daily load
WHO	World Health Organization
WHO-ECEH	WHO European Centre for Environmental Health
WHO-IPCS	WHO International Programme on Chemical Safety

PCB abbreviations:

TCB	tetrachlorinated biphenyl
PeCB	pentachlorinated biphenyl
HxCB	hexachlorinated biphenyl
HpCB	heptachlorinated biphenyl
OCB	octachlorinated biphenyl
PCBs	polychlorinated biphenyls

PCDD abbreviations:

TCDD	tetrachlorinated dibenzo-*p*-dioxin
PeCDD	pentachlorinated dibenzo-*p*-dioxin
HxCDD	hexachlorinated dibenzo-*p*-dioxin
HpCDD	heptachlorinated dibenzo-*p*-dioxin
OCDD	octachlorinated dibenzo-*p*-dioxin
PCDDs	polychlorinated dibenzo-*p*-dioxins

PCDF abbreviations:

TCDF	tetrachlorinated dibenzofuran
PeCDF	pentachlorinated dibenzofuran
HxCDF	hexachlorinated dibenzofuran
HpCDF	heptachlorinated dibenzofuran
OCDF	octachlorinated dibenzofuran
PCDFs	polychlorinated dibenzofurans

1. INTRODUCTION

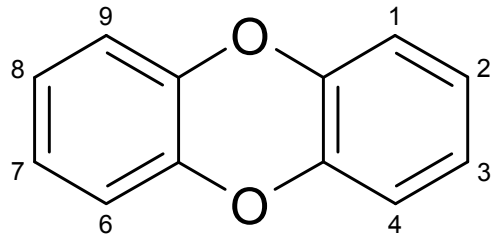
Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) (Figure 1) are persistent bioaccumulative contaminants that are found ubiquitously in environmental matrices, including tissues of fish, birds, and mammals. The most well-studied chemical in this group is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Demonstrated toxic effects of 2,3,7,8-TCDD in fish, birds, and mammals include adverse effects on reproduction, development, and endocrine functions; wasting syndrome; immunotoxicity; and mortality. Several PCDDs, PCDFs, and PCBs have been shown to cause toxic responses similar to 2,3,7,8-TCDD, in both laboratory and field situations. In this document, the term "dioxin-like effects" is used to refer to those effects that are similar to those caused by 2,3,7,8-TCDD, and the term "dioxin-like chemicals" is used to refer to chemicals that exert such effects through binding to the aryl hydrocarbon receptor (AHR). For further information regarding dioxin-like effects observed specifically in wildlife species, refer to U.S. EPA (1993, 2001b) and references therein. It should be noted that a number of chemicals other than PCDDs, PCDFs, and certain PCBs may also exert dioxin-like effects through binding to the AHR (see Section 2.1). Although these chemicals are not specifically addressed in this framework, if they meet the criteria discussed in Section 2 they may be included in assessments that apply the toxicity equivalence methodology.

Presently, evidence is sufficient to conclude that a common mechanism of action, involving binding of the chemicals to the AHR as the initial step, underlies 2,3,7,8-TCDD-like toxicity elicited by these PCDDs, PCDFs, and PCBs (Van den Berg *et al*., 1998, 2006; Hahn, 2002a). PCDDs, PCDFs, and PCBs present in the environment are generally found as complex mixtures such that assessment of ecological risk requires a means of quantifying their combined effects.

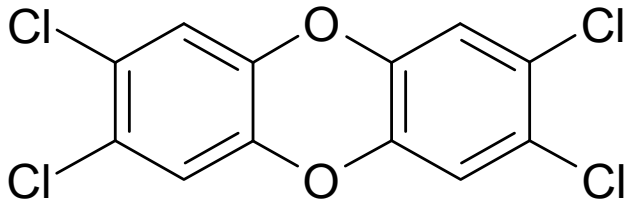
The purpose of this framework is to describe a methodology for assessing risks associated with exposure to complex mixtures of PCDDs, PCDFs, and dioxin-like PCBs. It is not a comprehensive guide for conducting a risk assessment for PCDDs, PCDFs, and dioxin-like PCBs, rather it describes how to apply a specific tool, the toxicity equivalence methodology, within the broader context of an ecological risk assessment. Accordingly, the intended audience for this framework is risk assessors who have a working knowledge of EPA's Guidelines for Ecological Risk Assessment (U.S. EPA, 1998) and are familiar with issues related to conducting risk assessments for dioxin-like chemicals (U.S. EPA, 1993, 2001a). This framework provides informed risk assessors with a summary of technical insights and recommendations from a variety of documents and expert workshops on the topic of toxicity equivalence methodology and its application in ecological risk assessment (U.S. EPA, 1987, 1989, 1991, 2000a, 2001a). The framework also provides ecological risk assessors with an understanding of the uncertainties associated with the application of the methodology in general and with situation-specific decisions made in applying the methodology within their risk assessments.

In this framework, definitions and a description of how the methodology has evolved are described in Chapter 1. Chapter 2 summarizes the toxicity equivalence methodology. Chapter 3 provides ecological risk assessors with an understanding of issues to consider when applying the toxicity equivalence methodology in ecological risk assessments. Chapter 3 is organized according to the phases of ecological risk assessment (planning, problem formulation, analysis, and risk characterization). Chapter 4 concludes by summarizing important benefits, implications, and uncertainties of the toxicity equivalence methodology as one of several methods within the broader context of ecological risk assessment.

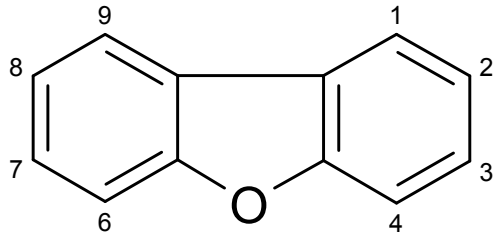
General Structure | **Representative Examples**



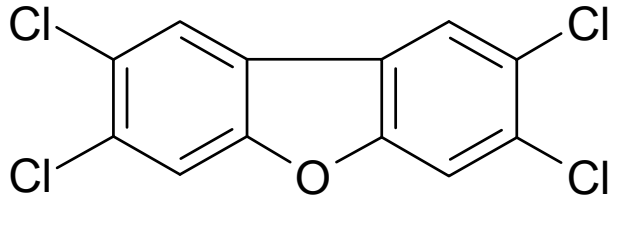
Polychlorinated dibenzo-*p*-dioxins (PCDDs)



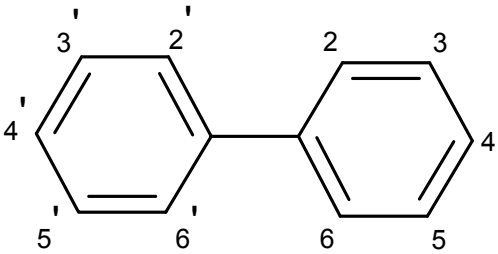
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)



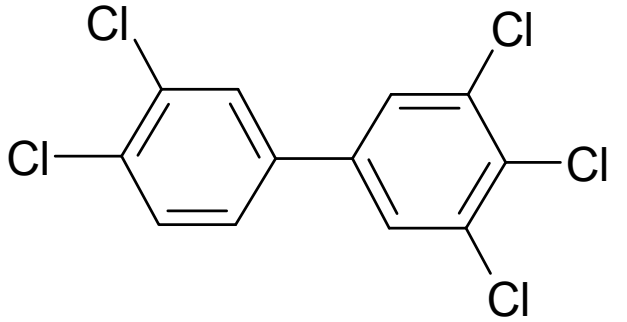
Polychlorinated dibenzofurans (PCDFs)



2,3,7,8-Tetrachlorodibenzofuran (TCDF)



Polychlorinated biphenyls (PCBs)



3,3',4,4',5-Pentachlorobiphenyl (PeCB)
(PCB 126)

Figure 1. Chemical structure of PCDDs, PCDFs, and PCBs.
Numbers by aromatic ring carbons in general structures represent potential chlorine substitutions.

1.1. DEFINITIONS

To date, many different terms and acronyms have been used to describe the potency, or the strength to cause toxic effects, of individual PCDDs, PCDFs, and PCBs relative to 2,3,7,8-TCDD (see Text Box 1). For example, a Toxicity Equivalence Factor (or TEF) has been used to describe the relative potency of dioxin-like chemicals to affect a single endpoint in a single study as well as to describe a relative potency value based on the results of several studies. Inconsistency in the use of various terms and abbreviations associated with the toxicity equivalence methodology can contribute to confusion and misunderstanding, and has led to recommendations to further clarify terminology and acronyms (U.S. EPA, 2001a). In response, this framework establishes a clear, systematic, and unified terminology scheme for the toxicity equivalence methodology, building on the terminology adopted at the World Health Organization European Centre for Environmental Health (WHO-ECEH) international consultation (Van den Berg *et al*., 1998).

Text Box 1. Clarification of terminology.

Acronym used in this framework	Analogous acronyms found in the literature
ReP	REP, ReP, RP, RPF, TEF
RPF	REP, ReP, RP, RPF, TEF
TEF	IEF, I-TEF, TEF-WHO, REP, RPF, RP
TEC	TEqC, TEQ, TEq

Term used in this framework	Analogous terms found in the literature
Toxicity equivalence	Toxicity Equivalency, Toxicity Equivalent, Toxic Equivalency, Toxic Equivalent

This framework employs the following definitions:

Relative Potency (ReP) – Estimate of the potency, relative to 2,3,7,8-TCDD, of an individual chemical to cause a particular AHR-mediated toxic or biological effect in an individual organism, cellular, or biochemical assay. The relative potency estimate for a given chemical must be derived from a single *in vitro* or *in vivo* study, that is, a study in which the potencies of a PCDD, PCDF, or PCB congener and a reference chemical (2,3,7,8-TCDD or PCB 126) to cause a particular effect are measured in a single experiment or by the same authors using the same study design in both experiments. Such an ReP may be suitable for use in risk assessment, becoming an RPF. Furthermore, some TEFs are currently based on RePs.

Relative Potency Factor (RPF) – Estimate based on one or more studies of the potency, relative to 2,3,7,8-TCDD, of an individual chemical to cause AHR-mediated toxicity or biological effects, determined using careful scientific judgment after considering all available relative potency data. The ReP database used to derive an RPF for a chemical may include multiple endpoints, species, and/or *in vitro* or *in vivo* studies. RPFs may be used as alternatives to TEFs when more specific data for the species, endpoint, and/or site conditions are judged to improve the accuracy of the risk assessment. If the RPF is based on a single ReP, the RPF is equal to the ReP.

Toxicity Equivalence Factor (TEF) – Estimate of the potency, relative to 2,3,7,8-TCDD, of an individual polychlorinated dibenzo-*p*-dioxin, dibenzofuran, or biphenyl congener, determined using careful scientific judgment after considering all available relative potency data. EPA presently applies this term only to TEFs derived through an international scientific consensus-building process supported by the World Health Organization (Van den Berg *et al*., 1998, 2006).

Toxicity Equivalence Concentration (TEC) – The TEC is the product of the TEF or RPF multiplied by the concentration for an individual dioxin-like chemical. The total TEC for a mixture is calculated as the sum of 2,3,7,8-TCDD equivalence concentrations of all dioxin-like chemcials present in the mixture.

The WHO-ECEH consultation report (Van den Berg *et al*., 1998) clarified the terminology used in the toxicity equivalence methodology to distinguish between REPs and TEFs. The term *relative potency* was introduced to refer to estimates of the potencies of individual PCDDs, PCDFs, and PCBs congeners, relative to 2,3,7,8-TCDD, to cause a particular toxic or biological effect as determined in a single study. This framework adopts the WHO-ECEH terminology and definition, except that the acronym "ReP" is used rather than "REP" to be consistent with use of lower case letters when two or more letters in an acronym represent a single word. This framework also adopts the WHO-ECEH definition of TEFs as estimates of the relative potencies of individual dioxins, furans, and PCBs, relative to 2,3,7,8-TCDD, derived using careful scientific judgment after considering all available data. TEFs are used to convert concentrations of individual dioxin-like chemicals in tissues or diet to 2,3,7,8-TCDD toxicity equivalent concentrations.

Additionally, this framework extends the WHO-ECEH terminology by introducing the term *relative potency factor*, abbreviated RPF, as an intermediate between ReP and TEF. An RPF refers to an estimate based on one or more studies of the potency, relative to 2,3,7,8-TCDD, of an individual chemical to cause AHR-mediated toxicity or biological effects. Hence, the term RPF is directly analogous to TEF, but an RPF is derived in the context of a specific risk assessment rather than by international expert consensus. It is hoped that adoption of these more logically consistent and grammatically correct terms will ultimately aid in understanding and use of the methodology.

1.2. EVOLUTION OF THE TOXICITY EQUIVALENCE METHODOLOGY

In the 1970s and 1980s, human health risk assessments of complex mixtures of PCDDs and PCDFs were generally performed including only 2,3,7,8-TCDD or assuming that all dioxin-like chemicals were equally potent to 2,3,7,8-TCDD (U.S. EPA, 1987, 1989). A review of the scientific information currently available clearly demonstrates that both of these assumptions were inaccurate. While many PCDD and PCDF congeners act through a common mechanism of action (binding and activation of the AHR) and induce similar biochemical and toxicological effects, the relative potency of individual dioxin-like chemicals to induce such effects has been shown to vary.

The first use of a toxicity equivalence-like method for risk assessment purposes was described by Eadon *et al*. (1986) as a means to estimate potential human health risks associated with a PCB transformer fire in Binghamton, New York. In an examination of the initial human health risk assessment methodologies designed to address the emission of dioxins and furans from waste incinerators, EPA also concluded that TEFs were the best available interim scientific

policy for dealing with complex mixtures of these contaminants. Hence, in 1987, EPA adopted an interim procedure, based on TEFs, for estimating the hazard and dose-response of complex mixtures containing PCDDs and PCDFs in addition to 2,3,7,8-TCDD (U.S. EPA, 1987).

Following adoption of the toxicity equivalence methodology in the United States and Canada, the North Atlantic Treaty Organization/Committee on the Challenges of Modern Society (NATO/CCMS) examined the methodology and concluded that it was the best available interim method for PCDD/PCDF human health risk assessment (NATO, 1988a, b). The TEFs proposed for the different dioxin-like chemicals were refined by the NATO/CCMS based on inclusion of more recent data sets, resulting in a greater number of the TEFs being based on toxicity observed *in vivo*. The NATO/CCMS panel assigned TEFs to octachlorinated dibenzo-*p*-dioxin (OCDD) and octachlorinated dibenzofuran (OCDF), and removed TEFs for all congeners lacking chlorine in the 2,3,7,8-positions. Although it was indicated that, theoretically, it may be possible to detect nearly all of the 210 PCDD/PCDF isomers in the environment, only the seventeen 2,3,7,8-substituted PCDD and PCDF congeners were known to significantly bioaccumulate (Table 1). EPA officially adopted the revised TEFs in 1989 (TEFs-$NATO_{89}$), with the caveat that the methodology remain interim and continued revisions be made (U.S. EPA, 1989; Kutz *et al*., 1990). The use of the toxicity equivalence methodology for human health risk assessment and risk management purposes has since been formally adopted by a number of other countries (*e.g.,* Canada, Germany, Italy, the Netherlands, Sweden, and the United Kingdom) (Yrjänheiki, 1992).

Table 1. Number of polychlorinated dioxin, furan, and biphenyl congeners

Chemical Class	Number of Congeners	Dioxin-like Chemicals
Dioxins (PCDDs)	75	7
Furans (PCDFs)	135	10
Biphenyls (PCBs)	209	12

During the initial development of the toxicity equivalence methodology for PCDDs/PCDFs, a number of researchers were also examining the structure-activity relationships for PCBs (see reviews by Safe and co-workers, Leece *et al*., 1985; Safe 1990; 1994). These studies revealed that only PCB congeners substituted in the *meta* and *para* positions (Figure 2) were approximate stereo isomers of 2,3,7,8-TCDD and induced dioxin-like biochemical and toxicological effects. PCBs with a single chlorine substitution in an *ortho* position on the biphenyl (mono-*ortho*) have diminished dioxin-like activity. In some organism classes, (*e.g.,* fish), the reduction in dioxin-like activity is substantial.

In 1991, EPA convened a workshop to consider TEFs for PCBs (Barnes *et al*., 1991; U.S. EPA, 1991). From the workshop it was concluded that a small subset of the PCBs displayed dioxin-like activity and met the criteria for inclusion in the methodology. It was also noted that the PCBs not included in the toxicity equivalence methodology (*i.e.,* the non-dioxin-like PCBs) are not a single class of chemicals and have multiple toxicities with separate structure-activity relationships (Barnes *et al*., 1991).

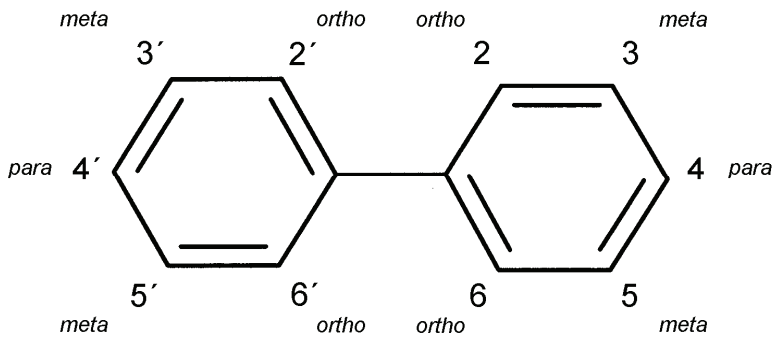


Figure 2. Structure of PCB molecule and positions for chlorine substitution.

In the years since initial adoption of the toxicity equivalence methodology, additional data have accumulated on the toxicological potency of individual PCDDs, PCDFs, and PCBs relative to 2,3,7,8-TCDD. A joint project to harmonize toxicity equivalence methodologies for dioxin-like chemicals, conducted by the WHO-ECEH and the International Programme on Chemical Safety (IPCS), resulted in the development of a database consisting of all relevant toxicological data for dioxin-like chemicals available through 1993. Following a review of almost 1,200 peer-reviewed publications, 146 were selected and analyzed to derive TEFs for PCBs (TEFs-WHO_{94}). Based on the reported results for 14 different biological and toxicological parameters from a total of 60 articles, a panel of experts from eight countries recommended interim TEFs for 13 dioxin-like PCBs (Ahlborg *et al*., 1994). Application of this methodology in human health risk assessment was reaffirmed in EPA's Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (U.S. EPA, 2000a).

At a second WHO-ECEH consultation in 1997, the TEFs for PCDDs, PCDFs, and PCBs were reviewed and the toxicity equivalence methodology expanded, based on availability of additional data, to include class-specific TEFs for mammals, birds, and fish. TEFs for seven PCDD, 10 PCDF and 12 PCB congeners for mammals, birds, and fish (TEFs-WHO_{98}; Table 2) were included in the resulting report (Van den Berg *et al*., 1998). At the WHO-ECEH consultation, the TEFs previously assigned to PCB 170 and PCB 180 were withdrawn and a TEF for PCB 81 was established, such that the number of PCB congeners with TEFs assigned was reduced from 13 to 12 (Van den Berg *et al*., 1998). It should be noted that the species and endpoints examined for assignment of TEFs varied among individual dioxin-like chemicals. Van den Berg *et al*. (1998) also provide greater documentation on how the expert panel at the WHO-ECEH consultation selected studies for consideration, derived relative potency factors from individual studies, and developed TEFs from the existing database. Although a number of uncertainties associated with the toxicity equivalence methodology have been identified (Van den Berg *et al*., 1998), it was the conclusion of the WHO-ECEH consultation that an additive toxicity equivalence methodology remained the most appropriate risk assessment method for assessing complex mixtures of dioxin-like PCDDs, PCDFs, and PCBs.

In 1998, EPA and DOI sponsored a meeting entitled: *"Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and Wildlife."* The major objective of the workshop was to address uncertainties associated with the use of the toxicity equivalence methodology in ecological risk assessment. Thirty-one experts from academia, government, industry, and environmental groups participated in the workshop. General conclusions regarding application of the toxicity equivalence methodology in ecological risk assessment included:

- The toxicity equivalence methodology is technically appropriate for evaluating risks to fish, birds, and mammals associated with AHR agonists and it can support risk analyses beyond screening-level assessments.

- The methodology entails less uncertainty and is less likely to underestimate risks than are methods based on single chemicals. Specifically, because the methodology takes into account the possible effects of the suite of dioxin-like chemicals found in complex environmental mixtures, it is less likely to underestimate risk than methods based on only one of these chemicals (*i.e.,* 2,3,7,8-TCDD). Further, because total PCBs in the environment can be comprised of many chemicals that vary in concentration and relative potency as AHR agonists, the toxicity equivalence methodology provides a means for accounting for their variable potency.

- The uncertainties associated with using the methodology are not thought to be larger than other sources of uncertainty within the ecological risk assessment process (*e.g.,* dose-response assessment, exposure assessment, and risk characterization).

For a thorough understanding of the technical issues discussed and conclusions drawn from the EPA/DOI workshop, refer to U.S. EPA (2001a).

In 2005, the WHO International Programme on Chemical Safety held another expert meeting during which the 1998 mammalian TEFs-WHO_{98} were re-evaluated. Preceding this meeting, a one-day public hearing was convened at which members of the expert panel discussed various aspects of the TEF concept with stakeholders and interested parties. The 2005 WHO re-evaluation relied extensively on the mammalian TEF database recently published by Haws *et al*. (2006); however, the expert panel used all available RePs, including those from studies published since 1997, in making their assessments. Changes made to the mammalian TEFs-WHO_{98} are reflected in Table 2 and designated as TEFs-WHO_{05}. This expert panel also concluded that additivity, an important pre-requisite of the TEF concept, was further confirmed by recent *in vivo* mixture studies by Walker *et al*. (2005) and Van den Berg *et al*. (2006).

In 2006, the National Research Council of the National Academies (NRC) re-affirmed the scientific basis and credibility of the use of the toxicity equivalence methodology in risk assessment. As part of their evaluation of the draft *Exposure and Health Reassessment of 2,3,7,8-TCDD and Related Compounds* (U.S. EPA 2003a), the NRC reviewed EPA's use of the Toxicity Equivalence Methodology in assessing risks from dioxin-like compounds. The NRC concluded that, "...the toxic equivalency factor methodology provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative potency of DLCs" (DLC = dioxin-like compounds; NRC, 2006). In addition, the NRC Committee examined a number general of issues that have been raised regarding the assumptions underlying the use of the toxicity equivalence methodology. One important conclusion of the Committee that is particularly relevant to this document is that addressing the additivity assumption. The Committee concluded that "from an overall perspective, this assumption appears valid, at least in the context of risk assessment" (NRC, 2006).

Table 2. World Health Organization TEFs for mammals, birds, and fish

Congener	TEF		
	Mammals[1]	Birds[2]	Fish[2]
Dioxins			
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	0.05	0.5
1,2,3,6,7,8-HxCDD	0.1	0.01	0.01
1,2,3,7,8,9-HxCDD	0.1	0.1	0.01
1,2,3,4,6,7,8-HpCDD	0.01	<0.001	0.001
OCDD	0.0003	0.0001	<0.0001
Furans			
2,3,7,8-TCDF	0.1	1	0.05
1,2,3,7,8-PeCDF	0.03	0.1	0.05
2,3,4,7,8-PeCDF	0.3	1	0.5
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	0.0003	0.0001	<0.0001
Non-*ortho* PCBs			
3,3',4,4'-TCB (77)	0.0001	0.05	0.0001
3,4,4',5-TCB (81)	0.0003	0.1	0.0005
3,3',4,4',5-PeCB (126)	0.1	0.1	0.005
3,3',4,4',5,5'-HxCB (169)	0.03	0.001	0.00005
Mono-*ortho* PCBs			
2,3,3',4,4'-PeCB (105)	0.00003	0.0001	<0.000005
2,3,4,4',5-PeCB (114)	0.00003	0.0001	<0.000005
2,3',4,4',5-PeCB (118)	0.00003	0.00001	<0.000005
2',3,4,4',5-PeCB (123)	0.00003	0.00001	<0.000005
2,3,3',4,4',5-HxCB (156)	0.00003	0.0001	<0.000005
2,3,3',4,4',5'-HxCB (157)	0.00003	0.0001	<0.000005
2,3',4,4',5,5'-HxCB (167)	0.00003	0.00001	<0.000005
2,3,3',4,4',5,5'-HeCB (189)	0.00003	0.00001	<0.000005

Source: [1]Van den Berg *et al*., 2006; [2]Van den Berg *et al*., 1998.

2. TOXICITY EQUIVALENCE METHODOLOGY

The toxicity equivalence methodology is a tool for assessing the cumulative toxicity of a complex mixture of dioxin-like PCDDs, PCDFs, and PCBs. To apply the methodology to such a mixture, the following activities need to be performed for each chemical present in the mixture:

- Verify that the chemical is known to act through the AHR-mediated mechanism of action.
- Review potency estimates of the chemical relative to 2,3,7,8-TCDD based on *in vivo* or *in vitro* studies.
- Select or derive an appropriate relative potency estimate (ReP, RPF, TEF) for the chemical.
- Measure or predict the concentration of the chemical in the appropriate tissues or diet of each species being assessed.
- Apply the relative potency estimate for the chemical to calculate its TEC.

Extensive research efforts and numerous expert workshops have resulted in both verification that certain PCDDs, PCDFs, and PCBs act by the AHR-mediated mechanism of action and derivation of relative potency estimates for these dioxin-like chemicals. These efforts are summarized and references are provided in Sections 1.2 and 2.1 of this document. The selection or derivation of the appropriate relative potency estimates and the calculation of a TEC are required for each ecological risk assessment that uses the toxicity equivalence methodology. These activities are summarized in Sections 2.2 and 2.3 and discussed further in Chapter 3.

2.1. AHR-MEDIATED MECHANISM AND ASSIGNMENT OF RELATIVE POTENCY

Inherent in the toxicity equivalence methodology are the assumptions that the effect of individual AHR agonists act via the same AHR-mediated mechanism and that their combined effects are additive. The general basis for the methodology is the observation that the AHR mediates most if not all biological and toxic effects induced by AHR agonists (Safe, 1990; Okey *et al*., 1994; Birnbaum, 1994; Hankinson, 1995; NRC, 2006). Recent advances in molecular biology have provided techniques to verify that a functional AHR is required for dioxin-like toxicity to be elicited. In organisms and cells that have been engineered such that the expression of a functional AHR is reduced or eliminated, toxicity following exposure to 2,3,7,8-TCDD and other AHR agonists has been reduced or eliminated. Hence, dioxin-like chemicals exert their activity by binding with the AHR (Sewall and Lucier, 1995; DeVito and Birnbaum, 1995). However, just because a congener can bind to the AHR, does not necessarily mean that it is able to "activate" all of the processes which underlie the development of toxic effects in an organism. Hence, none of the current WHO-TEFs are based on AHR binding alone.

The scientific defensibility of the second assumption – that the combined effects of AHR agonists are additive – has been raised since the onset of the use of TEFs. Arguments challenging this assumption include the presence of competing agonists or antagonists in various complex mixtures from environmental sources, interactions based on non-dioxin-like activities (antagonism or synergism), and the fact that dose-response curves for various effects may not be

parallel for all AHR agonists assigned TEFs. Despite these concerns, empirical data support the use of the additivity concept.

A substantial effort has been made to test the assumptions of additivity and the ability of the toxicity equivalence methodology to predict the effects of mixtures of AHR agonists. These efforts have included environmental, commercial, and laboratory derived mixtures. Studies in fish and wildlife species of mixtures of PCDDs, PCDFs, and PCBs support the additivity assumption (Zabel *et al*., 1995; Walker *et al*., 1996; Tillitt and Wright, 1997). Further, numerous studies that have examined the effects of environmental mixtures in marine mammals and avian species show a correlation between toxic effects and dietary concentrations (Ross *et al*., 1996; Summer *et al*., 1996a, b; Giesy and Kannan, 1998; Restum *et al*., 1998; Shipp *et al*., 1998a, b; Ross, 2000). More recently, the 2005 WHO-IPCS expert panel (Van den Berg *et al*. 2006) re-visited the additivity assumption issue and found that additivity, an important pre-requisite of the TEF concept, was further confirmed by recent *in vivo* mixture studies by Walker *et al*. (2005). Likewise, the NRC review of EPA's *Exposure and Health Reassessment of 2,3,7,8-TCDD and Related Compounds* included an evaluation of the additivity assumption. The NRC Committee concluded that "from an overall perspective, this assumption appears valid, at least in the context of risk assessment" (NRC, 2006).

Several criteria have been developed that are deemed requisite for including a chemical in the toxicity equivalence methodology. These criteria were first employed in assigning TEFs for PCBs (Ahlborg, 1994) and were affirmed in the process of assigning taxonomic class-specific TEFs (Van den Berg *et al*., 1998; 2006). The criteria are:

- Structural similarity to 2,3,7,8-TCDD;
- Demonstrated binding to the AHR;
- Demonstrated ability to elicit an AHR-mediated toxic or biochemical effect; and
- Persistence and bioaccumulation in the food chain.

Using these inclusion criteria, the expert panel at the WHO-ECEH consultation developed a TEF scheme (TEFs-WHO_{98}) that includes 7 PCDD, 10 PCDF, and 12 PCB congeners (Table 2).

In June 2005, a WHO-IPCS expert meeting was convened at which the mammalian TEFs-WHO_{98} were re-evaluated. For the re-evaluation, the refined mammalian TEF database published by Haws *et al*. (2006) was used as a starting point. The expert panel used all available RePs, whether or not they were included in this database, and made decisions based on a combination of ReP distributions from the database, expert judgment, and point estimates (Van den Berg *et al*., 2006). Changes in TEF values were determined by the expert panel for one dioxin (OCDD), three furans (2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF, and OCDF), two non-*ortho* PCBs, and all relevant mono-*ortho*-substituted PCBs (Van den Berg *et al*., 2006). These recent changes in the mammalian TEFs (TEFs-$WHO_{98/05}$) are represented in Table 2 and in the relevant examples provided in this document.

For PCBs, the toxicity equivalence methodology applies only to dioxin-like PCBs. Other PCBs, sometimes referred to as "non-dioxin-like PCBs," are not a single class of chemicals and may have an additional spectrum of toxicological properties that are not accounted for in the toxicity equivalence methodology. Although current evidence indicates that the greatest potential for effects on ecological endpoints of most concern (*e.g.,* growth, survival, reproduction) from exposure to PCB mixtures is from the AHR agonists (Giesy and Kannan, 1998; Rice *et al*.,

2002), risk estimates based solely on the 12 dioxin-like PCBs may underestimate the total PCB risk. Hence, because PCB mixtures contain both dioxin-like and non-dioxin-like congeners, assessing ecological risks posed by both types of congeners may be warranted.

A dual analysis of risks based on total PCBs and on toxicity equivalence for dioxin-like PCBs is an approach that may be taken to assess PCB mixtures (Beltman *et al*., 1997; Brunstrom and Halldin, 2000; Finley *et al*., 1997; Giesy and Kannan, 1998; U.S. EPA 2005; note, however, that only Giesy and Kannan incorporated the 1998 taxa-specific TEFs-WHO_{98} in their analysis). EPA currently recommends this combined approach for assessing PCB cancer risks to humans (U.S. EPA, 1996). As more information becomes available about the toxicity mechanisms and relative potency of specific non-dioxin-like PCB congeners, alternative methods for assessing their risk will likely emerge.

In addition to the PCDDs, PCDFs, and PCBs that are the subject of this framework, a wide variety of structurally diverse anthropogenic chemicals are capable of interacting with the AHR (Denison and Nagy, 2003). These chemicals also have a broad range of potencies at inducing dioxin-like effects in experimental systems. Other chemicals that bind and activate the AHR include industrial chemicals (*e.g.,* polyhalogenated biphenyls, halogenated naphthalenes, chlorinated paraffins), pesticides (*e.g.,* hexachlorobenzene), combustion products (*e.g.,* unsubstituted polycyclic aromatic hydrocarbons (PAHs)), and flame retardants and their byproducts (*e.g.,* brominated dioxins, dibenzofurans, biphenyls, diphenyl ethers, and naphthalenes). The expert panel at the 1997 WHO consultation concluded that "at present, insufficient environmental and toxicological data are available to establish a TEF value" for these other chemicals (Van den Berg *et al*., 1998), and the 2005 WHO-ECEH meeting came to similar conclusions (Van den Berg *et al*., 2006). Likewise, the NRC Committee concluded that currently, insufficient toxicological and environmental distribution studies and lack of consensus TEFs may hinder consideration of these chemicals in risk assessments but that EPA should include these chemicals in TEC calculations when TEFs are developed (NRC, 2006).

Conceptually, a methodology based on toxicity equivalence can be applied to other chemicals that share a common mechanism of toxicity and to which aggregate exposure may occur. For example, EPA has recently issued guidance based on the toxicity equivalence concept for assessing cumulative health risks of pesticides that have a common mechanism of action (U.S. EPA, 2002). In ecological risk assessment, application of toxicity equivalence to chemicals other than those that interact with the AHR has been more limited. The government of Canada has recently used a toxicity equivalence approach in assessing certain nonylphenol ethoxylates (Environment Canada and Health Canada, 2001). Toxicity equivalence and common mechanism of action also provide the foundation for recent efforts to develop water quality values for mixtures of type I narcotic chemicals in general and PAHs in particular (DiToro *et al*., 2000; DiToro and McGrath, 2000). Many of the principles described in this framework may be applicable to other chemical mixtures, but risk assessors should take care in deciding whether a toxicity equivalence approach is appropriate for their mixture of concern (U.S. EPA, 2000a).

2.2. SELECTION OF THE APPROPRIATE RELATIVE POTENCY FACTORS

One of the most important considerations to be made when applying the toxicity equivalence methodology to ecological risk assessment is what relative potency value to use for each dioxin-like chemical. One approach is to use the TEFs-$WHO_{98/05}$. Alternatively, ReP data from a single study or from multiple relevant studies may be selected as the basis for deriving an RPF to be used in lieu of a TEF. A clear understanding of the difference between RePs, RPFs,

and TEFs is critical for making this decision and is thus described here. The issues to consider when selecting an estimate are described in Section 3.3.2 of this framework.

The relative potency of a dioxin-like chemical may be determined from a variety of effect concentrations; for example, effective concentration (EC_x), effective dose (ED_x), lethal dose (LD_x), no-observed adverse Effect Level (NOAEL), lowest observed adverse effect level (LOAEL), benchmark dose, or entire dose-response curves have all been used. To date, RePs have most commonly been determined as the EC_{50}, ED_{50}, or LD_{50} of 2,3,7,8-TCDD divided by the EC_{50}, ED_{50}, or LD_{50} of the individual dioxin-like chemical. RePs have been derived from *in vitro* and *in vivo* studies and include endpoints ranging from biochemical changes (*e.g.,* cytochrome P4501A induction) to mortality. An RPF may be derived from a database of ReP values that includes multiple endpoints, species, and *in vitro* or *in vivo* studies. Both RePs and RPFs may be derived and used as alternatives to TEFs when more specific data for the species, endpoint, and/or site conditions are judged to improve the accuracy of a risk assessment. An ReP or RPF may also be derived and used for dioxin-like chemicals not currently assigned a WHO-TEF, but for which data are judged sufficient to include in an assessment of AHR-mediated risks. Risk assessors can learn more about other halogenated chemicals that meet the criteria for inclusion in the TEF methodology in Van den Berg *et al*. (1998; 2006) and the references therein.

Values of the TEFs-$WHO_{98/05}$ reproduced in Table 2 were determined based on the consensus judgment of the experts present at the WHO consultations (Van den Berg *et al*., 1998; 2006). The TEFs-$WHO_{98/05}$ were derived from considering all available RePs and then rounded up or down to the nearest half-order of magnitude for fish and bird TEFS (Van den Berg *et al.*, 1998) and one order of magnitude for mammal TEFs (Van den Berg *et al*., 2006). A summary, through 1996, of available RePs can be found in the Karolinska Institute database. A link to this database is available at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=55669 (Haws *et al*., 2006). Additional data from which to determine RePs and/or derive RPFs have been reported in the literature since 1996, and it is expected that more will be available in the future.

2.3. TOXICITY EQUIVALENCE CONCENTRATION

The 2,3,7,8-TCDD TEC is the primary expression of exposure to an organism in an ecological risk assessment involving complex mixtures of PCDDs, PCDFs, PCBs, and/or any other AHR agonists which may contribute to the toxicity. While the TEC is best based on dioxin-like chemical concentrations in tissues of organisms at risk, in ecological risk assessments it has often been based on concentrations in the diet.

$$TEC = \sum_{n=1}^{k} C_n * TEF_n \qquad (2\text{-}1)$$

Where: C_n = concentration of dioxin-like chemical *n* in an organism or its diet
TEF_n = toxicity equivalence factor for dioxin-like chemical *n*
(*Note:* An RPF can replace the TEF term)
k = number of toxic dioxin-like chemicals in mixture

When TECs in organisms of concern or their diet are unknown, they may be calculated from dioxin-like chemical concentrations in water, sediment, or soil <u>only</u> if bioaccumulation

(*e.g.,* BAFs, BSAFs, or bioaccumulation modeling) is appropriately incorporated to relate the concentrations of each dioxin-like chemical in the media to concentrations in the organism or its diet (see Sections 3.3.1.3 and 3.3.1.4 for further discussion).

3. APPLICATION OF THE TOXICITY EQUIVALENCE METHODOLOGY IN ECOLOGICAL RISK ASSESSMENT

In this framework, application of the toxicity equivalence methodology is presented in the context of each phase of the ecological risk assessment paradigm: planning, problem formulation, analysis, and risk characterization (See Figure 3). Note that this framework focuses on providing specific information necessary for applying the toxicity equivalence methodology within an ecological risk assessment involving PCDDs, PCDFs, and PCBs, but does not discuss the many other aspects necessary for conducting such a risk assessment. Risk assessors may refer to the *Guidelines for Ecological Risk Assessment* for additional information on components of ecological risk assessment (U.S. EPA, 1998). Issues beyond the toxicity equivalence methodology that are pertinent to problem formulation, analysis (*i.e.,* characterization of exposure and effects), and risk characterization for dioxin-like chemicals have been described in depth previously (U.S. EPA, 1993; 1995b, c; 2001b; 2003a, 2005). Risk assessors are referred to these publications to address broader issues associated with conducting a risk assessment involving PCDDs, PCDFs, and PCBs.

3.1. CONSIDERATIONS IN PLANNING

Under the *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998), the problem formulation phase of a risk assessment is preceded by a dialogue among risk managers, risk assessors, and other interested parties. During this planning phase, risk managers and risk assessors develop management goals and determine the size and scope of the ecological risk assessment that is needed to support the risk management decision.

Planning involves a determination of the likely chemicals of concern and the method(s) for estimating risks from exposure to these chemicals. Multiple factors (cost, time, data adequacy, scientific uncertainty, political or social conditions) may be considered in selecting methods and measurements. The cost of analytical methods or measurements will vary depending on the complexity of the matrix and the data quality objectives for each project. Each method has its source of uncertainty due to lack of knowledge and variability. The risk assessor needs to define these for each method to provide the proper foundation for selection of the method appropriate for the particular decision. The risk assessor and others interested in evaluating the risks should review all methods carefully to ascertain the most appropriate method for their specific situation. Text Box 2 provides questions that should be considered during planning.

Text Box 2. Questions for planning

- Are there chemicals of concern other than those with dioxin-like activity?
- Is evaluation of "dioxin-like" toxicity risks necessary to meet risk management objectives?
- Is the accuracy provided by a congener-specific chemical analysis and toxicity equivalence methodology necessary for making risk management decisions at the site?
- Will TEFs-$WHO_{98/05}$ or more specific RPFs be needed to make risk management decisions?
- Will multiple lines of evidence (bioanalytical results, field studies) be used to inform the risk management decision?

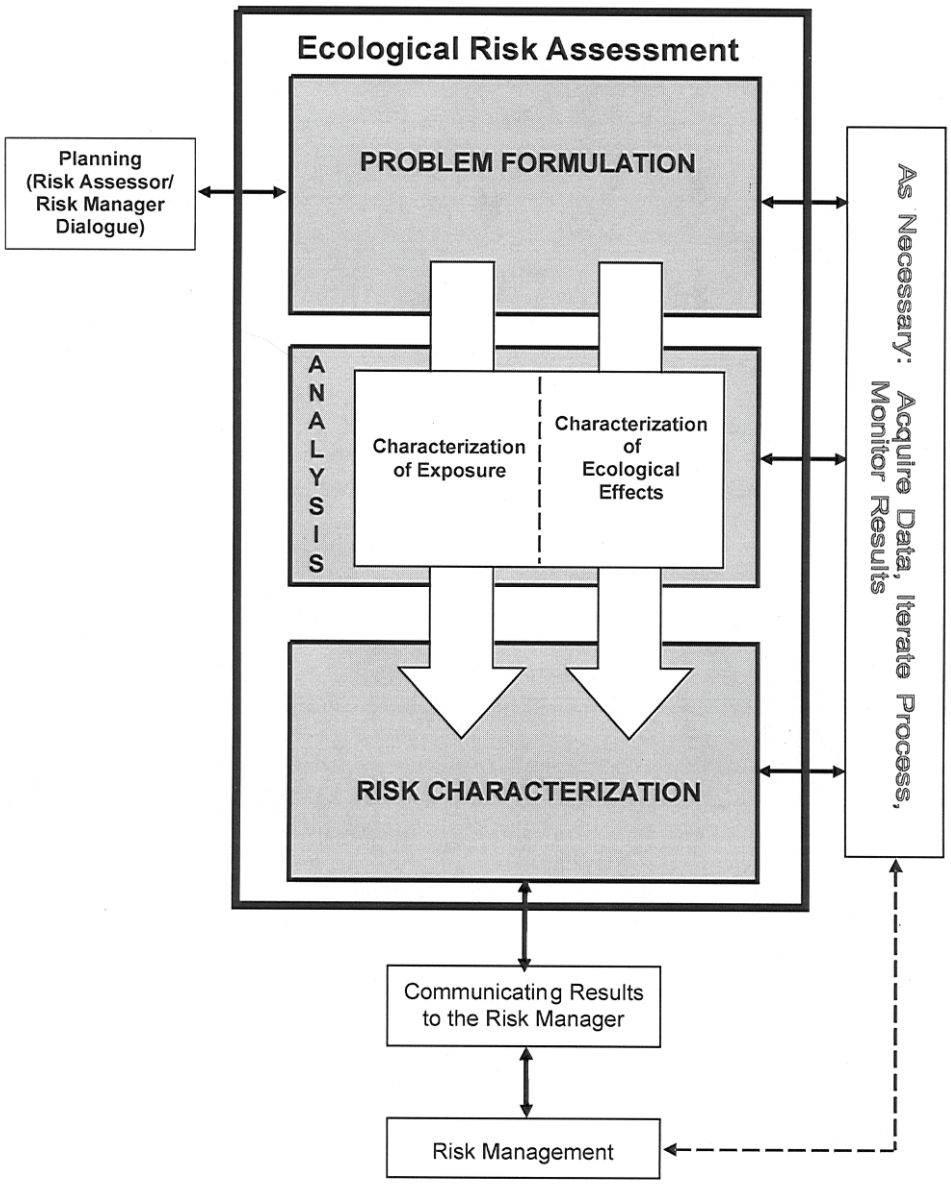


Figure 3. The framework for ecological risk assessment
Source: U.S. EPA, 1998

For example, it is possible that even though dioxin-like chemicals are present, risks posed by another chemical(s) may be expected to exceed risks posed by the dioxin-like chemicals (*e.g.,* trace amounts of low potency dioxin-like chemical are present vs. large amounts of another toxic chemical). If achieving the management goal for the primary chemical(s) of concern also results in the removal of risks posed by the dioxin-like chemical(s), it may be prudent to use methods that are less resource intensive than congener-specific chemical analysis and the toxicity equivalence methodology to estimate risks from and/or monitor concentrations of the dioxin-like chemicals.

Risk assessors should consider the following when selecting the most appropriate analytical measurement for estimating risks from dioxin-like chemicals:

1. Environmental PCB mixtures often cannot be adequately described by referencing Aroclor standards due to the subjective assignment of congeners (*i.e.,* Aroclors are identified on the basis of congener profiles present in the original formulation, but environmental weathering may significantly change these congener profiles such that subjective judgment is used to determine which Aroclor the environmental mixture resembles after weathering).

2. Homolog (level-of-chlorination) analysis can overestimate the total PCDD, PCDF, and PCB concentrations because the congeners measured in a specific homolog group analysis may overlap (*i.e.,* also be measured in another homolog group analysis).

3. Measurements of mixture concentrations (*e.g.,* Aroclors, homologs, and total PCBs) are not amenable to fate and transport or bioaccumulation modeling.

4. Large uncertainty may be introduced in assessing exposure and effects by assuming that congener profiles present in commercial mixtures used in toxicity tests (*e.g.,* Aroclors) are representative of PCB profiles in weathered environmental samples (either exposure media or biota).

5. The toxicity equivalence methodology <u>cannot</u> be directly applied to homolog groups, Aroclors, or total PCBs.

6. Uncertainty associated with application of TEFs to PCB congener concentrations estimated from Aroclor or homolog analyses is probably large due to differential weathering and fate and transport processes.

7. A dual analysis of risks based on total PCBs and on toxicity equivalence for dioxin-like PCBs is an approach that may be taken to assess dioxin and non-dioxin-like effects of PCB mixtures.

8. Regardless of the measurements or models used in the risk assessment, the chemical measurements should be reported in a manner that is transparent to the risk managers, including a characterization of the uncertainties associated with undetected chemicals.

9. In any risk assessment the dose metric (*i.e.,* the measurement or prediction of chemical concentrations) should be consistent between the exposure assessment and the effects assessment. For example, if the dose-response relationship used in the effects assessment is based on tissue concentrations, exposure estimates would also need to be based on concentrations in tissue of the species of concern.

3.1.1. Benefits of the Toxicity Equivalence Methodology

This framework is designed to address those risk assessments where PCDDs, PCDFs, and PCBs are present and the toxicity equivalence methodology is the appropriate method for estimating the AHR-mediated risks. In these cases, use of the toxicity equivalence methodology results in more accurate exposure and effects analysis for dioxin-like chemicals. Consequently,

risk managers may better formulate risk management strategies and evaluate risk management alternatives to mediate the effects of such chemical stressors.

The toxicity equivalence methodology is appropriate and applicable in ecological risk assessments involving both aquatic and terrestrial systems (U.S. EPA, 2001a). The toxicity equivalence methodology is well accepted in the scientific community, the international risk assessment community, and EPA for human health risk assessment (Ahlborg *et al*., 1994; Barnes *et al*., 1991; Eadon *et al*., 1986; U.S. EPA, 1987; 1989; 1991; 2001a; NATO, 1988a, b; NRC, 2006; Van den Berg *et al*., 1998; 2006; Yrjänheiki, 1992). Certain aspects related to application of the methodology (*e.g.,* bioaccumulation) have been better described and studied in aquatic systems, but the same principles apply to terrestrial systems.

In addition to being applicable to risk assessments of different levels of complexity, the toxicity equivalence methodology can be applied to assessments that evaluate the likelihood that effects were caused by past exposure to chemical stressors (retrospective assessments) and assessments that predict the likelihood of future adverse effects (prospective assessments). An example of the former is an aquatic system where adverse effects have been observed in fish and fish-eating birds and mammals, and the ecological risk assessor wishes to determine the degree to which existing sediment contamination from dioxin-like chemicals may be responsible. An example of the latter is the evaluation of the potential impacts of an industrial facility anticipated to discharge dioxin-like chemicals into an aquatic system. In both examples, when coupled with techniques to estimate dioxin-like PCDD, PCDF, and PCB fate, transport, and accumulation in living organisms, the toxicity equivalence methodology could be used to estimate the cumulative toxicity of dioxin-like chemicals to species of concern.

3.1.2. Methodological Considerations

As with any method, the ecological risk assessor should understand and verify that assumptions inherent in applying the toxicity equivalence methodology are valid for the specific situation to which the methodology is being applied (*e.g.,* the chemicals of concern are AHR agonists; organisms are sensitive to an AHR-mediated mechanism of toxicity; congener-specific exposure data are available). The toxicity equivalence methodology described in this framework only accounts for ecological effects associated with dioxin-like chemicals. Additional methods must be employed to account for other effects associated with dioxins, furans, and PCBs as well as other chemicals that may be present.

If the toxicity equivalence methodology is selected, the managers and risk assessors must decide whether to use the default TEFs-$WHO_{98/05}$ or more specific RPFs or RePs. Use of the TEFs-$WHO_{98/05}$ has several advantages, including: 1) minimal effort required on the part of the risk assessor in selecting and/or reviewing relative potency studies; and 2) consistency in approach used across sites. However, it may be decided to increase accuracy by selecting RePs and deriving RPFs that are more specific for species and/or endpoints of concern. The decision to use TEFs-$WHO_{98/05}$ or more specific RePs and RPFs will depend on the risk management goal, the resources available to complete the risk assessment, site specific conditions, and availability of ReP data for the species and/or endpoint of concern. The benefits of deriving assessment-specific RPFs are described in Section 3.3.2.

These are some, but not all, of the variables that should be considered when selecting the appropriate method for chemical analyses and estimating risks from exposure to dioxin-like chemicals.

There are several other methods (bioanalytical tools, field surveys) that may provide additional lines of evidence to support the risk estimate derived from using the toxicity

equivalence methodology. Bioanalytical tools have the advantage of measuring the integrated effects of complex mixtures of AHR agonists. Such bioanalytical tools have the potential of accounting for, in biological response, chemicals that act via the AHR which would not be identified by a chemical analysis that measures only PCDDs, PCDFs, and PCBs. TECs determined by bioanalytical means can typically be obtained more quickly and at a lower cost than TECs determined by chemical analysis. However, due to current technical limitations, lack of standard testing procedures, and lack of established quality criteria associated with existing bioanalytical tools, the experts at the EPA/DOI workshop concluded that such bioanalytical tools should not be used as an alternative to congener-specific analysis and the toxicity equivalence methodology (U.S. EPA 2001a). Rather, these bioanalytical analyses are complementary tools, particularly useful for defining the spatial extent of contamination, for prioritizing remedial actions, and for providing a relative measure of TEC between different environmental media (U.S. EPA, 2001a; Van den Berg *et al*., 1998). The uncertainties associated with bioanalytical methods are discussed in Section 3.4.2.

3.2. CONSIDERATIONS IN PROBLEM FORMULATION

Problem formulation, which follows planning, provides the foundation for the entire risk assessment (U.S. EPA, 1998). During problem formulation, preliminary hypotheses about why ecological effects have occurred, or may occur, as a consequence of exposure to dioxin-like PCDDs, PCDFs, and PCBs are generated and evaluated. Problem formulation also involves selecting assessment endpoints that are relevant to risk management decisions (Section 3.2.1), developing conceptual models that describe the key relationships between dioxin-like PCDDs, PCDFs, and PCBs and assessment endpoints (Section 3.2.2), and preparing an analysis plan (Section 3.2.3). Text Box 3 shows questions that should be considered during problem formulation.

Text Box 3. Questions for problem formulation.

- Are the chemicals of concern dioxin-like PCDDs, PCDFs, and PCBs?
- Assessment Endpoint - Has the initial evaluation of ecological setting identified species that are both exposed to and sensitive to “dioxin-like” toxicity?
- Conceptual Model - Does the conceptual model describe the relationship and linkages between sources, fate & transport, bioaccumulation of dioxin-like chemicals, and exposures to identified assessment endpoint entities?
- Are congener-specific exposure data available or obtainable?

3.2.1. Assessment Endpoints

Assessment endpoints are “explicit expressions of the environmental values that are to be protected, operationally defined as an ecological entity and its attributes” (U.S. EPA, 1998). Three principal criteria are used to select assessment endpoints: susceptibility to known or potential chemical stressors, ecological relevance, and relevance to management goals. Susceptibility involves two major factors: sensitivity (how readily an organism is affected by these chemicals) and exposure (the frequency, duration, and intensity of contact between an organism and these chemicals). This section considers the unique characteristics and effects of dioxin-like PCDDs, PCDFs, and PCBs in identifying the organisms and attributes that may be candidates for assessment endpoints under the first two criteria, susceptibility and ecological

relevance. The third criterion, relevance to management goals, is discussed only briefly, since it relates to the values placed on different assessment endpoints rather than particular characteristics of dioxin-like chemicals.

3.2.1.1. ***Susceptibility: Sensitivity***

Because of the fundamental role played by the AHR in toxicity caused by dioxin-like chemicals, presence of the AHR is an important indicator of an organism's potential susceptibility to toxicity from these chemicals. One or more forms of the AHR have been identified in numerous mammalian, avian, and fish species (for a review, see Hahn, 1998, 2002a). Accordingly, dioxin-like toxicity is clearly elicited by various PCDDs, PCDFs, and PCBs in a variety of mammals, birds, and fish (Peterson *et al*., 1993; Theobald *et al*., 2003; U.S. EPA, 1993; U.S. EPA, 2001b). Species- and class-specific differences in AHR number and function have been identified in fish and birds (Hahn *et al*., 1997; Karchner *et al*., 1999; Abnet *et al*., 1999; Hansson *et al*., 2003) illustrating that the issue regarding presence or absence of an AHR homolog is complex. For example, different fish species can have multiple AHR homologs; zebrafish and Atlantic killifish both have AHR1 and AHR2. However, while both homologs are functional in killifish (*i.e.,* bind 2,3,7,8-TCDD and cause effects), only AHR2 is active in zebrafish. It is not yet clear whether these differences in AHR diversity and function play a role in species differences in sensitivity to dioxin-like toxicity.

Homologs of the AHR have also been identified in other classes of organisms, including one reptile and one amphibian species (Hahn, 2002a). It has been demonstrated that several marine species have cytosolic proteins that bind a dioxin analog (Brown *et al*., 1997). Further analysis reveals that the amino acid sequence of these proteins is closely related to vertebrate AHRs. However, these binding proteins do not bind the prototypical vertebrate AHR ligands, 2,3,7,8-TCDD and β-naphthoflavone, which distinguishes them from vertebrate AHRs (Butler *et al*., 2001). It is not yet known whether any of these invertebrate proteins have any role in producing toxic responses. Effects data, described below, are extremely limited for amphibians, reptiles, and invertebrates and resulted from exposure to relatively high chemical concentrations. A summary of effects that have been observed in various animal species is presented in Table 3.

Reproductive and developmental effects are generally among the most sensitive toxicity endpoints elicited by dioxin-like PCDDs, PCDFs, and PCBs in mammals, birds, and fish. Developmental effects are manifested in embryonic or early life stages and hence these life stages are generally more sensitive than juvenile or adult stages in susceptible mammals, birds, and fish. In addition, reproductive and developmental effects are often considered among the most relevant toxicity endpoints in ecological risk assessment as these toxicity endpoints may lead to adverse impacts on wildlife populations (U.S. EPA, 1993, U.S. EPA, 1995a).

The relative sensitivity to dioxin-like toxicity among species that possess the AHR varies greatly, even within taxonomic class. Inter-species differences in sensitivity exist even when considering only developmental toxicity or mortality endpoints. A variety of mammals, including laboratory rodents, monkeys, and mink, have been shown to be sensitive to 2,3,7,8-TCDD-induced reproductive and developmental toxicity and prenatal or early life stage mortality, although it is often difficult to quantify the cross-species range in sensitivity in mammals due to differences in exposure regimens (Peterson, *et al*., 1993). When administered doses are converted to body burden concentrations to facilitate cross-species and cross-endpoint comparisons among mammals, the lowest doses resulting in significant effects on a variety of non-cancer endpoints are quite similar among rodents and monkeys, with only an approximately 10-fold range in LOAELs (DeVito, *et al*., 1995; WHO, 1998; van Leeuwen, 2000). The

Table 3. Effects of 2,3,7,8-TCDD and related chemicals in different animal species

Effect	Fish	Birds		Mammals		
		Avian Wildlife	Chicken	Aquatic Mammals	Mink	Laboratory Mammals*
Presence of AHR	+ [1,2]	+ [1,2,27]	+ [1,2]	+ [1,2]		+ [1,2]
Binding of 2,3,7,8- TCDD:AHR Complex to the DRE (enhancer)	+ [3-6]	+ [28]	+ [3,43,44]	+ [60]		+ [3,71]
Enzyme induction	+ [7-11]	+ [29-33]	+ [29-31,44-49]	+ [61]	+ [63]	+ [72-80]
Acute lethality	[12,13]	+ [34]	+ [50]		+ [64]	+ [81-89]
Wasting syndrome	+ [14]	+ [34]	+ [50]		+ [64-66]	+ [90,91]
Hepatotoxicity (pathology, hyperplasia, hypertrophy, porphyria)	+ [13,15-17]	+/- [35-37]	+ [50-53]			+ [92-101]
Endocrine effects	+ [18,19]	+/- [38-40]				+ [102-103]
Immunotoxicity	+ [20]			0 [62]		+ [104-107]
Carcinogenicity	+ [21]					+ [92, 108,109]
Developmental and Reproductive Toxicity (mortality, teratogenesis, embryo-fetal toxicity, including neurological, immunological and/or endocrine effects during perinatal period)	+ [15,16,22-26]	+ [22, 31,32,41, 42]	+ [22, 32, 50, 54-59]		+ [66-70]	+ [22, 110-117]

+ = observed; +/– = observed to limited extent, or +/– results; 0 = not observed; blank cell = no data.

* Selected references representative of some effects in various laboratory mammals are provided in the table. Health effects of 2,3,7,8-TCDD and related chemicals are more comprehensively reviewed in U.S. EPA (2003a).

[1] Hahn, 1998; **[2]** Hahn, 2002a; **[3]** Bank et al., 1992; **[4]** Abnet, et al., 1999; **[5]** Karchner, et al., 1999; **[6]** Tanguay, et al., 1999; **[7]** Janz and Metcalfe, 1991; **[8]** Parrott et al., 1995; **[9]** Clemons et al., 1994; **[10]** Clemons et al., 1996; **[11]** Andreasen et al., 2002; **[12]** Kleeman, et al., 1988; **[13]** Spitsbergen et al., 1988; **[14]** Carvalho et al., 2004; **[15]** Spitsbergen, et al., 1991; **[16]** Henry, et al., 1997; **[17]** Hahn and Chandran, 1996; **[18]** Adams, et al., 2000; **[19]** Palace, et al., 2001; **[20]** Duffy, et al., 2002; **[21]** Johnson, et al., 1992; **[22]** Peterson, et al., 1993; **[23]** Walker, et al., 1991 **[24]** Walker and Peterson, 1991; **[25]** Elonen, et al., 1998; **[26]** Hill, et al., 2003; **[27]** Yasui, et al., 2004; **[28]** Yasui, et al., 2007; **[29]** Sanderson and Bellward, 1995; **[30]** Kennedy et al., 1996; **[31]** Brunstrom and Halldin, 1998; **[32]** Hoffman, et al., 1998; **[33]** Kennedy et al., 2003; **[34]** Nosek et al., 1992; **[35]** Elliott, et al.,

1990; **[36]** Elliott, et al., 1991; **[37]** Elliott, et al., 1997; **[38]** Janz, and Bellward, 1997; **[39]** Janz and Bellward, 1996a; **[40]** Janz and Bellward, 1996b; **[41]** Nosek, et al., 1993; **[42]** Powell, et al., 1997; **[43]** Denison, et al., 1988; **[44]** Walker et al., 2000; **[45]** Hamilton, et al., 1983; **[46]** Brunstrom and Andersson, 1988; **[47]** Brunstrom, 1991; **[48]** Bentivegna, et al., 1998; **[49]** Gilday, et al., 1998; **[50]** El-Sabeawy, et al., 2001; **[51]** Sano, et al., 1985; **[52]** Sinclair, et al., 1986; **[53]** Lambrecht, et al., 1988; **[54]** Powell, et al., 1996a; **[55]** Powell, et al., 1996b; **[56]** Blankenship, et al., 2003; **[57]** Bruggeman, et al., 2003; **[58]** Goff, et al., 2005; **[59]** Henshel, et al., 1998; **[60]** Jensen and Hahn, 2001; **[61]** Garrick, et al., 2006; **[62]** DeGuise, et al., 1998; **[63]** Gillette et al., 1987; **[64]** Hochstein, et al., 1988; **[65]** Hochstein, et al., 1998; **[66]** Hochstein, et al., 2001; **[67]** Aulerich, et al., 1988; **[68]** Render, et al., 2000; **[69]** Render, et al., 2001; **[70]** Beckett, et al., 2008; **[71]** Zhou, et al., 2003; **[72]** Kitchin, et al., 1979; **[73]** Nebert, 1989; **[74]** Poland, et al., 1982; **[75]** Hook, et al., 1975; **[76]** Liem, et al., 1980; **[77]** Beatty and Neal, 1977; **[78]** Håkansson, et al., 1994; **[79]** Gasiewicz, et al., 1986; **[80]** Kruger, et al., 1990; **[81]** Beatty, et al., 1978; **[82]** Neal, et al., 1982; **[83]** Chapman and Schiller, 1985; **[84]** Schwetz, et al., 1973; **[85]** McConnell, et al., 1978b; **[86]** DeCaprio, et al., 1986; **[87]** Henck, et al., 1981; **[88]** Olson, et al., 1980; **[89]** McConnell, et al., 1978a; **[90]** Peterson et al., 1994; **[91]** Kelling, et al., 1985; **[92]** Kociba, et al., 1978; **[93]** Cantoni, et al., 1981; **[94]** Goldstein, et al., 1982; **[95]** van Birgelen, et al., 1996b; **[96]** Jones and Sweeney, 1980; **[97]** van Birgelen, et al., 1996a; **[98]** Shen, et al., 1991; **[99]** Birnbaum, et al., 1990; **[100]** Seefeld, et al., 1980; **[101]** Bombick, et al., 1985; **[102]** van Birgelen, et al., 1995a; **[103]** van Birgelen, et al., 1995b; **[104]** Smialowicz, et al., 1994; **[105]** Smialowicz, et al., 1996; **[106]** Hong, et al., 1989; **[107]** Thomas and Hinsdill, 1978; **[108]** National Toxicology Program, 1982; **[109]** Rao, et al., 1988; **[110]** Roman and Peterson, 1998; **[111]** Couture, et al., 1989; **[112]** Abbott, et al., 1987b; **[113]** Abbott et al., 1987a; **[114]** Eriksson, et al., 1998; **[115]** Giavini, et al., 1982; **[116]** Wolf, et al., 1999; **[117]** Arnold, et al., 1997.

reduction in variability realized by conversion of administered dose to body burden concentrations demonstrates how analyses based on internal dose or concentration can reduce variability among toxicity data. Accordingly, development and application of risk assessment approaches based on internal dose or concentration (body burdens) would also reduce uncertainties associated with extrapolating data, such as relative potency estimates (van den Berg *et al*., 2005).

Although data for 2,3,7,8-TCDD-induced reproductive and developmental toxicity are lacking for mammalian wildlife species, mink are considered to be among the most sensitive mammals to dioxin-like toxicity based on studies with adult animals, PCBs, and endpoints other than reproduction/development (Hochstein *et al*., 1998; Aulerich *et al*., 1988; U.S. EPA, 2001b). The sensitivity of tested bird species to 2,3,7,8-TCDD-induced embryo mortality varies by about 200-fold, with the domestic chicken generally more sensitive than wildlife species (Hoffman *et al*., 1996). Of purely aquatic species, fish are more sensitive than other aquatic species. Among tested freshwater fish species, sensitivity to 2,3,7,8-TCDD-induced early life stage toxicity ranges approximately 50-fold, with salmonids being the most sensitive and zebrafish the least sensitive species (Walker and Peterson, 1994; Henry *et al*., 1997; Elonen *et al*., 1998; Tanguay *et al*., 2003).

The relative sensitivity of animal classes is not constant across chemical classes. For example, fish are generally quite sensitive to PCDD and PCDF toxicity, as are birds and mammals. However fish are very insensitive, if sensitive at all, to mono-*ortho*-substituted PCBs, whereas these PCB congeners are toxic to birds and mammals. These differences in species sensitivity to particular dioxin-like chemicals may create differences in exposure susceptibility associated with variations in the chemical mixture composition in food webs and demonstrates the utility of congener-specific site characterization data during problem formulation.

Although AHR homologs have been identified in amphibians and primitive fish (Hahn, 1998), their toxicological significance is uncertain. Amphibians, reptiles, and primitive fish (*e.g.,* lamprey, hagfish) are relatively insensitive to dioxin-like chemicals. Frogs and toads are at least 100- to 1000-fold less sensitive to 2,3,7,8-TCDD-induced early life stage mortality than fish

(Jung and Walker, 1997; U.S. EPA, 1993). A very limited number of studies demonstrating that PCBs induce dioxin-like biochemical effects (*e.g.,* CYP1A induction) in a few frog and turtle species (Huang *et al*., 1998; Yawetz *et al*., 1997) provide some evidence that the AHR-mediated toxicity pathway is nominally functional in some amphibians and reptiles. Gutleb *et al*. (1999) have reported effects of PCBs on development in two frog species, but it is unclear whether these effects are mediated via the AHR. In summary, data demonstrating dioxin-like effects in amphibians and reptiles are extremely limited, and effects have been observed at relatively high concentrations.

It has been demonstrated that a wide variety of invertebrates including amphipods, cladocerans, midges, mosquito larvae, sandworms, oligochaete worms, snails, clams, and grass shrimp are insensitive to 2,3,7,8-TCDD-induced toxicity (West *et al*., 1997; Barber *et al*., 1998; Van Beneden *et al*., 1998; see U.S. EPA, 1993 and 2001b for summaries and references prior to 1998). Likewise, dioxin-like PCBs (*e.g.,* congeners 77 and 118) generally have little effect on survival, growth, and reproduction in the cladoceran, *Daphnia magna*, and the purple sea urchin (U.S. EPA, 2001b). The insensitivity of invertebrates to dioxin-like toxicity is consistent with the recent finding that several invertebrate AHR homologs lack the ability to bind the prototypical AHR ligands, 2,3,7,8-TCDD and β-naphthoflavone (Powell-Coffman, *et al*., 1998; Butler *et al*., 2001). Given these data, the toxic equivalence methodology is generally not applicable to invertebrates. However, invertebrates may be vulnerable to these chemicals via other non-dioxin-like toxicological effects. It is notable, for example, that PCBs measured as Aroclors have been shown to be chronically toxic to *daphnids* at low ppb levels (Maki and Johnson, 1975; Nebeker and Puglisi, 1974).

Limited data indicate that freshwater plants likewise are relatively insensitive to 2,3,7,8-TCDD. Despite significant accumulation of 2,3,7,8-TCDD in algae and duckweed (*i.e.,* μg/g concentrations), no adverse effects were observed (U.S. EPA, 1993).

Given the known differences in sensitivity among species and endpoints, risk assessors should consider the uncertainty introduced when extrapolating from a species or endpoint for which sensitivity has been established to a species or endpoint of unknown sensitivity. (See U.S. EPA, 1998 and Section 3.4.3 for a discussion of dealing with uncertainty.) This uncertainty, which will affect the choice of the threshold or action level to which the calculated TEC is compared (effects characterization), should be handled in a manner similar to any other chemical for which interspecies extrapolations need to be performed (*e.g.,* consideration of taxonomic relatedness).

3.2.1.2. *Susceptibility: Exposure*

When evaluating the relative susceptibility of species on the basis of exposure, risk assessors may need to consider three alternative expressions of exposure: (1) concentrations of PCDDs, PCDFs, and PCBs in water, sediment, and diet associated with the species; (2) concentrations of PCDDs, PCDFs, and PCBs in the whole body of the species; or (3) concentrations of PCDDs, PCDFs, and PCBs in specific tissues of the species. As indicated in Section 3.2.1.1, the relative sensitivity of species is better measured on the basis of concentrations of PCDDs, PCDFs, and PCBs in tissue(s) of the species than on an external concentration or administered dose. Thus, assessment endpoints should include species that are not only susceptible on the basis of sensitivity, but are exposed through bioaccumulation of dioxin-like PCDDs, PCDFs, and PCBs. Species with greatest bioaccumulation of dioxin-like chemicals are generally those located at higher trophic levels because these hydrophobic chemicals have a strong potential for biomagnification (*i.e.,* the increase in concentration of a

chemical in the tissue of organisms along a series of predator-prey associations, primarily occurring through the mechanism of dietary accumulation).

Temporal and spatial aspects of exposure should also be considered when selecting species with the highest exposure and bioaccumulation. For example, many PCDDs, PCDFs, and PCBs are known to biomagnify such that their concentrations in the tissues of fish-eating birds and mammals may be greater than their concentrations in the tissues of the fish that the birds or mammals eat. However, if birds and mammals move in and out of contaminated areas this exposure scenario may actually result in bioaccumulation that is less than would be observed for animals that feed exclusively from the contaminated area.

PCDDs, PCDFs, and PCBs are not metabolized to a large extent by invertebrates. Therefore, invertebrate tissues tend to be at equilibrium with the water or sediments in which they live (Thomann, 1989; Gobas, 1993). The strong propensity of PCDDs, PCDFs, and PCBs to partition to organic carbon, combined with the fact that their freely dissolved concentration in the water column is extremely low, results in the concentration in sediments usually exceeding the concentration in surface waters (*i.e.,* sediment concentrations are not at equilibrium with surface water concentrations). Thus, organisms whose food chains are linked to contaminated sediments through benthic invertebrates will have greater exposures than those with food chains linked to surface water through pelagic invertebrates (Burkhard *et al*., 2003a).

Unlike invertebrates, vertebrates metabolize PCDDs, PCDFs, and to a limited extent some PCBs. PCDDs and PCDFs that do not possess chlorines at all four 2, 3, 7 and 8 positions do not bioaccumulate significantly in vertebrates. Although metabolism of PCDDs and PCDFs with chlorine substitution at the 2, 3, 7, and 8 positions (the most toxic congeners) occurs to a lesser extent than those without, this metabolism of PCDDs and PCDFs results in significantly less bioaccumulation in comparison to PCBs with the same degree of chlorination (Endicott and Cook, 1994). See Section 3.3.1 for discussion of BAFs and food chain models that are needed to account for competing mechanisms of bioaccumulation and metabolism.

In addition, since the ability to metabolize dioxin-like chemicals, which would enhance elimination and thus reduce bioaccumulation, varies across species and by dioxin-like chemical, the differences in TECs for different species can depend on the relative composition of the PCDD-PCDF-PCB mixture to which each species is exposed. Thus, selection of susceptible species should be specific to the exposure conditions associated with each ecological risk assessment. Examples of how EPA has previously identified predaceous fish (lake trout), piscivorous birds (belted kingfisher, herring gull, bald eagle), and mammals (river otter, mink) as appropriate species in regional (*i.e.,* Great Lakes) and national assessments of potential risks posed by 2,3,7,8-TCDD to aquatic life and associated wildlife can be found in EPA reports (U.S. EPA, 1993; U.S. EPA, 1995a, U.S. EPA, 1995b).

3.2.1.3. *Susceptibility: Integration of Sensitivity and Exposure Considerations*

Susceptibilities related to species sensitivity and exposures are not independent. Generally, species that are highly sensitive and experience high exposure and bioaccumulation will generally be species at greatest risk. However, as explained in Section 3.2.1.2, species with the greatest dietary exposure do not always achieve the greatest tissue concentrations of PCDDs, PCDFs, and PCBs because of inter-species differences in bioaccumulation and metabolism. For example, species with high exposure may be less vulnerable to toxicity than species with lower exposure if the latter is more sensitive and/or has higher levels of bioaccumulation. Hence, it is important to consider both sensitivity and exposure in determining species susceptibility.

Spatial and temporal gradients in environmental concentrations of PCDDs, PCDFs, and PCBs can complicate determinations of species at greatest risk, especially when both species sensitivity and potential population effects are being considered. Timing of exposure with respect to sensitive life stages may make a difference. Fish and bird embryos with maternal exposures that occur outside areas of contamination are probably at greatly reduced risk of early life stage mortality despite subsequent rearing in contaminated ecosystems.

Variations in the composition of dioxin-like chemical mixtures across sites can influence relative susceptibilities of phyla. Sensitive fish species tend to be more vulnerable at sites with large PCDD and PCDF concentrations, whereas birds and mammals are relatively more sensitive than fish at sites with large dioxin-like PCB concentrations. Even within sites, differences in the relative concentration of PCDD, PCDF, and PCB in chemical mixtures in food chains may influence which species are at greatest risk. When overall susceptibility is unclear, determination of TECs and consequent levels of risk for multiple species is advisable.

3.2.1.4. *Ecological Relevance*

The *Guidelines for Ecological Risk Assessment* define ecologically relevant assessment endpoints as those that reflect important characteristics of an ecosystem and are functionally related to other endpoints (U.S. EPA, 1998). Given the taxonomic diversity and number of species that have been shown to be sensitive to dioxin-like toxicity, it is likely that most ecological risk assessments would include multiple "dioxin-sensitive" species. Since ecologically relevant endpoints may be represented at any level of biological organization, many, if not all, of the species or groups of species that are sensitive to dioxin-like toxicity may also be relevant to sustaining the natural structure, function, and biodiversity of the ecosystems under consideration. For example, in aquatic ecosystems, fish may represent an important class of ecologically relevant species, owing either to their role as keystone species or because they serve as a functional link between trophic levels within the food web. Hence, fish would represent both a sensitive and an ecologically relevant assessment endpoint in many, if not most, aquatic ecological risk assessment scenarios.

The ecological relevance of an assessment endpoint is further defined by the potential for adverse effects as a result of exposure to one or more stressors (*i.e.,* susceptibility). The *Guidelines for Ecological Risk Assessment* identify five criteria for evaluating adverse changes in assessment endpoints: nature of effects, intensity of effects, spatial scale of effects, temporal scale of effects, and potential for recovery (U.S. EPA, 1998). With respect to the effects of dioxin-like chemicals on wildlife, each of these criteria is meaningful. As summarized in Table 3, 2,3,7,8-TCDD and related chemicals are known to cause, among other effects, reproductive toxicity, developmental toxicity, and mortality in a wide variety of species. These effects are particularly relevant ecologically because they have the potential to lead to reduced populations of fish, birds, and mammals and to subsequent changes in the structure, function, and biodiversity of ecosystems. 2,3,7,8-TCDD and related chemicals are also particularly significant ecologically because they are among the most, if not the most, potent reproductive and developmental toxicants known (*i.e.,* the intensity of effects has the potential to be great). Further, dioxin-like PCDDs, PCDFs, and PCBs are found ubiquitously in environmental matrices, and they persist in the environment for ecologically relevant time periods, making the spatial and temporal scale for effects potentially large. Finally, because the critical effects of AHR agonists occur during developmental stages of sensitive organisms, there is little or no opportunity for recovery.

3.2.2. Conceptual Model

A conceptual model in problem formulation is a written description and visual representation of predicted relationships between ecological entities and the chemical stressors to which they may be exposed (U.S. EPA, 1998). In the case of ecological risk assessments involving 2,3,7,8-TCDD and related chemicals, a conceptual model might depict the hypothesized movement of these chemicals from a source into the environment; the subsequent exposure of ecological entities from media such as soils, sediments, or the water column; further exposure through the food web (bioaccumulation); and finally the hypothesized direct and secondary ecological effects from these exposures. Figure 4 illustrates exposure to these chemicals through sediment and the water column and resulting exposure through an aquatic food web (source and effects information are omitted for simplicity).

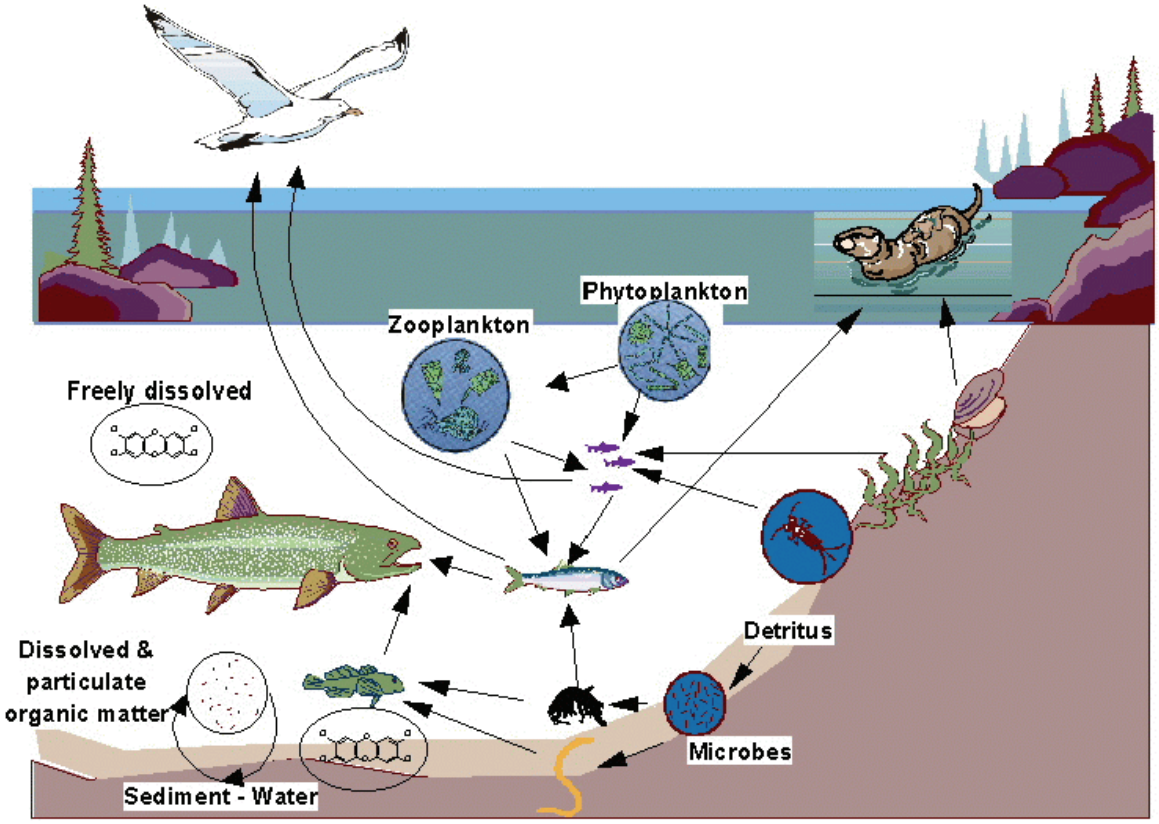


Figure 4. An aquatic food web depicting hypothesized bioavailability and trophic transfer of 2,3,7,8-TCDD through sediment and the water column.

The toxicity equivalence methodology fits well within such a conceptual model. The methodology serves as a bridge between exposure and effects by accumulating exposures to a number of different chemicals into a single value (expressed as a 2,3,7,8-TCDD toxicity equivalence concentration, TEC). A hypothetical model for exposure to PCDDs, PCDFs, and PCBs is illustrated in Figure 5, with areas of application for the toxicity equivalence methodology noted. The items in the boxes making up the flow diagram (left-side) represent the measured or calculated values that will be necessary to perform a toxicity equivalence-based assessment. The items listed on the right side of the diagram are pertinent issues that should be

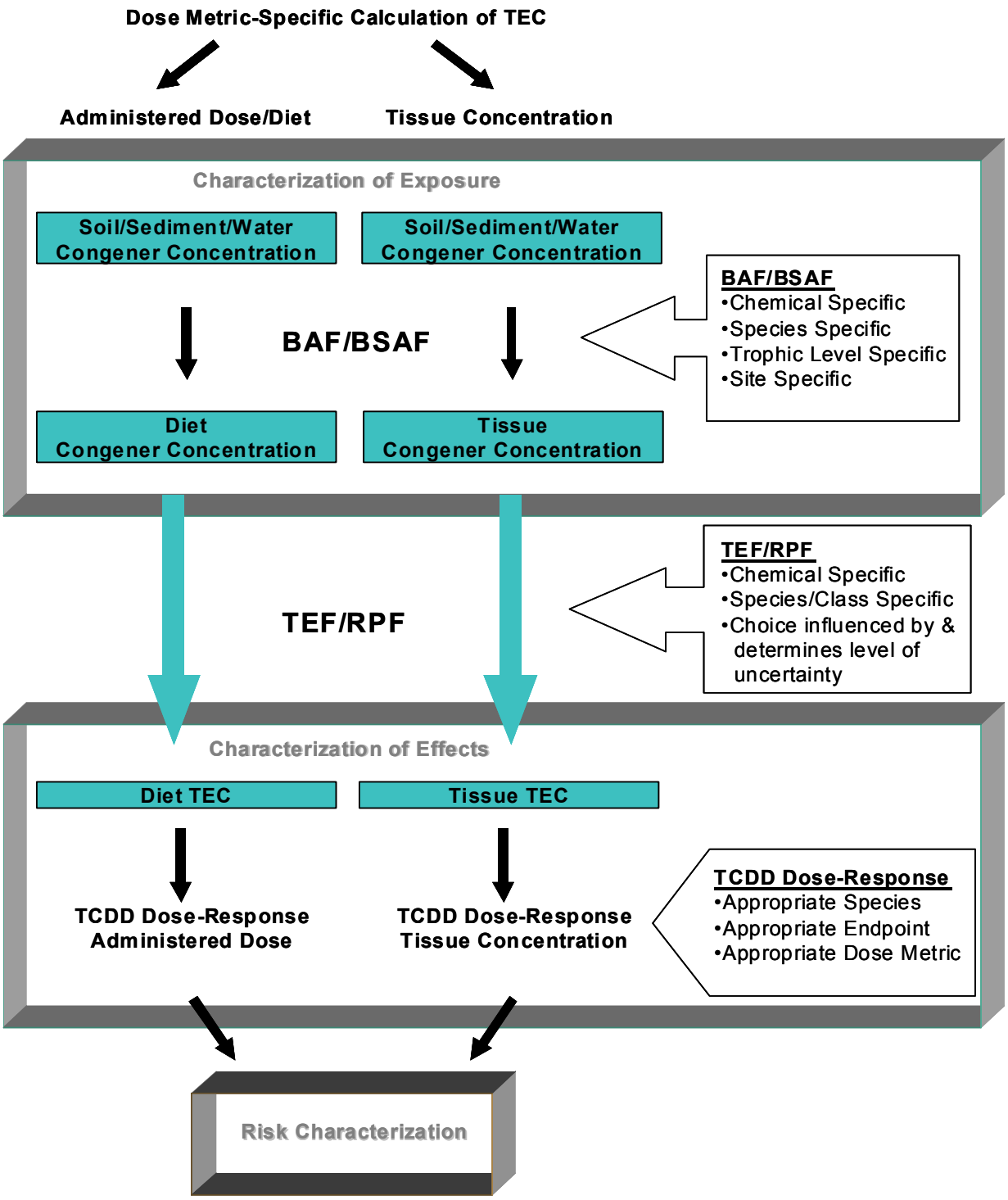


Figure 5. Application of the toxicity equivalence methodology in ecological risk assessment for exposure to PCDDs, PCDFs, and PCBs.

considered in selecting or obtaining the values in the flow diagram. The elements of Figure 5 are discussed in more detail in Section 3.3.

3.2.3. Analysis Plan

The methods for conducting the analysis phase of the risk assessment and estimating risks are described in the analysis plan (U.S. EPA, 1998). The analysis plan provides the risk assessor the opportunity to review for managers and other interested individuals the methods that will be used to complete the risk assessment. The plan includes an assessment of the available data, additional data needs, the methods for collecting these data (including analytical methods), and the method for estimating risks. The uncertainties associated with the data gaps are also described to provide decision makers with a means of determining the resources needed to complete the assessment or realistic expectations about the likely outcome of the assessment.

In the application of the toxicity equivalence methodology to risk assessment, the analysis plan should describe, at a minimum, the method(s) for:

1. Measuring PCDD, PCDF, and PCB concentrations in media and/or biota and how to account for non-detects.

2. Estimating or measuring exposure (duration, frequency, and intensity).

3. Selecting consensus TEFs or deriving assessment-specific RPFs.

4. Estimating or measuring toxicity effects (laboratory or field studies).

5. Estimating risk.

6. Characterizing uncertainties.

The analysis plan should give anyone involved in the risk assessment a clear understanding of the strengths and limitations associated with each of the methods, as well as a clear and transparent description of the assumptions inherent in each of the methods.

Text Box 4. Questions for analysis.

- Have I selected appropriate analytical methods and data quality objectives for measuring individual dioxin-like chemical concentrations in the media of interest?
- Do I have environmental fate and transport information for the PCDDs, PCDFs, and PCBs known or believed to be present?
- Have I determined a method for determining bioaccumulation for individual PCDDs, PCDFs, and PCBs that are relevant to the assessment endpoints?
- Am I applying the TEFs or RPFs to the appropriate tissues or dietary components?
- Are the reasons for selection of TEFs or RePs and derivation of RPFs for the assessment clear and well-supported?
- Are effects of PCDDs, PCDFs, and PCBs in the target or related species of interest documented?

3.3. CONSIDERATIONS IN ANALYSIS

Analysis is a process that examines the two primary components of risk (*i.e.,* exposure and effects), and their relationships between each other and ecosystem characteristics (U.S. EPA, 1998). Important considerations for characterizing exposure to PCDDs, PCDFs, and PCBs are described in Section 3.3.1. The selection of TEFs or

RPFs, which is important in linking exposure and effects, is described in Section 3.3.2. Aspects of the characterization of effects relevant to the toxicity equivalence methodology are presented in Section 3.3.3. Questions to consider during analysis are provided in Text Box 4.

3.3.1. Characterization of Exposure

Characterization of exposure (U.S. EPA, 1998) includes a description of the actual or potential contact of a receptor species with chemical stressors or co-occurring chemical stressors, as in chemical mixtures. The objective of an exposure characterization is to produce a summary exposure profile that identifies the exposed ecological entity (organism), describes the exposure pathway, and estimates the dose of each chemical received by the organism. Important components of an exposure profile for dioxin-like chemicals include: (1) measurements and/or predictions of individual chemical concentrations in water, sediment, soil, and diet; (2) an accounting for the differential fate and transport of PCDDs, PCDFs, and PCBs in the ecosystem; (3) measurements and/or predictions of the bioaccumulation for individual dioxin-like chemicals; and (4) calculation of TECs that are consistent with the dose metrics of the toxicity data being used to determine risks (refer to Figure 5).

3.3.1.1. *Congener-Specific Analyses*

The toxicity equivalence methodology is inherently congener-specific. That is, RePs, RPFs, and TEFs are derived from and applied to data for individual and specific dioxin-like chemicals rather than to homolog groups or commercial mixtures (*e.g.,* Aroclors). Effects, bioaccumulation, and chemical fate and transport models all require input and output of congener-specific data. Only the species-specific, effect endpoint-specific, spatially and temporally-specific toxicity equivalence exposure values which result from the completion of the analysis may be expressed as a TEC. Thus, a prerequisite for using the methodology is chemical characterization that is high-quality and congener-specific. The toxicity equivalence methodology <u>cannot</u> be directly applied to homolog groups or to total PCBs. Uncertainty for application of TEFs to PCB congener concentrations estimated from Aroclor or homolog analyses is probably large because of the wide range of possible congener mixtures, even within homolog groups.

Analytical detection levels for dioxin-like chemicals should be lower than concentrations at which important biological effects may occur. In some cases analytical detection limits for specific chemicals may be too high to allow measurement of concentrations which would significantly add to the TEC. In such cases, options exist for calculating the TEC. For example, concentrations for undetected chemicals may be set equal to zero (no contribution to TEC) or calculated based on either one half the detection limit or the whole detection limit. Alternatively, the TEC may be reported as the range of possible values based on the options. If the TECs are reported in a manner that is transparent to the risk managers, the uncertainties associated with undetected chemicals will be understood. The best method for handling non-detects in a particular risk assessment should be determined through consultation between risk assessors and risk managers early in the risk assessment process (planning/problem formulation phase).

3.3.1.2. *Chemical Fate of PCDDs, PCDFs, and PCBs*

As indicated in Section 3.3.1.1, modeling or monitoring the environmental fate and transport of PCDDs, PCDFs, and PCBs requires chemical-specific data and models. PCDDs, PCDFs, and PCBs are persistent in the environment because they are resistant to chemical and biological degradation. Affinity for organic carbon and lipids, and relatively low volatility,

allows these chemicals to be retained in soils, sediments, and biota for long periods of time. Transport on particles through the atmosphere or waterways are important mechanisms for redistribution of PCDDs, PCDFs, and PCBs and temporal and spatial changes in mixture composition. PCBs tend to partition from water to air to a greater extent than PCDDs and PCDFs, which are more subject to photodegradation (U.S. EPA, 2001c). Hydrophobicity is the most important chemical property that controls bioavailability from water, sediment, and soils, and can be related to measurements of the octanol-water partition coefficient, K_{ow}. PCDDs, PCDFs, and PCBs for which dioxin-like toxicity is established have log K_{ow} values that increase with the degree of chlorination from approximately 6 to 9. This high degree of hydrophobicity makes measurement of concentrations in water very difficult, especially for PCDDs and PCDFs, which are present in the environment in much smaller amounts than PCBs. Conversely, concentrations in surficial sediments or soils are often measurable and can be used effectively to reference each chemical's distribution to abiotic and biotic components of the ecosystem.

While physical and chemical properties of PCDDs, PCDFs, and PCBs as a group can be generalized as above, the differences among the individual chemicals result in different profiles for distribution, fate, and transport and thus temporal and spatial changes in the composition of chemical mixtures in the environment. Properties such as bioavailability, bioaccumulation, metabolism, and biomagnification also differ among PCDDs, PCDFs, and PCBs such that the relative concentration of the individual chemicals in organisms varies with species and trophic level. Therefore, concentrations of individual PCDDs, PCDFs, and PCBs in abiotic media usually do <u>not</u> reflect the chemical concentration profile observed in the tissues of wildlife. TEFs-$WHO_{98/05}$ or RPFs should only be applied to the specific chemical mixtures to which the organisms are exposed. Thus, it is imperative that chemical concentrations in abiotic media be converted to concentrations in either the tissues of organisms being assessed or their food through use of appropriate bioaccumulation factors or models <u>prior to</u> applying TEFs-$WHO_{98/05}$ or RPFs to calculate TECs (refer to Figure 5). For example, BAFs can be applied to PCDD, PCDF, and PCB concentrations in media to obtain predicted concentrations in organisms (as described in the following section and illustrated in Figure 6). It follows that TECs should generally not be directly based on water, sediment, or soil, since these media are inconsistent with the dosimetry basis for the toxicity equivalence model. In cases where direct ingestion of contaminated media (*e.g.,* soil, sediment, or water) is a reasonable and significant exposure pathway, the appropriate exposure dose metric (*i.e.,* administered dose) as described in Section 3.3.1.3, must be considered.

3.3.1.3. *Choices for the Exposure Dose Metric*

In any risk assessment the dose metric (*i.e.,* the measurement or prediction of chemical concentrations) should be consistent between the exposure assessment and the effects assessment. For example, if the dose-response relationship used in the effects assessment is based on toxicity as a function of concentrations of 2,3,7,8-TCDD in tissue, exposure estimates would also need to be based on concentrations in tissue of the species of concern. When incorporating the toxicity equivalence methodology into an exposure assessment, the dose metric basis for TEFs-$WHO_{98/05}$, RPFs, or RePs should be selected to provide consistency in bridging the exposure assessment to the effects assessment (*i.e.,* exposure dose metric = TEF-$WHO_{98/05}$, RPF, or ReP dose metric = effects dose metric). When this is not possible (*e.g.,* TEF or RPF is based on concentration of chemical in tissues, and dose-response for effects is based on administered dose in the diet), the risk assessor should describe, to the extent possible, the direction and magnitude of the errors that may be introduced.

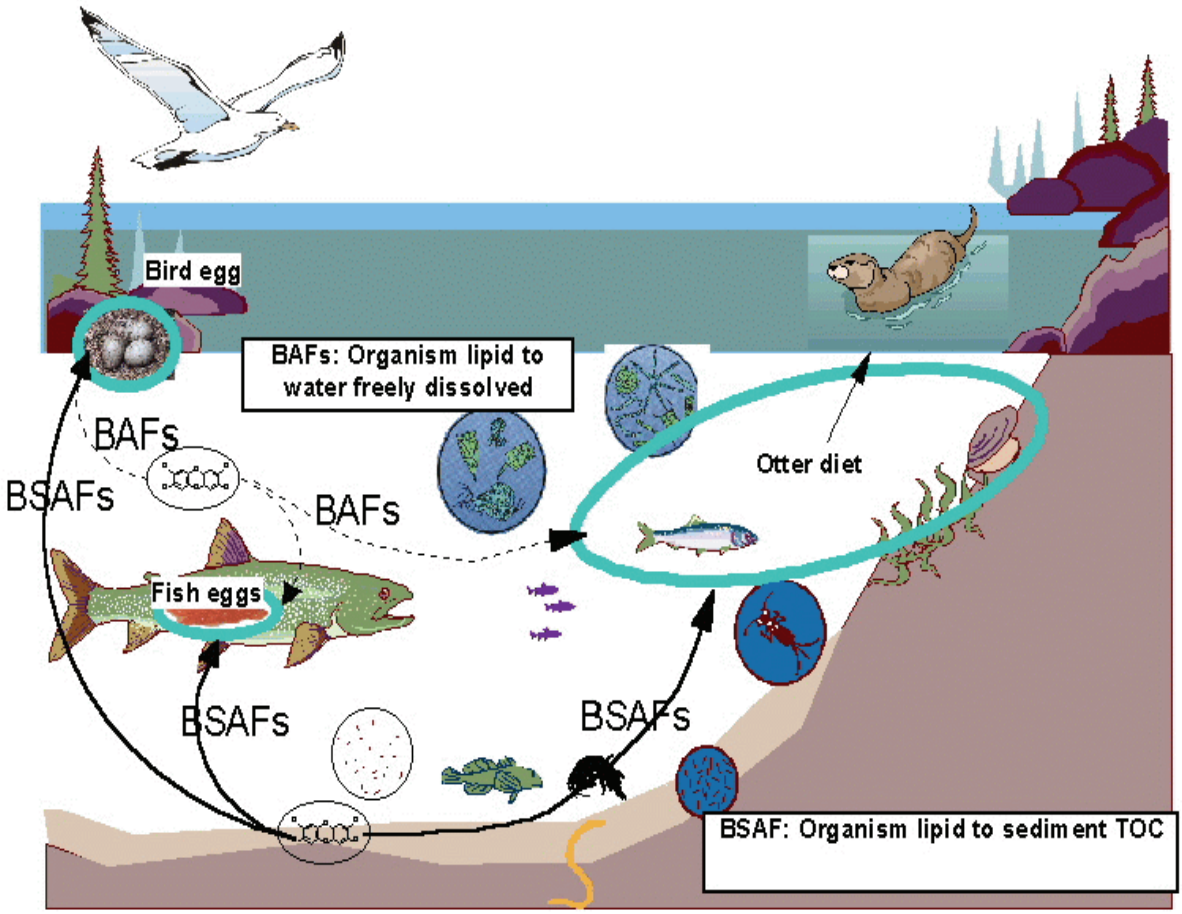


Figure 6. Estimating chemical concentrations in eggs and diet by applying BAFs and BSAFs for PCDDs, PCDFs, and PCBs.

The TEFs-WHO_{98} and RePs for fish and birds are generally based on the potencies of dioxin-like chemicals within cells, organs, or whole organisms, with the concentration in tissue used as the dose metric. The dose metric for 2,3,7,8-TCDD-induced developmental toxicity in fish and birds is also often expressed as a concentration in tissue (*i.e.,* egg or embryo), which is desirable. Hence, the dose metrics for fish and bird TEFs-WHO_{98}, RPFs, and RePs are often consistent with the dose metrics used for the toxicity relationship and allow for an internally consistent exposure and effects assessment based on concentration of chemicals in the organism's tissues. TECs based on measurements or estimates of PCDD, PCDF, and PCB concentrations in tissues are presently most accurate for assessment of effects in fish and birds, with concentrations in whole embryos used to assess early life stage effects. If concentrations in tissue are unavailable, they may be estimated from environmental media based on BAFs or models (as described in Section 3.3.1.4 and Cook *et al*., 2003) or bioaccumulation from the diet if dietary intake and concentrations can be estimated.

In contrast to fish and birds, the dose metric used for mammalian TEFs-$WHO_{98/05}$ and RePs is generally administered dose. Application of the mammalian TEFs-$WHO_{98/05}$ to dietary exposures, rather than concentrations measured or predicted for specific tissues, is presently more accurate and will minimize uncertainty associated with the risk assessment. While data are

available for derivation of RePs or RPFs based on potencies of dioxin-like chemicals in mammalian cells or organs (*e.g.,* CYP1A induction), such relative potency data are subject to variability associated with toxicokinetic differences between chemicals for absorption, distribution, metabolism, and elimination. For example, the mouse hepatic ethoxyresorufin-O-deethylase (EROD) RePs based on administered doses for 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF are less than the RePs based on concentration of the chemicals in the mouse liver that result from the administered dose (DeVito *et al*., 1997). The difference in RePs occurs because both 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF are more rapidly metabolized than 2,3,7,8-TCDD, and greater administered doses are required to attain 2,3,7,8-TCDD equivalent concentrations in the liver (DeVito *et al*., 1997, Santostefano *et al*., 1998). Until tissue concentration-based RePs for mammals are fully developed for application to dietary or *in vivo* dose metrics, potential systematic errors associated with using such relative potency estimates in conjunction with exposure and effects data, based on an administered dose metric, should be recognized and documented in the risk assessment.

3.3.1.4. *Bioaccumulation of PCDDs, PCDFs, and PCBs*

Because TECs should be based on concentrations in tissues of organisms (or their diet) rather than in abiotic media, risk assessors should consider how they will measure or predict concentrations of PCDDs, PCDFs, and PCBs in tissues or diets. If measured concentrations in tissues of the species associated with assessment endpoints are available for all dioxin-like chemicals of concern, then TECs may be calculated directly as presented in Equation 2-1. In many cases, however, measured concentrations in organisms will not be available. Furthermore, even if concentrations in organisms have been measured, there may be a need to relate them to ambient concentrations of PCDDs, PCDFs, and PCBs in water, sediment, or soil over time in order to quantify the connections between contaminant sources and exposure as is necessary to meet remedial action goals. Therefore, it will frequently be necessary to estimate or measure bioaccumulation for PCDDs, PCDFs, and PCBs in risk assessments involving the toxicity equivalence methodology.

One traditional method for estimating bioaccumulation is through the use of bioconcentration factors (BCFs), but BCFs have poor applicability to PCDDs, PCDFs, and PCBs. BCFs, which are measured under laboratory conditions, describe uptake of the chemical by aquatic organisms only from water through respiration (*i.e.,* via the gills). Thus, for very hydrophobic chemicals, BCFs tend to underestimate bioaccumulation, which is the net uptake and retention of a chemical through all routes of exposure, uptake, and elimination. Additional complicating factors for PCDDs and PCDFs in aquatic food chains are: (1) uncertainty/difficulty associated with measuring or estimating the fraction bioavailable in water; (2) strong influence of benthic (sediment associated) food chains; (3) metabolism rates in vertebrates that may be sufficient to greatly reduce the impact of dietary exposure; and (4) significant time periods required to reach approximate steady-state levels in tissues as occurs with most environmental exposures.

Alternatives to BCFs, water-based BAFs and biota-sediment accumulation factors (BSAFs) are obtained from direct measurements in the environment or prediction of uptake and elimination rates of each chemical as a result of all routes of exposure. As shown in Figures 5 and 6, BAFs and BSAFs are the essential connectors of concentrations of PCDDs, PCDFs, and PCBs in the environment with concentrations in the diet or relevant tissues of organisms of concern. Typically, BAFs and BSAFs are determined and applied for conditions that

approximate steady-state of the organism with respect to water and sediments, respectively. Fluctuating concentrations of these chemicals in water can often be effectively handled by using average concentrations over time because their bioaccumulation rates are relatively insensitive to short term fluctuations in water (Burkhard, 2003a). Thus, BAFs and BSAFs are the appropriate quantitative expressions for the relationships between concentrations of PCDDs, PCDFs, and PCBs in the environment (water, sediment, soil) and concentrations in an organism's tissues. BAFs have been used explicitly to define water quality standards, as in the *Great Lakes Water Quality Initiative* (GLWQI) (U.S. EPA, 1995a) and the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (U.S. EPA, 2000b).

Text Box 5. Key to symbols and notations used in equations 3-1 to 3-9.

Symbol	Representation	Common units
TEF	toxicity equivalence factor	ng TCDD/ng chemical
C	concentration	ng/kg
TEC	toxicity equivalence concentration	ng/kg
k	number of chemicals in mixture	
$n = i$	individual chemical in mixture	
superscript fd	freely dissolved chemical	
superscript t	total chemical	
subscript w	in water	
subscript soc	in sediment organic carbon	
subscript t	in tissue	
subscript ℓ	in lipid	
subscript r	reference chemical	
subscript i	individual chemical of interest	
BAF	bioaccumulation factor	L water/kg organism
BAF_{ℓ}^{fd}	BAF, lipid normalized and based on freely dissolved chemical in water	L water/kg lipid
$BSAF$	biota-sediment accumulation factor	kg sediment/kg organism
C_w	C of total chemical in water	ng/L water
C_w^{fd}	C of chemical freely dissolved in water	ng/L water
C_s	C of total chemical in sediment	ng/kg sediment
C_{soc}	C of chemical in sediment organic carbon	ng/kg organic carbon
C_{ℓ}	C of chemical in lipid	ng/kg lipid
C_t	C of chemical in tissue	ng/kg tissue
f_{ℓ}	fraction lipid in the organism	kg lipid/kg organism
f_{soc}	fraction organic carbon in sediment	kg oc/kg sediment
K_{ow}	octanol-water partition coefficient	L water/L octanol
$\prod_{socw}$	sediment-water concentration quotient	L water/kg organic carbon
$D_{i/r}$	ratio between values of $\prod_{socw}$ for reference chemical and chemical of interest	unitless

Concentrations in biota, sediments, and water used to calculate BAFs and BSAFs need to accommodate variability in bioavailability conditions and express bioaccumulation on a thermodynamic basis (degree of equilibrium/disequilibrium between biota, water, and sediments). Thus, the concentration of the chemical in the organism's tissues (C_t) is normalized to lipid content (C_{ℓ}) with the fraction lipid (f_{ℓ}) in the organism's tissues, and the concentration of

the chemical in sediment (C_s) is normalized to organic carbon content (C_{soc}) with the fraction of organic carbon in the sediment (f_{soc}). The concentration of the bioavailable chemical in water is defined as the concentration of freely dissolved chemical (C_w^{fd}), which is calculated with the fraction of chemical that is freely dissolved (f^{fd}) as estimated from concentrations of particulate organic carbon (POC) and dissolved organic carbon (DOC) in the water (U.S. EPA, 1995a, 2000b, and 2003b). Thus there are two basic forms of bioaccumulation factors in current use: for water, the bioaccumulation factor, $\mathrm{BAF}_\ell^{\mathrm{fd}}$, and for sediment, the biota sediment accumulation factor, BSAF:

$$BAF_\ell^{fd} = \frac{C_\ell}{C_w^{fd}} = \frac{C_t \bullet 1/f_\ell}{C_w^t \bullet f^{fd}} \tag{3-1}$$

$$BSAF = \frac{C_\ell}{C_{soc}} = \frac{C_t \bullet 1/f_\ell}{C_s \bullet 1/f_{soc}} \tag{3-2}$$

For a visualization and sensitivity analysis of the critical determinants of site-specific BAF and BSAF values and their connection to tissue-based toxicity risk criteria, see Burkhard *et al*. (2003a). If tissue concentrations are not available for the species and/or ecosystem of concern in a risk assessment, it may be possible to estimate BAFs and BSAFs by extrapolation from other species or ecosystems, as discussed in Section 3.3.1.5 and Burkhard *et al*. (2006), which describes a hybrid modeling approach for extrapolating BAFs and BSAFs. A high quality BSAF data set for PCDDs, PCDFs, and PCBs has been reported for lake trout in Lake Michigan (Burkhard *et al*., 2004). In addition, EPA has recently compiled an extensive data set of approximately 20,000 biota-sediement accumulation factors (BSAFs) from 20 locations (mostly Superfund sites) for nonionic organic chemicals (e.g., PCBs, PCDDs, PCDFs). This data set can be downloaded at http://www.epa.gov/med/Prods_Pubs/bsaf.htm.

While TEFs-$\mathrm{WHO}_{98/05}$ (or RPFs/RePs) cannot be used to calculate TECs directly from concentrations of PCDDs, PCDFs, and PCBs in water or sediments, they may be combined with BAF_ℓ^{fd}s or BSAFs and the fraction lipid in the organism (f_ℓ) to determine a wet weight TEC for an organism as shown in the following two equations:

$$TEC = \sum_{n=1}^{k} \left(C_w^{fd}\right)_n \left(BAF_\ell^{fd}\right)_n \left(f_\ell\right)\left(TEF_n\right) \tag{3-3}$$

$$TEC = \sum_{n=1}^{k} \left(C_{soc}\right)_n \left(BSAF\right)_n \left(f_\ell\right)\left(TEF_n\right) \tag{3-4}$$

Risk assessments, which are concerned with ecological effects as a consequence of loadings of PCDDs, PCDFs, and PCBs to aquatic ecosystems, must be designed to consider the

masses, and thus the concentrations, of these chemicals in both water and sediments. In these cases the risk analysis will, either directly or indirectly, involve specific values of BAF_{ℓ}^{fd} and BSAF for each chemical. BAF_{ℓ}^{fd} values can be measured for many PCB congeners but are difficult to measure directly for PCDDs, PCDFs, and the most toxic PCB congeners because concentrations in water fall below detection limits. Nevertheless, it may be necessary to calculate BAF_{ℓ}^{fd} values, such as for water quality criteria development and application, even if the BAF_{ℓ}^{fd} values are not needed for calculating TECs. Any risk management decision based on future chemical mass balances associated with reducing concentrations of chemicals in sediments and/or external sources has to address concentration changes in biota, water, and sediment compartments, regardless of whether measured concentrations are available for each compartment at any point in time.

To calculate BAF_{ℓ}^{fd} values for such purposes, EPA presently uses measured BSAFs for PCDDs, PCDFs, and co-planar PCBs, combined with estimates of sediment-water concentration quotients ($\prod_{socw}$ as defined by equation 3-5) for reference chemicals which have measurable concentrations in water (U.S. EPA, 1995a; 2000b). The BSAF method, as described by equation 3-6, has provided accurate predictions of BAF_{ℓ}^{fd} values for PCBs in several different ecosystems (L. Burkhard, personal communication; U.S. EPA, 2003b). Since $(\prod_{socw})_i$ is difficult to measure for PCDDs, PCDFs, and PCBs assigned TEFs, a measured value for a reference chemical *r* (usually a PCB congener of similar K_{ow}) may be used to estimate $(\prod_{socw})_i$. The ratio of $(\prod_{socw})_i$ for the PCDD, PCDF, or PCB of interest to $(\prod_{socw})_r$ for the reference chemical (factor $D_{i/r}$ in equation 3-6) accounts for observed or predicted chemical specific differences in $\prod_{socw}$. High quality measurements of $\prod_{socw}$ values for PCBs, PCDDs, and PCDFs in Lake Michigan have been obtained (Burkhard *et al*., 2006) and can be used as a source of measured values for $D_{i/r}$. Even with $D_{i/r}$ set as 1.0, the BSAF method robustly captures congener-specific differences in bioavailability and metabolism in the food chain through use of BSAFs as indicators of relative bioaccumulation potentials for the dioxin-like chemicals. The method also highlights the necessity of linking biota to both water and sediment when quantitative ecological risk assessments are required. For more details see U.S. EPA (2000b).

$$\prod_{socw} = \frac{C_{soc}}{C_w^{fd}} = \frac{BAF_{\ell}^{fd}}{BSAF} \tag{3-5}$$

$$\left(BAF_{\ell}^{fd}\right) = \left(BSAF\right)_i \frac{\left(D_{i/r}\right)\left(\Pi_{socw}\right)_r \left(K_{ow}\right)_i}{\left(K_{ow}\right)_r} \tag{3-6}$$

BSAFs are advantageous for describing and predicting bioaccumulation of PCDDs, PCDFs, and PCBs because they can be measured at a site to capture effects of food web structure, bioavailability, and metabolism. BSAFs also tend to integrate fluctuations of chemical concentrations in the water and accommodate spatial gradients in sediment (Burkhard *et al*., 2003b; U.S. EPA, 2003b). When risks are to be assessed and managed on the basis of approximate steady state conditions expected in the future, the predictive power of BSAFs

depends on adjustments to account for expected changes in these conditions. In reality, these adjustments are relatively small and similar for each AHR agonist (Cook *et al*., 2003). Because BSAFs are very good quantitative measures of the relative bioaccumulation potentials of AHR agonists in aquatic ecosystems, they are especially useful for calculating TECs. Note that use of a measured or extrapolated set of BSAFs for a specific site and trophic level in calculating a TEC under equation 3-4 accommodates chemical-specific differences in bioaccumulation in a manner that often may equal or exceed the specificity and accuracy for the relative potencies available. That AHR-mediated toxicity risks can be predicted accurately and relate to the historical elimination of lake trout as a keystone species in Lake Ontario (Cook *et al*., 1997; 2003) demonstrates that the net risks associated with both bioaccumulation and relative potency differences can be effectively assessed through input of appropriate values into equation 3-4.

3.3.1.5. *Examples of TEC Calculations for Fish, Birds, and Mammals*

Calculations of TECs are conceptualized in Figure 7. Examples of estimating 2,3,7,8-TCDD TECs in fish eggs and bird eggs (TEC_{eggs}) from average values of measured PCDD, PCDF, and PCB congener concentrations in sediments are presented in Tables 4 and 5. Table 6 presents an example of estimating 2,3,7,8-TCDD TECs in mink diet. The tables are followed by detailed descriptions of the calculations. The hypothetical sediments are representative of a moderately contaminated ecosystem.

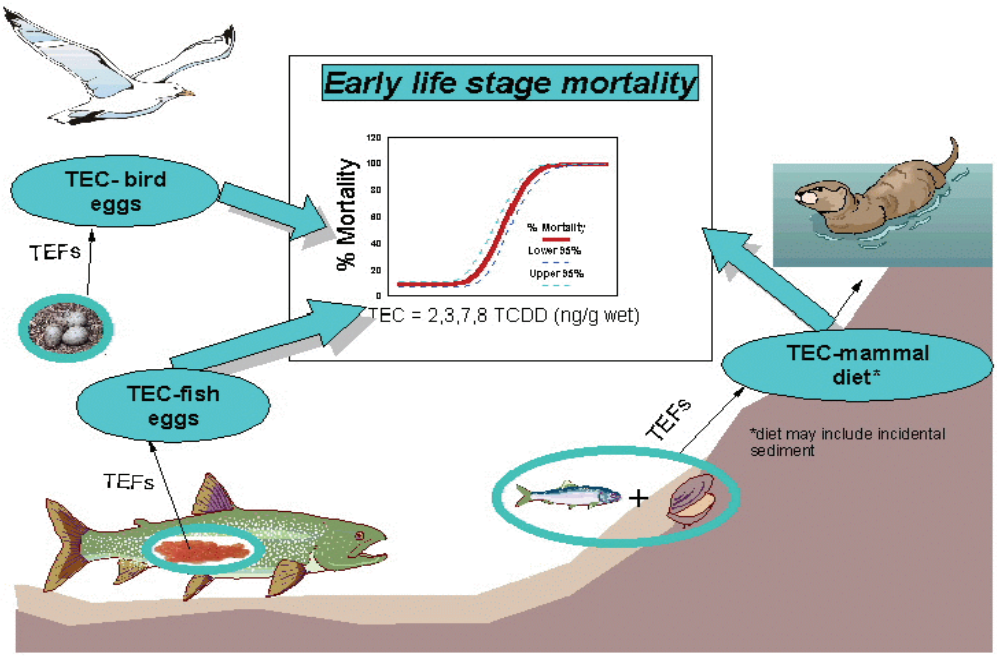


Figure 7. PCDDs, PCDFs, and PCBs: effects on vertebrates. TECs are calculated from concentrations in bird eggs, fish eggs, or mammal diet.

The important risk question associated with these examples is whether the chemicals have accumulated sufficiently to cause significant mortality of lake trout and herring gulls during early life stages. BSAFs, based on Lake Ontario data for sediments (U.S. EPA, 1995a), lake trout eggs (Guiney *et al*., 1996), and herring gull eggs (Government of Canada, 1991), are used here to

illustrate how concentrations of the dioxin-like chemicals in biota may be estimated from contaminated sediment data. The following relationships are used to calculate concentrations in trout and gull embryos from BSAFs (for an actual, more specific, and rigorous assessment example involving lake trout, see Cook *et al*., 2003):

$$C_{trout\ egg} = \frac{C_s}{f_{soc}} \bullet BSAF_{trout\ egg} \bullet (f_\ell)_{trout\ egg} \tag{3-7}$$

$$C_{gull\ egg} = \frac{C_s}{f_{soc}} \bullet BSAF_{gull\ egg} \bullet (f_\ell)_{gull\ egg} \tag{3-8}$$

The fraction of organic carbon (f_{soc}) is measured for sediments, in association with concentrations of each dioxin-like chemical in sediments (C_s), and the fraction of lipid (f_ℓ) in trout or gull eggs that would exist at the site is predicted from literature values. Finally, concentrations of PCDDs, PCDFs, and PCBs in tissue are multiplied by the appropriate fish and bird TEFs-WHO_{98} (see Table 2) and the products summed to estimate total TECs for trout and gull embryos, respectively, as indicated by equation 2-1 (note that this is equivalent to use of equation 3-4). As summarized in Table 4, the trout egg TEC is reported as a range (3.82-10.46 ng/kg trout egg) reflecting the use of both 0 and 0.000005 as the TEF for the mono-*ortho* PCBs (TEF<0.000005). Table 5 reports the gull egg TEC as a single value of 703.2 ng/kg gull egg because the avian TEFs for mono-*ortho* PCBs biphenyls are discrete values. In this hypothetical example the non-*ortho* PCBs contribute 2.06 ng/kg trout egg and 419.62 ng/kg gull egg in contrast to 1.76 ng/kg trout egg and 10.58 ng/kg gull egg for PCDDs and PCDFs.

Figures 8 and 9 illustrate the relative contributions to the TECs made by PCDDs and PCDFs in comparison to PCBs for trout and gull eggs, respectively. In this example PCDDs and PCDFs make approximately equal contributions with PCBs to the trout egg TEC, whereas the PCBs make a much greater contribution to the gull egg TEC. This is a consequence of both PCB TEFs and BSAFs being greater for birds than fish. The right half of the graphs also illustrate the consequences of calculating a TEC based on concentrations in sediments rather than in the eggs. In the fish example, the sediment-based TEC is somewhat greater than the egg-based TEC, but the PCDD/PCDF contribution is magnified greatly in comparison to the PCB contribution because the sediment-based TEC does not account for effects that impact bioaccumulation (e.g., bioavailability, food chains, biomagnification, and metabolism). In the gull egg example, the sediment-based TEC is much less than the egg-based TEC because the effects associated with exposure and bioaccumulation are not included when TEFs are applied to concentrations in sediment. More importantly, the gull egg TEC, which is approximately one hundred times greater than the trout egg TEC, does not necessarily indicate that the gulls are at greater risk than trout. The risk for lake trout can be greater if the trout are more than one hundred fold more sensitive to 2,3,7,8-TCDD than herring gulls on the basis of TEC_{egg} values. This in fact appears to have been the case for lake trout and herring gull populations in Lake Ontario during the last century (Cook *et al*., 2003).

Table 4. An example of estimating TECs in fish eggs from average concentrations of PCDD, PCDF, and PCB congeners measured in surface sediment samples of a reservoir.

Note: All data (with the exception of TEFs) in this table are for illustrative purposes only. They are not recommended default values for all risk assessments.

	Concentration in Sediment (ng/kg)	Trout Egg BSAF[1]	Concentration in Trout Egg (ng/kg egg)	TEFs-WHO_{98} Fish TEF	Trout Egg TEC Predicted Concentration (ng/kg egg)
2,3,7,8-TCDD	0.30	0.149	0.22	1	0.22
1,2,3,7,8-PeCDD	1.20	0.121	0.73	1	0.73
1,2,3,4,7,8-HxCDD	1.10	0.018	0.10	0.5	0.05
1,2,3,6,7,8-HxCDD	4.70	0.007	0.17	0.01	0.002
1,2,3,7,8,9-HxCDD	2.90	0.010	0.15	0.01	0.002
1,2,3,4,6,7,8-HpCDD	78.20	0.002	0.78	0.001	0.0008
OCDD	530.00	0.0007	1.96	<0.0001	< 0.002
2,3,7,8-TCDF	1.10	0.069	0.38	0.05	0.02
1,2,3,7,8-PeCDF	0.92	0.009	0.04	0.05	0.002
2,3,4,7,8-PeCDF	1.40	0.162	1.13	0.5	0.57
1,2,3,4,7,8-HxCDF	4.10	0.0045	0.09	0.1	0.009
1,2,3,6,7,8-HxCDF	1.60	0.007	0.06	0.1	0.006
1,2,3,7,8,9-HxCDF	0.30	0.020	0.03	0.1	0.003
2,3,4,6,7,8-HxCDF	1.00	0.002	0.01	0.1	0.001
1,2,3,4,6,7,8-HpCDF	2.70	0.001	0.01	0.01	0.0001
1,2,3,4,7,8,9-HpCDF	133.00	0.023	15.30	0.01	0.15
OCDF	2.40	0.001	0.01	<0.0001	<0.00001
Sum PCDD and PCDF					**1.76**
3,4,4',5-TCB (81)	60	0.95	285	0.0005	0.14
3,3',4,4'-TCB (77)	1623	0.29	2353	0.0001	0.24
3,3',4,4',5-PeCB (126)	16	4.18	334	0.005	1.67
3,3',4,4',5,5'-HxCB (169)	4.8	5.58	134	0.00005	0.007
2,3,3',4,4'-PeCB (105)	5370	2.54	68199	<0.000005	< 0.341
2,3,4,4',5-PeCB (114)	4170	5.22	108837	<0.000005	< 0.544
2,3',4,4',5-PeCB (118)	35658	4.66	830831	<0.000005	<4.154
2',3,4,4',5-PeCB (123)	538	3.80	10222	<0.000005	<0.051
2,3,3',4,4',5-HxCB (156)	8413	5.87	246921	<0.000005	<1.2346
2,3,3',4,4',5'-HxCB (157)	917	7.89	36175	<0.000005	<0.1809
2,3',4,4',5,5'-HxCB (167)	705	2.03	7156	<0.000005	<0.0358
2,3,3',4,4',5,5'-HpCB (189)	1876	2.07	19416	<0.000005	<0.0971
Sum PCB					**2.06 - 8.70**
Sum all					**3.82 - 10.46**

[1]BSAFs for trout eggs are based on 7% lipid in eggs and 1.4% organic carbon in sediment.

Table 5. An example of estimating TECs in bird eggs from average concentrations of PCDD, PCDF, and PCB congeners measured in surface sediment samples of a reservoir.
Note: All data (with the exception of TEFs) in this table are for illustrative purposes only. They are not recommended default values for all risk assessments.

	Concentration in Sediment (ng/kg)	Gull Egg BSAF[1]	Concentration in Gull Egg (ng/kg egg)	TEFs-WHO_{98} Avian TEF	Gull Egg TEC Predicted Concentration (ng/kg egg)
2,3,7,8-TCDD	.30	1.2188	1.83	1.0	1.83
1,2,3,7,8-PeCDD	1.20	1.0313	6.19	1.0	6.19
1,2,3,4,7,8-HxCDD	1.10	0.0368	0.20	0.05	0.01
1,2,3,6,7,8-HxCDD	4.70	0.2321	5.46	0.01	0.055
1,2,3,7,8,9-HxCDD	2.90	0.0102	0.15	0.1	0.015
1,2,3,4,6,7,8-HpCDD	78.20	0.0016	0.63	<.001	< 0.0006
OCDD	530.00	0.0018	4.75	0.0001	0.0005
2,3,7,8-TCDF	1.10	0.0250	0.14	1.0	0.14
1,2,3,7,8-PeCDF	0.92	0.0221	0.10	0.1	0.01
2,3,4,7,8-PeCDF	1.40	0.3068	2.15	1.0	2.15
1,2,3,4,7,8-HxCDF	4.10	0.0181	0.37	0.1	0.04
1,2,3,6,7,8-HxCDF	1.60	0.0893	0.71	0.1	0.07
1,2,3,7,8,9-HxCDF	0.30	0.0174	0.03	0.1	0.003
2,3,4,6,7,8-HxCDF	1.00	0.1200	0.60	0.1	0.06
1,2,3,4,6,7,8-HpCDF	2.70	0.0001	0.001	0.01	0.00001
1,2,3,4,7,8,9-HpCDF	133.00	0.0027	1.78	0.01	0.02
OCDF	2.40	0.0002	0.002	0.0001	0.0000002
Sum PCDD and PCDF					**10.58**
3,4,4',5-TCB (81)	60	3.41	1024	0.1	102.40
3,3',4,4'-TCB (77)	1623	0.178	1445	0.05	72.24
3,3',4,4',5-PeCB (126)	16	30.6	2446	0.1	244.62
3,3',4,4',5,5'-HxCB (169)	4.8	15	360	0.001	0.36
2,3,3',4,4'-PeCB (105)	5370	15.9	426118	0.0001	42.61
2,3,4,4',5-PeCB (114)	4170	24	505919	0.0001	50.59
2,3',4,4',5-PeCB (118)	35658	46.4	8270925	0.00001	82.71
2',3,4,4',5-PeCB (123)	538	22.3	60000	0.00001	0.60
2,3,3',4,4',5-HxCB (156)	8413	18.8	790822	0.0001	79.08
2,3,3',4,4',5'-HxCB (157)	917	32.1	147148	0.0001	14.72
2,3',4,4',5,5'-HxCB (167)	705	24.8	87420	0.00001	0.87
2,3,3',4,4',5,5'-HpCB (189)	1876	19.4	181972	0.00001	1.82
Sum PCB					**692.62**
Sum all					**703.20**

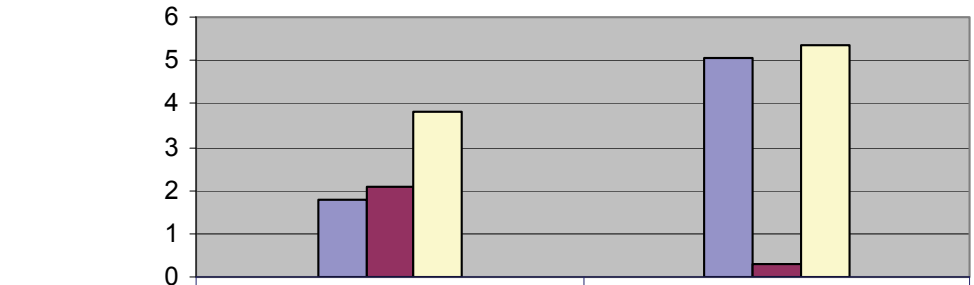
[1]BSAFs for gull eggs are based on 7% lipid in eggs and 1.4% organic carbon in sediment.

Table 6. An example of estimating TECs in the diet of otter from average concentrations of PCDD, PCDF, and PCB congeners measured in surface sediment samples of a reservoir.

Note: All data (with the exception of TEFs) in this table are for illustrative purposes only. They are not recommended default values for all risk assessments.

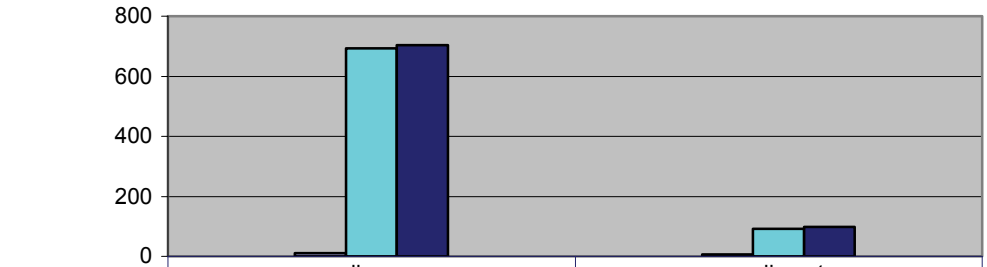
	Concentration in Sediment (ng/kg)	Forage Fish BSAF[1]	Concentration in Otter Diet (ng/kg fish)	TEFs-WHO_{05} Mammalian TEF	Otter Diet TEC Predicted Concentration (ng/kg fish)
2,3,7,8-TCDD	0.30	0.20	0.133	1	0.1330
1,2,3,7,8-PeCDD	1.20	0.18	0.479	1	0.4790
1,2,3,4,7,8-HxCDD	1.10	0.03	0.073	0.1	0.0073
1,2,3,6,7,8-HxCDD	4.70	0.02	0.209	0.1	0.0209
1,2,3,7,8,9-HxCDD	2.90	0.02	0.129	0.1	0.0129
1,2,3,4,6,7,8-HpCDD	78.20	0.008	1.389	0.01	0.0139
OCDD	530.00	0.0005	0.588	0.0003	0.0002
2,3,7,8-TCDF	1.10	0.12	0.293	0.1	0.0293
1,2,3,7,8-PeCDF	0.92	0.01	0.020	0.03	0.0006
2,3,4,7,8-PeCDF	1.40	0.33	1.026	0.3	0.3078
1,2,3,4,7,8-HxCDF	4.10	0.01	0.091	0.1	0.0091
1,2,3,6,7,8-HxCDF	1.60	0.01	0.036	0.1	0.0036
1,2,3,7,8,9-HxCDF	0.30	0.04	0.027	0.1	0.0027
2,3,4,6,7,8-HxCDF	1.00	0.05	0.111	0.1	0.0111
1,2,3,4,6,7,8-HpCDF	2.70	0.001	0.006	0.01	0.0001
1,2,3,4,7,8,9-HpCDF	133.00	0.03	8.858	0.01	0.0886
OCDF	2.40	0.001	0.005	0.0003	0.000002
Sum PCDD and PCDF					1.1200
3,4,4',5-TCB (81)	60	0.35	46.6	0.0003	0.0140
3,3',4,4'-TCB (77)	1623	0.25	901	0.0001	0.0901
3,3',4,4',5-PeCB (126)	16	0.92	32.7	0.1	3.2678
3,3',4,4',5,5'-HxCB (169)	4.8	1.08	11.5	0.03	0.3450
2,3,3',4,4'-PeCB (105)	5370	0.85	10133	0.00003	0.3040
2,3,4,4',5-PeCB (114)	4170	1.41	13052	0.00003	0.3916
2,3',4,4',5-PeCB (118)	35658	1.57	124282	0.00003	3.7285
2',3,4,4',5-PeCB (123)	538	1.02	1218	0.00003	0.0365
2,3,3',4,4',5-HxCB	8413	1.66	31004	0.00003	0.9301
2,3,3',4,4',5'-HxCB (157)	917	2.08	4234	0.00003	0.2170
2,3',4,4',5,5'-HxCB (167)	705	1.09	1706	0.00003	0.0512
2,3,3',4,4',5,5'-HpCB (189)	1876	1.26	5248	0.00003	0.1574
Sum PCB					9.4454
Sum all					10.5654

[1]BSAFs for forage fish in diet of otter are based on 3.11% lipid in forage fish and 1.4% carbon in sediment.



	trout eggs	sediment
PCDD+PCDF	1.76	5.06
PCB	2.06	0.27
TEC (Total)	3.82	5.34

Figure 8. Fish TECs calculated with TEFs-WHO$_{98}$ appropriately from concentrations in eggs versus inappropriately from concentrations in sediment.



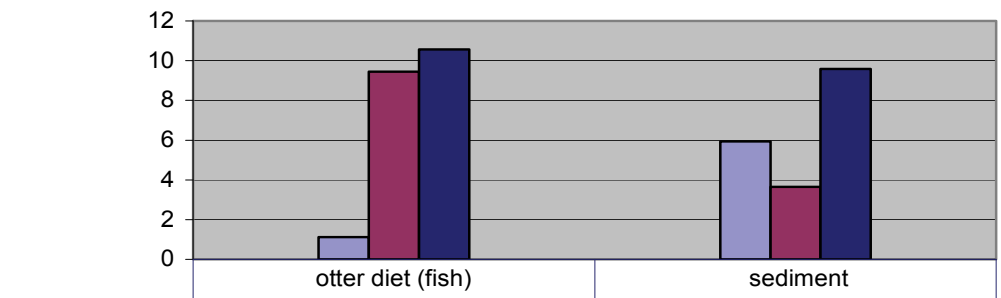
	gull eggs	sediment
PCDD+PCDF	10.58	6.59
PCB	692.62	91.03
TEC (Total)	703.2	97.62

Figure 9. Bird TECs calculated with TEFs-WHO$_{98}$ appropriately from concentrations in eggs versus inappropriately from concentrations in sediment.

A third example of a TEC calculation, also conceptualized in Figure 7, is based on chemical concentrations in the mammalian diet associated with contaminated sediment rather than chemical concentrations in the mammal's tissue. The forage fish-based dietary exposure calculation utilizes equation 3-9, and the TEC calculations are reported in Table 6. Although the concentrations of individual PCDDs, PCDFs, and PCBs in vulnerable tissues are the most relevant dose metric for understanding biological responses, it is often impractical or impossible to define dose on a tissue-specific basis. Thus, the mammalian TEFs-WHO$_{05}$ are largely based on relative potency data associated with the administered doses in the diet of test animals. Section 3.3.1.3 provides further discussion.

$$C_{otter\ diet} = \frac{C_s}{f_{soc}} \bullet BSAF_{forage\ fish} \bullet \left(f_\ell\right)_{forage\ fish} \tag{3-9}$$

Unlike the gull egg example (Figure 9), Figure 10 shows that calculation of the TEC based on sediments using mammalian TEFs-WHO_{05} does not necessarily significantly underestimate the value of the TEC calculated based on the otter diet. However, note that, although the diet- and sediment-based otter TECs are similar, the relative contributions of PCDDs and PCDFs versus PCBs would significantly impact the extent to which these two TEC calculations differ at a particular site due to differential bioaccumulation among PCBs, PCDDs, and PCDFs.



	otter diet (fish)	sediment
PCDD+PCDF	1.12	5.93
PCB	9.44	3.65
TEC (Total)	10.56	9.58

Figure 10. Mammal TECs calculated with TEFs-WHO_{05} appropriately from concentrations in diet versus inappropriately from concentrations in sediment.

TEC calculations for terrestrial birds and mammals exposed through food chains connected to contaminated soils should proceed in a manner parallel to the aquatic examples in Tables 5 and 6. The principal exposure pathway is soil to insect to mammal/bird through diet. Dietary uptake from ingestion of plant foods or soil through preening may in some cases provide important exposures. However, unlike aquatic systems in which respiration from water is an important exposure route, for terrestrial mammalian species, by analogy with humans, respiration of air is unlikely to be a significant direct exposure route for terrestrial organisms (ATSDR, 1998; http://www.cfsan.fda.gov/~lrd/dioxinqa.html#g4).

Although the TEC calculations are straightforward and fairly simple, multiple decisions need to be made beforehand. Some of these are described in Text Box 6. Decisions and assumptions used in the examples described in Tables 4, 5, and 6 include using measured BSAFs for Great Lakes trout and gulls (which assumes Great Lakes exposure and food web conditions are sufficiently representative of the aquatic system to be assessed), and selecting values for percent lipid for organisms and percent organic carbon for sediments.

Text Box 6. Questions when calculating TECs.

- Have I selected the appropriate species and identified a percent lipid for the whole organism, specific tissues of the organism, and/or the diet of the organism?
- Have I selected appropriate analytical methods for measuring concentrations of chemicals in sediment or water?
- Have I decided how to handle chemicals that have concentrations below the detection limit?
- Have I selected appropriate methods for measuring or estimating the fraction of organic carbon in the sediment at the site of interest?
- Have I measured or selected appropriate BAFs or BSAFs that will be used to estimate concentrations of each chemical in the organism's tissue or diet?
- Have I considered implications of biomagnification for higher trophic level organisms?
- Have I selected and applied the TEFs, RPFs, or RePs in a transparent fashion? (See Sections 3.3.1.3.)

Measured BAFs from one site, such as the Lake Ontario values used in the GLWQI (U.S. EPA, 1995a), the high-quality BSAF values from Lake Michigan (Burkhard *et al*., 2004), or EPA's BSAF data set (available at http://www.epa.gov/med/Prods_Pubs/bsaf.htm) may be extrapolated to another assessment site where similar measurements are either not possible (*e.g.,* chemicals not detectable in water) or feasible (*e.g.,* insufficient time, resources) (Burkhard *et al*., 2006). When the trophic level, food web, and the sediment-water concentration quotient, $\prod_{socw}$, are similar for two ecosystems, direct extrapolation of BAF_{ℓ}^{fd} values or BSAFs from one ecosystem to the other can be accurate if concentrations of chemicals in water or sediments are defined and measured in a consistent way for both sites. When conditions are not comparable, as often is the case, BAF_{ℓ}^{fd} values or BSAFs can be adjusted, using a basic food chain model, such as that of Gobas *et al*. (1993; 1998), for known differences in trophic level, food web, and/or $\prod_{socw}$. This should increase accuracy of measured BAF_{ℓ}^{fd} values or BSAFs when applied to an unmeasured system. An initial demonstration of such a "hybrid modeling approach" appears promising (Burkhard *et al*., 2006).

The case studies used for the 1998 EPA/DOI workshop (U.S. EPA, 2001a) present additional and more detailed examples of exposure characterizations. Many practical exposure and bioaccumulation assessment concerns were incorporated into these case studies, including how to employ the toxicity equivalence methodology in setting total maximum daily loads (TMDLs).

3.3.2. Three Dimensional Relative Potency Matrix – A Tool for Visualization and Selection of RePs or Derivation of RPFs

When applying the toxicity equivalence methodology an important consideration to be made is what relative potency factors to use for each dioxin-like chemical. One expected approach is to use the TEF-$WHO_{98/05}$ values, unless there is a need for more site- or species-specific calculations. When confronted with a lack of ReP data for the specific species and endpoint of concern, choices from alternative RePs or RPFs and the TEFs must be made.

The ideal RPF is species-specific for the effect endpoint of concern and based on dose metrics that best describe the toxicity data available, while effectively relating the dose-response relationship to environmental exposures. Data limitations do not negate the need to consider uncertainties and make optimum ReP/RPF/TEF-$WHO_{98/05}$ selection decisions for the particular problem formulation, species, and effects of concern. To this end, the three dimensional matrix depicted in Figure 11 provides risk assessors with a conceptual tool for selecting ReP values for derivation of assessment-specific RPFs. It provides an approach for evaluating the applicability of different ReP data associated with either the TEFs-$WHO_{98/05}$ or other RPFs that may be available (or that could be derived from the ReP data) and the types of uncertainty inherent to each. The rationale behind this hierarchal methodology is the mechanistic understanding of AHR-mediated toxicity as well as empirical data that support the extrapolation of relative potency data across endpoints and species. Using this concept, selection of RePs or derivation of RPFs can be based on a three-dimensional hierarchal approach involving use of the best available information relative to the ideal choice – a species-specific RPF for the endpoint of concern based on optimum dose metrics. Currently, the primary value of the three-dimensional matrix is to allow a visualization of the complex factors that influence the applicability of potentially diverse relative potency data for specific risk assessment scenarios. It could also facilitate efforts to describe uncertainties associated with ReP selections and/or RPF derivations. The matrix may also be helpful in describing and guiding research needs, and ultimately may lead to the development of more quantitative methods and further guidance for selecting RePs and deriving RPFs or proposing revisions to the TEFs-$WHO_{98/05}$.

The issues of species, endpoint, or dose metric differences in ReP data are separate from that of species differences in sensitivity to 2,3,7,8-TCDD. Two species that differ widely in their sensitivity to 2,3,7,8-TCDD can have relatively similar RePs for most dioxin-like chemicals. For example, chickens are 119-fold more sensitive than ducks to *in vitro* effects of 2,3,7,8-TCDD, yet for TCDF and PCB congeners 126 and 81, the *in vitro*-based RPFs differ less than 5-fold between these species (Kennedy *et al*., 1996). Similarly among fish, salmonids are the most sensitive species and zebrafish are the least sensitive species to the early life stage toxicity caused by 2,3,7,8-TCDD, with salmonids approximately 40-fold more sensitive than zebrafish (Elonen *et al*., 1998), yet RePs based on zebrafish *in vitro* endpoints (*i.e.,* CYP1A induction in liver) are generally within 5-fold of RePs determined in a variety of rainbow trout *in vitro* systems when the <u>same</u> endpoint in the <u>same</u> tissues are compared (Henry *et al*., 2001). Limited ReP data for fish embryos (bull trout, lake trout, rainbow trout, and medaka) suggest that species sensitivity to 2,3,7,8-TCDD is associated with small differences in RePs for PCB 126 when based on early life stage mortality. These differences in RePs are less than proportional to the differences in species sensitivity. Analysis of rainbow trout and zebrafish RePs suggests that uncertainties surrounding application of the toxicity equivalence methodology are likely to be greater when applying TEFs-WHO_{98} values or RPFs across tissues or endpoints than across fish species (Henry *et al*., 2001). At this time, data are lacking for making RPF comparisons between

sensitive and insensitive species based on *in vivo* toxicity of greatest concern. In summary, there are presently insufficient data to determine definitively if there is any association between sensitivity to 2,3,7,8-TCDD and RePs for different species.

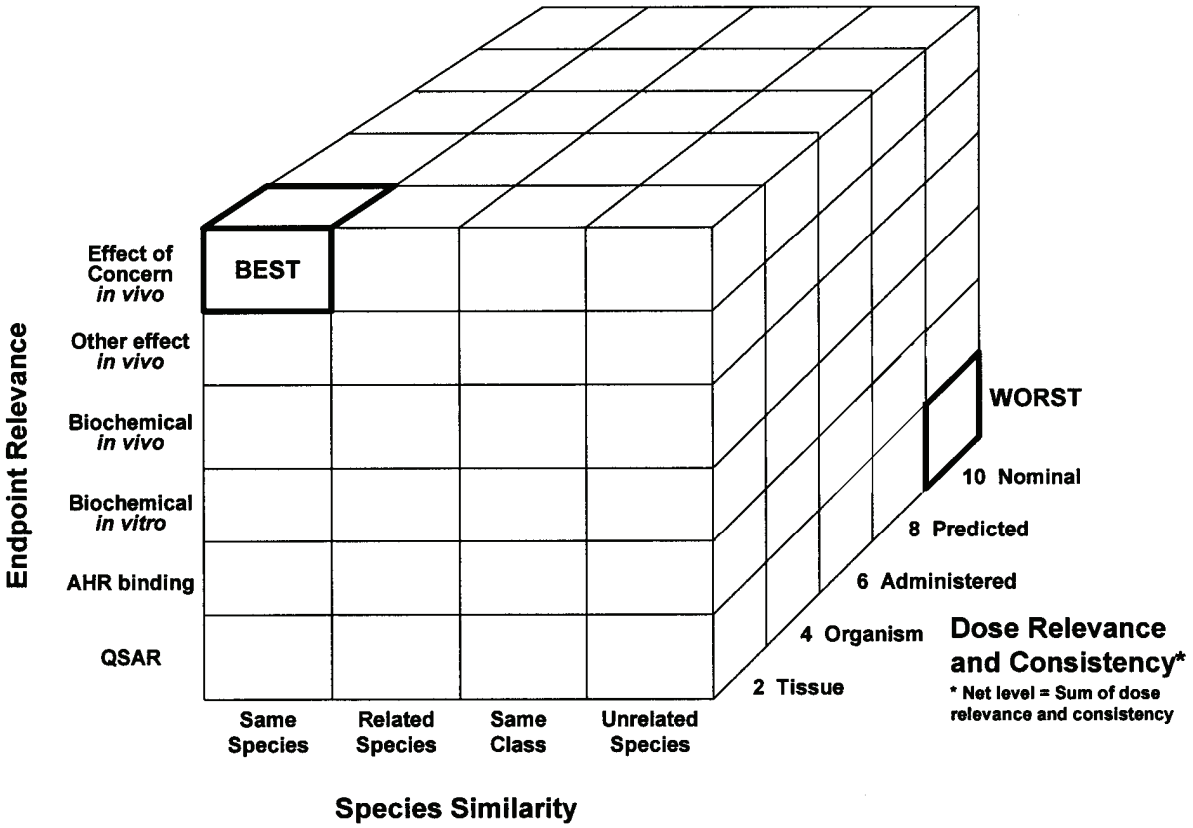


Figure 11. Three dimensional relative potency matrix for selection of RePs and derivation of RPFs for risk assessment.

Selection involves consideration of how relevant a toxicity test endpoint is to the endpoint of concern (y-axis); how similar a tested species is to the species of concern (x-axis); and how relevant the dose metric for an ReP value is to the optimum dose metric, while being consistent with the dose metric of 2,3,7,8-TCDD dose-response relationship to be used (z-axis). ReP values with the closest association to the species and toxic effect of concern, and based on doses measured in the tissues that are targets for toxic effects, should best minimize uncertainty while maximizing relevance ("BEST" cube).

3.3.2.1. *Endpoint Relevance*

The y-axis of the matrix represents six levels that correspond to the various *in vivo*, *in vitro*, and biochemical endpoints used currently to determine relative potency of dioxin-like chemicals. The levels from bottom to top represent a preferential ranking of endpoint categories based on probable increasing relevance to the species of concern for which RPFs are to be derived. The order of preference is similar to that used in deriving the TEFs-$WHO_{98/05}$ for fish, birds, and mammals (Van den Berg *et al.*, 1998; 2006). The highest preference is given to RePs determined for *in vivo* toxicity endpoints.

In this matrix, Level 1 is reserved for *in vivo* toxicity data for the endpoint of concern (*e.g.,* early life stage mortality or reproductive failure). Level 2 is for other *in vivo* toxicity endpoints that may be less directly connected to the assessment endpoint of concern (*e.g.,* growth or behavior). Biochemical effect endpoints, such as CYP1A1 induction, are distinguished as Level 3 for effects *in vivo* and Level 4 for effects *in vitro* because *in vitro* data tend to be less toxicokinetically realistic than *in vivo* data. Level 5 is assigned to RPFs based on binding affinity to the AHR, which is a biochemical endpoint considerably upstream from toxicities of concern, and thus more distantly related to typical ecological assessment endpoints. Consistent with the TEFs-$WHO_{98/05}$ selection process (Van den Berg *et al*., 1998; 2006), Level 6 is reserved for quantitative chemical structure-activity relationships (or QSARs) that may be more or less quantitative in comparing AHR agonist potencies to 2,3,7,8-TCDD for a variety of endpoints.

3.3.2.2. *Species Similarity*

The x-axis in the matrix is a scale for the phylogenetic similarity of the species of concern to the species for which RePs are available. It is divided into four levels, reflecting different degrees of uncertainty, with uncertainty increasing from left to right. If RePs are available for the species of concern (Level 1), little uncertainty related to interspecies sensitivity is involved in using the RePs to calculate an RPF. If ReP data are available for a closely related species (Level 2), for example, a species within the same genus or family, uncertainty is greater. The TEFs-$WHO_{98/05}$, although based in some cases on species-specific data, are based on class generalizations and are thus represented in Level 3. In cases when the TEFs-$WHO_{98/05}$ are based on a single species the same as or closely related to the species of concern, the TEFs-$WHO_{98/05}$ may equate to Level 1 or Level 2, respectively. If ReP data are from a more distantly related species within the same class, uncertainty increases (Level 4). When ReP data for a dioxin-like chemical is available for multiple species, the magnitude of the difference or similarity in the RePs across the levels can be used to gauge the uncertainty associated with using RePs for those dioxin-like chemicals having only one level of data available.

The basis for reflecting phylogenetic similarity in the matrix is both theoretical and empirical. The assumption that two species that are more closely related phylogenetically will have RPFs (determined for the same endpoint) that are similar or even identical is supported by data. For example, RePs for PCB 126-induced early life stage mortality in lake trout and rainbow trout vary by less than a factor of two (Zabel *et al*., 1995). However, it is clear that more data on the relative potency of dioxin-like chemicals to produce various effects in additional species are necessary to more systematically test this assumption.

3.3.2.3. *Dose Relevance for Effect and Consistency with Dose-Response Relationship*

The z-axis of the matrix represents the degree to which the dose data, associated with different sets of RePs, are related to the effect of concern (dose relevance) and are consistent with the specific 2,3,7,8-TCDD dose-response relationship chosen for the assessment (dose consistency). All effects of concern are assumed to be best related to concentrations of dioxin-like chemicals, at relevant times, and in specific tissues associated with the mechanism of action (Level 1). Concentrations in whole bodies of affected organisms (Level 2) are more commonly the best available and relevant dose metric. Administered doses (Level 3) include injection and dietary exposures. Level 4 includes doses that are predicted based on mechanisms of fate and uptake during exposures from water, sediments, or other exposure media from which uptake is less certain than for Level 3. Level 5 includes doses that are based simply on the nominal mass of the chemical used in the toxicity test, rather than on measurement during the test. Most *in*

vitro effects-based ReP dose data probably fall into Level 5 because concentrations of the chemicals are often not measured in cell cultures.

The extent to which the dose-metric of the 2,3,7,8-TCDD dose-response relationship is relevant to the endpoint and species associated with the ReP data may be best considered in tandem with the dose relevance of the ReP data. The matrix illustrates a strategy for doing this by setting the z-axis scale from 2 to 10 to allow ReP dose relevance and 2,3,7,8-TCDD dose-response consistency to be summed. For example, if avian ReP data for PCB 126 involve chicken embryo mortalities based on doses measured as concentrations of PCB 126 or 2,3,7,8-TCDD in the diets of female chickens (Level 3 - administered dose), but a 2,3,7,8-TCDD dose-response relationship is available for the risk assessment based on the concentration of 2,3,7,8-TCDD in embryos associated with embryo mortality for a closely related bird species (Level 1 - dose measured in tissue), the net dose metric relevance and consistency level for selecting the ReP data could be set at 4 (3+1) on a scale of 2 to 10. This example should not be regarded as a prescription, but only as an illustration of how the uncertainties associated with combined multiple dose expressions associated with the toxicity data might be considered in choosing the most appropriate ReP values for a particular assessment.

A third dose-related concern is the specificity and accuracy of the analytical methodology used for the available relative potency data. Because dose metrics impact ReP choices, evaluation of potential systematic errors associated with the analytical methodology should be considered as a final quality assurance step in choosing RePs. Dose data suspected of having significant errors that increase uncertainty associated with RPFs effectively place the RPF in a lower dose specificity level. An example of data that could fall into this category is relative potency determined in the presence of potent impurities or synthetic byproducts in test chemicals that could cause or contribute to the observed effects. For example, certain PCDFs are known to contaminate PCB congener standards (Goldstein *et al*., 1978; Elliott *et al*., 1997; National Toxicology Program, 2006; U.S. EPA, 2001a). Contamination of test samples usually becomes a problem when the contaminant causes the relative potency of the test chemicals to be overestimated. Other sources of dose measurement errors may be related to limitations of analytical methods.

3.3.2.4. *Application of Three Dimensional Relative Potency Matrix – Examples of ReP Data Prioritization Choices for Deriving RPFs*

The matrix (Figure 11) is not a purely quantitative and unambiguous model. Therefore, any number of questions concerning specific data may arise with its use in risk assessments. A few examples of such questions are presented here to assist in understanding how the approach can be used to consider and select RePs or derive RPFs from the types of ReP data available.

The three examples should be regarded as illustrative of the variety of considerations that may be involved in selecting RePs or deriving RPFs for specific applications. Choices are suggested primarily to complete the illustrations, not as prescriptions for specific applications. The complexities involved in evaluating RPFs as alternatives or adjuncts to TEFs illustrate the value of using TEFs-$WHO_{98/05}$, which are based on expert opinion, in an assessment.

3.3.2.4.1. *Example 1: Incomplete ReP data sets.*

As ReP data sets are often incomplete, it is appropriate to derive RPFs from different ReP data sets in order to calculate a TEC for a specific species. For example, in performing an ecological risk assessment for lake trout based on early life stage mortality, the only ReP that exists specifically for lake trout is for PCB 126. For other dioxin-like chemicals, RePs exist only

for rainbow trout or other fish species. The PCB 126 ReP for lake trout is based on early life stage mortality, with the dose measured as the concentration in the embryo. Therefore, it is appropriate to choose the lake trout ReP for PCB 126 and rainbow trout RePs for the other congeners. In this specific case, since PCB 126 is the most potent PCB, choosing a more species-specific ReP probably increases accuracy of the TEC for lake trout, at least in situations where PCBs are a predominant proportion of the TEC. Insufficient data exist to determine if use of rainbow trout based TEFs for the other congeners may over- or underestimate the TEC for lake trout (with respect to the 2,3,7,8-TCDD dose-response relationship based on lake trout).

3.3.2.4.2. *Example 2: Species similarity versus endpoint similarity.*

Selecting RePs or deriving RPFs on the basis of species similarities versus endpoint similarities, in the absence of data that would allow one to quantify the uncertainty in each, creates difficult questions. For example, early life stage mortality risks for Caspian terns, using measured, congener-specific concentrations of PCDDs, PCDFs, and PCBs in tern eggs, cannot be assessed with RePs specifically based on early life stage mortality in Caspian terns because such RePs do not exist. The only bird early life stage mortality data for 2,3,7,8-TCDD (*i.e.,* dose-response data for conducting the effects assessment) are for chickens and pheasants. It is well established that chickens are exceptionally sensitive to 2,3,7,8-TCDD induced embryo mortality relative to other bird species. Assume, based on knowledge of population responses of Lake Ontario Caspian terns to historical 2,3,7,8-TCDD exposures, that the terns are significantly less sensitive than chickens. Therefore pheasant, rather than chicken, early life stage mortality data for 2,3,7,8-TCDD was chosen for application in the effects assessment for Caspian terns.

Assume there are RePs for (A) *in vitro* CYP1A induction in liver cells of Caspian terns, (B) *in vivo* early life stage mortality in domestic chickens (used to establish the TEFs-WHO_{98}) and (C) *in vivo* CYP1A induction in embryos of common terns, a closely related species. Table 7 illustrates the positions these three types of data would have in the species-endpoint specificity matrix. Which of these three sets of ReP data would provide the most accurate estimate of the embryo TEC for a population of Caspian terns? The TEFs-WHO_{98}, based largely on chicken embryo mortality, might be regarded as preferable because the endpoint used is more relevant to the effect of concern. However, differences between TEFs-WHO_{98} and tern RePs could indicate some fundamental difference between terns and chickens in the relative potencies of dioxin-like chemicals. Under these conditions, the greater species specificity of tern CYP1A induction based RePs might be considered more relevant than the higher endpoint specificity of most of the chicken based TEFs. Since Caspian terns are very closely related to common terns, RePs or RPFs based on *in vivo* CYP1A induction in embryos of common terns should be preferred over the RePs or RPFs based on *in vitro* CYP1A induction in liver cells of Caspian terns due to greater endpoint relevance. One option when confronted with such difficult choices is to calculate TECs with both sets of RePs or RPFs. The comparison may indicate both the magnitude and sources of the uncertainty (*e.g.,* specific dioxin-like chemicals with large differences in RePs).

Table 7. ReP selection matrix for Caspian terns (example 2).

In this example, the risk assessor is faced with choosing from (A) RePs based on *in vitro* effects in the species of concern, (B) RePs based on *in vivo* effects of concern in an unrelated species, or (C) RePs based on *in vivo* effects in a related species.

Endpoint	Taxonomic Relationship to Species of Concern			
	Same Species	**Related Species (*e.g.*, same genus or family)**	**Class-Specific TEFs-WHO_{98}**	**Unrelated Species**
Effect of Concern *in vivo*	*No data*		(B) Chicken early life stage mortality data	
Other Toxic Effect *in vivo*				
CYP1A induction *in vivo*		(C) Common Tern data		
CYP1A induction *in vitro*	(A) Caspian Tern data			
AHR binding				
Structure Similarity				

3.3.2.4.3. ***Example 3: Dose-response and exposure relationships.***

As described in Section 3.3.1.3 of this report, the dose metric used in an exposure analysis should be consistent with the dose metric associated with the dose-response relationship chosen for the risk assessment. It follows that the dose metric basis for the ReP (RPF or TEFs) selected in an assessment should be as consistent as possible with the dose metrics for both the exposure analysis, as reflected in the dose specificity axis of Figure 11, and the dose-response relationship. Example 3 illustrates how the choice of a dose-response relationship and options for the exposure assessment may influence the choice of RePs.

The case is founded on a study by Tillitt *et al*. (1996), who assessed risk of reduced mink kit survival as a consequence of exposure of female mink through a diet of contaminated fish. Concentrations of PCDDs, PCDFs, and PCBs in both the fish fed to mink and in the livers of the exposed mink dams were measured as alternative exposure expressions.

Two sets of RPFs, the TEFs-WHO_{94} (the TEFs-WHO_{94} <u>for mammals</u> are essentially the same as the TEFs-WHO_{98}) and a set of RPFs based on rat liver H4IIE cell CYP1A induction, were used to estimate alternative TECs that represent kit survival thresholds. The result was four separate kit survival threshold TECs:

<u>Diet-Based TECs</u>:

- 1.9 pg 2,3,7,8-TCDD equivalence/g diet based on TEFs-WHO_{94}.
- 4.4 pg 2,3,7,8-TCDD equivalence/g diet based on rat liver H4IIE cell-RPFs.

Tissue-Based TECs:

- 60 pg 2,3,7,8-TCDD equivalence/g mink dam liver based on TEFs-WHO_{94}.
- 70 pg 2,3,7,8-TCDD equivalence/g mink dam liver based on rat liver H4IIE cell-based RPFs.

Note that the dose-response relationship between exposure to 2,3,7,8-TCDD alone and kit survival was not examined in the Tillitt *et al*. (1996) study. Only the mixture of PCDDs, PCDFs, and PCBs present in the fish diet and mink livers were evaluated.

Consider a risk assessment that involves the effects of fish contamination on mink kit survival based on a field data set that includes concentrations of PCDDs, PCDFs, and PCBs both in several species of fish and in livers of mink from the area. The Tillitt *et al*. (1996) paper is the logical source for the dose-response relationship because it involves both the species of concern and the endpoint of concern, particularly given that no reproductive effects data for 2,3,7,8-TCDD have been reported for mink or any other mammalian wildlife species. Selection of both the exposure metric and the RePs for the assessment should be consistent with the dose-response relationship used. Hence, if a TEC based on mink dam liver is selected from the study by Tillitt *et al*., then clearly using the field data set from the mink liver would be a more comparable exposure dose metric or diet data. Conversely, if a mink diet TEC from the Tillitt *et al*. study is chosen for the effects characterization, then exposure should be based on the field data set based on fish contamination and RPFs based on dietary administration.

Which exposure metric would be preferable, the fish diet or the mink dam liver concentrations? In this case the mink dam liver chemical residue data probably provide a more direct and precise measure of exposure than would reconstruction of the average dietary exposure from the fish monitoring data. Theoretically, the net effect of metabolism and biomagnification on the mixture composition *in vivo* is better accommodated by basing the TEC on concentrations in the mink dam liver, rather than as administered in the diet. The question then becomes, which ReP set has the greater dose specificity if mink dam liver based exposure data are chosen? Both TEFs-WHO_{05} and rat liver H4IIE cell-RePs are based on administered doses and thus cannot be used in a manner completely consistent with the dose metric (measured concentrations in liver tissue) for the liver dose-response relationships available (Tillitt *et al*., 1996). However, since the rat liver H4IIE cell-RePs are based on administered dose to liver cells, they circumvent potential errors associated with biomagnification that would affect RePs based on doses administered through diet. If rat liver H4IIE cell-RePs are used to derive a TEC for this risk assessment, then they should also be used in deriving the threshold TEC from the Tillitt *et al*. study (*i.e.,* the selected threshold TEC would be 70 pg 2,3,7,8-TCDD equivalence/g mink dam liver).

A third choice of liver exposure RePs exists: a partial set of RePs based on hepatic EROD induction in female mice following sub-chronic exposures characterized as measured concentrations in liver of PCDDs and PCDFs (DeVito *et al*., 1997) and PCBs (DeVito *et al*., 2000). The mouse liver EROD-based ReP data for PCDDs and PCDFs are similar to both TEFs-WHO_{05} and rat liver H4IIE cell-RPFs. However, the mouse liver EROD-based ReP data for PCBs are more similar to the rat liver H4IIE cell-RePs than TEFs-WHO_{05}. Since the mouse liver EROD RePs are based on measured concentrations in the livers as well as *in vivo* responses, they are more dose-specific than TEFs-WHO_{05} or the rat liver H4IIE cell-RePs to the chemical concentrations measured in mink dam livers. Therefore, the best choice for RePs in this case is

probably those based on mouse liver EROD RePs, supplemented with rat liver H4IIE cell RePs for dioxin-like chemicals without mouse liver EROD RePs.

If the risk assessor chooses to use fish diet as the exposure measure, it would be more consistent to employ RePs or RPFs based on administered dose. In that case, the TEFs-WHO_{05} probably would be preferable to the rat liver H4IIE cell-RPFs or mouse liver EROD-RePs. This in turn would necessitate selection of the threshold TEC of 1.9 pg 2,3,7,8-TCDD equivalence/g diet based on TEFs-WHO_{94} from Tillitt *et al*. (1996).

When choices for RePs or RPFs must be made for alternative dose-response relationships as well as alternative dose expressions for ReP data (as summarized for example 3 in Table 8) to what extent can one determine which set of RePs or RPFs is the most accurate? Lacking a site-specific mink bioassay, there is insufficient information to be sure which set provides a more accurate result, but maintaining consistency in the selection of the dose-response relationship, the exposure metrics, and the RePs reduces the potential for systematic errors. As pointed out in example 2, comparison of calculations using the alternative RePs may be helpful in describing the range of possible risk values. In the case of Tillitt *et al*. (1996), differences between the alternative RePs for the PCBs were most responsible for the differences in TECs for the TEFs-WHO_{05} versus the rat liver H4IIE cell-RePs (PCBs were responsible for about 60% of the TECs for the TEFs-WHO_{94} compared with 10% for the rat liver H4IIE cell-RePs). Therefore, applications of the RePs or RPFs that are inconsistent with the choice of TEC-effect relationship would likely have a more significant effect on the final risk estimates at sites where PCBs are present at high concentrations, relative to PCDD and PCDF concentrations.

Table 8. ReP selection matrix for mink (example 3).

The risk assessor is seeking to select RePs, RPFs, or TEFs that are most consistent with the species, endpoint, and dose metrics used for each of four possible dose-response relationships from Tillitt *et al*. (1996). The advantages and disadvantages of alternative sets must be considered.

TEF or RPF	**Characteristics of optimal mink RPFs** If using the dose-response relationships and exposure metrics presented in Tillitt *et al*. (1996)		**Characteristics of available TEFs/RePs from which to select**		
			TEFs-WHO_{94}	**Rat liver H4IIE cell**	**Mice liver (partial set)**
Species	Mink		Mammals as a class (based primarily on rodents)	Rats	Mice
End point	Kit survival		Vary depending on the dioxin-like chemical; includes subchronic or chronic effects *in vivo* and *in vitro*	EROD induction *in vitro*	EROD induction *in vivo*
Dose	TEC in diet based on concentrations in fish	TEC in mink dams based on concentrations in liver	For *in vivo* endpoints, based on concentrations in diet	As added to cell culture	Measured in liver tissue

3.3.2.5. *Summary of Selection of TEFs, RPFs, or RePs*

When applying the toxicity equivalence methodology an important consideration to be made is what relative potency factors to use for each dioxin-like chemical. One expected approach is to use the TEF-$WHO_{98/05}$ values, unless there is a need for more site- or species-specific calculations. When confronted with a lack of ReP data for the specific species and endpoint of concern, choices from alternative RePs or RPFs and the TEFs must be made. This necessary choice may be used to minimize uncertainty based on differences in species, endpoints, and/or dosimetry associated with specific relative potencies. Uncertainties associated with the use of TEFs and RPFs or RePs are separate from the species differences in sensitivity to 2,3,7,8-TCDD. The former affects the accuracy associated with exposure characterization (*i.e.,* the 2,3,7,8-TCDD TEC to which the species is exposed), whereas the latter impacts the effects characterization (*i.e.,* the species-specific dose response for 2,3,7,8-TCDD). While data are currently insufficient to determine definitively the type of uncertainty that is greater, a larger uncertainty for species response to 2,3,7,8-TCDD does not reduce the need to minimize uncertainties associated with calculation of exposure and, therefore, the selection of RePs, RPFs, and TEFs.

A best available information methodology using the three dimensional matrix (Figure 11) is recommended for ReP selection. Species specificity, endpoint specificity, and dose specificity/consistency are the three factors to consider when creating a hierarchy of possible ReP data for each chemical. To the extent dose specificity is related to the endpoint and species associated with each candidate set of RePs, it may be best considered after characterizing the endpoint and species specificity of available RePs. When relative potency data for a mixture of chemicals lack consistency for species, endpoint, or dose metric, systematic errors associated with excluding chemicals with inconsistent RePs from the TEC analysis may well exceed any errors associated with use of the weak relative potency data. However, in the absence of more specific RePs or RPFs for the species and endpoint of concern, the vertebrate class-specific TEFs-$WHO_{98/05}$ are expected, in most cases, to be used for the assessment. In other cases with more ReP data choices, final selection of ReP may involve use of sensitivity analysis based on TECs calculated using alternative RePs.

Through the three examples that illustrate application of the ReP matrix, several additional considerations were identified:

- Species specificity for ReP/RPF selection/derivation should be based on the species being assessed, not the species on which the dose-response relationship is based.

- RePs/RPFs based on *in vivo* CYP1A induction in a closely related species may be preferable to RePs/RPFs based on a more endpoint-specific effect in an unrelated species, especially when significant differences in the RePs/RPFs may be attributable to differences in toxicokinetic or toxicodynamic factors in the species.

- The dose metrics for the RePs, RPFs, or TEFs used should be as consistent as possible with the dose metrics for both the dose-response relationship and the exposure analysis.

- Accuracy of TECs is probably increased when more species-specific and endpoint-specific RePs/RPFs are used for a key chemical.

- In some cases the most applicable dose-response relationship may be based on TECs, determined with a specific set of RePs for a complex mixture, rather than concentration of 2,3,7,8-TCDD alone (*e.g.,* the Tillitt, *et al*. (1996) study derived a TEC-based toxicity reference value).

- The choice of a specific dose-response relationship may be influenced by the ReP data available and the nature of exposure measurements available.

3.3.3. Characterization of Ecological Effects

An ecological effects analysis includes an examination of all data describing the effects of the specific chemicals of concern. This analysis concludes with a stressor-response profile. PCDDs, PCDFs, and PCBs present in the environment are generally found as complex mixtures. An assessment of their ecological risk requires both quantifying their individual exposures and developing a stressor-response profile for their cumulative effects. Figure 7 includes a dose-response curve illustrating the relationship between early life stage mortality and exposure to 2,3,7,8-TCDD, one example of a relationship that can be used in developing a stressor-response profile.

Demonstrated toxic effects of 2,3,7,8-TCDD in wildlife species include adverse effects on reproduction, development, cardiovascular, and endocrine functions; wasting syndrome; immunotoxicity; and mortality. Effects in fish larvae exposed to 2,3,7,8-TCDD include pericardial, yolk sac, and meningeal edema; impaired jaw development; impaired heart development and function; reduced trunk blood flow; anemia; hemorrhage; growth retardation; and mortality. While 2,3,7,8-TCDD is by far the most studied of the dioxin-like chemicals, a number of other PCDDs, PCDFs, and PCBs have been shown to cause toxic responses similar to 2,3,7,8-TCDD in both laboratory and field situations. A summary of effects associated with exposure to 2,3,7,8-TCDD and related chemicals in different fish, bird, and mammalian species is presented in Table 3. For further information regarding effects observed specifically in wildlife, refer to U.S. EPA (1993, 2001b) and references therein. Many of the toxicological studies used in generating RePs, RPFs, and TEFs are also the critical studies that provide a basis for evaluating the causal connection between exposure to dioxins and potential effects.

A stressor-response profile for the cumulative effects of PCDD, PCDF, and PCB mixtures is typically based on the stressor-response profile for 2,3,7,8-TCDD. This is because it is often the only or best available data for endpoints of concern for this chemical. Recall that in applying the toxicity equivalence methodology, TEFs or RPFs ‘convert’ the various dioxin-like chemical concentrations into a ‘common currency,’ the TEC, which is a 2,3,7,8-TCDD equivalent concentration. If sufficient data are available, however, it may be possible to develop stressor-response profiles for chemicals other than 2,3,7,8-TCDD. Such an approach has been employed when particular dioxin-like chemicals other than 2,3,7,8-TCDD dominate the estimated TEC (*e.g.,* PCBs).

3.4. CONSIDERATIONS IN RISK CHARACTERIZATION

In risk characterization, the final phase of ecological risk assessment, the exposure profile and stressor-response profile developed during the analysis phase are combined to realize the final estimate of risk. Development of a risk estimate using the toxicity equivalence methodology is described in Section 3.4.1. Lines of evidence including field and laboratory studies and process models are discussed in Section 3.4.2. The uncertainties in the methodology and its application to ecological risk assessment are summarized in Section 3.4.3. Text box 7 identifies important questions to consider for risk characterization.

Text Box 7. Questions for risk characterization.

- Have I clearly presented the assumptions and uncertainties associated with applying the toxicity equivalence methodology and in preparing the risk estimates based on TECs?
- Have I considered multiple lines of evidence, such as bioanalytical tools, bioassays, field surveys, or other relevant RPFs?
- Have I considered the evidence for causality associated with each line of evidence?

3.4.1. Risk Estimation

When the toxicity equivalence methodology is used, exposure is expressed by the TEC, which reflects the combined contribution of the individual dioxin-like chemicals that comprise the mixture. Effects are usually estimated based on studies of the toxicity of 2,3,7,8-TCDD. TEC values for the ecological risk assessment are compared to available 2,3,7,8-TCDD toxicity values to estimate the likelihood and magnitude of effects. The type of comparison depends on the nature of both exposure and effects information. The simplest risk estimation method is the quotient method. It is the ratio of the toxicity equivalence exposure point concentration divided by a toxicity reference value; with quotients exceeding "one" qualitatively suggesting an increased likelihood for effects:

$$Risk\ Estimate = \frac{TEC}{2,3,7,8 - TCDD\ Toxicity\ Reference\ Value} \tag{3-10}$$

The quotient method for estimating risk has a number of limitations. As a single point estimate of risk for one species or endpoint, it does not provide a means of quantitatively expressing the probability of risk or uncertainty. Numerous approaches for estimating risk and describing uncertainty are available and should be examined before selecting one method for combining exposure and effects data. For example, more sophisticated models may be used to combine the exposure and toxicity information into distributions that may allow for the development of probability density functions, if data are adequate. Additional discussion of stressor-response profiles and methods for risk estimation in ecological risk assessment are available in the *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998).

3.4.2. Lines of Evidence

This framework presents considerations for the application of RePs, RPFs, and TEFs-$WHO_{98/05}$ in the development of a line of evidence to complete an ecological risk assessment for dioxin-like chemicals. Risk assessments may, however, also include other lines of evidence derived from bioanalytical tools, field surveys, or similar data that can be incorporated into the risk characterization (Text Box 7). For example, field studies may be available that evaluate

mortality and reproductive success of fish, birds, and mammals likely to be affected by dioxin-like chemicals, thereby offering a means to compare risks estimated using the toxicity equivalence methodology to observed field effects. The toxicity equivalence methodology has recently been applied using both historical field data and laboratory toxicity data in a retrospective assessment of risks posed by dioxin-like chemicals to lake trout in Lake Ontario (Cook *et al*., 2003).

Additional lines of evidence that may be appropriate for evaluating TECs in environmental samples may be derived from a variety of bioanalytical tools developed for this purpose. For example, measurement of chemically activated gene expression via CYP1A1 (*e.g.,* EROD) or luciferase [*e.g.,* chemical-activated luciferase gene expression (CALUX)] activity (Garrison *et al*., 1996; Sanderson *et al*., 1996; Richter *et al*., 1997) in a variety of wild-type or recombinant mammalian (*e.g.,* H4IIE rat hepatoma, Hepa 1c1c7 mouse hepatoma) and fish (RTH-149 rainbow trout hepatoma) cell lines has been used to characterize total dioxin-like activity in environmental samples. Examples include:

1) bird eggs (Tillitt *et al*., 1991; Williams *et al*., 1995);

2) mink liver (Tillitt *et al*., 1996);

3) sediments and pore water (Murk *et al*., 1996);

4) newspapers (Seidel *et al*., 2000); and

5) combustion gas, fly ash, PCB oil, and animal feed (Behnisch *et al*., 2002).

Several reviews summarize the strengths and limitations associated with these bioanalytical tools (Behnisch *et al*., 2001; Seidel *et al*., 2000; Denison *et al*., 1999; Giesy *et al*., 2002; Hahn, 2002b). These bioanalytical tools have the advantage of integrating the total activity of complex mixtures of AHR agonists. Also, bioanalytically derived TECs can typically be obtained more quickly and at a lower cost than TECs obtained by chemical analysis. Several potential problems are associated with these tools, however (see Behnisch *et al*., 2001 for detailed discussion). They may overestimate the toxic potency of chemicals that are rapidly metabolized *in vivo* and are therefore not a replacement for *in vivo* tests (Van den Berg *et al*., 1998; 2006). Experts at the EPA/DOI workshop (U.S. EPA, 2001a) concluded that the potential for generating false positive responses was high in situations where potent EROD-inducing, non-dioxin-like chemicals (*e.g.,* PAHs) are abundant. Another important shortcoming of these bioanalytical tools is that they are not chemical specific (Schmitz *et al*., 1996) and so cannot be used to show causality for individual chemicals or classes of chemicals in environmental samples nor can the results derived from them be used in fate and transport or food chain modeling.

Due to current technical limitations, lack of standard testing procedures, and lack of established quality criteria associated with existing bioanalytical tools (for summary see Behnisch *et al*., 2001), the experts at the EPA/DOI workshop concluded that such bioanalytical tools should *not* be used as an alternative to congener-specific analysis and the toxicity equivalence methodology. Rather, these bioanalytical tools may be considered as additional lines of evidence for characterizing ecological effects of PCDDs, PCDFs, and PCBs.

The availability and utility of additional lines of evidence, whether they be bioanalytical tools, field data or surveys, or other relevant information, should be discussed and described during the planning and problem formulation phases of the ecological risk assessment.

3.4.3. Summary of Uncertainties

One of the components of a successful risk assessment is identifying and quantifying uncertainties. This section provides a summary of both the uncertainties inherent to the toxicity equivalence methodology and the uncertainties associated with the application of the methodology in ecological risk assessment. Uncertainties associated with the TEFs-$WHO_{98/05}$ and their application to ecological risk assessment are only briefly discussed here, but are described in detail in Van den Berg *et al*. (1998; 2006) and U.S. EPA (2001a). Uncertainties associated with interpreting the ecological significance of toxicity from dioxin-like chemicals are not discussed in this framework, but may be found in U.S. EPA (1993; 1995b, c; 2001b).

3.4.3.1. *Uncertainty Associated With the Toxicity Equivalence Methodology*

While there are uncertainties associated with the application of the toxicity equivalence methodology, they are believed to be, in aggregate, less significant than those associated with other aspects of the risk assessment process and those associated with other approaches for assessing risks of dioxin-like chemicals. Uncertainties in the toxicity equivalence methodology are related to the assumptions and procedures used to derive the TEFs-$WHO_{98/05}$, RPFs, or RePs, as well as the relative potency data underlying these values.

3.4.3.1.1. *AHR ligands.*

The TEFs-$WHO_{98/05}$ include only those PCDDs, PCDFs, and PCBs known to elicit AHR-mediated responses. Currently there are consensus TEFs for 29 PCDD, PCDF, and PCB congeners. Derivation of RPFs for other dioxin-like chemicals is possible based on existing or emerging ReP values (Villeneuve *et al*., 2000). Field surveys or bioanalytical tools may provide another line of evidence regarding whether dioxin-like toxicity risks are fully represented by the TEFs-$WHO_{98/05}$.

3.4.3.1.2. *Additivity assumption.*

The fundamental assumption of the toxicity equivalence methodology is that exposure concentrations of PCDDs, PCDFs, and PCBs are additive when expressed as toxicity equivalence concentrations. Section 2.1 describes the theoretical and empirical basis for the assumption of additivity. Van den Berg *et al*. (1998; 2006) and the NRC (2006) concluded that use of an additive toxicity model is the most plausible approach for assessing combined risks from dioxin-like chemicals, despite the fact that some non-additive interactions among chemicals have been reported (Van Birgelen *et al*., 1996b). Antagonistic effects are usually seen above environmentally relevant doses. Therefore, the assumption of additivity in the toxicity equivalence methodology is unlikely to result in large errors when antagonists are present (Van den Berg, 1998; 2006). Considerable experimental data for ecologically relevant exposures and toxicity endpoints support the additivity assumption, with no evidence of antagonism or synergism (Walker and Peterson, 1991; Walker *et al*., 1996; Zabel *et al*., 1995; Tillitt *et al*., 1996). The assumption of additivity was further supported by recent experimental data from Walker *et al*. (2005) that showed that the TEFs-WHO_{98} adequately predicted the increased incidence of liver tumors in mammals with exposure to a mixture of TCDD, 2,3,7,8- PeCDF, and PCB 126. Likewise, the NRC review of the draft *Exposure and Health Reassessment of 2,3,7,8-*

TCDD and Related Compounds (U.S. EPA 2003a), included an evaluation of the additivity assumption (NRC, 2006). The NRC Committee concluded that "from an overall perspective, this assumption appears valid, at least in the context of risk assessment" (NRC, 2006).

3.4.3.1.3. *Relative potency data.*

Inaccuracies in individual dose-response studies used to determine relative potencies of dioxin-like chemicals, as well as the variability among alternative ReP values, are sources of uncertainty in TEFs-$WHO_{98/05}$, RPFs, and RePs. Accuracy of relative potency estimates may be attributed to factors such as purity of the test chemicals, study design (*e.g.,* exposure regimens and endpoints measured), and measurement errors. Variability in relative potency data may be attributable to factors such as precision of dose and effects measurements, the calculation technique (*e.g.*, ED_{50} or LD_{50} ratios, LOEL or NOEL ratios, NOEC or LOEC ratios, benchmark dose ratios) used and the natural variability among organisms of the same species in their response to dioxin-like chemicals. In deriving the TEFs-$WHO_{98/05}$, the expert panel preferred RePs derived from ED_{50} or LD_{50} ratios; when full dose-response relationships were not available (precluding calculation of ED_{50} or LD_{50}), RePs based on LOELs or benchmark doses were deemed usable, but were considered to have more uncertainty associated with them (van den Berg *et al.*, 2006). Because relative potency data sets are inherently heterogeneous, uncertainties in the data used to select TEFs-$WHO_{98/05}$, RPFs, or RePs should be analyzed on a case-by-case basis.

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

3.4.3.1.4. *Point estimates.*

The TEFs-$WHO_{98/05}$ and RPFs are point estimates even though the experimental data from which they are derived may range over several orders of magnitude. Hence, TEFs-$WHO_{98/05}$ and RPFs include uncertainty in the individual RePs, as well as the uncertainty in the method used to aggregate the data to derive the TEF-$WHO_{98/05}$ or RPF. Because of the multiple biological models used for deriving ReP values for a particular chemical, it is difficult to estimate the variability or uncertainty of a TEF-$WHO_{98/05}$ or RPF point estimate. However a qualitative assessment of uncertainties associated with the use of TEFs-$WHO_{98/05}$ or RPFs is possible. When evaluating uncertainties associated with use of TEFs-$WHO_{98/05}$ or RPFs the following should be considered:

- Qualitative judgments, based on expert opinion, of data quality and confidence in ReP values are embodied in establishment of the TEFs-$WHO_{98/05}$.

- Rounding TEFs to harmonize results across vertebrate classes (Van den Berg *et al.*, 1998; 2006) may have introduced systematic errors in the TEFs-$WHO_{98/05}$ (U.S. EPA, 2001a).

- Multiple RePs will provide a means of assessing the uncertainty associated with the ReP, and by extension, with an RPF derived from multiple RePs.

- In a few cases, standard errors associated with RePs (*i.e.*, variability around ReP estimates) have been reported in the literature (Henry *et al*., 2001). To date they have not been widely reported in ReP publications or routinely carried over to the TEFs-$WHO_{98/05}$, but if available, could be used to describe variability around point estimates.

- Meta-analyses or Monte Carlo techniques have been proposed as methods for providing quantitative uncertainty descriptors for certain TEFs-WHO_{98} or RPFs (Finley *et al*., 1999). However, these approaches deal only with uncertainties associated with the precision of the data. They do not address the gap in knowledge regarding the toxicity of these chemicals.

A recent review by Haws *et al*. (2006) of the underlying mammalian data for 28 of the 29 dioxin-like chemicals contained in the relative potency database (ReP_{1997} database) used to derive the TEFs-$WHO_{98/05}$ provides suggestions for refinements of this database that could support quantitative uncertainty analyses. Haws *et al*. (2006) acknowledge that the mammalian data in the ReP_{1997} database are based on qualitative or subjective judgment. Therefore, Haws *et al*. (2006) and others (Van den Berg *et al*., 1998) conclude that the mammalian data are not amenable to determining percentiles or distributions of RePs. However, using a set of criteria for excluding data (duplicate study, duplicate endpoint, single dose level, etc.), Haws *et al*. (2006) selected those RePs that could be included in a new improved database (ReP_{2004} database) that would be amenable to quantitative uncertainty analysis. Haws *et al*. (2006) illustrate their proposal with percentiles (10th, 25th, etc.) to characterize the mammalian data distributions. It is anticipated that continued refinement of existing ReP data, as well as the addition of new studies, will provide more data for future iterations that could include distributions of RePs and eventually probability density distributions. If such approaches are to be employed, existing guidance on the application of probabilistic analysis in risk assessment should be consulted (U.S. EPA, 1997a, b).

In the interim, the three dimensional matrix provided of this framework (Figure 11) for assessing the quality of the mammalian, fish, or bird ReP, RPF, and TEFs-$WHO_{98/05}$ data is an existing tool that can be used to identify data that could be included in quantitative uncertainty analyses.

3.4.3.2. *Uncertainty Associated With Application of the Toxicity Equivalence Methodology in Ecological Risk Assessment*

In addition to uncertainties inherent in the toxicity equivalence methodology, application of the methodology involves a number of uncertainties common to any ecological risk assessment. This section provides a summary of these uncertainties. In general, uncertainties in any risk assessment include natural variability in chemical concentrations, interspecies differences in sensitivity to exposure, errors in field and laboratory measurements of exposure and effects, lack of knowledge regarding pathways and routes of exposure, and errors in models of effects and exposure. Quantifying uncertainties in ecological risk assessments for PCDDs, PCDFs, and PCBs is not discussed in great detail in this framework. It is clearly a challenge with multiple chemical exposures. Each chemical must be treated as a discrete entity with its own

variance. This requires high-quality data relevant to exposure, toxicity, and the derivation of the TEFs-$WHO_{98/05}$.

3.4.3.2.1. *Other methods.*

Other methods for addressing risks from exposure to PCDDs and PCDFs include assuming TCDD is the only toxic congener or assuming that all AHR-agonists are equipotent to TCDD. These approaches underestimate or overestimate risks, respectively. Methods for assessing risks from PCBs are more complicated (U.S. EPA, 2005). There are 209 PCB congeners, but only 12 are AHR-agonists. Therefore, the risk assessor should utilize multiple methods to address all the risks due to PCB exposure. In addition, the methods for measuring PCBs in the environment are based on the original formulations of Aroclors. Much of the historical data on PCB toxicity and exposure for mammals is based on studies of Aroclors. While there are more laboratory studies with PCB congeners for fish and birds, most of the field surveys are based on Aroclor or total PCB measurements. The uncertainty associated with these chemical mixture techniques may result in an overestimation or underestimation of the AHR-mediated effects depending on the site-specific chemical matrix. Currently, many site-specific risk assessments rely on measurements of individual dioxin-like chemicals with some type of chemical mixture. This provides the risk assessor with a body of evidence that is comparable to past assessments and contributes to developing a more robust congener-specific database.

3.4.3.2.2. *Uncertainties in characterization of exposure.*

Measurements of chemical concentrations and fate and transport modeling of individual dioxin-like chemicals are essential for application of the toxicity equivalence approach. The risk assessor needs to be aware that appropriate data need to be collected for each dioxin-like chemical considered in the risk assessment, and appropriate models modified to include each dioxin-like chemical. Variability in chemical concentrations may appear to be a concern with the toxicity equivalence methodology because of the number of dioxin-like chemicals involved. However, this same variability occurs when any group of chemicals are considered in estimating exposures. Furthermore, the incremental contribution of each chemical to overall variability in a TEC is proportional to the fraction of the TEC associated with the chemical. Analytical measurement errors associated with current chemical-specific methods, if conducted to meet appropriate data quality objectives, need not be a major source of uncertainty associated with the exposure assessment (U.S. EPA, 2001a).

Because there are multiple chemicals involved in the toxicity equivalence methodology, minimizing the uncertainty associated with detection limits for each chemical will add some complexity to the risk assessment. It is important that appropriate detection limits are selected for each individual chemical during the planning and problem formulation phase of the risk assessment. Detection limits that are relevant to the toxicity endpoint are important, since this will reduce the uncertainty associated with risk estimates that are driven by chemicals that were "not detected." There are numerous procedures for estimating concentrations that are below the detection limit (U.S. EPA, 2006).

The bioaccumulation potential of PCDDs, PCDFs, and PCBs is influenced by several site- and species-specific factors (*e.g.,* trophic level, benthic/pelagic food chain, sediment organic carbon, organismal lipid, and sediment-water concentration quotient) as discussed in detail in Section 3.3.1.4. Hence, extrapolation of BAFs or BSAFs from one ecosystem to another is a source of uncertainty. When BAFs or BSAFs must be extrapolated, the uncertainty associated with this approach can be reduced by selecting factors for conditions that are most similar to the

species and ecosystem of interest. Adjustments for lipid and organic carbon are built into BAFs and BSAFs. Adjustments for other key differences can be made on the basis of food chain parameters (see Burkhard *et al*., 2006). Uncertainties for the actual site-specific point estimates for each chemical can be reduced by determining BAFs or BSAFs that are specific for the risk assessment being conducted. Choosing fixed reference sites for sampling organisms, sediment, and water for all aspects of the risk assessment and future monitoring is an important step in reducing uncertainty in relating risks to concentrations in water and sediments over time.

Consistency in applying the correct dose metric for estimating the exposure point concentration is critical. Applying TEFs-WHO_{98}, RPFs, or RePs directly to concentrations of chemicals in abiotic media for fish and birds introduces significant errors and uncertainties into risk assessments (see Section 3.3.1.5). Since the TEFs-WHO_{98} for fish and birds are based on tissue measurements, concentrations in abiotic media should be converted to concentrations in tissue using bioaccumulation factors and models as discussed in Section 3.3.1.4. Risk assessments for mammalian species may be derived directly from diet that may include water or soils, since the mammalian TEFs-$WHO_{98/05}$ are based on administered dose.

3.4.3.2.3. *Uncertainties in characterization of ecological effects.*

Use of the toxicity equivalence methodology in ecological risk assessments requires that 2,3,7,8-TCDD dose-response relationships be used to characterize adverse effects. An impetus for development of the toxicity equivalence approach is the fact that 2,3,7,8-TCDD has been the most well-studied, dioxin-like chemical and, hence, dose-response relationships for a number of effects have been well characterized. Some uncertainty may be introduced in using 2,3,7,8-TCDD dose-response relationships to characterize toxicity of all dioxin-like chemicals. For example, it is well established that fish are less sensitive than birds and mammals to *ortho*-substituted PCBs. Species differences in sensitivity to 2,3,7,8-TCDD are also sources of uncertainty in the measures of effect (*i.e.,* extrapolating from species of known sensitivity to 2,3,7,8-TCDD to a species of unknown sensitivity). However, reduction of this type of uncertainty was the impetus for deriving class-specific TEFs-$WHO_{98/05}$ (Van den Berg *et al*., 1998; 2006).

3.4.3.2.4. *Uncertainties in risk estimation.*

The risk estimate, which is derived from a toxicity equivalence concentration, has similar uncertainties to other methods of estimating risks for multiple chemicals. The inherent uncertainties in the methods of estimating risks such as the quotient method (see Section 3.4.1) are not unique to the application of the toxicity equivalence methodology to risk assessment.

If data are sufficient, the uncertainty in the risk estimate may be quantified. The reliability of the data distributions should be clearly described. In particular, the uncertainty describing the variability in the data should be distinguished from the uncertainty due to lack of knowledge. The risk assessment should include a complete disclosure of all the assumptions and the statistical conventions that were used to define the uncertainty associated with the risk estimates.

4. CONCLUSIONS

A number of PCDDs, PCDFs, and PCBs have been shown to cause toxicity to mammals, birds, and fish through a common mechanism of action mediated by the AHR. Although these chemicals can be collectively described as persistent and bioaccumulative in the environment, their specific environmental profiles and potencies relative to 2,3,7,8-TCDD differ, in some cases substantially. PCDDs, PCDFs, and PCBs frequently occur in the environment as mixtures; hence, ecological risk assessments involving these chemicals should consider their cumulative impacts. As described in this framework, the toxicity equivalence methodology offers a means to derive a single exposure estimate, the TEC, from multiple chemical concentrations found in such environmental mixtures. Although not without uncertainties, the toxicity equivalence methodology has several advantages compared with alternative methods for estimating risks from mixtures of these chemicals.

There is a growing body of evidence that the use of congener-specific analyses decreases the overall uncertainty associated with assessing the risks posed by mixtures of PCDDs, PCDFs, and PCBs (U.S. EPA, 2005). Certainly, a congener-specific approach is far less uncertain compared to assessment methods based only on 2,3,7,8-TCDD that were used previously. For example, assessing only 2,3,7,8-TCDD does not take into account the effects of the various other dioxin-like chemicals often found in environmental mixtures and therefore would underestimate risk. Alternatively, assuming that all dioxin-like chemicals found in the environment have toxicity potency equal to 2,3,7,8-TCDD would significantly overestimate risk posed by environmental mixtures of dioxin-like chemicals. In the assessment of PCBs, a congener-specific approach, including the toxicity equivalence methodology, is more accurate than either an Aroclor- or homolog-based approach for a number of reasons (U.S. EPA, 2005). A significant uncertainty associated with Aroclor analysis is that environmental PCB mixtures often cannot be adequately described by reference Aroclor standards due to the subjective assignment of Aroclor congeners. In addition to these analytical uncertainties, there is great uncertainty introduced in assuming that Aroclors or homolog groups are representative of environmentally weathered PCB profiles. Hence, measurements of PCB concentrations, bioaccumulation model predictions, and estimates of exposures (using the toxicity equivalence methodology) are all likely to be more accurate if based on congener-specific data, rather than total PCBs as determined by either Aroclor or homolog methods.

The use of the toxicity equivalence methodology has several implications for ecological risk assessment. The primary implication is that the ecological risk assessor must select appropriate relative potency factors for PCDDs, PCDFs, and PCBs. As demonstrated in this framework, practical approaches exist for selecting relative potency factors. International TEFs-$WHO_{98/05}$ have been established for mammals, birds, and fish vertebrate classes, and they represent reasonable values for estimating the TEC. This framework also presents a matrix to facilitate the selection of assessment-specific RePs or RPFs as alternatives or adjuncts to TEFs that may enhance the accuracy of risk estimates using the toxicity equivalence methodology. The selection matrix is a useful tool in optimizing the application of the toxicity equivalence methodology and encouraging the appropriate use of new relative potency information as it becomes available.

The relative importance of the uncertainties inherent to the toxicity equivalence methodology versus those endemic to all risk assessments depends on the particular assessment. For example, inaccuracies among individual dose-response studies used to determine relative potencies of dioxin-like chemicals, as well as the variability among alternative ReP values, are

sources of uncertainty in TEFs and RPFs. Section 3.4.3 summarizes uncertainties inherent to the toxicity equivalence methodology and the uncertainties associated with applications in ecological risk assessment. The decision matrix for selection of RePs, described in Section 3.3.2 and Figure 11, provides some considerations for ordering the uncertainties underlying particular elements of the methodology.

While there are uncertainties associated with the application of the toxicity equivalence methodology, they are believed to be in aggregate less significant than those associated with other aspects of the risk assessment process (U.S. EPA, 2001a). Furthermore the NRC has concluded that even with the inherent uncertainties, the toxicity equivalence methodology provides a reasonable, scientifically justifiable, and widely accepted method to estimate the relative potency of dioxin-like chemicals (NRC, 2006). Nonetheless, it is important to note that the methodology should only be applied in a manner consistent with its underlying assumptions; that is, it should only be used for the appropriate chemicals, media, and target species. Furthermore, since the toxicity equivalence methodology is applied by combining toxicity data for specific effects, exposure relationships involving different media, and species-related toxicokinetic and toxicodynamic factors, it is important to ensure (to the extent possible) that the data and calculations are consistent through each step.

In summary, the benefits of the toxicity equivalence methodology can best be realized by understanding its strengths, limitations, and its role as one of several methods within the broader context of ecological risk assessment. The goal of this framework has been to foster such understanding and to encourage future developments in the assessment of ecological risks from exposure to PCDDs, PCDFs, and PCBs.

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GLOSSARY OF TERMS

Administered Dose: External to the whole organism; concentrations in the diet of test animals rather than concentrations in cells or tissues

Aroclor: PCB mixtures manufactured in the United States carried the trademark "Aroclor" followed by a four digit number. The first two digits are 12 and the last two digits indicate the percent chlorine content by weight. Aroclor 1016 is an exception to the rule. It contains approximately 41 percent chlorine. The chemical characterization of PCB mixtures as Aroclors is an imprecise method. Human error (qualitative) and quantitative errors can arise from judgments used in interpreting results from gas chromatography/mass spectrometry (GC/MS) analysis. Specifically, GC/MS methods involve comparing chromatographic peaks from environmental mixtures to "standard" Aroclor peaks. If there is no comparable peak in the environmental mixture, the sample is assumed to be without Aroclors, even though congeners may be present. PCB determination by the Aroclor method is subject to systematic and computational errors that may result in over or under estimation of the true PCB concentration (Alford-Stevens *et al*., 1985; Alford-Stevens *et al*., 1986; Sather *et al*., 2003).

Aryl Hydrocarbon Receptor (AHR): A ligand-activated transcription factor involved in the regulation of several genes, including those for xenobiotic-metabolizing enzymes such as cytochrome P450 1A and 1B. Ligands for the AHR include a variety of halogenated aromatic hydrocarbons including chlorinated dioxins, furans, and biphenyls. The endogenous function and ligand(s) for the AHR have not been fully elucidated at this time.

AHR Homolog: A protein with structure similar to the AHR.

AHR Ligand: A chemical that stereo-specifically binds to the AHR.

Bioaccumulation: The net accumulation of a substance by an organism as a result of uptake from all environmental sources.

Bioconcentration: The net accumulation of a substance by an aquatic organism as the result of uptake directly from the ambient water, through gill membranes or other external body surfaces.

Biomagnification: The increase in tissue concentration of a chemical in organisms at successive trophic levels through a series of predator-prey associations, primarily through the mechanism of dietary accumulation.

Bioaccumulation Factor (BAF): The ratio of the concentration of a substance in tissue of an organism to its concentration in the ambient exposure media (*e.g.,* water or soil) in situations where both the organism and its food are exposed and the ratio does not change substantially over time. For aquatic organisms, the BAF is the ratio of the concentration of chemical in the organism to its concentration in water, expressed in L/kg. For terrestrial organisms, the BAF is the ratio of the concentration of chemical in the organism to its concentration in soil.

Biota-Sediment Accumulation Factor (BSAF): A specific type of bioaccumulation

factor, defined as the ratio of the lipid-normalized concentration of a substance in tissue of an aquatic organism to its organic carbon-normalized concentration in surface sediment (expressed as kg of sediment organic carbon per kg of lipid).

Chlorine Substitution: Each biphenyl molecule consists of two benzene (6-carbon) rings with one carbon-carbon chemical bond joining each ring. Dibenzo-*p*-dioxin and furan have two *ortho* bridging oxygen atoms joining two benzene rings to form the central *p*-dioxin ring. Dibenzofurans have one bridging oxygen plus one carbon-carbon bond joining each benzene ring to form the central furan ring. Chlorine can be bound (substituted for a hydrogen atom) to any of the other 10 carbons for biphenyl or other 8 carbons for dibenzo-*p*-dioxin and dibenzofuran.

Coplanar: A molecule's two rings can twist on the bond joining them; the molecule is coplanar if the two benzene rings are aligned in the same plane. See ***Planar***.

Cytochrome P450 1A (CYP1A): An enzyme (of the cytochrome P450 family) found in a variety of tissues, predominantly liver, that metabolizes xenobiotic (foreign) chemicals in addition to numerous endogenous chemicals; because its production is induced by exposure to dioxin-like chemicals, CYP1A induction can be used to estimate potency of various dioxin, furan, and PCB congeners relative to 2,3,7,8-TCDD.

Effective Concentration (EC_{50}): The concentration of a substance required to produce 50% of maximal effect in an individual test unit (*e.g.,* cell culture) or to produce a response in 50% of a population of test organisms.

Effective Dose (ED_{50}): The dose of a substance required to produce 50% of maximal effect in an individual test unit (*e.g.,* cell culture) or to produce a response in 50% of a population of test organisms.

Isomers: Molecules that have the same formula but may have different structures.

Lethal Concentration (LC_{50}): The concentration of a substance required to cause lethality in 50% of test units (*e.g.,* cells in a culture; organisms in a population).

Lethal Dose (LD_{50}): The dose of a substance required to cause lethality in 50% of test units (*e.g.,* cells in a culture; organisms in a population).

Polychlorinated Biphenyls (PCBs): A family of 209 congeners, the polychlorinated biphenyls, of which 13 (listed in Table 2) are thought to have dioxin-like toxicity. PCBs are no longer manufactured in the United States but formerly were widely used as coolants and lubricants in electrical equipment.

PCB Congeners: Chemicals with a common carbon molecular structure such as chlorinated biphenyls, dibenzo-*p*-dioxins, or dibenzofurans, regardless of exact molecular formula. There are 209 possible arrangements of chlorines attached to the ten available carbons on the biphenyl molecule. The International Union of Pure and Applied Chemists (IUPAC) has adopted a system for numbering PCB congeners sequentially from 1 to 209, starting with a single chlorine and proceeding to ten chlorines on a biphenyl molecule.

Polychlorinated dibenzo-*p*-dioxins (PCDDs): A family of 75 congeners of which 7 (listed in Table 2) are thought to have dioxin-like toxicity. The polychlorinated dibenzo-*p*-dioxin structure consists of two benzene rings joined by two *ortho* oxygen atoms and varying degrees of chlorine atom substitution on the remaining carbon atoms in the rings. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is the prototypical chemical in this class. PCDDs have not been commercially produced but are produced inadvertently by a number of industrial chemical processes and combustion of waste materials.

Polychlorinated dibenzofurans (PCDFs): A chemical class containing 135 congeners of which 10 (listed in Table 2) are thought to have dioxin-like toxicity. The polychlorinated dibenzofuran structure consists of two benzene rings joined by one oxygen atom *ortho* to a carbon-carbon bond linkage and has varying degrees of chlorine atom substitution on the remaining carbon atoms in the rings. PCDFs, like the PCDDs, are not produced intentionally but occur as inadvertent by-products in chemical production processes as well as waste combustion and PCB degradation reactions.

Planar: Relating to or lying in a plane two-dimensional in quality. See ***Coplanar***.

Polarity: The particular degree to which a molecule's electron density is anisotropically distributed between two opposing poles.

Quantitative Structure-Activity Relationship (QSAR): Mathematical models that use non-empirical structural descriptors (*e.g.,* stereoelectronic indices) and/or empirical parameters (*e.g.,* octanol/water partition coefficients) to estimate biological activity (*e.g.,* toxicity, enzyme induction, lethality, etc.). QSARs are context specific, *i.e.,* chemical structural similarity is defined in the context of a well-defined biological endpoint that is being modeled.

Relative Potency (ReP): Estimate <u>based on a single study</u> of the potency, relative to 2,3,7,8-TCDD, of an individual chemical to cause a particular AHR-mediated toxicity or biological effect in an individual organism, cellular, or biochemical assay.

Relative Potency Factor (RPF): Estimate <u>based on one or more studies</u> of the potency, relative to 2,3,7,8-TCDD, of an individual chemical to cause AHR-mediated toxicity or biological effects. The ReP database used to derive an RPF for a chemical may include multiple endpoints, species, and *in vitro* or *in vivo* studies. RPFs may be used as alternatives to TEFs when more specific data for the species, endpoint, and/or site conditions are judged to improve the accuracy of the risk assessment. If the RPF is based on a single ReP, the RPF is equal to the ReP.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD): The PCDD congener that has been most extensively studied and is used as the prototypical AHR agonist. Also commonly referred to simply as TCDD, it is the congener to which all other dioxin-like congeners (dioxin, furan, and PCB) are compared to determine their ReP for producing a particular AHR-mediated toxicity or biological effect. When this is done, the ReP of 2,3,7,8-TCDD is assigned a value of 1.0.

TEFs-WHO$_{98}$: Toxicity Equivalence Factors established at a WHO-ECEH consultation (Van den Berg *et al*., 1998); the TEFs scheme built upon previous international efforts

establishing TEFs for humans and added TEFs-WHO_{98} values for fish and birds.

TEFs-WHO_{05}: Toxicity Equivalence Factors established at a WHO-IPCS consultation (Van den Berg *et al*., 2006); based on review of only the mammalian TEFs-WHO_{98} this consultation resulted in revision to the TEF values for one dioxin (OCDD), three furans (2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF, and OCDF), two non-*ortho*-substituted PCBs (PCB 81 and PCB 169), and all relevant mono-*ortho*-substituted PCBs (Van den Berg *et al*., 2006).

Toxicity Equivalence: The concept of translating the concentrations of dioxin-like congeners (dioxin, furan, PCB) in fish, birds, or mammals to a 2,3,7,8-TCDD equivalence concentration. This is done by multiplying the vertebrate class-specific and congener-specific RPFs or TEFs by whole body or tissue concentrations of the individual dioxin-like congeners in a fish, bird, or mammal, respectively, to give a corresponding 2,3,7,8-TCDD equivalence concentration for each congener. These concentrations are then summed for all dioxin-like congeners present in the fish, bird, or mammal to yield a total 2,3,7,8-TCDD equivalence concentration.

Toxicity Equivalence Factor (TEF): Estimate of the potency, relative to 2,3,7,8-TCDD, of an individual polychlorinated dibenzo-*p*-dioxin, dibenzofuran or biphenyl congener, using careful scientific judgment after considering <u>all available</u> relative potency data. EPA presently applies this term only to relative potency factors derived through an international scientific consensus-building process supported by the World Health Organization (Van den Berg *et al*., 1998; 2006).

Toxicity Equivalence Concentration (TEC): The TEC is the product of the TEF or RPF multiplied by the concentration for an individual congener. The total TEC for a mixture is calculated as the sum of 2,3,7,8-TCDD equivalence concentrations of all congeners present in the mixture.

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