



# **Workshop on the Use of Available Data and Methods for Assessing the Ecological Risks of 2,3,7,8- Tetrachlorodibenzo-p- Dioxin to Aquatic Life and Associated Wildlife**



**RISK ASSESSMENT FORUM**

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**WORKSHOP ON THE USE OF AVAILABLE DATA AND METHODS  
FOR ASSESSING THE ECOLOGICAL RISKS OF  
2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN TO AQUATIC  
LIFE AND ASSOCIATED WILDLIFE**

**Risk Assessment Forum  
U.S. Environmental Protection Agency  
Washington, DC 20460**

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## ACKNOWLEDGMENTS

This report is based on discussions and presentations at a workshop held in Minneapolis, MN, on September 14 and 15, 1993. Dr. Robert Huggett (Virginia Institute of Marine Science, The College of William and Mary) chaired the workshop, which included work groups led by Dr. William Adams (ABC Laboratories), Dr. Charles Menzie (Menzie-Cura & Associates), Dr. Randall Wentzel (U.S. Army), and Dr. Huggett. Scientists from the U.S. Environmental Protection Agency's (EPA's) Duluth Environmental Research Laboratory, including Dr. Philip Cook, Dr. Russell Erickson, Dr. Robert Spehar, Dr. Steven Bradbury, and Dr. Gerald Ankley, prepared the report that served as the primary background document for the workshop.

Dr. William van der Schalie and Dr. John Gentile of EPA compiled this report using materials generated at the workshop and assisted the Duluth EPA scientists in preparing the hypothetical TCDD ecological risk assessment scenario. The workshop was organized with the assistance of Ms. Helen Murray and Ms. Arlene Levin of Eastern Research Group. Mr. John Bergin provided final editing of this report.

## FOREWORD

In April 1991, EPA initiated efforts to conduct a scientific reassessment of the human health and environmental risks of TCDD and related compounds. The first document to address ecological effects developed under this reassessment is the *Interim Report on Data and Methods for the Assessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Risks to Aquatic Life and Associated Wildlife* (U.S. EPA, 1993a) (the *Interim Report*), which was originally peer reviewed in the fall of 1992 and published in March 1993. The *Interim Report* compiles and critically reviews current scientific literature concerning toxicity and exposure data for TCDD ecological risks to aquatic and wildlife species.

This report summarizes the discussions of a peer panel workshop that evaluated the utility of the information in the *Interim Report* for future ecological risk assessments. In addition to the *Interim Report*, workshop participants used a hypothetical scenario and the EPA report *Framework for Ecological Risk Assessment* (U.S. EPA, 1992) as a context for identifying significant issues, discussing major uncertainties, and recommending research needs for future assessments. The focus of the workshop was on the problem formulation, or scoping phase, of the ecological risk assessment process. The resulting "conceptual model," including comments and suggestions on the transport, fate, and effects of TCDD and related compounds, should serve as a valuable resource for risk assessors planning to evaluate the ecological risks of TCDD.

Dorothy E. Patton, Ph.D.  
Executive Director and Chair  
Risk Assessment Forum

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This report summarizes the discussions of a peer panel workshop that evaluated the utility of the information in the Interim Report for future ecological risk assessments. In addition to the Interim Report, workshop participants used a hypothetical scenario and the EPA report *Framework for Ecological Risk Assessment* to identify significant issues, discuss major uncertainties and recommend research needs for future assessments. The focus of the workshop was on the problem formulation or scoping phase of the ecological risk assessment process. Participants evaluated a "conceptual model" from the hypothetical scenario and provided comments and suggestions on the transport, fate, and ecological effects of TCDD and related compounds.

## 1. INTRODUCTION

### 1.1. Background and Workshop Objectives

For several years, the U.S. Environmental Protection Agency (EPA) has been evaluating studies on the health and environmental risks associated with exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Scientists at EPA's Duluth Environmental Research Laboratory issued a report that summarizes and analyzes scientific information about the effects of TCDD on aquatic life and associated wildlife. Following a peer review in the fall of 1992, this report, entitled *Interim Report on Data and Methods for the Assessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Risks to Aquatic Life and Associated Wildlife* (U.S. EPA, 1993a)(the *Interim Report*) was published in March 1993.

While the *Interim Report* provides guidance on data and models for evaluating the exposure and effects of TCDD on aquatic life and wildlife, identifies major uncertainties, and outlines some risk assessment issues, the report was not intended to constitute a risk assessment or even to serve as guidance for conducting a risk assessment. Thus to examine how the data and methods in the *Interim Report* could be applied in future ecological risk assessments, EPA held a workshop in Minneapolis, Minnesota, on September 14 and 15, 1993. EPA asked the workshop peer panel to discuss scientific uncertainties, identify related research needs, and consider how risk assessments might be planned.

Several important topics were beyond the scope of the workshop and were not addressed. These included consideration of EPA policy on TCDD, revisions to EPA's ecological risk framework, formulation of a complete TCDD risk assessment, and issues related to the health effects of TCDD.

### 1.2. Workshop Organization and Source Materials

Prior to the workshop, the 20 participants (table 1) received copies of a scenario describing the development of a "conceptual model" for a hypothetical risk assessment of TCDD introduction to the Omigoshsee Reservoir (appendix A), questions on the major aspects of the scenario (appendix B), and the EPA report *Framework for Ecological Risk Assessment* (EPA, 1992)(*Framework Report*), which proposes terminology and offers a structure for conducting ecological risk assessments. As described in the *Framework Report*, a conceptual model is developed during the "problem formulation" stage of the risk assessment process (figure 1). This initial planning and scoping stage has several elements, including stressor characterization and ecological effects and endpoint selection (figure 2). The *Framework Report's* definitions of key terms are shown in text box 1 (page 5).

As a starting point for the workshop, EPA scientists extracted several major findings from the *Interim Report* and posed questions concerning the relationship of these findings to the risk assessment approach described in the hypothetical scenario. Each panelist prepared written premeeting comments addressing the questions concerning the scenario as well as other issues identified by the participants. Panelists were asked to comment on the use of the *Interim Report's* findings in the scenario as well as to propose any changes or additional information that might be necessary for conducting risk assessments. The comments were collated and distributed

**Table 1**

**LIST OF WORKSHOP PARTICIPANTS**

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**Workshop Chair**

- Robert Huggett, Virginia Institute of Marine Science, The College of William and Mary

**Workshop Work Group Leaders**

- William Adams, ABC Laboratories
- Charles Menzie, Menzie-Cura & Associates
- Randall Wentzel, U.S. Army Edgewood Research, Development, and Engineering Center

**Other Participants**

- Nigel Blakely, Washington State Department of Ecology
  - Peter Chapman, EVS Environment Consultants
  - Keith Cooper, Rutgers University
  - G. Michael DeGraeve, Great Lakes Environment Center, Inc.
  - Joseph DePinto, University of New York at Buffalo
  - John Giesy, Michigan State University
  - Wayne Landis, Western Washington State University
  - Derek Muir, Freshwater Institute
  - Thomas O'Connor, National Oceanic and Atmospheric Administration
  - Robert Pastorok, PTI Environmental Services
  - Richard Peterson, University of Wisconsin
  - Paul Rodgers, LTI Limno-Tech
  - Thomas Sibley, University of Washington
  - John Stegeman, Woods Hole Oceanographic Institution
  - John Sullivan, Wisconsin Department of Natural Resources
  - Bill Williams, Ecological Planning and Toxicology, Inc.
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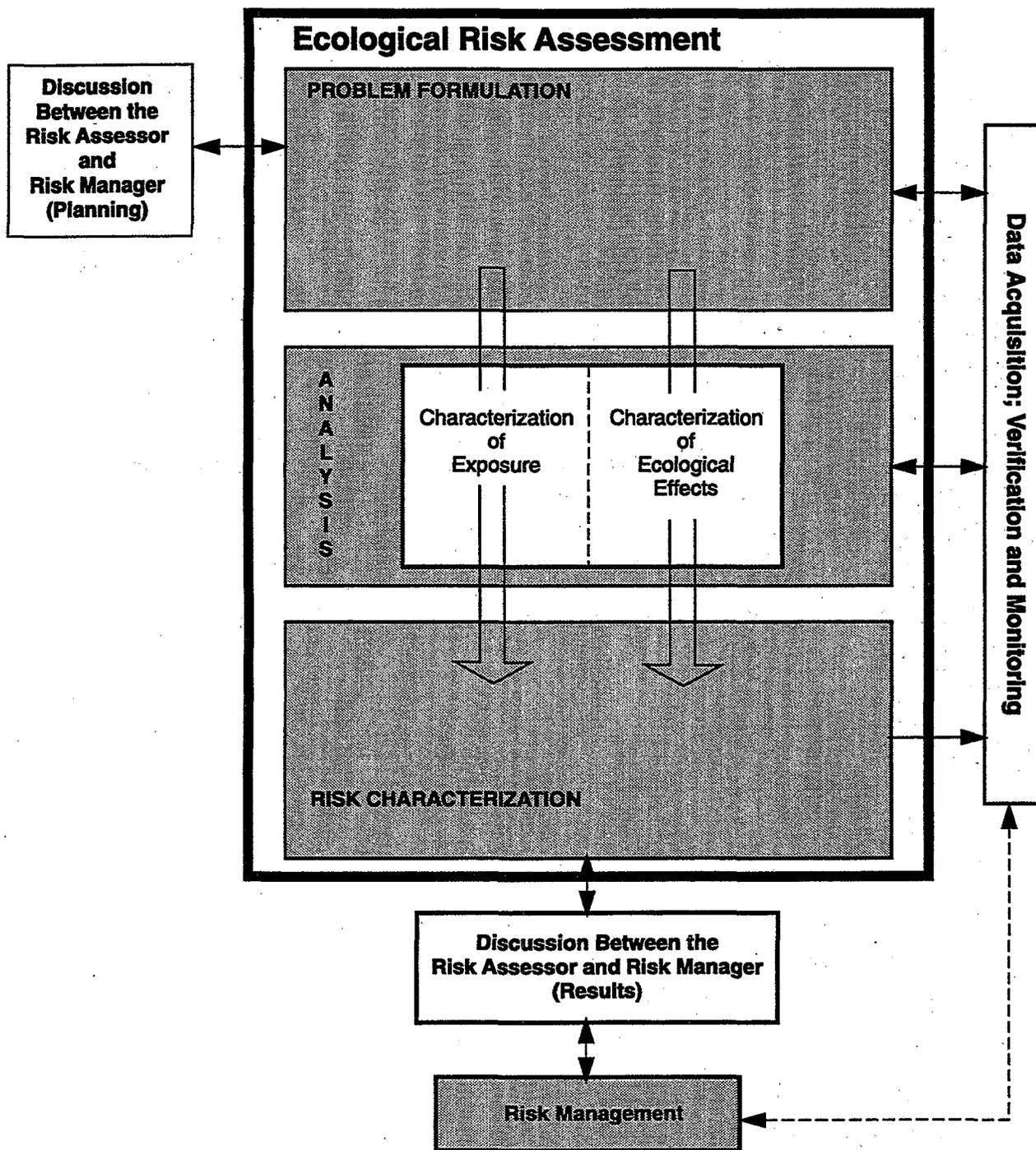


Figure 1. Ecological risk assessment framework. The risk assessment process, shown within the heavy line, includes three phases: problem formulation, analysis, and risk characterization. Each phase includes the elements of the exposure to and the effects of stressors. (EPA, 1992)

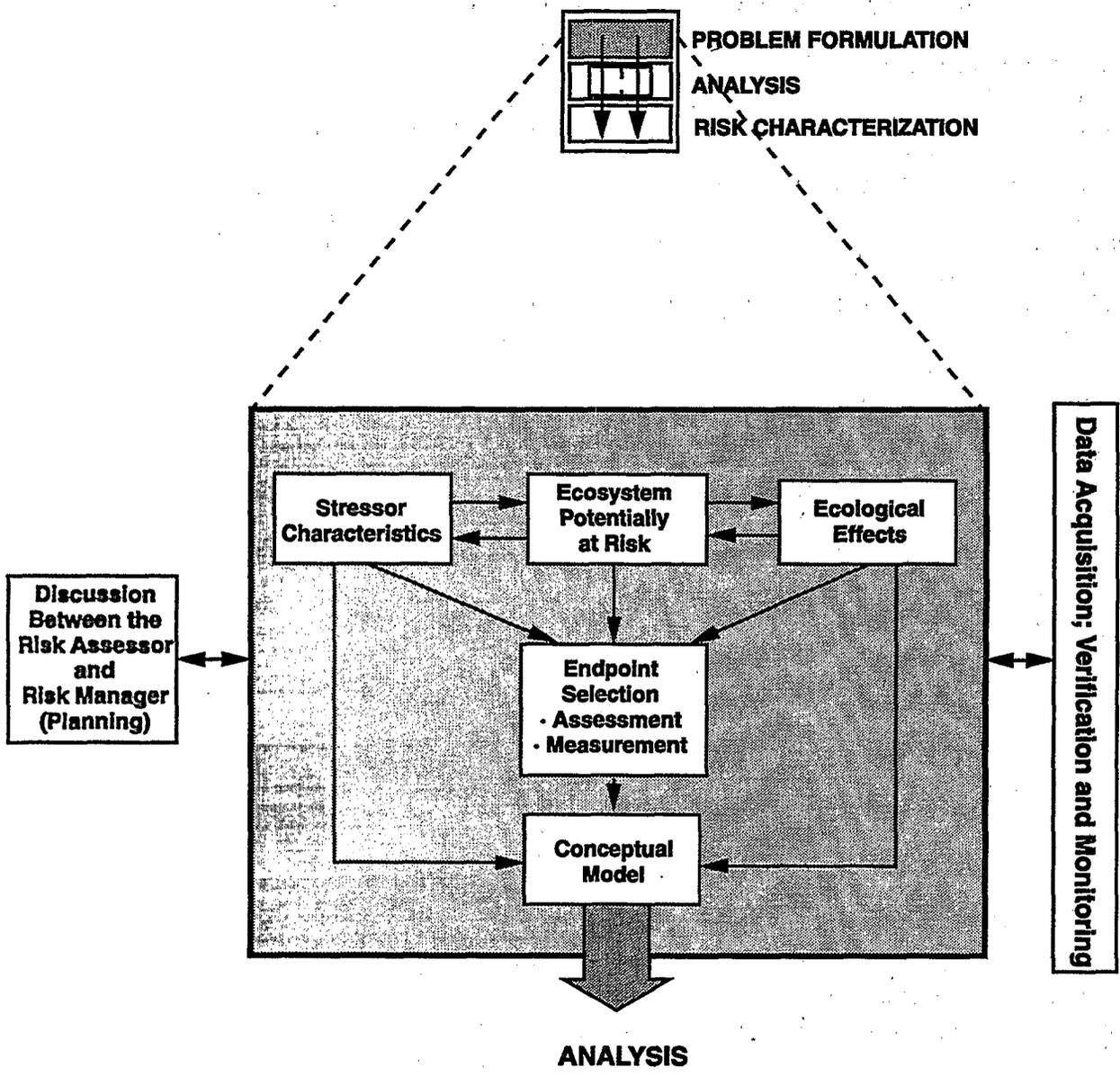


Figure 2. Ecological risk assessment framework: problem formulation. Problem formulation, an initial planning and scoping activity, has several elements, including stressor characterization, ecosystem characterization, and ecological effects and endpoint selection, all leading to a conceptual model for the risk assessment. (EPA, 1992)

to each participant prior to the meeting (appendix C).

At the workshop, panelists were divided into three work groups assigned to consider the three major aspects of the scenario: ecological effects and endpoint selection; stressor characterization; and the conceptual model development (appendices D-F). Work group leaders summarized the written premeeting comments relevant to their topics in plenary session, then led discussions of issues in breakout groups. Finally, breakout group comments and recommendations were discussed in plenary session. This report is organized around the material developed by the three work groups and is generally presented as responses to EPA's questions concerning the hypothetical scenario (appendix A). Many comments and recommendations were made, both individually and jointly, by the panelists. This document attempts to convey those inputs.

**Text Box 1. Framework Report Terminology (EPA, 1992)**

**assessment endpoint**—An explicit expression of the environmental value that is to be protected.

**conceptual model**—The conceptual model describes a series of working hypotheses of how the stressor might affect ecological components. The conceptual model also describes the ecosystem potentially at risk, the relationship between measurement and assessment endpoints, and exposure scenarios.

**ecological component**—Any part of an ecological system, including individuals, populations, communities, and the ecosystem itself.

**ecological risk assessment**—The process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors.

**exposure**—Co-occurrence of or contact between a stressor and an ecological component.

**exposure scenario**—A set of assumptions concerning how an exposure may take place, including assumptions about the exposure setting, stressor characteristics, and activities that may lead to exposure.

**measurement endpoint**—A measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint. Measurement endpoints are often expressed as the statistical or arithmetic summaries of the observations that comprise the measurement.

**stressor**—Any physical, chemical, or biological entity that can induce an adverse response.

### **1.3. General Summary**

As noted above in section 1.2, workshop discussions were organized around major elements of the problem formulation phase of an ecological risk assessment, including evaluating ecological effects and stressor characteristics, selecting endpoints, and developing a conceptual model for the assessment. While panelists felt that the information contained in the *Interim Report* would be useful for conducting ecological risk assessments, they identified a number of areas for which additional research should be conducted.

#### **1.3.1. Ecological Effects and Endpoint Selection**

Ecological effects attributable to TCDD are identified in the *Interim Report* and in the scenario that was provided to the workshop panelists. Data on these effects are available from both laboratory and field sources. Data on ecological effects and other information are used to help select assessment endpoints for the risk assessment. Assessment endpoints may be defined as explicit expressions of the actual environmental value that is to be protected (EPA, 1992). Panelists commented on the utility of existing ecological effects data for risk assessments and on the assessment endpoints proposed for the scenario.

Most panelists felt that the report's focus on early life-stage effects in fish as representing the most sensitive aquatic effect was appropriate given the present state of scientific knowledge, in spite of the uncertainties. Some panelists, however, felt that additional data should be generated on the Ah receptor and its role in TCDD toxicity for a wider range of species than have been tested. Such data would help evaluate interspecies differences in sensitivity to TCDD.

Given the difficulty in measuring TCDD levels in water, most panelists felt that evaluating TCDD effects based on tissue levels was appropriate, although linkages between residue levels and effects and residue levels and TCDD loadings need to be established.

Panelists also discussed whether "single species" data from laboratory tests are appropriate for solving a complex ecological problem. While most panelists agreed that existing data on single species could be used to make a reasonable assessment of ecological risk, others felt that it would be more appropriate to use other types of test data, such as multispecies tests. Several panelists pointed out that a high probability exists for unanticipated, indirect effects in a "real world" situation that could not be addressed by typical laboratory tests.

Assessment endpoints selected by the panelists included maintenance of the aquatic community; maintenance of sport fishery populations; and maintenance of piscivorous wildlife (i.e., birds, mammals, and reptiles such as turtles or alligators). Panelists made several suggestions for developing additional data to support measurement endpoints (see section 1.4).

#### **1.3.2. Stressor Characterization**

Important characteristics of a stressor include type (chemical or physical), intensity, duration, frequency, and scale (EPA, 1992). Stressor characteristics are clearly important for

identifying the ecosystems potentially at risk from the stressor as well as for anticipating likely ecological effects (figure 2). Panelists reviewed the available data on TCDD and related compounds and evaluated their applicability in risk assessment.

TCDD is a highly hydrophobic chemical and a member of a broad group of non-ionic organic chemicals. As such, TCDD will be associated primarily with suspended and bedded solids in the reservoir described in the scenario. Moreover, the fate and transport of solids is critical to predicting the distribution of TCDD within the reservoir. The key parameter is the organic carbon/water partition coefficient ( $K_{oc}$ ) for TCDD, along with other physicochemical characteristics (e.g., Henry's Law constant, octanol/water partition coefficient [ $K_{ow}$ ]) that influence partitioning. Since available fate-and-transport models vary widely in their complexity and resource requirements, a tiered approach was suggested whereby the risk assessor would start with simple but conservative models and proceed toward more complex (and expensive) models as far as required by the goals (and resources) of the risk assessment.

Given the high  $K_{oc}$  values for TCDD, major routes of exposure will be through contact with sediments or through ingestion of contaminated food. Both the biota-sediment accumulation factor (BSAF) and food web approaches were suggested for evaluating exposure. BSAFs will be most appropriate for lower trophic level species associated with the sediments. Panelists felt that it may be premature to conclude that biomagnification of TCDD does not occur among aquatic species, although this is suggested based on available data. Biomagnification may occur due to food chain transfer of TCDD from aquatic to avian and from aquatic to mammalian species. Present data, however, do not allow this possibility to be evaluated.

### 1.3.3. Conceptual Model

The conceptual model is the culmination of the problem formulation stage of an ecological risk assessment (figure 2). Ideally, the conceptual model includes a discussion of potential exposure pathways, effects of the stressor on ecological components, descriptions of the ecosystem potentially at risk, and identification of endpoints. Exposure scenarios are developed that provide "... a qualitative description of how the various ecological components co-occur with or contact the stressor" (EPA, 1992). Panelists evaluated and modified the conceptual model proposed in the Omigoshie Reservoir scenario (see appendix A).

Panelists indicated that a risk assessment for TCDD must emphasize the importance of physicochemical fate-and-transport modeling to link exposure to loadings. Application of equilibrium models would introduce less uncertainty than time-variable models, but the latter must be used if system response time is critical to the management questions being asked in the risk assessment. For the hypothetical scenario, models that predict concentrations in bedded sediments and on suspended sediments make the most sense. Linking sediment concentrations and possibly water concentrations of TCDD to fish body burdens can be done using either appropriate factors (e.g., BSAFs) and/or a food chain model. Residue levels, in turn, should be linked to reproductive and other effects and, if possible, these effects should be expressed in terms of changes at the population level.

## 1.4. Identification of Research Needs

It is important to note that while the panelists recognized the usefulness of the additional research described below, they also felt that TCDD risk assessments could be conducted at present using the data and methods described in the *Interim Report*.

### 1.4.1. Toxicological Considerations

The following research needs were identified by individual panelists and listed in no particular order:

- Collect Ah receptor information from a wider range of species (e.g., amphibians, reptiles, invertebrates).
- For avian and mammalian species, evaluate feeding behavior, dietary uptake, and long-term effects on survival and reproduction as a function of whole-body as well as tissue-specific residues.
- Conduct complete life cycle testing with fish.
- Investigate effects on marine mammals and ecosystems, aquatic plants, and detrital communities in oligotrophic lakes.

### 1.4.2. Fate, Transport, and Exposure Considerations

The following recommendations were considered high priorities (they are not listed in any particular order):

- Improve the  $K_{oc}$  estimate for TCDD.

$K_{oc}$  is used to estimate sediment/soil partitioning and is a key parameter in assessing TCDD transport and fate in aquatic and terrestrial ecosystems. The  $K_{oc}$  estimate could be improved by measuring  $K_{ow}$  and predicting  $K_{oc}$  from the  $K_{ow}$  for TCDD. The methodology currently being used to obtain  $K_{ow}$ s for sediment quality criteria should be employed (i.e., the slow-stir equilibrium method). Attempts to remeasure  $K_{ow}$  using standard sediment partitioning experiments will not improve current estimates. Too many operational variables are *extremely difficult* to control.

- Standardize methods for lipid and organic carbon measurements.

Several methods are currently being used to measure both tissue lipid content and organic sediment/soil organic carbon content. It would be fairly easy to review the methods, run comparisons, and standardize single approaches for lipid and for organic carbon measurements.

- Compile a library of bioconcentration factor (BCF), bioaccumulation factor (BAF), BSAF, and biota-suspended solids accumulation factor (BSSAF) values. (Of greatest interest would be BSAF and BSSAF values from field data).

The need for a compilation of these values stems from the position that for highly hydrophobic compounds approaches are being derived to assess risk that are not dependent on measurements of the specific chemical in the water column. These methods have been favorably reviewed by the panelists. Methods include the use of parameters such as the BSAF, BSSAF, and BAF. Issues that have arisen with the use of these estimators include whether the values can be translated from site to site (even with carbon and lipid normalization) or whether the values are site specific. A compilation of these values would allow this important question to be answered. Universal application of the concepts is desirable.

Another recommendation was considered important but not of the highest priority:

- Spatial and temporal patterns of dietary uptake of contaminated prey need to be investigated.

Adequate characterization of food webs and an understanding of the transport of TCDD and similar compounds through various food webs are critical for ecological risk assessments of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

The last recommendation was considered of a somewhat lower priority, although still important:

- Standardize the method(s) for measuring  $K_{oc}$  for highly lipophilic compounds.

This recommendation is of a lower priority because  $K_{oc}$  can be estimated from  $K_{ow}$  and because the problems that have to be solved to standardize an approach for extreme hydrophobes are enormous.



## **2. SIGNIFICANCE OF WORKSHOP DISCUSSIONS FOR ECOLOGICAL RISK ASSESSMENT**

Although the peer panelists focused their attention primarily on assessing ecological risks associated with TCDD, their discussions also raised points that are more generally relevant to ecological risk assessment. This section describes some of the more important conclusions for risk assessors concerned with both TCDD-specific and more general evaluations of ecological risk.

### **2.1. Ecological Risk Assessments for TCDD**

Peer panelists generally found the information in the *Interim Report* to be sound. Nonetheless, they suggested that attention be given to a number of important research needs (section 1.4) and recommended approaches to using the information in the risk assessment process. Panelists indicated that methodologies for TCDD risk assessment may be more generally applicable to other hydrophobic organic chemicals (HOCs). This suggests that it may be possible to develop a generic aquatic ecological risk assessment model that could be adjusted for the characteristics of the particular chemicals and ecosystems involved.

The overall approach to developing a sound conceptual model for the ecological risk assessment involves establishing and evaluating the logical linkages among TCDD sources (in this case, the paper mill), fate and transport of TCDD, uptake by biota, effects on biota, and the consequences of these effects at the population level (and higher). Many tools are available for determining TCDD fate and transport, and their selection in the analysis phase will depend on the management questions being asked and the resources available. BSAF and food chain approaches can both be used to help predict residue levels in organisms, which in turn can be related to effects. Yet, predicting biological effects at the population level and above may be difficult given data and methodological limitations at this time—an aspect of ecological risk assessment that is not necessarily unique to TCDD.

It is important to note that the scenario used at this workshop was simplified in the sense of having only one stressor (TCDD) and one primary source (the paper mill). Interpretation of data in situations where TCDD is already present are frequently much more complicated because of multiple sources and the presence of other stressors (i.e., chemical, physical, and biological). In these cases, the emphasis may be less on prediction of effects and more on establishing causality between an observed effect (e.g., decline of a sport fishery population) and a presumed cause(s) (e.g., TCDD, overfishing, habitat destruction).

### **2.2. General Ecological Risk Assessment Considerations**

EPA's Risk Assessment Forum recently published a set of 11 peer-reviewed case studies that evaluated a wide range of ecological assessments from a risk assessment perspective (EPA, 1993b). A number of common themes concerning the nature of ecological risk assessment emerged during the development and evaluation of these case studies and many of the "lessons learned" from the case studies were reflected in the peer panel discussions of the hypothetical TCDD scenario. Three of these recurring themes are described below.

***Formulating the Problem and Developing the Scope Are Critical First Steps.***

Several of the studies reviewed for the case studies report involved difficulties that could be attributed to inadequate problem formulation. Indeed, the peer panel found shortcomings in the conceptual model for the TCDD case study and provided many suggestions for revision and expansion of the conceptual model. The final product of these discussions provides a better basis for proceeding to the analysis phase of the risk assessment. Clearly, an essential factor for success in the problem formulation stage of an ecological risk assessment is a sound understanding of both the stressor and the ecosystem involved.

***Defining Assessment and Measurement Endpoints Focuses the Scope of the Risk Assessment.***

Selection of the proper assessment endpoints for the TCDD risk assessment generated considerable discussion among panelists. For example, some felt that a focus on the sport fishery population was appropriate, while others found this to be too narrow a focus. The presence of rare or endangered species in the area also could have influenced the selection of assessment endpoints. Panelists emphasized the importance of selecting assessment endpoints that can be used for decision-making and that at the same time address ecological concerns.

***Clearly Stated Risk Scenarios Help Structure the Assessment.***

Risk scenarios developed as part of the conceptual model reflect the risk assessor's judgment concerning which stressors, ecological components, and pathways are likely to be the most significant in the risk assessment. Such scenarios are critical since resources frequently limit the range of possibilities that can be explored and the process of problem formulation must reduce the risk scenarios to a manageable number. Identification of important TCDD pathways and species that are sensitive to TCDD were helpful in scenario development for the reservoir.

### 3. COMMENTS ON ECOLOGICAL EFFECTS AND ENDPOINT SELECTION

Peer panel members were asked to use information in the *Interim Report* as source material to address seven issues concerning ecological effects and endpoint selection raised by the scenario. The *Interim Report* focuses on the effects of TCDD on freshwater aquatic organisms and associated wildlife (*Interim Report*, chapter 4). Available data on TCDD effects on fish are provided in section 4.2.1 and are summarized in section 4.2.3 of the *Interim Report*, while effects on aquatic-associated wildlife are provided in section 4.3.1 and summarized in section 4.3.3.

#### 3.1. Issue 1—Focus on Fish Species

*The lack of Ah receptors in some species (Interim Report, section 4.1 [U.S. EPA, 1993a]) along with the results of a limited number of laboratory studies suggest that amphibians, invertebrates, and plants are less sensitive to TCDD than fish, birds, and mammals. Fish appear to be most sensitive in early life stages. Because of this range in sensitivity, productivities of fish species were selected as assessment endpoints for the scenario. Comment on whether this focus on fish species will result in adequate protection for the rest of the aquatic community in the reservoir from the direct or indirect effects of TCDD.*

Most panelists felt that the report's focus on fish early life stage effects as representing the most sensitive aquatic effect/species early life stage was appropriate, given the present state of scientific knowledge, in spite of the uncertainties. One panelist thought that this approach put too much emphasis on a single-species-type solution to a complex ecological problem. Some panelists proposed that measurement endpoints should be relevant to population-level effects and should include both reproductive and developmental measurements. Ideally, mink data would be used (in addition to available rat data) for estimating mammalian wildlife effects. Also, data on a wild bird species (including F1 reproductive effects) would be useful. Additionally, panelists discussed behavioral effects and the need for a complete fish life cycle test.

Panelists discussed the relative merits of empirical and mechanistic data. Some panelists felt that additional data should be generated on the Ah receptor and its role in TCDD toxicity for a wider range of species than presently have been tested in order to help evaluate interspecies differences in sensitivity to TCDD. Others were concerned that there could be a problem relating molecular-level receptor information back to ecological changes.

#### 3.2. Issue 2—TEFs and BSAFs

*Section 4.1 of the Interim Report (and the scenario) describe the use of toxicity equivalency factors (TEFs) for TCDD-like compounds. Section 3.5 of the Interim Report discusses the use of TCDD biota-sediment accumulation factors (BSAFs) for calculating bioaccumulation equivalency factors for other related compounds. Comment on the use of these approaches for evaluating the effects and bioaccumulation of dibenzodioxins and dibenzofurans in the paper mill effluent.*

TEFs available for fish early life stage effects are based on rainbow trout mortality data. The data appear to be valid for chlorinated dioxins, chlorinated furans, and polychlorinated

biphenyl (PCB) congeners. Additivity is shown where compounds are full agonists, but may not occur where compounds may be only partial agonists. Compounds have been shown to differ in this respect between fish, birds, and mammals when *in vitro* hepatocyte cultures were tested. While the assumption of additivity has not been adequately addressed for avian and mammalian species, it is an appropriate assumption given the present state of knowledge. For the future, it would be desirable to examine TEFs for other endpoints and for species other than rainbow trout.

Panelists found the use of BSAFs to be a reasonable approach given the present state of knowledge, but one limitation is that data are available for only two field sites—Lake Ontario and the Fox River. While the correlation between these sites was good, panelists felt that BSAF data from additional field sites should be examined.

### **3.3. Issue 3—Use of Tissue Levels vs. Exposure Concentrations**

*Because of difficulties in extrapolating from various laboratory exposure conditions to observed effects, the Interim Report (section 4.2.3.1) emphasizes using tissue levels of TCDD (rather than exposure concentrations) to evaluate effects. Comment on the applicability of this approach to evaluating the risks of TCDD from the paper mill effluent.*

Certain panelists pointed out that toxic responses depended on TCDD concentrations at the target organ sites and that reliance on tissue levels for regulatory purposes was necessary given the difficulty of measuring TCDD levels in the water. Others recognized that general changes in TCDD tissue levels may not imply that a specific effect will occur and that calculating back from tissue levels to permit or discharge levels may be difficult for a risk assessor. In general, however, the panel was comfortable with using tissue levels to evaluate TCDD effects as described in the *Interim Report*.

### **3.4. Issue 4—Use of Laboratory Data to Predict Field Effects**

*The Interim Report uses both laboratory and field information to predict levels of TCDD in fish and wildlife tissues that will cause adverse effects. The scenario proposes to use laboratory test data at the individual level of organization to predict population changes in fish and wildlife. Comment on the utility of available laboratory data to predict effects on field populations and discuss the associated uncertainties.*

Certain panelists contended that available laboratory test data are sufficient to allow reasonable decisions to be made, while others maintained that because of the potential importance of subtle population and community effects, other types of tests, such as multiple species tests, should be developed. Gaining a better understanding of the implications of laboratory data also requires an understanding of the ecosystem (e.g., keystone species, population control mechanism).

Several points made concerning extrapolation from laboratory test species to resident species in the reservoir are listed below:

- Extrapolation should be performed using all existing data on TCDD effects and dose-response relationships in species in the same taxa.
- Assessment of the relationship of effects to tissue concentrations should take into account residues in the whole body and all organs for which data are available.
- Effects to be examined should be of any type, ranging from mortality to cellular and early molecular changes. Reproductive failure or changes directly linked to *reproductive success* would be most relevant.
- Markers of change for which dose-response relationships have been demonstrated, particularly those having a known mechanistic link to TCDD, may be valuable.

At present, the assessment most often will require extrapolation from *model species* to the species of concern. It is important that the assessment consider the *most sensitive model species* for which data are available. The lipid content of various organs and the biological variables influencing mobilization and deposition in organs that are sites of action should be considered. These would include *eggs* or *embryos* in the case of early life stage mortality, and *brain, liver, or gonad* in the case of reproductive effects.

In the future, new dose-response relationships and an understanding of the mechanism of TCDD action will provide additional model systems that could be incorporated into the assessment. Determination of specific effects from tissue residue levels or body burdens must be tied to estimated uptake (dietary) for each taxa (figure 3). Model species (or surrogate) data are used to determine most sensitive endpoints (no observed effect level [NOEL]) for each category of concern (figure 3). The allowable dietary uptake (or tissue residue, if available) can be back-calculated to determine maximum allowable media concentrations.

### 3.5. Issues 5 and 6—Concentration-Response Curves and Data Extrapolations

Issue 5. *The Interim Report sites data that indicate a very steep concentration-response curve for TCDD effects in fish and wildlife. Discuss the implications of this observation for evaluating ecological effects in the scenario.*

Issue 6. *The general summary of effect levels for aquatic species and associated wildlife (Interim Report boxes 1 and 2, section 4) is based on extrapolations from a limited number of test species and from tests that do not span complete reproductive cycles. Associated uncertainties are summarized in section 5.1.3. Discuss the utility of these data and uncertainties for evaluating ecological effects.*

Panelists felt that these issues did not require further discussion, since both areas were adequately addressed during the discussion of other issues.

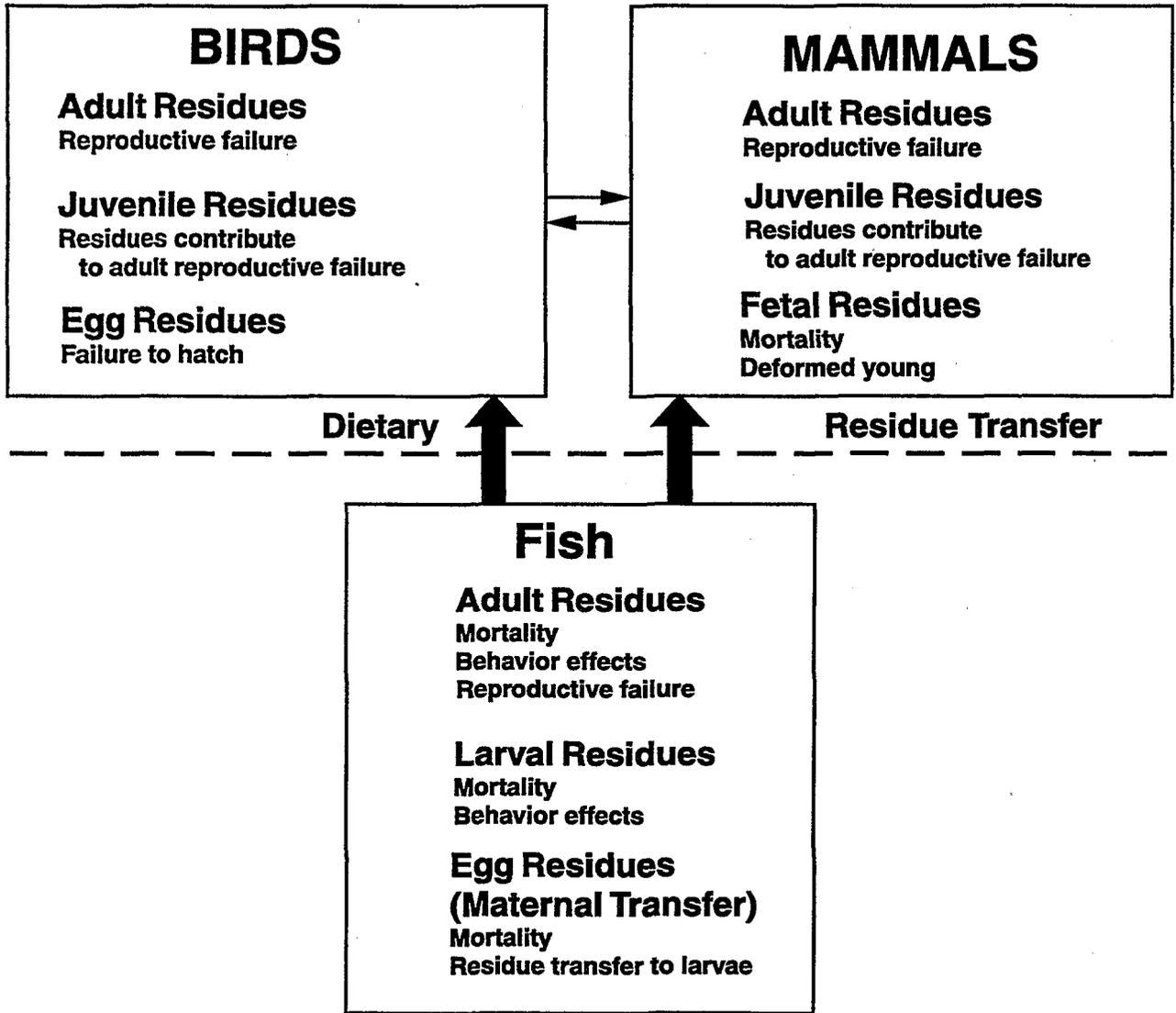


Figure 3. Residue vs. effects. Consumption of contaminated food by birds, mammals, and fish will increase residues within the organisms, causing a range of effects that depend on both the residue level and life stage of the organism.

### 3.6. Issue 7—Additional Effects Data Not Considered in the Scenario

*As discussed in the Interim Report, few data on the effects of TCDD on estuarine and marine organisms have been reported (section 4.2.1.5), and no data were found in the literature for TCDD effects on reptiles or marine mammals. Although all the current wildlife toxicity data were reviewed, an analysis to establish an effects profile for terrestrial organisms was beyond the scope of the report. Describe other effects data not identified in the Interim Report or the scenario that will be important for future ecological risk assessments.*

Panelists generally agreed that a reasonable risk assessment could be conducted with existing data. Nevertheless, obtaining additional data in specific areas could strengthen the risk assessment. A number of data gaps were pointed out along with suggestions for use in future TCDD ecological risk assessments:

- Ah receptor information from a wider range of species (e.g., amphibians, reptiles, invertebrates) is needed.
- Residue-effects data for a wide variety of species are needed.
- Aquatic plant effects are needed.
- Complete life cycle testing with fish should be conducted.
- Data relevant to effects on marine mammals are needed.
- Marine ecosystem effects in general are lacking.
- Effects on detrital communities in oligotrophic lakes should be assessed.

Panelists recommended that additional research be directed toward reptiles, specifically snapping turtles and alligators that may represent top predators in many aquatic ecosystems, particularly in the southern United States. Given the potential influence of external variables (e.g., temperature) on sex ratios in reptiles such as turtles, panelists considered development of a measurement endpoint sensitive to changes in sex ratios to be important.

### 3.7. Assessment and Measurement Endpoints

Although not specifically raised as an issue area, panelists discussed the suitability of the assessment and measurement endpoints proposed in the hypothetical scenario. Panelists suggested three assessment endpoints for the scenario:

- maintenance of the aquatic community;
- maintenance of sport fishery populations; and
- maintenance of piscivorous wildlife (birds and mammals).

Measurement endpoints were discussed for both "real" situations (using existing data) and "ideal" situations (where new data could be obtained). Measurement endpoints involving real data include reproductive effects in fish (early life stage tests), birds (pheasant data), and mammals (rat data). Some panelists were concerned that there was an inadequate basis for extrapolation from the available data to the species and effects of concern in the scenario. Some discussion also took place about the adequacy of TCDD data for the rat and chicken.

Ideally, measurement endpoint data could be expanded using information listed in section 3.6. Additional data needs might include a multispecies test system that could factor in direct and indirect effects on other species such as benthic invertebrates. Additional single species tests would include developmental as well as male and female reproductive toxicity tests for fish, birds, and mammals (e.g., mink). Also, full life cycle tests (including exposure of F1 generation) would be very useful. Tissue levels of TCDD could be measured in organisms, or eggs, for instance. Test organisms should include resident species in the reservoir, if at all possible.

#### 4. COMMENTS ON STRESSOR CHARACTERIZATION

Peer panel members were asked to use information in the *Interim Report* as source material to address eight issues concerning stressor characterization raised by the scenario. Available information on TCDD physicochemical properties and exposure characteristics are described in chapter 2 of the *Interim Report*, while bioaccumulation is described in chapter 3.

##### 4.1. Issue 8—Uncertainties in $K_{ow}$ and $K_{oc}$

*The Interim Report (sections 2.1 and 2.2) indicates that there is considerable uncertainty in the estimates of parameters including  $K_{ow}$  and  $K_{oc}$  and the partitioning of TCDD onto organic matter. This uncertainty results in part from difficulties in analytical measurements of various fractions of TCDD in water. Since these limitations may affect predictions of TCDD partitioning and exposure, please address how they should be handled in stressor characterization and conceptual model development for this scenario.*

HOCs are known to partition to solids as a result of their organic carbon content (i.e., lipophilic characteristics). Thus, HOCs will generally distribute themselves among a soluble phase and a particulate phase. TCDD is among those HOCs that have a relatively high tendency to partition themselves in the particulate phase. This characteristic of TCDD results in the fate and transport of TCDD being tied to the fate and transport of solids in aquatic systems. Experience with simulating HOCs in aquatic systems has revealed that if one succeeds in quantifying the fate and transport of solids (including bulk transport, settling, resuspension, burial, and internal primary production), then a substantial portion of HOC fate and transport has been defined. This observation is programmatically fortunate since monitoring programs to support site-specific HOC exposure assessment will be well served by an emphasis on the measurement of solids and solids transport phenomena, a less-costly approach than a nearly exclusive emphasis on chemical measurements.

Knowledge of the dominant role that solids dynamics play in determining the fate and transport of highly partitioning HOCs has contributed greatly to the ability to develop models that are not chemical specific, but depend instead on measurable physicochemical characteristics such as  $K_{oc}$ ,  $K_{ow}$ , and Henry's Law constant. Therefore, it is necessary to continue to develop a library of these physicochemical characteristics for the HOCs of concern. It may not be necessary, however, to treat each HOC, homolog, or congener with a chemical-specific paradigm of fate and transport.

The panelists agreed that there should be a standardization of protocols to be used when determining both total organic carbon (TOC) and the partitioning to organic carbon ( $K_{oc}$ ). A lack of standard methods will result in different values based on the method selected. The panelists also agreed on the need to characterize the type of organic carbon, since from the Green Bay study there is limited evidence for different  $K_{oc}$  values for sediment and algal carbon. Both laboratory and field validation for the  $K_{oc}$  for selected isomers at concentrations ranging from background to environmental levels are needed. This will answer the question of whether laboratory-spike-type studies are providing results similar to those from field (or site specific) situations.

Once these data are produced, a central database library listing the results should be created. This information should be screened for compliance with QA/QC criteria. If the data do not meet such criteria, they could be listed with a footnote noting any irregularities. This data base also could list field data with  $K_{ow}$  and tissue concentrations on a lipid normalized basis. Such information would allow for the screening of species-specific information in similar ecosystems. These data could be used by the risk manager to get information for particular species of concern and could help prevent duplication of field and laboratory studies.

#### 4.2. Issue 9—Exposure Routes

*The Interim Report (section 2.4) indicates that most TCDD exposures will arise from food consumption and contact with sediments or suspended solids, with the water pathway being less important. Address the implications of this information relative to the exposure routes in the conceptual model.*

Given the high  $K_{ow}$  values for TCDD and many PCDD/PCDF congeners, the main routes of exposure for most receptors will be through food ingestion and contact with or ingestion of sediment or suspended solids. Implications of this for development of the conceptual model and the risk assessment include:

- Food webs and sediment exposure pathways need to be characterized in detail, including possible spatial and seasonal variations.
- Assimilation of PCDDs/PCDFs from key food items and sediment needs to be determined for receptors of concern.
- BSAFs, BSSAFs, and BAFs are important, while BCFs may be negligible (except for algal species).
- While the *Interim Report* indicates that BSAFs apply as much to pelagic organisms as to benthic organisms, workshop panelists felt that the use of an empirical BSAF/BSSAF-based approach is more applicable for benthic food webs. Panelists indicated that food web models may be needed for other systems where higher trophic level fish species depend on pelagic food webs or algal foods.

Adequate characterization of food webs is critical for ecological risk assessments of PCDDs/PCDFs, especially assessments based on predictive models such as those of Thomann et al. (1992a). The conceptual model for the hypothetical scenario needs to include key food web species for each receptor of concern, as well as ingestion of sediment or suspended solids by some species. Three types of food webs (Paine, 1980) should be considered.

- A connective web, which includes essentially all possible web linkages, should be considered for any receptors that are linked or for rare, threatened, or endangered species.

- A materials-flow web, which emphasizes the relatively important linkages for transfer of PCDDs/PCDFs, should be the basis for an exposure assessment for all receptors of concern.
- A functional web, which emphasizes linkages among strongly interacting species (e.g., keystone predators feeding on competitive dominants), should be considered to prioritize receptors for detailed consideration in the risk assessment and to develop risk hypotheses about effects of exposure on community structure.

Trophic compartments in food webs may be defined on the basis of species, life stage, sex, etc. (i.e., trophic species rather than simple taxonomic species). (Terrestrial organisms may also be key prey items in food webs for many aquatic receptors.) For "ideal" assessments, spatial and seasonal variability in food webs should be included.

If dynamic food chain or food web models are desired, assimilation of PCDDs and PCDFs in ingested prey and sediments by key receptors may need to be measured in laboratory experiments. The relative importance of this data gap should be evaluated further through sensitivity analysis of food web exposure models. Assimilation efficiency will likely vary with prey species and the nature of the organic carbon.

Empirical determination of BSAFs and BSSAFs from site-specific measurements will be an appropriate alternative to detailed food web modeling, especially for lower trophic level species associated with sediments. The nature of organic carbon in different sediment types will likely affect BSAF and BSSAF values. Modeling should still play a role for higher trophic level species dependent on pelagic food webs and for cases where examination of the temporal disequilibria is a management objective. Since empirical measurements of BAFs are not available at present, estimates of BAFs will be needed for modeling. BCFs will not be useful except in the case of algae, which form the base of many aquatic food chains.

#### 4.3. Issue 10—Fate-and-Transport Models

*Fate and transport models are beyond the scope of the Interim Report, but are clearly critical for risk assessment. In stressor characterization and development of the conceptual model, they will be necessary for linking TCDD source loads to concentrations in different compartments of the reservoir.*

- a. *Comment on the availability of fate and transport data/models suitable for use with TCDD.*
- b. *Discuss the applicability of available transport models for predicting the deposition of particulate-bound TCDD in the reservoir.*

Panelists considered the availability of fate-and-transport models for quantitatively relating the source loadings of TCDD to TCDD concentrations in various compartments of an aquatic system such as the reservoir in the case study. The simple answer was that mass balance models simulating the fate and transport of HOCs have been developed and applied for a variety of chemicals in a variety of environmental settings. These models range in their spatial,

temporal, and kinetic sophistication from relatively simple steady-state, screening-level models (e.g., Endicott et al., 1991) to more "state-of-the-science" models (e.g., Bierman et al., 1992; DePinto et al., 1994) that address the problem at a higher resolution of space, time, and kinetic formulation.

The panelists recognized that no single model is best for all applications. The level of complexity (or resolution) of a model required for a given application is determined by two basic factors: the complexity and resolution of the management questions being asked, and the resources (mainly in terms of data acquisition) available to support the model. (In general, increased model complexity requires increased data/resources for application.)

The panelists felt that for a typical situation, such as in the Omigoshsee Reservoir scenario, where the questions being asked range from simple to complex, a tiered approach is recommended. Initially, a simple but conservative calculation would be used, progressing toward a more complex modeling approach that provides the optimum reliability (i.e., utility) for the most complex questions and for the resources available. Panelists noted that it is possible to see a decrease in reliability (model certainty) if a model is *more* complex than warranted by the questions posed and resources at hand.

When contemplating the use of models to calculate the chemical exposure in aquatic systems under various management scenarios, it is essential that the prospective user has a clear picture of the levels of models that are available, their corresponding features and data requirements, and the management or investigation questions for which the models can provide answers or guidance. Table 2 summarizes this information for aquatic models that are capable of simulating exposure levels for HOCs. Specifically, these models compute environmental concentration(s) resulting from alternative loads of the chemical to the system.

The first two models in the table (level 0 and 1a) simulate only the total chemical in the water column, while the remainder of the models simulate HOCs in the particulate and the dissolved phase and in the sediment layer (except for level 1b). Therefore, many of these models supply information regarding the environmental distribution of a chemical loaded to an aquatic system in four environmental compartments, namely: (1) dissolved phase in the water column; (2) particulate phase in the water column; (3) dissolved phase in the sediment layer; and (4) particulate phase in the sediment layer. Beyond these features, the levels of the models reflect increasing capabilities to simulate HOCs at increasing levels of spatial resolution and as a function of time. The last level (level 3) represents a highly variable group of models referred to here as complex models that may address specific additional areas of concern, such as quantitative characterization of model uncertainty, more complex hydrodynamics (e.g., estuaries and three dimensional phenomena, such as saline or temperature layers), and more refined representations of chemical, biological, or population relationships.

The listed model features, data needs, and management answers represent a progression in characteristics and needs. Several guidance documents have been published by EPA and others to aid the user in model selection, use of specific aquatic chemical models, collection of supporting field data, and evaluation of model inputs (e.g., Delos et al., 1984).

The panelists noted that it would be particularly useful if future risk assessment guidelines included a protocol for applying the tiered approach along with criteria (aspects of

Table 2

PROGRESSIVE LEVELS OF AQUATIC CHEMICAL MODELS

Level	Features	Data Needs	Answers
0	Dilution model, yields initial complete mix concentration	Effluent design flow, critical low flow in receiving water or allowable mixing radius/zone, upstream chemical concentration, effluent load or ambient standard—model solves for missing parameter	Worst case ambient concentration in the water column following mixing; additional calculations using $K_{oc}$ yields information on the expected phase distribution (particulate or dissolved)
1a	Steady-state model, simple one-dimensional (1-D) segmentation, first-order loss from the water column	River physiography, chemical concentration versus river mile and/or knowledge of first-order loss rates	More realistic estimate of concentration as a function of distance from the effluent, rough estimate of the chemical retained in the system
1b	Steady-state model, 1-D segmentation, partitioning to solids, net settling links water to sediment	Solids loads, solids versus river mile, solids characteristics, and partitioning coefficient	Chemical distribution in particulate and dissolved phases in the water column
1c	Steady-state model, 1-D segmentation, partitioning, full solids dynamics	Literature and site-specific analysis of resuspension and gross settling rates	Provides chemical levels in the sediment and the water compartments
1d	Steady-state model, 1-D segmentation, partitioning, separation of abiotic and biotic solids	Information on water column abiotic-biotic solids origin and transport rates	More accuracy, better differentiation of biotic component
2a	Time-variable model, 1-D segmentation, partitioning, full solids dynamics	Time variable loads and environmental conditions, better vertical solids transport rates	Response as a function of time and distance from the source(s)
2b	Steady-state model, 2-D segmentation, partitioning, full solids dynamics	Hydraulic transport or routing, more spatially distributed field data	Spatially distributed (2-D) results, better representation of certain systems, a broader range of questions addressable to correspond to locations of specific interest
2c	Time-variable model, 2-D segmentation, partitioning, full solids dynamics	Typically more highly resolved data (time and space)	Temporal and spatially related questions
3	More hydraulic (3-D), sorbent, chemical, or biological complexity	Additional problem-specific site data and potentially supporting research	More complex questions of source, chemical interaction, fate, transport, or effects

complexity of management questions) for governing the progression toward higher resolution (spatial, temporal, kinetic) modeling approaches.

#### 4.4. Issue 11—Additional Exposure Issues

*List some of the major exposure issues not relevant for the paper mill scenario that may be encountered in future ecological risk assessments (e.g., marine/estuarine, terrestrial).*

Panelists identified the following exposure issues (not relevant for the paper mill scenario) in both premeeting comments and workshop discussions.

- Terrestrial exposure information for wildlife species is generally lacking. Often information is insufficient regarding relative source strength (e.g., atmospheric, point and nonpoint) and input to terrestrial ecosystems to perform terrestrial risk assessments.
- Evaluation of exposure in marine systems, which was not conducted for the present scenario, is particularly difficult due to the openness of marine systems. Additional confounding issues include the problem of determining the percentage of a population that is exposed, the migratory and behavioral patterns of marine species, the role of recruitment and recovery, time-variable exposures to mobile species, and spatial heterogeneity resulting from the multiple source inputs characteristic of estuaries.
- Fish exposure time is rarely well known, often time-variable, and generally confounded by a lack of understanding of how fish interact with sediment to accumulate chemical contaminants.
- Information on spatial and temporal patterns of dietary uptake of contaminated prey that may be particularly important for TCDD and related contaminants is needed.
- Recovery time is not systematically evaluated, particularly in regard to the impact of catastrophic or rare meteorological events.
- The paper mill example does not explicitly discuss the role of a mixing zone, which may be appropriate given the nature of the endpoints.

Panelists suggested that there are substantial exposure issues for both aquatic (particularly marine/estuarine) and terrestrial systems that are outside the paper mill conceptual model but that will need to be addressed in other site-specific problem settings.

#### 4.5. Issue 12—BCFs and BSAFs

*The Interim Report (sections 3.2-3.5) summarizes available data on TCDD bioconcentration, bioaccumulation, biomagnification, and biota-sediment accumulation factors from*

*laboratory experiments and field measurements. Discuss the applicability of these factors to stressor characterization for the paper mill scenario.*

Panelists noted that BCFs suffer from two limitations: the need for accurate measurements of dissolved TCDD concentrations in the water, and the use of data from laboratory studies that often lack realism under site-specific conditions. Further, the ability to obtain accurate measures and/or predictions of dissolved TCDD is confounded by uncertainties associated with  $K_{ow}$  measurements. For example, the BCF- $K_{ow}$  relationship departs from linearity when  $\log K_{ow}$ s exceed 6, which includes the range of  $K_{ow}$ s for TCDD and related compounds.

Panelists felt that BSAFs should play an important role in TCDD risk assessment. BSAFs are likely to have a lower uncertainty, and they may serve as a steady-state, upper-bound body-burden estimation, particularly for lower trophic levels (e.g., plankton and invertebrates). Since BSAF values are calculated using concentrations in muscle, tissue-dose specificity may be much higher than indicated by typical body burdens. Finally, if BSAFs are to be used extensively, a library of BSAF values, representing different species, lipid, organic carbon, sediment types, and ecosystems, will be necessary.

#### **4.6. Issue 13—Applicability of Lake Ontario BAF Data**

*The Lake Ontario lipid-normalized bioaccumulation factor for dissolved TCDD ( $BAF_l^d$ ) may be useful as a predictor of residue levels in other systems if the concentration of freely dissolved TCDD in water ( $C_w^d$ ) can be estimated accurately (Interim Report, section 3.3). Comment on the applicability of this BAF for the paper mill stressor characterization.*

Panelists felt that extrapolation of the Lake Ontario data to other sites would be appropriate as a Tier I screening level assessment. This is an area where a library of values from different areas would be particularly useful. In applying this information, ecosystem differences have to be considered, with a key focus on the dietary habits of consumer organisms. Food chain length and lake trophic status can influence TCDD residue levels.

#### **4.7. Issue 14—Biomagnification**

*The Interim Report (section 3.4) indicates that biomagnification is significant between fish and fish-eating birds but not between fish and their food. Comment on the biomagnification pathway relative to stressor characterization and the conceptual model.*

Based on the properties of TCDD and related compounds, the panel felt that it may be premature to conclude that biomagnification does not occur among aquatic species (i.e., between plants and animals, invertebrates and fish, or forage fish and piscivorous fish) despite the available data that suggest that biomagnification is not occurring. Although the panel found the available information to support or refute the occurrence of biomagnification to be limited, it acknowledged—based on existing data—that biomagnification does not appear to be a significant process within aquatic food webs excluding mammals and birds.

The panelists felt that biomagnification did appear to occur between fish and piscivorous mammals and birds. The significance of this phenomenon for risk assessment was thought to depend, in part, on how risks are estimated. At present, risks to mammals and birds can be estimated from dietary levels of TCDD and related compounds. The effects data are not based on the body burdens in these animals and, therefore, the issue of biomagnification from fish body burdens to mammal and bird body burdens is somewhat moot. If effects data are developed in the field and laboratory in relation to body burdens, then information on biomagnification processes would be helpful for relating body burdens and associated effects to dietary levels.

#### 4.8. Issue 15—BAF Uncertainties

*Uncertainties associated with bioaccumulation factors are discussed in section 5.1.2 of the Interim Report. Discuss the relevance of these uncertainties to the prediction of TCDD residues in Omigoshie Reservoir biota.*

Previous panel comments on uncertainties about the procedures used to estimate BAFs apply here as well. The primary concern is the inability to measure freely dissolved TCDD concentrations in the water phase.

#### 4.9. Key Research Needs in Stressor Characterization

Panelists considered the following areas to be critical topics for future research:

- Improve the  $K_{oc}$  estimate for TCDD.
- Standardize the methods(s) for measuring  $K_{oc}$  for highly lipophilic compounds.
- Standardize methods for lipid and organic carbon measurements.
- Compile a library of BCF, BAF, BSAF, and BSSAF values. Of greatest interest would be BSAF and BSSAF values from field data.

## 5. COMMENTS ON THE CONCEPTUAL MODEL

Peer panel members were asked to use information in the *Interim Report* as source material to address four issues concerning conceptual model development raised by the scenario. Different approaches to bioaccumulation are indicated in the scenario and are discussed in chapter 3 of the *Interim Report*; in particular, BSAFs are discussed in section 3.5. In addition to responding to the individual issues, panelists suggested revisions and additions to the conceptual model.

### 5.1. Responses to Conceptual Model Issues

#### 5.1.1. Issue 16—Conceptual Model Focus on Fish and Piscivorous Wildlife

*Consistent with the Interim Report, the conceptual model focuses on effects on fish and wildlife that consume fish. Comment on whether this approach captures the full range of potential ecological effects for this scenario.*

Panelists differed in their perspectives on this topic. Some felt that the conceptual model's focus on fish and piscivorous wildlife probably captures the most important ecological effects and is a reasonable attempt to ensure environmental protection by concentrating on "worst case" situations. Others felt that the degree of protection for non-target components of the ecosystem would be better understood only through long-term monitoring programs and that there could be subtle effects on trophic structure. In particular, the possibility of effects on invertebrates was raised, although the assumption is that these animals do not possess the Ah receptor.

Some panelists were more pessimistic, maintaining that a wide range of unanticipated effects are likely to occur because of the present lack of data and information. In this view, indirect effects have not been adequately considered. Examples might include effects on a forage fish (e.g., a minnow) or an invertebrate (e.g., a crayfish) upon which other fish or mammalian species (e.g., the otter) rely for food.

#### 5.1.2. Issue 17—Linking TCDD Loadings to Tissue Residues

*The Interim Report emphasizes using tissue residue levels to estimate the adverse effects of TCDD. To conduct the risk assessment outlined by the conceptual model, however, it will be necessary to link predicted loadings of TCDD in the paper mill effluent to residues in the organisms as identified in the assessment endpoints. Discuss the utility of available risk assessment tools for accomplishing this goal.*

Analyses are most likely to be data limited, not model limited. Panelists noted that a range of models is available for estimating exposure fields in sediments and water. No single model is correct for all situations, but an approach that involves a progression from simple to more complex models makes the most sense. Panelists gave examples, listed below, of the wide range of models available.

- Simple box models
- Fugacity Level III
- Screening models
- Food and Gill Exchange of Toxic Substances (FGETS) model
- Exposure Analysis Modeling System (EXAMS)
- Water Analysis Simulation Program (WASP4)
- RIVER/FISH
- Green Bay model
- Thomann food chain-type model

Panelists indicated that a risk assessment for TCDD must put much more emphasis on the importance of physicochemical fate-and-transport modeling to link exposure to loadings. For the hypothetical scenario, models that predict concentrations in sediments and on suspended sediments make the most sense. Linking these concentrations and possibly water concentrations of TCDD to fish body burdens can

be done using either appropriate factors (e.g., BSAFs) or a food chain model. One possible approach is discussed in text box 2. While a model that relies heavily on partitioning coefficients might be a shaky "house of cards," it may be the best that can be achieved at the present time.

#### **Text Box 2. Predicting TCDD Concentrations in the Hypothetical Scenario**

A two-step modeling approach is needed to predict TCDD concentrations in fish. The first step is to use a fate-and-transport model to link effluent concentrations of TCDD to concentrations in bed and suspended sediments (also dissolved organic carbon [DOC] and dissolved) in the depositional zones of the reservoir. The model could be a simple steady-state model consisting of single water column and sediment compartments. A more realistic simulation of the transport and sedimentation of TCDD from the paper mill effluent would require a multisegmented model run at either steady state (e.g., constant flow, sedimentation rates) or in the dynamic mode. A critical parameter in either model is the  $K_{oc}$  value. Ideally,  $K_{oc}$  values are required for the paper mill effluent, the suspended solids in the river, and algae in the lake and bed sediments, because there is evidence that  $K_{oc}$  values can vary depending on the type of carbon (and whether algae/phytoplankton are in a growth phase). For an initial screening of TCDD transport/fate, a single  $K_{oc}$  value could be used with the steady-state single water/sediment compartment model.

The second step in the modeling approach would be to apply a food chain model or BSAFs to predict concentrations in bed sediments. Because pharmacokinetic parameters for TCDD in invertebrates are uncertain, a possible approach is to use BSAFs to predict concentrations in lower food chain organisms, then use a food chain model to predict concentrations in fish. Pharmacokinetic parameters for TCDD in fish are readily available, although information on assimilation and depuration by larger fish is limited. The food chain model has the advantage of being able to accommodate multiple age classes and feeding relationships, whereas the BSAF approach would require a lot of empirical data to generate the same "library" of relationships between fish and sediment. Given monitoring results from paper mills as well as studies such as Rassmussen et al. (1990) on lake trout, we know that the food chain relationships of various fish species and their age classes will be important in predicting tissue concentrations. Gobas (1992) essentially used a combined BSAF/food chain approach for modeling concentrations of PCBs in Lake Ontario. This approach differs from that used by Thomann et al. (1992b), which uses pharmacokinetics for all trophic levels.

Panelists suggested examining other similar systems with existing sources to help in developing empirical relationships. Where possible, incorporation of site-specific (experimental) data also would help reduce uncertainty in estimates. Use of whole body tissue levels may be inappropriate for assessing effects, and there may be a need to estimate doses to specific target organs. In any case, monitoring will play an important role in checking the predictions of the assessment.

### **5.1.3. Issue 18—Applicability of BAFs and BSAFs to the Conceptual Model**

*The Interim Report describes the limited field data that are available for estimating BAFs and BSAFs. Discuss the applicability of these factors to the Omigoshsee Reservoir conceptual model.*

Panelists felt that BSAFs (and BAFs) provide simple, straightforward models and may be the best available technique for estimating tissue concentrations. Panelists also felt that it would be helpful to compare characteristics of the reservoir to areas for which these factors have been developed.

The utility of the BSAF and BAF approaches depends to a large degree on the accuracy of organic carbon and lipid measurements. In addition, BSAF and BAF values will be site specific as well as spatially and temporally variable. Uncertainty could be decreased by making measurements in a variety of systems and for a variety of environmental conditions.

Panelists suggested using BSAFs for lower trophic levels and perhaps combining this approach with food chain models for piscivorous fish. Another suggestion was to use the relationships established for PCB BSAFs as a tool for estimating TCDD BSAFs, given the larger data sets available for PCBs.

### **5.1.4. Issue 19—Temporal Dynamics of TCDD in the Reservoir**

*The temporal dynamics and disequilibrium situations commonly associated with TCDD are mentioned in the Interim Report (section 2.3). Comment on how these aspects should be considered in establishing (1) the time course for the build-up of TCDD levels following initiation of the paper mill discharge, and (2) the time course for the decrease of TCDD levels and recovery of biota should the paper mill cease operation.*

Panelists felt that the relevant time course depends on the area under consideration. For purposes of estimation, steady-state or quasi-steady state conditions should be relied upon with a temporal constraint being the operational life of the paper mill facility. Attempting to introduce kinetics would involve so much uncertainty that it would be better to use equilibrium models. If there are management questions related to system response time, however, then a time-variable (not steady state) set of models *must* be used. Analysis of effects could use either the time profile for exposure or a time-weighted average of calculated chemical concentrations. The analysis should include a "worst case" scenario.

Important information for the hypothetical scenario includes TCDD loading, reservoir volume and flushing rate, hydraulic retention time, overflow rate (ratio of mean depth to

hydraulic retention time), organic carbon levels, and lipid concentrations in fish. The time course for build-up will depend on hydrodynamics and especially on sediment transport dynamics. Examination of data from existing facilities (including those that have changed operations) will assist in determining the likely time course for build-up and decrease of TCDD levels.

While media contamination may be a straightforward input-output with spatial/temporal variability due to lake morphology, tissue concentrations will more likely follow bounded chaotic dynamics since tissue concentrations depend heavily on a variety of biotic and abiotic factors.

## 5.2. Comments on the Conceptual Model

Panelists modified the proposed conceptual model for the hypothetical scenario in several ways. Panelists rewrote the text (appendix G) and developed additional figures to provide a more complete description of the conceptual model. The overall approach involved:

- linking chemical loadings to water and sediment levels;
- relating these exposure levels to residues in aquatic organisms;
- relating organism residue levels to reproductive and other effects (see section 3.4); and
- relating expected effects to changes at the population level.

The overall approach relating effluent discharge levels, fate-and-transport considerations, and tissue residues and effects is shown in figure 4. The problem may be viewed as establishing the effects of concern to the system, then working backwards to determine release levels from the paper mill that will prevent adverse effects from occurring (figure 5). Further details on TCDD fate-and-transport relationships are given in figure 6. Models used to evaluate fate and transport are likely to be data limited; the best available information would have to be used, whether from experimental data or from the literature. As mentioned earlier (see sections 4.3 and 5.1.2), modeling these relationships can be performed with varying degrees of sophistication. It may be necessary to divide the reservoir up into several segments (e.g., main channel vs. tributary arms) to deal with spatial heterogeneity. If a quantitative analysis of uncertainty is possible, model outputs can be expressed in probabilistic terms (e.g., figures 7a and 7b).

Calculating sediment and water concentrations of PCDDs and PCDFs from allowable residue levels in fish can be carried out using either an empirical BSAF ( $C_f/C_{oc}$ ) or a food chain model. Table 3 lists some of the considerations involved with these two approaches.

Panelists also discussed how to relate anticipated effects at the individual level to population level effects. Qualitatively, one could assume that changes in a measurement endpoint adequately reflect potential changes in the assessment endpoint without further extrapolation; however, more quantitative approaches also are possible. For example, population models such as life table models or individual-based models could be used. The main drawback is the amount of data required to successfully apply these models. Another approach is a "rule

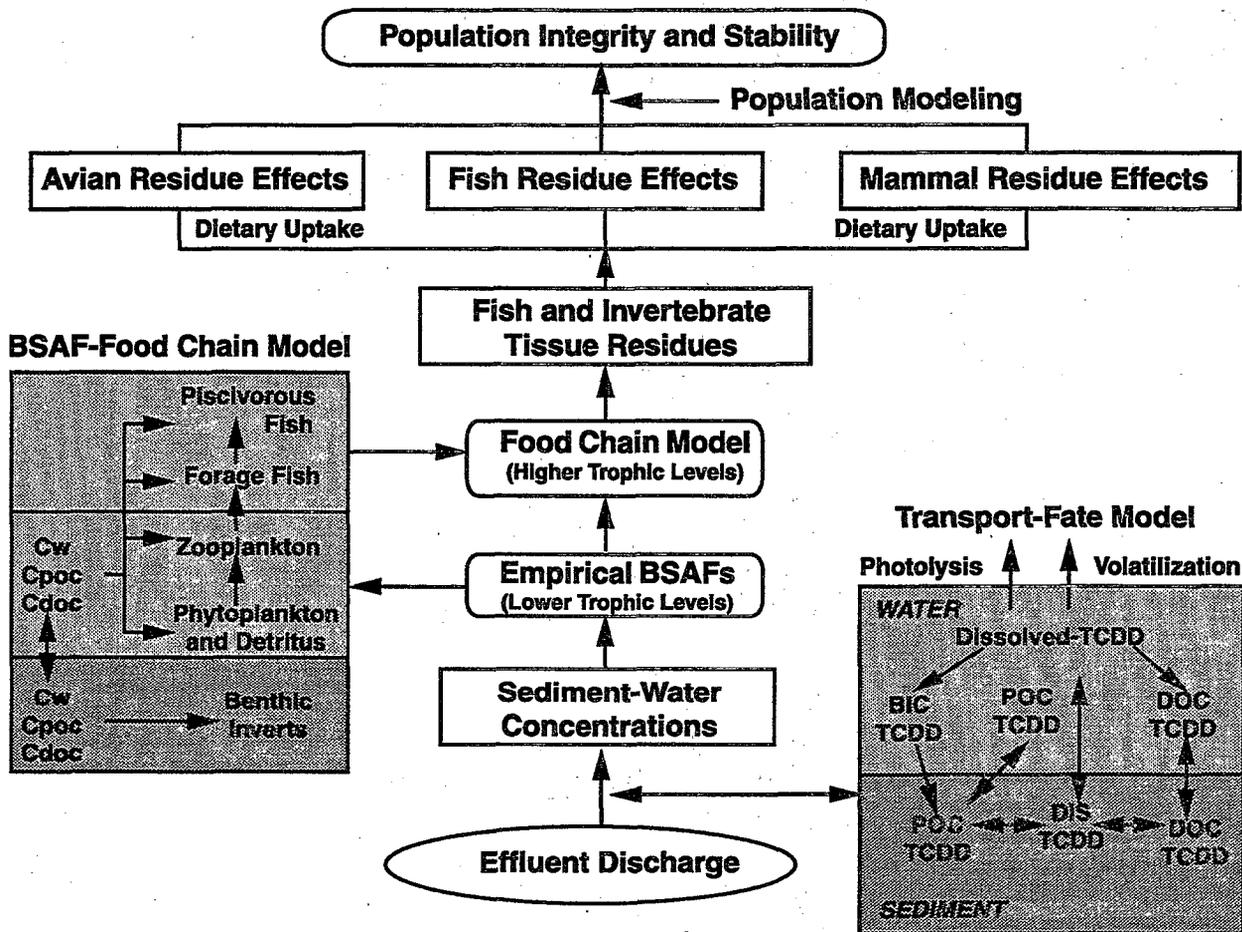


Figure 4. Overall model. The risk assessment needs to establish the linkages between TCDD loadings in the effluent, TCDD fate and transport within the reservoir, residue levels in biota, and effect on biota.

Key: BIC = biological carbon; Cw = TCDD concentration in water; Cdoc = TCDD concentration associated with dissolved organic carbon; Cpoc = TCDD concentration associated with particulate organic carbon; DIS = dissolved; POC = particulate organic carbon.

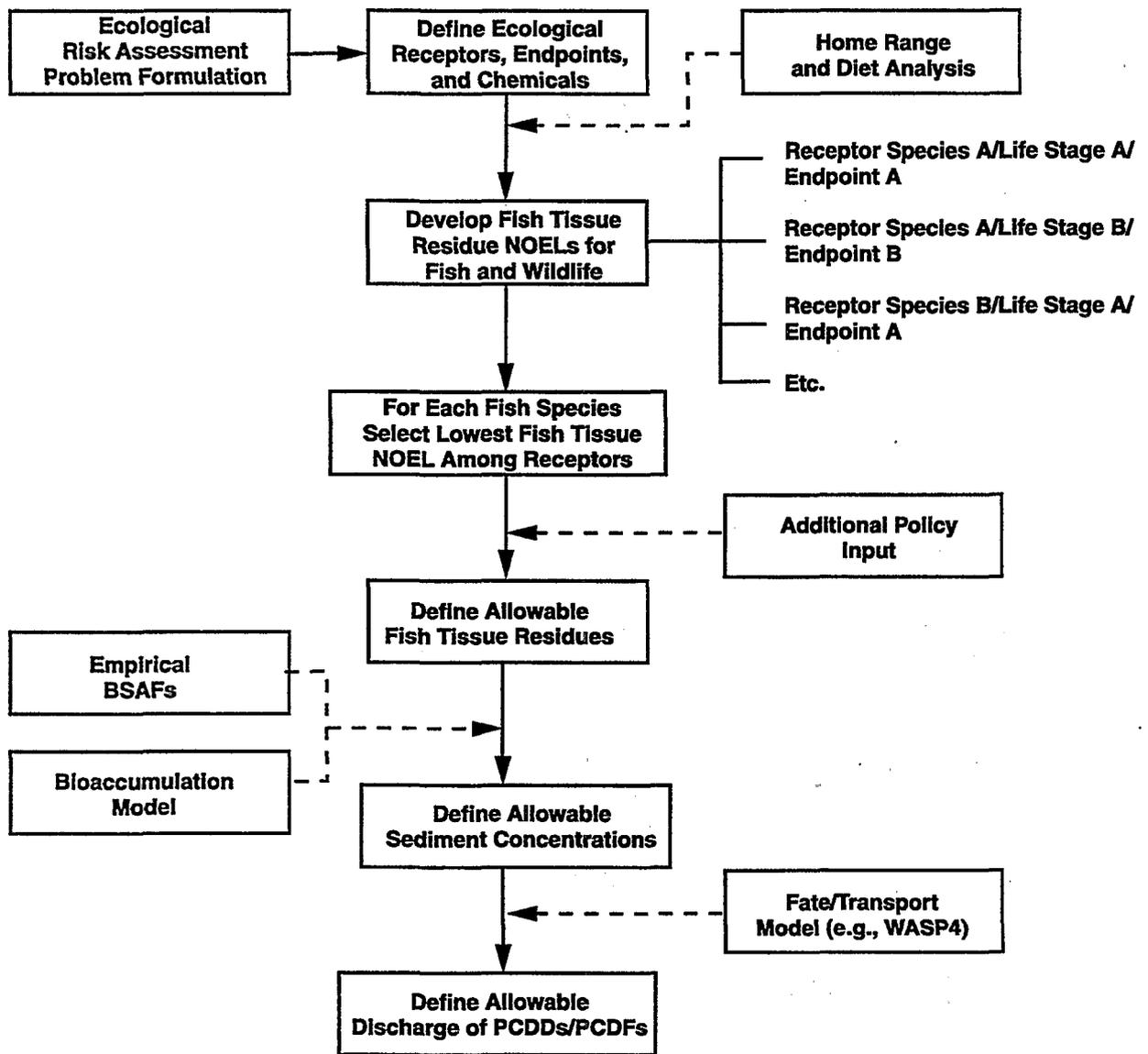


Figure 5. Developing discharge permit limits based on ecological risk assessment. (R.A. Pastorok, PTI, Premeeting Comments. See appendix C.)

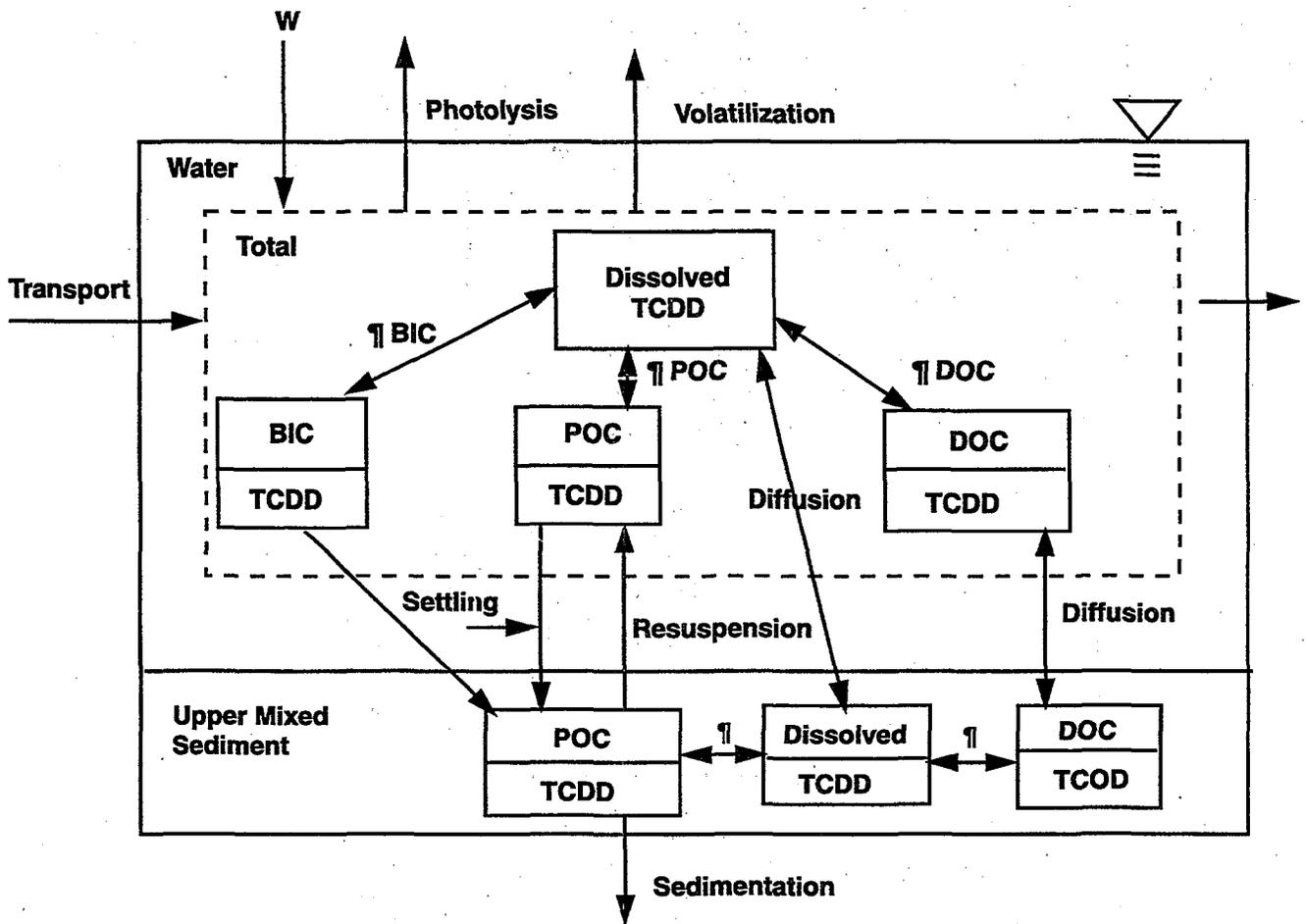


Figure 6. Fate-and-transport diagram. This figure provides a more detailed view of the linkages shown in figure 4. Pathways for partitioning of TCDD between water, sediment, and biota are shown. DOC—dissolved organic carbon; POC—particulate organic carbon; BIC—biota.

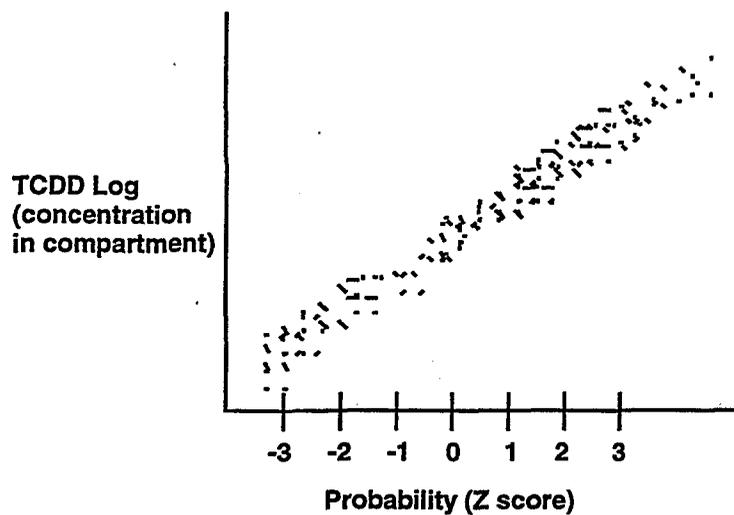


Figure 7a. Example fate model outputs—TCDD (log [concentration in compartment]) vs. probability.

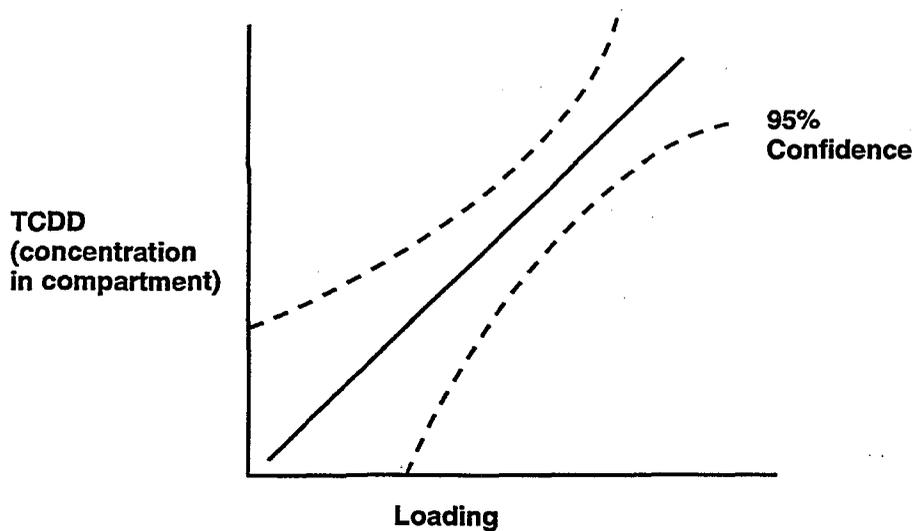


Figure 7b. Example fate model outputs—TCDD (concentration in compartment) vs. loading.

Table 3

COMPARISON OF BSAFs AND FOOD CHAIN MODELS

Category	BSAF	Food Chain Model
<b>Ecoreceptors</b>		
■ Fish	catfish	bass, crappie, bluegill
■ Wildlife food	fish and benthic invertebrates	fish—pelagic
<b>Data Types</b>	$C_p, C_{oc}$	population age structure, diet by age class
	lipid, TOC	lipid, TOC
	BSAFs by species, age, tissue	$K_{ow}, K_{oc}$
	wildlife diet	assimilation efficiency
	allowable $C_1$	allowable $C_p, C_{oc}$
<b>Analytical Tools</b>	method of standardization	model structure
	data bases	software
	framework for predicting BSAFs (figure 8)	
<b>Uncertainty Analysis</b>	joint probability	Monte Carlo
	within-site variance	
	between-site variance	
	extent of disequilibrium	
	nature of TOC, lipid	
<b>Validation</b>	ground truth with PCBs	ground truth with PCBs

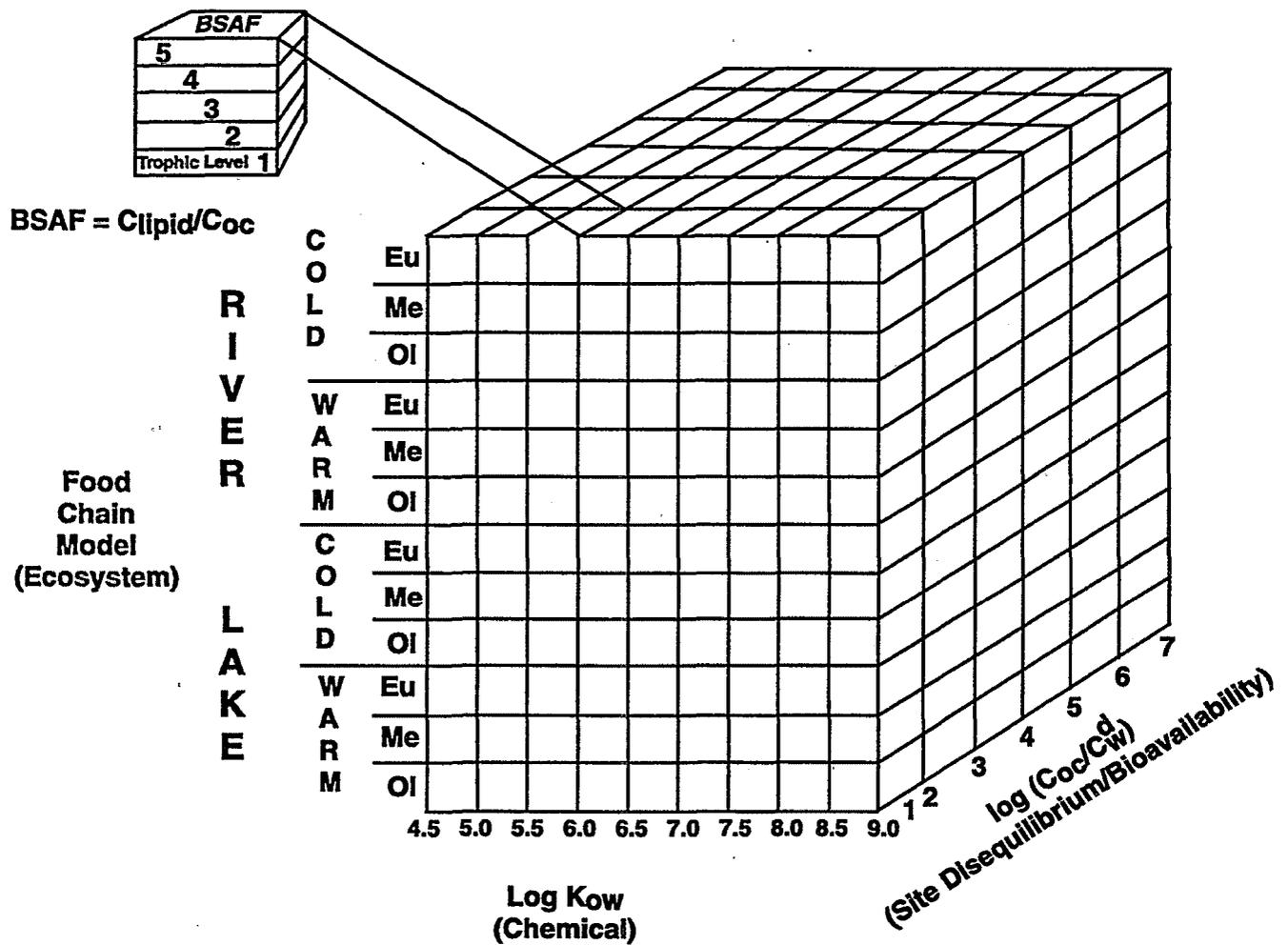
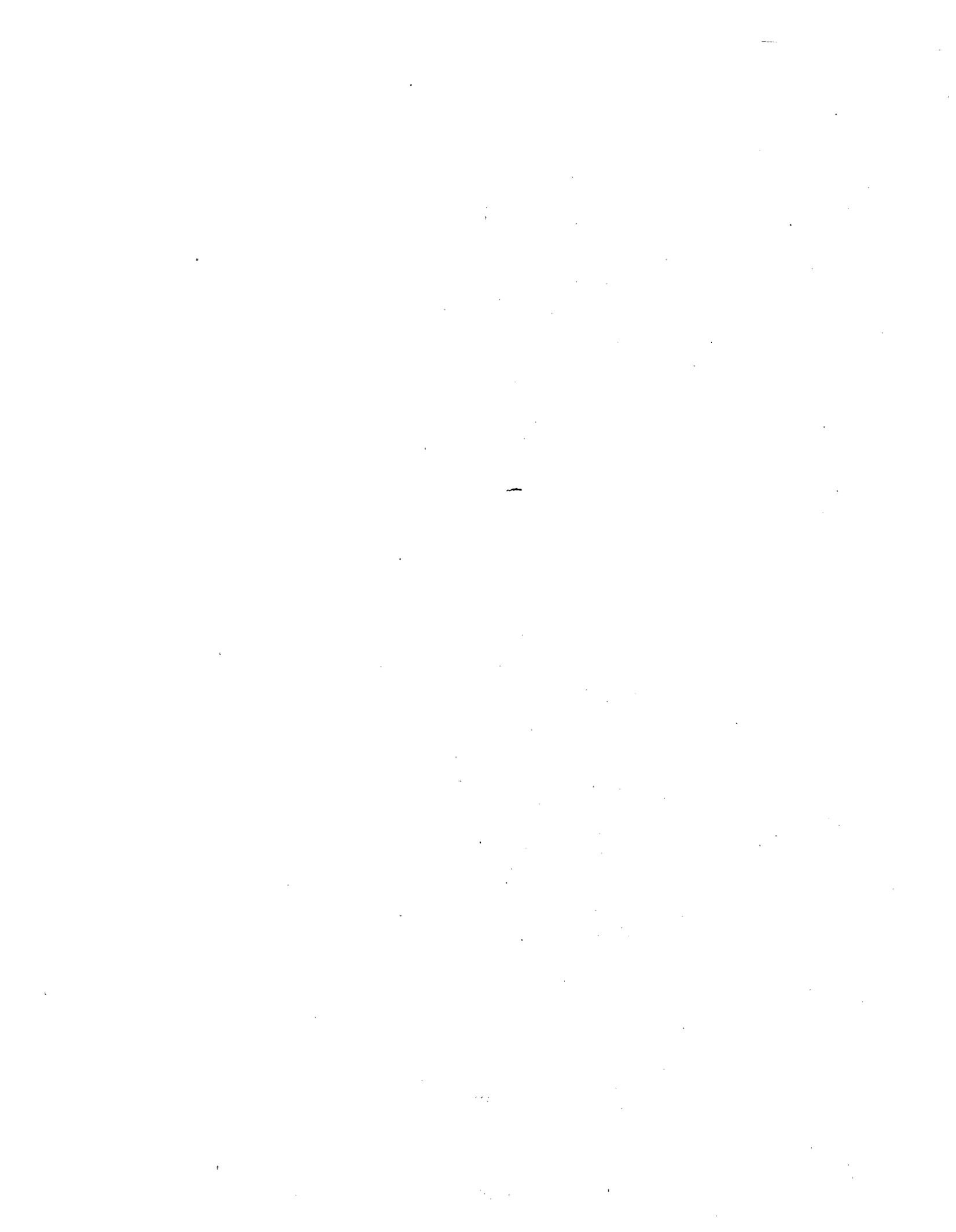


Figure 8. Framework for predicting BSAFs applicable to different aquatic ecosystems. Although developed for BAFs, substituting  $C_{oc}$  for  $C_w$  in this figure shows relationships appropriate for BSAFs. Variables include site-specific bioavailability,  $K_{ow}$  for the chemical(s) of concern, and food chain structure (river vs. lake and trophic status). Eu—eutrophic; Me—mesotrophic; Ol—oligotrophic. (Cook et al., 1991)

of thumb" method. One panelist indicated that EPA uses a 20 percent effect level to distinguish "acceptable" from "unacceptable" mortality. The population effect of a 20 percent decrement, however, will clearly depend on the overall fecundity of the population.



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**APPENDICES A-C**  
**PREWORKSHOP MATERIALS**



**APPENDIX A**

**A PRELIMINARY PROBLEM FORMULATION FOR A DIOXIN SCENARIO:  
PROPOSED PAPER MILL ON A SOUTHERN RESERVOIR**



## A PRELIMINARY PROBLEM FORMULATION FOR A DIOXIN SCENARIO: PROPOSED PAPER MILL ON A SOUTHERN RESERVOIR

Note: EPA scientists have created the attached scenario to supplement the Interim Report for the workshop exercises on TCDD risk assessment. The scenario provides background information for a hypothetical reservoir and presents issues in three areas (stressor characterization, ecological effects and endpoint selection, and the conceptual model), with each area drawing on information in the Interim Report as a starting point. The scenario is presented to promote discussion on TCDD ecological risk assessment in general, EPA is not asking for guidance on how to assess the risk of TCDD discharges from paper mills.

### Background

Introduction. A paper mill is proposed on the Igotchyala River, 5 km upstream from the Omigoshee Reservoir in the southern United States. The reservoir is an important recreational area, supporting a large sport fishery and a variety of avian and mammalian wildlife on public land along its shores. No other industrial development has ever existed in the area and, although the adjacent land is mostly forested, no logging has occurred over the last 30 years. Chemical and biological surveys indicate that the watershed currently is essentially free from significant stress due to toxic contaminants.

Because the proposed mill will use chlorine in its bleaching process, the production and discharge of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other chlorinated dibenzodioxins and dibenzofurans is expected. Because low doses of TCDD can significantly effect egg viability and/or the survival of young fish, mammals, and birds in laboratory tests, the presence of TCDD and associated organic contaminants could impact fish and wildlife populations of the reservoir from this proposed facility.

Risk Management Goals. Under the authority provided by the Clean Water Act (307a) and federal regulations (49 FR 9016), the state is required to control the discharge of toxic pollutants to the Nation's surface waters through the NPDES permit limit process. Because of this mandate, operators of the facility seek to maintain discharges from the proposed facility to below levels expected to be detrimental to the fisheries and wildlife of the reservoir. This risk assessment will evaluate the adequacy of the proposed mill effluent treatment plan and the anticipated chemical discharges in relation to potential effects on fish and wildlife, based on the concentrations of chemicals at steady-state exposure conditions expected under average annual inputs of water and solids to the reservoir. Other stressors and endpoints are subjects of other assessments and will not be considered here. Results of the assessment will be used to determine final permit conditions and effluent treatment standards.

Ecosystem Description. The reservoir (Figure 1) is composed of a broad central basin with three large arms corresponding to the main channel of the river (circled 1 on Figure 1) and two major tributaries (circled 2 and 3 on Figure 1). The shoreline is highly irregular, with numerous coves and inlets of small tributaries. The average total suspended solids concentration in the main channel and major tributaries is 20 mg/L, with an organic carbon content of 10%. Each of the major arms of the reservoir retains about 50% of the sediment load from its respective rivers, with the remainder reaching the main basin of the reservoir. Sediments range from 2 to 15% organic carbon on a dry weight basis, with an average of 5%.

The reservoir has a substantial warm-water sport-fishery including largemouth bass, catfish, crappie, and bluegills. The reservoir is moderately productive, with diverse phytoplankton and littoral vegetation. There are healthy communities of pelagic and benthic invertebrates.

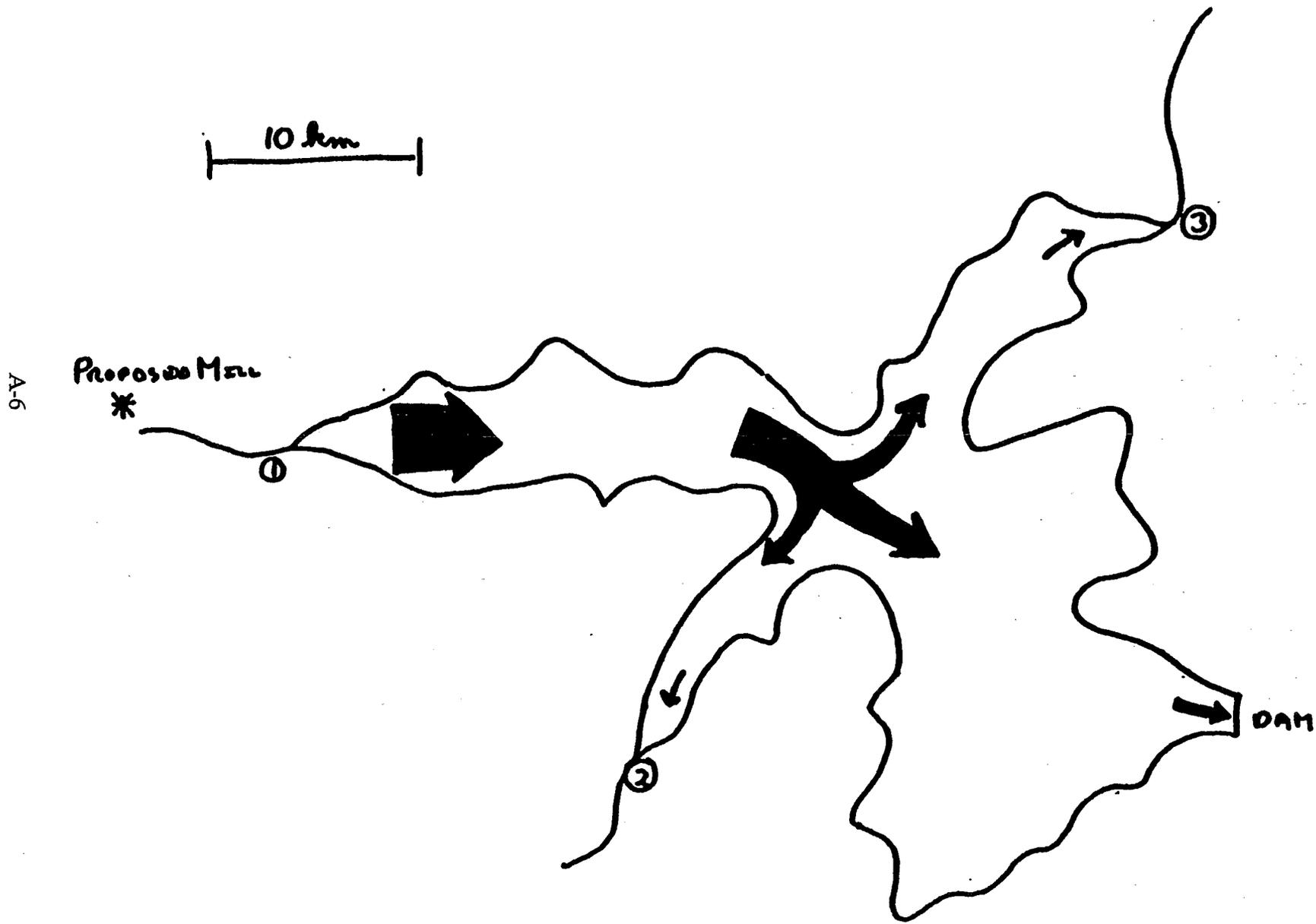
The shoreline supports a variety of avian wildlife, including many species of birds that are primarily piscivorous (e.g., herons and egrets; grebes, cormorants, and various diving ducks; osprey and bald eagle; several species of gulls and terns) and others that feed heavily on emergent aquatic insects (e.g., various fly-catching swallows and warblers). Mammals such as the river otter, muskrat, and mink are found along the shores of some of the coves and tributaries; however, the diet of the otter and mink are only partly from fish from the reservoir itself.

### Stressor Characterization for the Southern Reservoir

The effluent from the proposed paper mill will contain TCDD and other polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), which are formed in paper production which uses chlorine-bleaching. TCDD is highly hydrophobic and associates strongly with organic matter, distributing primarily into the sediments, suspended solids, and biota of an aquatic system. This results in low dissolved concentrations of PCDDs and PCDFs in water. The mill effluent, following the proposed treatment, is predicted to contain TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF. Other chemicals that are known to contribute to toxic effects through an Ah receptor mediated mode of action are not predicted to occur in the effluent. However, the role of other chemicals, including certain PCB congeners that are present in most aquatic ecosystems, in modifying the effect of TCDD through antagonism or synergism is unknown. There are currently no significant sources of TCDD and related compounds to this reservoir, the TCDD level in fish being <0.5 pg TCDD/g whole fish. Total PCB levels in fish are 500 ng/g (presumed to be from atmospheric deposition). There are no data for concentrations of bioaccumulative organic chemicals in water, sediments, aquatic life (other than fish) or wildlife associated with the Omigoshie Reservoir.

The river has no depositional zones that would result in significant loss of the chemical prior to reaching the reservoir and essentially 100% of the discharged chemicals will appear as a point source in the main arm of the reservoir (Figure 1). The discharge of these chemicals will be continuous and is expected to be relatively constant. Despite the use of expected steady-state conditions for the risk assessment, the exposure to aquatic life in the reservoir should be rather heterogeneous, with these chemicals being most concentrated in the main arm of the reservoir, somewhat less so in the central basin, and even less in the other arms where uncontaminated tributaries enter (Figure 1). The effect of sediment distribution and burial on availability are significant aspects of exposure that must first be evaluated with appropriate exposure models. Additional data and models for fate and transport of hydrophobic organic chemicals are provided in a recent EPA report on estimating exposure to dioxin-like compounds (U.S. EPA, 1992b) and the WASP4 model user's manual (Ambrose, 1988).

Figure 1. Diagram of Reservoir with Horizontal TCDD Transport. (Width of arrows denote relative magnitude of net horizontal TCDD flux.)



## Ecological Effects and Endpoint Selection for the Southern Reservoir

Ecological Effects of TCDD. TCDD has been demonstrated in the laboratory to be highly toxic to fish and to many warm-blooded vertebrates. Based on lethal doses, a variety of fish, mink, and gallinaceous birds are especially sensitive. The survival of early life stages of fish and reproduction in mammals and birds have been shown to be the most sensitive endpoints, with survival and growth of older organisms being significantly less sensitive. Other aquatic life (aquatic plants, invertebrates and amphibians) have been shown to be much more tolerant to TCDD than fish and thus would not be an endpoint of concern for this risk assessment. Ecological effects of greatest concern will be the survival of fish fry and the reproductive success of piscivorous wildlife. Doses of concern to piscivorous wildlife are particularly low because of biomagnification of TCDD, although they will not necessarily be feeding exclusively on the most contaminated fish in the reservoir.

The dose/response curves for TCDD and fish are so steep that there is likely little difference in exposures between no effects on populations and very severe effects. For both fish and wildlife the most sensitive and most heavily exposed species appear to be at the top of aquatic food webs. Therefore, this assessment could largely depend on information on effects to individuals. Because available toxicity information on early life stage survival is limited to a few species, a major uncertainty that must be considered is the variability among species and the extrapolation of available toxicity information to species of interest.

For aquatic associated-wildlife, effects will be based on concentrations in aquatic organisms in their diet. For some of the wildlife species, especially the piscivorous mammals, some portion of the diet will be from terrestrial sources or from aquatic animals in the uncontaminated tributaries. These feeding habits and movements must be considered in relationship to the expected contamination of food organisms in order to estimate expected doses. Dose/response relationships for receptor wildlife species or surrogates can then be applied to assess expected effects on individuals and then extrapolated as appropriate to expected effects on populations. As for fish, extrapolations among species of different sensitivities is a major uncertainty that must be addressed.

Ecological Effects of Related Compounds. A major consideration of this assessment is the joint behavior and toxic effects of TCDD and other planar chlorinated aromatic organic chemicals. Comparative toxicity information is available for the rainbow trout (see Walker et al., 1992) and for some mammals (e.g., see DeVito et al., 1993; Safe, 1990). Based on their relative toxicities and concentrations in paper mill effluent, other chemicals of significant concern are 1,2,3,7,8 PeCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF. Relative concentrations in the effluent will not be quantitatively repeated in residues in aquatic organisms due to differences in chemical fate, transport, bioavailability and bioaccumulation.

Toxicity equivalence factors (TEFs) appropriate for assessing the toxic potential of complex mixtures of PCDDs and PCDFs in trout sac fry are available (Walker et al., 1992). The assessment will have to address the fate and transport of these chemicals and their expected accumulation in the food chain in addition to their toxic potential relative to TCDD. The predicted safe tissue concentration of TCDD for each organism is equal to the total TCDD residue toxicity equivalence concentration (RTEC) of concern for the organism under the TCDD toxicity equivalence model (Safe, 1990) which assumes that each chemical's dioxin-like toxicity is additive. Relating the RTEC to concentrations of chemicals in the effluent is complicated by (1) the influence of bioaccumulation and chemical fate and transport phenomena on the composition of the chemical mixture and (2) the choice of appropriate TEFs. Note that there are considerable uncertainties regarding the choice of TEFs for different endpoints and for PCB congeners (DeVito et al., 1993). When the chemical composition of an effluent can be predicted, as in this scenario, the fate/transport and bioaccumulation models used for TCDD can be used to predict differences in the chemical mixture that bioaccumulates in comparison to the mixture in the effluent. If a biota-sediment accumulation factor (BSAF) approach is used, the RTEC can be related to sediment contaminant mixtures:

$$RTEC = \sum_i [(C_{oc})_i (BSAF)_i (f_l) (TEF)_i]$$

where  $(C_{oc})_i$  is the organic carbon-normalized concentration of the *i*th chemical in the surface sediment and  $f_l$  is the fraction lipid in the tissue represented by the BSAF. The chemical fate and transport model chosen to relate concentrations of chemicals in sediment and water to concentrations in the effluent can be used to determine changes in chemical mixture composition between sediment and effluent. The final expression of risk associated with concentrations of these chemicals in the effluent incorporates all of the above factors in a weighted fashion to represent combined effects.

**Assessment Endpoints.** Assessment endpoints of concern to the involved risk managers are the productivity of largemouth bass, catfish, crappie, and bluegill populations which are sought by sport anglers and populations of avian and mammalian wildlife along the shores of the reservoir. As stated above, invertebrate and plant populations are of less concern because of their demonstrated tolerance to TCDD in laboratory studies.

**Measurement Endpoints.** Measurement endpoints that are most relevant to these assessment endpoints are the effects that TCDD has on reproductive success (e.g., egg production and viability) and/or larval and offspring survival in laboratory tests. Because of the uncertainties in establishing the bioavailability of TCDD in aqueous solutions, measured TCDD concentrations in food or in the test organisms themselves, as opposed to aqueous TCDD concentrations, are a more useful metric of expressing and applying dose-response relationships.

Although several studies have been conducted to show reproduction and/or survival of early life stages are sensitive endpoints for TCDD toxicity, data are available for only a small number of species. Consequently, there are uncertainties in extrapolating measurement endpoints from tested species to the species of interest for the assessment endpoints. As stated previously, there are also few toxicity data available for these measurement endpoints with regard to other dibenzodioxins or dibenzofurans.

## Conceptual Model for the Southern Reservoir

The foundation for the conceptual model is the tissue residue approach contained in the interim TCDD report. Chemical residues in tissues of sensitive aquatic organisms exposed to persistent, hydrophobic, lipophilic organic chemicals, such as those predicted for the paper mill effluent, are the exposure metrics upon which the estimation of the potential for adverse effects to the organism must be based. In this case, Figure 2 shows the logical flow of assessment information when thresholds for adverse ecological effects or fish and wildlife population protection goals are to be related to safe chemical loadings to the ecosystem. This is a typical conceptual model for applications of water quality criteria to establishment of effluent permit conditions for single chemicals except that this model can be expanded to consider multiple stressors and provides for consideration of populations of multiple species and their interactions. Models for relating fish populations to chemical dose-toxic response relationships (Barnhouse et al., 1987) are adaptable to the tissue residue approach used for TCDD. The boxes in this conceptual model represent assessment endpoints which are generally quantitative. The arrows between boxes are specific types of models which are used to interrelate the assessment endpoints. All the models are reversible, hence the two-way arrows. The conceptual model applies equally to assessments which seek to determine risks associated with a known or predicted chemical loading (right to left flow of steps).

Figure 2 shows effects on aquatic organisms linked to exposure levels through chemical residue-based dosimetry. The same approach could be used for wildlife assessment, however the toxicity data available for TCDD and related chemicals at this time relate only to dietary dose. Since the known adverse effects of TCDD and related chemicals for fish are directly attributable to exposure of the embryo, the chemical residue levels in eggs is presently the exposure metric of primary interest. If, for example, male fertility should be determined to be a more sensitive endpoint, chemical residue levels in the testes might become an important exposure assessment endpoint. Care must be taken to insure that appropriate exposure and bioaccumulation models are chosen for relating each aquatic species' tissue residue level of concern to chemical concentrations in the water and sediment of the region they inhabit.

Figure 3 illustrates the pathways for TCDD exposures and bioaccumulation in Omigoshsee Reservoir biota. TCDD exposure to fish and wildlife in natural systems is expected to be primarily via contaminated food, and effects are often best referenced to accumulation in food or in the receptor organism itself. Bioaccumulation in aquatic organisms, and the distribution and bioavailability of TCDD in water and sediments, will therefore be of central concern in this assessment. Based on predicted concentrations of chemicals of concern for aquatic organisms, concentrations in the sediment, suspended solids, and water in various areas of the reservoir must be estimated using suitable bioaccumulation models. This can be accomplished via a

food chain model, such as that of Thomann et al. (1992) shown in Figure 4, or application of bioaccumulation factors for fish which describe accumulation relationships without explicitly considering trophic level transfers. The concentration of chemical predicted for the whole organism can be related to specific tissue concentrations through lipid normalization or a more specific toxicokinetic model. Bioaccumulation factors between fish and water are discussed in Chapter 3 of the interim report. The variability of these factors among different organisms and their relationship to organic carbon in suspended solids and lipid in organisms are major uncertainties that must be considered. A third bioaccumulation approach is to estimate chemical concentrations of concern in the surface sediments of the organism's habitat by application of measured or estimated biota-sediment accumulation factors (BSAF; see section 3.5 of the interim TCDD report) to the tissue residue-toxic response relationship for each species of concern. The BSAF approach has an advantage of using an accumulation factor which can be directly measured in contaminated ecosystems.

**Figure 2. Conceptual Model for Risk Assessments and Criteria Development Involving Determination of Safe Loadings of Bioaccumulative Chemicals to Aquatic Systems**

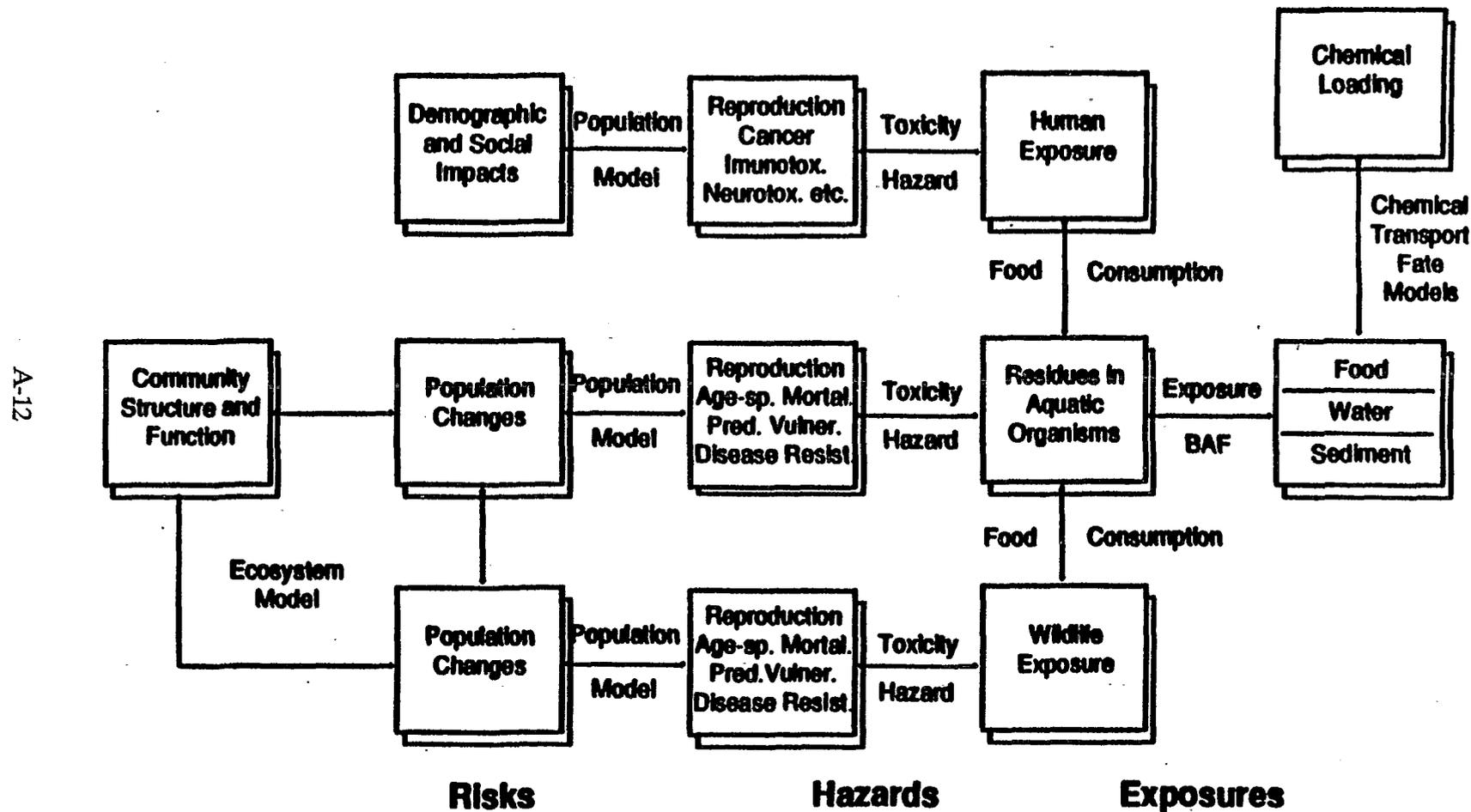


Figure 3. Pathways of TCDD Accumulation in Reservoir Biota.

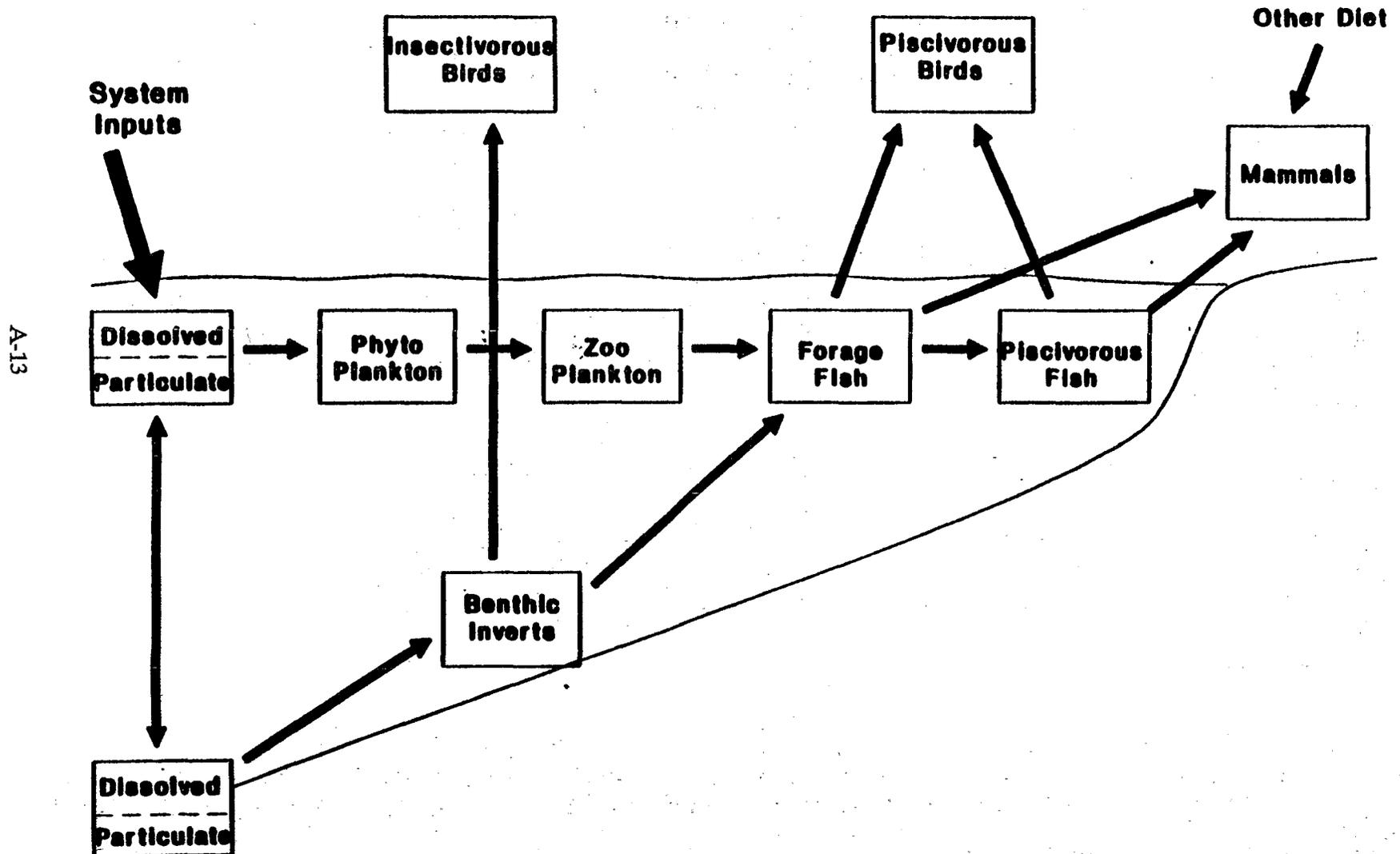
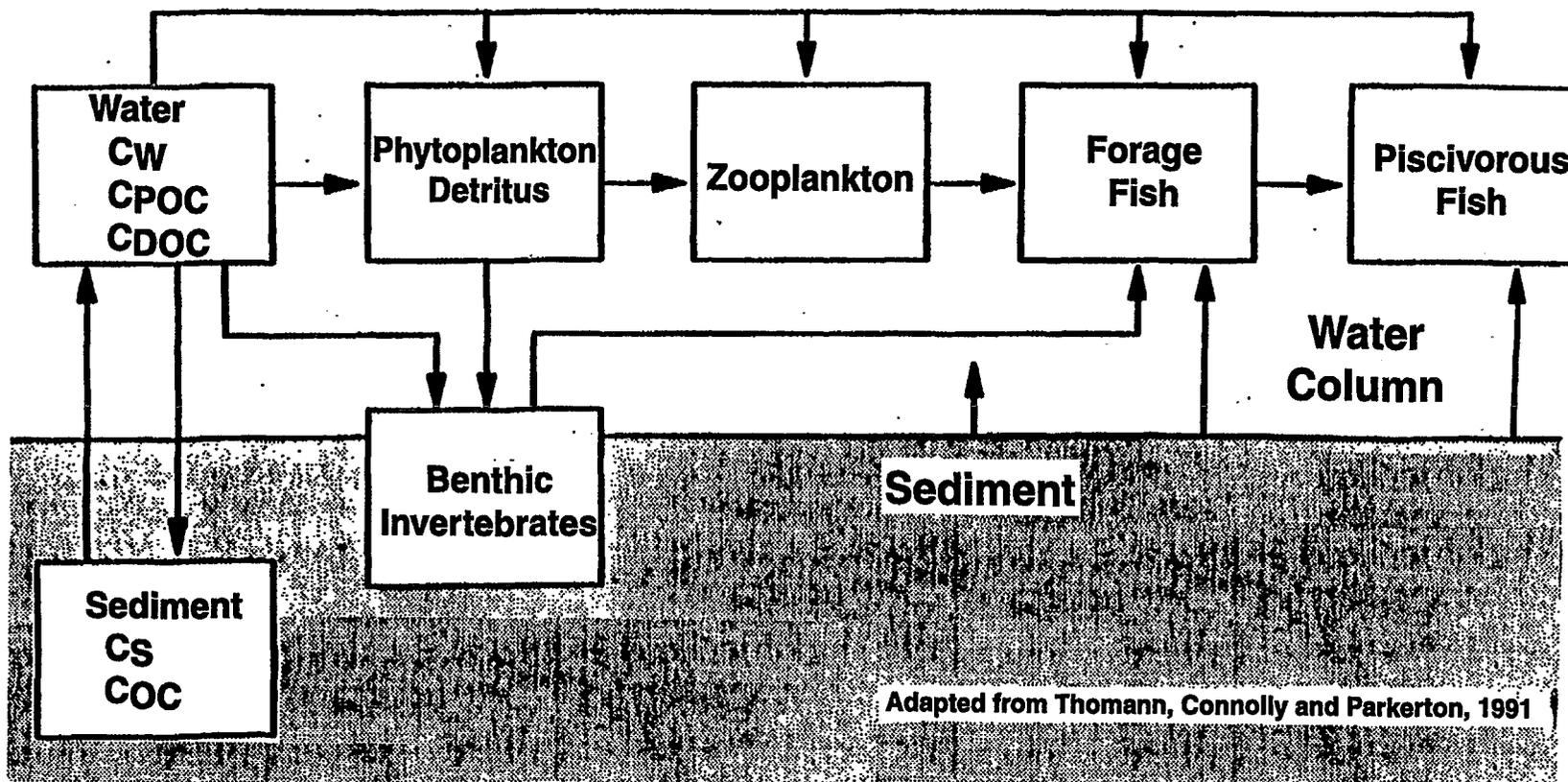
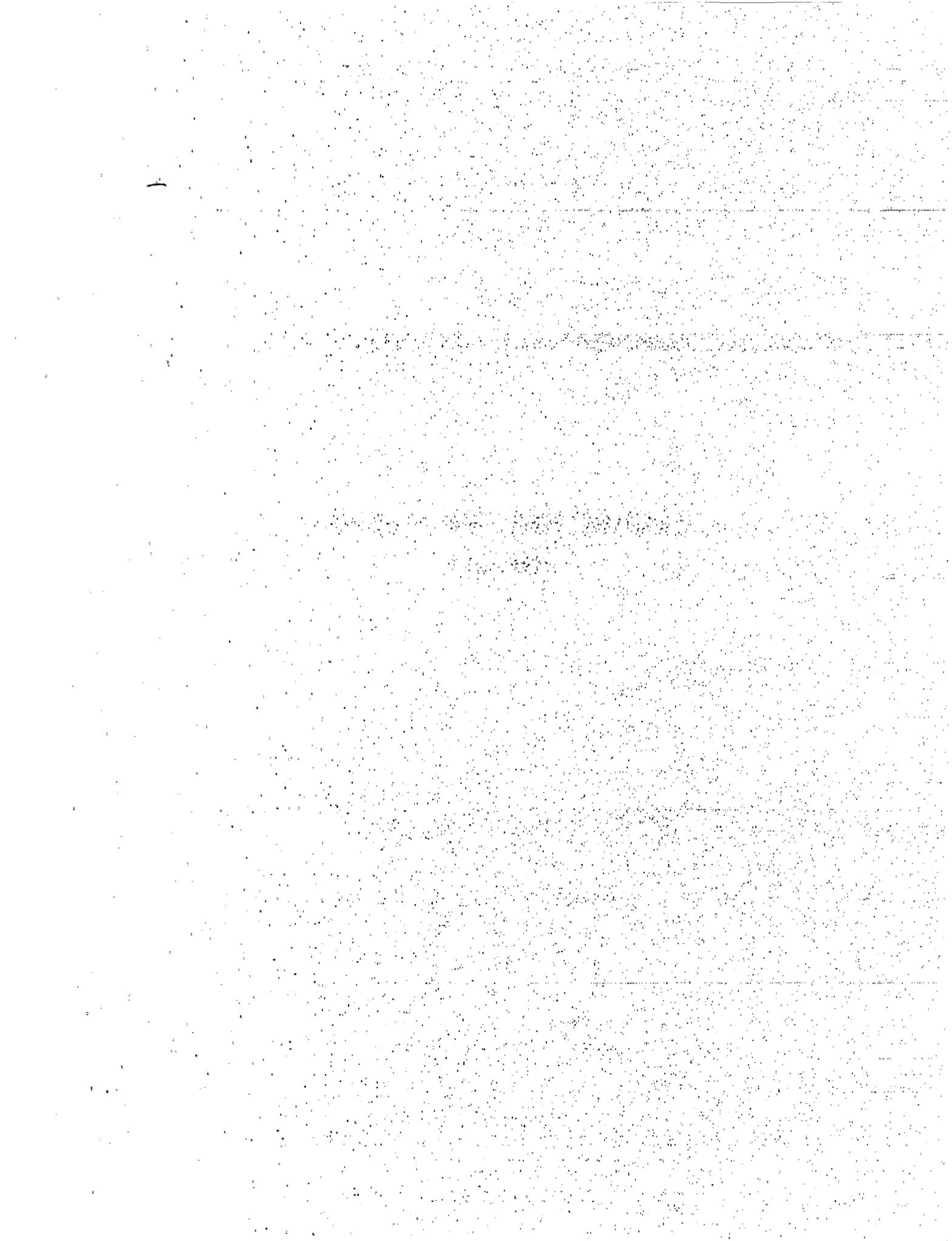


Figure 4. Coupled Benthic-Pelagic Food Chain Model for Bioaccumulation of Organic Chemicals



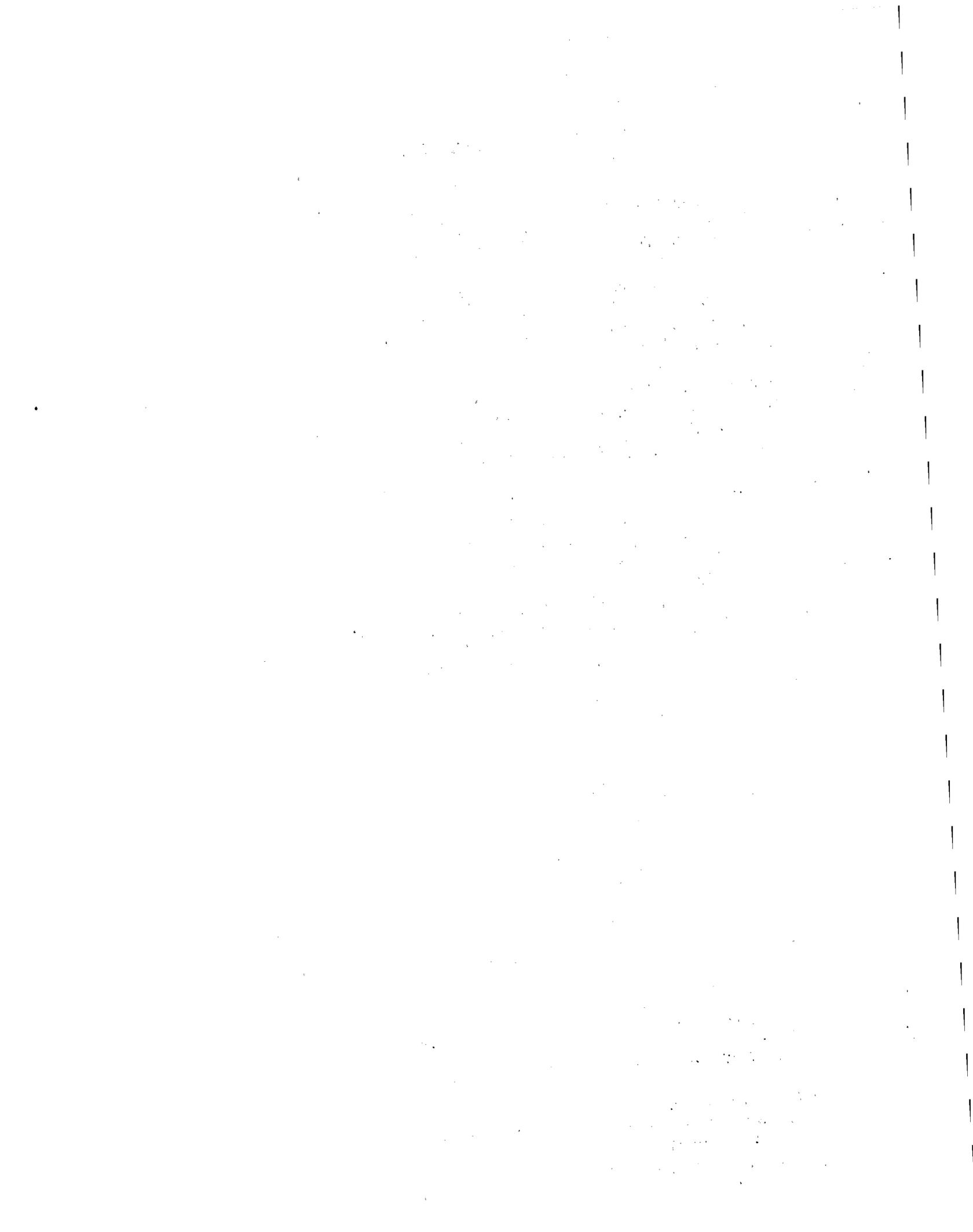
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**APPENDIX B**

**THREE WORKSHOP EXERCISES  
(QUESTIONS TO PANELISTS)**



### **THREE WORKSHOP EXERCISES**

The objective of the Minneapolis workshop is to use the Interim Report to evaluate and develop the conceptual model proposed in the attached scenario. As defined in EPA's Framework Report, the conceptual model is developed during the initial "problem formulation" stage of the risk assessment process. Problem formulation, an initial planning and scoping activity, has several elements, including stressor characterization, ecosystem characterization, and ecological effects and endpoint selection, all leading to a conceptual model for the risk assessment.

The workshop agenda includes three group exercises, each designed to use the attached materials to evaluate the suitability of the approaches described in the Interim Report for conducting ecological risk assessments for aquatic life and wildlife. In the first exercise, participants will review the suitability of available ecological effects information for risk assessment and will discuss endpoint selection, and in the second exercise, they will evaluate information for characterizing the stressor. In the final exercise, participants will discuss the feasibility of the risk assessment approach defined by the proposed conceptual model.

In each exercise, workshop participants are asked to develop approaches for both "ideal" risk assessments (those with appropriate existing scientific information and/or abundant resources -- data, time, funding, and expertise), and more "realistic" risk assessments (where data and/or resources are minimal, but scientifically acceptable). To focus each exercise, EPA has extracted some major findings and approaches from the Interim Report and included them in boxes on the following pages. In their pre-meeting comments, workshop participants are asked to comment on the use of the Interim Report findings in the scenario as well as to propose any changes or additional information that may be necessary for conducting risk assessments.

### **Exercise 1. Ecological Effects and Endpoint Selection**

Peer panel members are asked to use information in the Interim Report as source material to address the following issues concerning ecological effects and endpoint selection raised by the scenario. The Interim Report focuses on the effects of TCDD on freshwater aquatic organisms and associated wildlife (Interim Report, chapter 4). Available data on TCDD effects on fish are provided in section 4.2.1 and are summarized in section 4.2.3 of the Interim Report., while effects on aquatic-associated wildlife are provided in section 4.3.1 and summarized in section 4.3.3. Reviewers should consider using approaches applicable to both "ideal" and "realistic" risk assessments using the pulp mill scenario.

#### **Issues for Consideration**

1. *The lack of Ah receptors in some species (Interim Report, section 4.1) along with the results of a limited number of laboratory studies suggest that amphibians, invertebrates, and plants are less sensitive to TCDD than fish, birds and mammals. For fish, early life stages appear to be most sensitive. Because of this range in sensitivity, productivities of fish species were selected as assessment endpoints for the scenario. Comment on the whether this focus on fish species will result in adequate protection for the rest of the aquatic community in the reservoir from the direct or indirect effects of TCDD.*
2. *Section 4.1 of the Interim Report (and the scenario) describe the use of toxicity equivalency factors (TEFs) for TCDD-like compounds. Section 3.5 of the Interim Report discusses the use of TCDD biota-sediment accumulation factors (BSAFs) for calculating bioaccumulation equivalency factors for other related compounds. Comment on the use of these approaches for evaluating the effects and bioaccumulation of dibenzodioxins and dibenzofurans in the paper mill effluent.*
3. *Because of difficulties in extrapolating from various laboratory exposure conditions to observed effects, the Interim Report (section 4.2.3.1) emphasizes using tissue levels of TCDD (rather than exposure concentrations) to evaluate effects. Comment on the applicability of this approach to evaluating the risks of TCDD from the pulp mill effluent.*

## **Exercise 1. Ecological Effects and Endpoint Selection (Continued)**

### **Issues for Consideration**

4. *The Interim Report uses both laboratory and field information to predict levels of TCDD in fish and wildlife tissues that will cause adverse effects. The scenario proposes to use laboratory test data at the individual level of organization to predict population changes in fish and wildlife. Comment on the utility of available laboratory data to predict effects on field populations and discuss the associated uncertainties..*
5. *The Interim Report sites data that indicate a very steep concentration-response curve for TCDD effects in fish and wildlife. Discuss the implications of this observation for evaluating ecological effects in the scenario.*
6. *The general summary of effect levels for aquatic species and associated wildlife (Boxes 1 and 2, section 4) is based on extrapolations from a limited number of test species and from tests that do not span complete reproductive cycles. Associated uncertainties are summarized in section 5.1.3. Discuss the utility of these data and uncertainties for evaluating ecological effects.*
7. *As discussed in the Interim Report, few data on the effects of TCDD on estuarine and marine organisms have been reported (section 4.2.1.5), and no data were found in the literature for TCDD effects on reptiles or marine mammals. Although all the current wildlife toxicity data were reviewed, an analysis to establish an effects profile for terrestrial organisms was beyond the scope of the report. Describe other effects data not identified in the Interim Report or the scenario that will be important for future ecological risk assessments.*

## **Exercise 2. Stressor Characterization**

Peer panel members are asked to use information in the Interim Report as source material to address the following issues concerning stressor characterization raised by the scenario. Available information on the TCDD physico-chemical properties and exposure characteristics are described in chapter 2 of the Interim Report, while bioaccumulation is described in chapter 3. Reviewers should consider approaches applicable to both "ideal" and "realistic" risk assessments using the pulp mill scenario.

### **Issues for Consideration (Exposure)**

8. The Interim Report (sections 2.1 and 2.2) indicates that there is considerable uncertainty in the estimates of parameters including  $K_{ow}$  and  $K_{\infty}$  and the partitioning of TCDD onto organic matter. This uncertainty results in part from difficulties in analytical measurements of various fractions of TCDD in water. Since these limitations may affect predictions of TCDD partitioning and exposure, please address how they should be handled in stressor characterization and conceptual model development for this scenario.
9. The Interim Report (section 2.4) indicates that most TCDD exposures will arise from food consumption and contact with sediments or suspended solids, with the water pathway being less important. Address the implications of this information relative to the exposure routes in the conceptual model.
10. Fate and transport models are beyond the scope of the Interim Report, but are clearly critical for risk assessment. In stressor characterization and the conceptual model, they will be necessary for linking TCDD source loads to concentrations in different compartments of the reservoir.
  - a. Comment on the availability of fate and transport data/models suitable for use with TCDD.
  - b. Discuss the applicability of available transport models for predicting the deposition of particulate-bound TCDD in the reservoir.
11. List some of the major exposure issues not present in the paper mill scenario that may be encountered in future ecological risk assessments (e.g., marine/estuarine, terrestrial, etc.).

**Exercise 2. Stressor Characterization (Continued)**

**Issues for Consideration (Bioaccumulation)**

12. *The Interim Report (sections 3.2-3.5) summarizes available data on TCDD bioconcentration, bioaccumulation, biomagnification, and biota-sediment accumulation factors from laboratory experiments and field measurements. Discuss the applicability of these factors to stressor characterization for the paper mill scenario.*
13. *The Lake Ontario BAF<sub>f</sub> may be useful as a predictor of residue levels in other systems if C<sub>w</sub><sup>d</sup> can be estimated accurately (Interim Report, section 3.3). Comment on the applicability of this BAF for the paper mill stressor characterization.*
14. *The Interim Report (section 3.4) indicates that biomagnification is significant between fish and fish-eating birds but not between fish and their food. Comment on the biomagnification pathway relative to stressor characterization and the conceptual model.*
15. *Uncertainties associated with bioaccumulation factors are discussed in section 5.1.2 of the Interim Report. Discuss the relevance of these uncertainties to the prediction of TCDD residues in Omigoshie Reservoir biota.*

### Exercise 3. Conceptual Model

Peer panel members are asked to use information in the Interim Report as source material to address the following issues concerning conceptual model development raised by the scenario. Different approaches to bioaccumulation are indicated in the scenario and are discussed in Chapter 3 of the Interim Report. In particular, BSAFs are discussed in section 3.5. Reviewers should consider using approaches applicable to both "ideal" and "realistic" risk assessments using the pulp mill scenario.

#### Issues for Consideration

16. Consistent with the Interim Report, the conceptual model focuses on effects on fish and wildlife that consume fish. Comment on the whether this approach captures the full range of potential ecological effects for this scenario.
17. The Interim Report emphasizes using tissue residue levels to estimate the adverse effects of TCDD. However, to do the risk assessment outlined by the conceptual model, it will be necessary to link predicted loadings of TCDD in the paper mill effluent to residues in the organisms identified in the assessment endpoints. Discuss the utility of available risk assessment tools for accomplishing this goal.
18. The Interim Report describes the limited field data that are available for estimating BAF's and BSAF's. Discuss the applicability of these factors to the Omigoshsee Reservoir conceptual model.
19. The temporal dynamics and disequilibrium situations commonly associated with TCDD are mentioned in the Interim Report (section 2.3). Comment on how these aspects should be considered in establishing (1) the time course for the build-up of TCDD levels following initiation of the paper mill discharge and (2) the time course for the decrease of TCDD levels and recovery of biota should the paper mill cease operation.

**APPENDIX C**  
**PANELISTS PREMEETING COMMENTS**

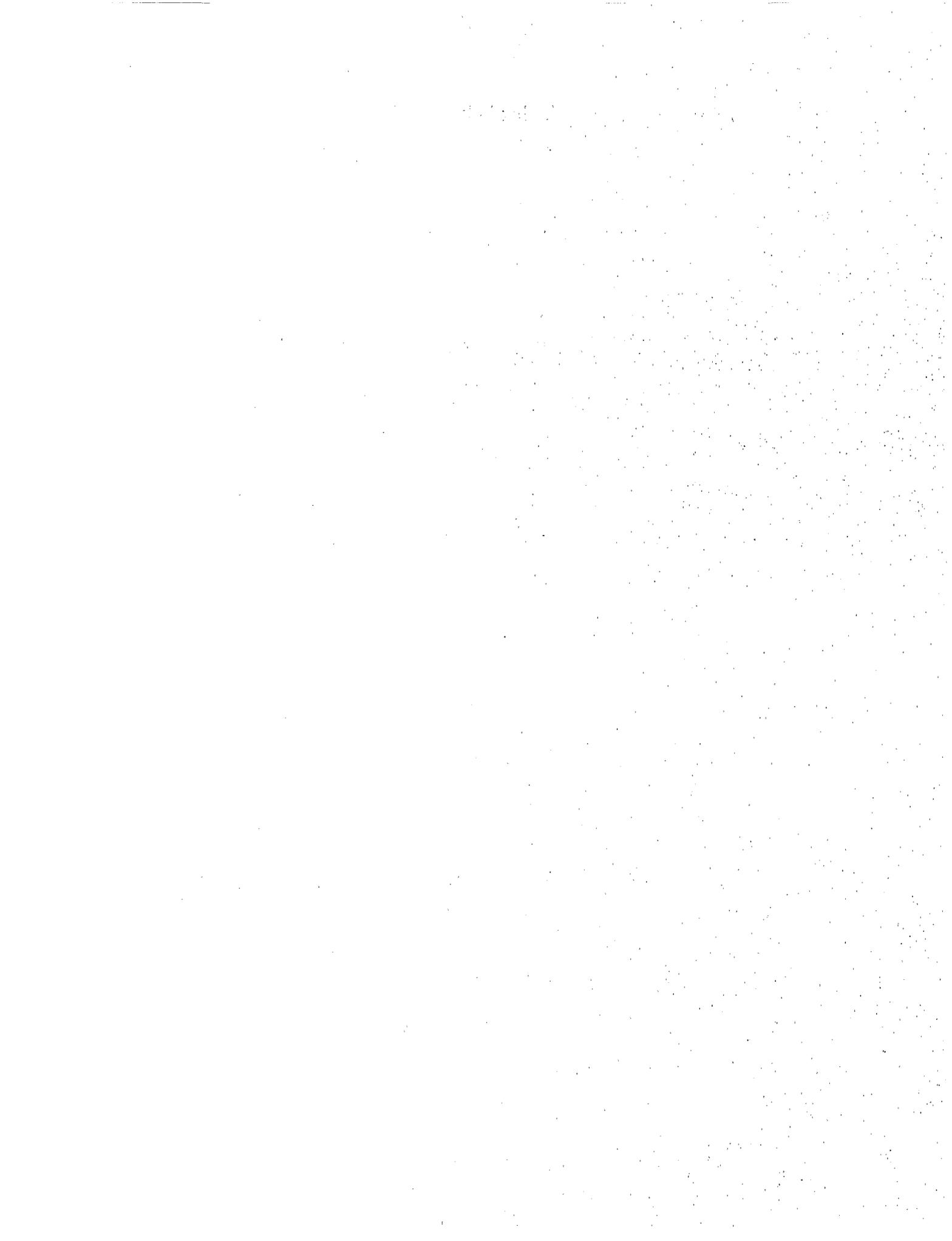


**United States  
Environmental Protection Agency**

**Workshop on  
Ecological Risk Assessment Issues for  
2,3,7,8,-Tetrachlorodibenzo-p-Dioxin**

**Premeeting Comments**

**Minneapolis, MN  
September 14-15, 1993**



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Section 1

**GENERAL COMMENTS**



**Peter Chapman**  
**EVS Environmental Consultants**

## QUESTION

1. The following table shows the number of people who attended a concert in each of the five years from 2000 to 2004.

Year

2000

2001

2002

2003

2004

The number of people who attended the concert in each of the five years from 2000 to 2004 is shown in the following table.

Year

2000

2001

2002

2003

2004

2005

2006

2007

2008

2009

2010

2011

2012

2013

2014

2015

2016

2017

2018

2019

2020

2021

2022

2023

**PREMEETING COMMENTS:**  
**WORKSHOP ON ECOLOGICAL RISK ASSESSMENT ISSUES FOR**  
**2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)**

**Prepared By:**

Peter M. Chapman  
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**General Comments**

The word "dioxin" is loaded with perception, which will inevitably complicate the scientific search for facts. This needs to be acknowledged up-front and a commitment made to conduct studies according to rigorous scientific methodologies, without regard to perceptions or fears associated with the word "dioxin".

In this regard, it is important to note that there is no record of human death or even life-threatening illness as a result of TCDD exposure. The only recorded effect, even with volunteer prisoners exposed to unrealistically high concentrations, or the Seveso accident (when cows, horses, rabbits, sheep and chickens died but humans did not despite identical exposures), is chloracne. TCDD is extraordinarily toxic to some animals, but the label "the most toxic substance known" only applies to guinea pigs. For instance, while TCDD has an LD50 of 0.6 ug/Kg to guinea pigs, hamsters are over three orders of magnitude less sensitive, with an LD50 of 3,000 ug/Kg. Clearly there is significant inter-species variability in toxicity, which is documented for aquatic species in the report.

To be scientifically credible, all studies of TCDD effects must be screened to eliminate those which do not control for confounding factors, those which include multiple comparisons which produce positive associations by chance alone, and those which do not establish clear exposure routes. This is clearly the "ideal" situation. More "realistic" situations may, depending on how far they are from the "ideal", not be scientifically credible.



Wayne Landis  
Institute of Environmental  
Toxicology and Chemistry  
Huxley College of Environmental Studies



Workshop on the Ecological Risk Assessment Issue for 2,3,7,8-Tetrachlorodibenzo-p-dioxin  
(TCDD) Radisson Hotel Metrodome, Minneapolis, MN. September 14-15, 1993  
Premeeting Comments-Issues for Consideration  
Wayne G. Landis

***General Comments***

The Interim Report is a well written and detailed document reflecting the state of the current Ecological Risk Assessment working paradigm. It reflects the single species approach, much like what occurs in human health risk assessment, to a multispecies problem. Dynamics at the population level or the interactions among the various organisms or guilds of the affected communities are difficult to extrapolate from the data presented in this report. This fact is not the fault of the report but perhaps a fault of the lack of a systematic program to ascertain the ecological effects of TCDD.

I would have liked to see a better description of the molecular biology and evolutionary derivation of the Ah receptor. Given a detailed description and some comparative molecular biology, some of the questions regarding potential sensitivities and additive effects would have been based on a better and more fundamental understanding of TCDD intoxication.

One approach to help facilitate the incorporation of data relevant to the evaluation of ecological datasets is presented below. Based on the factors determining competitive outcomes as described by Tilman (Tilman 1982, Landis 1986), the diagram attempts to highlight the factors that a toxicant impacts in an ecological framework.

On the physiological side, I have found the graphical representation of the physiological effects of a toxicant produced by the Wildlife Toxicology Group at USEPA Corvallis as a useful reference framework (Included as an enclosure). Immunological suppression, reproduction and other functions are delineated.

Another crucial factor that I would like to emphasize is the importance of the behavior of an organism and the impacts of TCDD intoxication on predator avoidance, mate selection, and foraging behavior. The outcomes of these alterations in behavior can have dramatic impacts on population dynamics and extinction rates. Organisms that cease to eat or that show lack of predatory avoidance are often dead in short order. Changes in behavior may also have dramatic impacts on survivorship of populations at risk.

Finally, and of course I am biased in this direction, the lack of multispecies toxicity tests (microcosms, mesocosms, microecosystems, eco-cores) is worrisome. Many of the questions points below may have been answered by the investigation of specific relationships using these types of tests. Admittedly, data analysis and extrapolation are difficult, but any more difficult than

extrapolating from clean water, laboratory cultured, limited time span single species toxicity tests? As an addendum I have included references and summaries of a variety of multispecies toxicity tests that may have proven useful in the evaluation of TCDD.

### Framework for Evaluating Ecological Effects Among Interacting Organisms

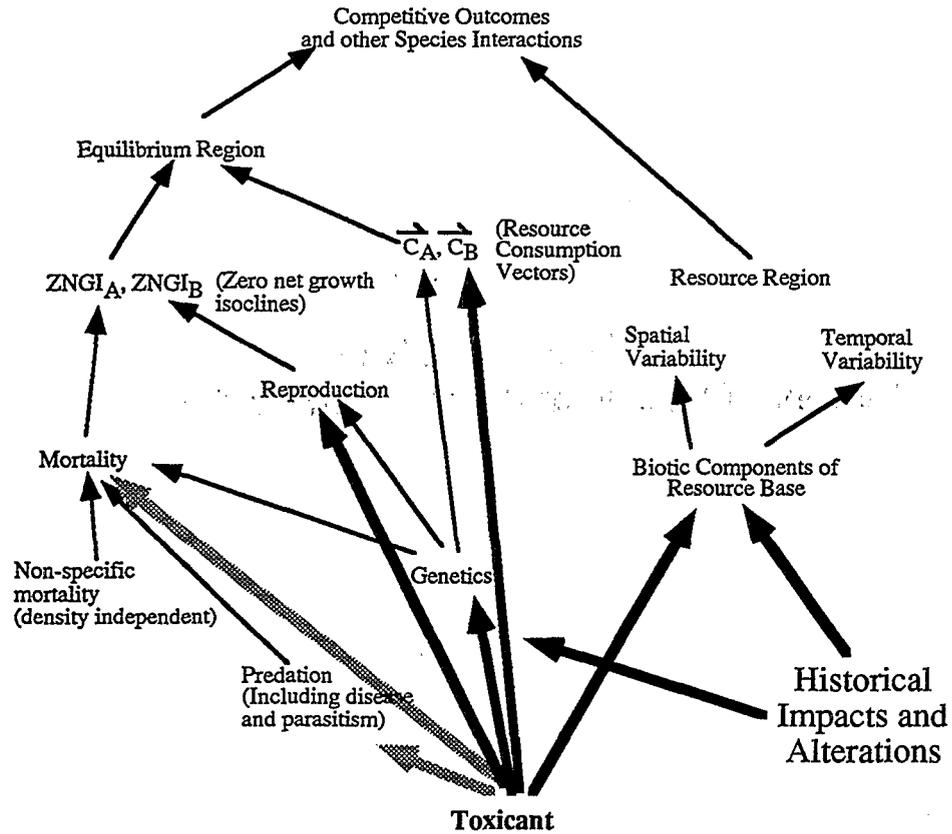


Figure 1. Resource Competition Theory as a Guide for Analysis of Populations and Ecosystems

**Thomas O'Connor**  
**National Oceanic and Atmospheric Administration**



## Comments on "Interim Report on Data and Methods for Assessment of 2,3,7,8-Tetrachlorodibenzo-p-dioxin risks to Aquatic Life and Associated Wildlife"

Thomas P. O'Connor NOAA N/ORCA21

I wrote these general comments before I realized that I was supposed to respond to a prescribed scenario. I did not envision the challenge of predicting the consequences of a future discharge of TCDD to a reservoir. Since some of what I was specifically asked to address is covered in these comments I will accept the embarrassment of leaving them in. In effect, I have called my own bluff.

### GENERAL COMMENTS

The summary of the Interim Report is Table 5.1. The first column contains TCDD concentrations in fish that are proposed to constitute risks to fish, terrestrial mammals and birds. As intended, and I think correctly, this column is central to the risk assessment. The other columns are calculated concentrations of TCDD in sediment or water that theoretically correspond to the fish concentrations. I will express some skepticism on this last point but, even if the calculations could be done, the risk assessment would still have to be based on TCDD concentrations in fish.

The calculated TCDD partitioning among organisms, sediment, and water are based on equilibrium assumptions that are useful in emphasizing the importance of lipid in organisms, organic carbon on sediment, and the octanol-water coefficient of TCDD. However, the calculations are only rough approximations. The report is replete with reasons for calculated partitioning not to conform with field data for dioxin. For other compounds for which there are much more field data, Bierman (1990) for example showed how much of a discrepancy exists between measured and predicted ratios of organic contaminants in sediments and in organisms.

The approximate nature of equilibrium assumptions appears in the EPA proposed Sediment Quality Criteria for neutral organics. Those criteria are based on the equilibrium assumption and calculations of interstitial water concentrations. The 1991 version of the "Proposed Technical Basis for Establishing Sediment Quality Criteria for Nonionic Organic Chemicals Using Equilibrium Partitioning" specifically points out that it is not technically justified to extend the calculations to body-burdens in fish. It is unjustified because of the long sequence of equilibrium assumptions needed to predict body-burdens in fish from concentrations on sediment.

If TCDD were a risk to the health of invertebrates there would be some incentive for extrapolating from sediment concentrations to body-burdens in benthic organisms. However, as the report explains in detail, invertebrates are not endowed with the Ah receptor necessary to initiate the sequence of reactions through which TCDD can affect health and reproduction, the concern is for birds, mammals and fish.

For birds and mammals, fish are the exposure-route, so TCDD concentrations in water and sediment are only indirectly relevant. For fish, of course, they are directly relevant but, it appears, that effects can be related to tissue concentrations instead of to concentrations in abiotic compartments.

To some extent that simplifies the risk assessment because it becomes one of assessing effects as a function of TCDD body burden in fish. It also provides insight to the spatial extent of possible risks because there are data plotted in this report as Figure 5.1 and in EPA (1992) on the distribution of TCDD concentrations in fish at 388 sites. Most of those site (314) were chosen to maximize chances of finding high levels of TCDD, but the remaining 74 are probably typical of most locations in the country. Concentrations in fish as high as 6 pg/g were rare, and were found near the discharges of paper mills using chlorine and at some industrial charges. Since 6 pg/g is the second lowest body-burden in Table 5.1, one would conclude that TCDD poses a threat to fish or to birds only in isolated "hot spots". While this does not argue against remediation, one could certainly question whether or not the spatial scales of such areas constitute ecosystems.

On the other hand, the lowest body-burden in Table 5.1 is 0.7 pg/g, a concentration found even at some of the 74 background sites. Since that fairly commonly found body-burden poses an hypothesized threat to reproduction among mink, one would expect feral mink populations to be on the decline. I have no idea whether that is happening but it seems worth a look. Similarly, experiments of the reproductive consequences on mink of ingesting 0.1 pg of TCDD per day seem to be in order.

I have obviously not considered this risk assessment in an absolutely objective sense. If the lowest TCDD concentration in fish thought to pose a risk to any organism is one that is rarely encountered, the assessment becomes very much an academic exercise. To use round numbers, the first question is whether or not TCDD body-burdens of 1 pg/g pose a threat to aquatic organisms or their predators. If there is threat does it constitute an ecological hazard?

I have nothing to add to the Interim Report that would cause me to consider such a concentration to be a threat to fish or birds. The interesting question concerns selecting mink reproduction as the critical ecological endpoint. I have no doubt that reproductive losses are an important endpoint. But I do question selecting the single most sensitive species. Three factors caused mink to be chosen 1) they are piscivorous, 2) the lethal TCDD dose to them is slightly less than that to guinea pigs but ten or more times less than it is for rats, rabbits, mice, or hamsters and 3) the level of TCDD ingestion shown not to affect Rhesus monkey reproduction was 0.13 pg/g/day and about ten times less than the corresponding value for Sprague-Dawley rats. It was therefore Hypothesized that the TCDD ingestion rate that would not cause reproductive damage to minks would be 0.1 pg/day or less.

This is a testable hypothesis but, assuming that fish body-burdens greater than 1 pg/g are a hazard to mink, are they an ecological hazard? Here the analysis gets really sticky so I started to reread the stuff sent to me. Then I found the scenario.



Section 2

**EXERCISE 1**

*Ecological Effects  
and Endpoint Selection*

**Workgroup Leader:**  
Randall Wentsel  
U.S. Army



## PREMEETING COMMENTS

RANDALL S. WENTSEL, Ph.D.

### INTERIM REPORT ON DATA AND METHODS FOR ASSESSMENT OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN RISKS TO AQUATIC LIFE AND ASSOCIATED WILDLIFE

I thought the Interim Report was well written and presented the data in a professional manner. The authors also did a good job in identifying data gaps and discussing their significance.

#### 1. a. Realistic

From the data available it appears that the focus on sensitive fish species will provide adequate protection for the aquatic community. It has been common in setting water quality criteria to protect the most sensitive species and through that protection infer protection of the aquatic community. Protection of the lake trout population is an important assessment endpoint in Great Lakes systems. The bioaccumulation of TCDD in tissues to produce a reduction in fecundity is an important pathway in the assessment of TCDD effects in aquatic systems.

Relating protection of fish species to the structure and function of an aquatic system is somewhat difficult. The reduction of a key fish species at the top of the food web will alter the structure of an aquatic system. The issue of the replacement of a sensitive species by a tolerant species may result in no functional change in the aquatic system. However, the loss of a sport fish population would reduce the value to society of the aquatic system.

#### b. Ideal

In an ideal situation the assessment endpoints would be at the community and ecosystem levels. The various direct and indirect pathways of TCDD to effect biota would be addressed.

2. Because data indicate that planar PCBs and other compounds act on the same site as TCDD, the use of toxic equivalents to reflect their additive effects is logical. Data on bioavailability of these compounds should be considered when toxic equivalents are calculated. The BSAF technique is a new application which requires additional validation before acceptance as a method. However, a technique is needed to relate sediment TCDD concentrations to toxic tissue concentrations and the BSAF can perform that function.

3. The major accumulation areas of TCDD in aquatic systems are in tissue and sediment. It makes sense to focus on those areas where measurable amounts of TCDD occur. Because of its hydrophobic properties TCDD binds with organic particles suspended in the water column or organic matter in the sediment. Measuring exposure concentrations for water are very difficult due to the very low detection limits required and the dynamic movement of TCDD out of the water column. The use of models would be difficult to validate with the data and techniques available. The use of tissue data to establish no effect levels is technically valid due to the relationship of tissue levels to reduced fecundity. Relating the sediment and tissue concentrations to the degree of risk for a given system will be necessary.

4. The use of laboratory data to predict effects in the field has been criticized for over and under estimating the impact to biota. In an ideal situation using laboratory data with field validation would be a preferred approach. For TCDD, the laboratory data identified a sensitive development phase and generated precise data on tissue concentration and effects on reproduction. A field study, under realistic conditions, could not have done this. The uncertainties will be associated with the bioavailability of the chemical in the laboratory compared to its bioavailability in the field. Useful information would include the distribution of TCDD in fish populations; with the percentage of the population predicted to be effected at given TCDD tissue concentrations.

5. Steep concentration response curves indicate a rapid change in effects versus small changes in exposure concentration. This situation necessitates increased protection factors for the aquatic system. For the scenario this would require increase precision in field sampling and monitoring efforts and/or additional protection factors.

6. Early life stage tests have been shown to produce results similar to complete reproduction cycle studies. The authors point out most of the weaknesses of the current data. Some discussion of the distribution of TCDD in tissue versus fry survival should be emphasized. Probability calculations to assess the degree of impact on a fish population are needed. Uncertainty is addressed in the report. Toxicity reference values could be used to address uncertainty when applying the data to other species. However, I would caution the user in applying uncertainty factors so that the result of the process is not a "safe" concentration below background levels of TCDD in the environment.

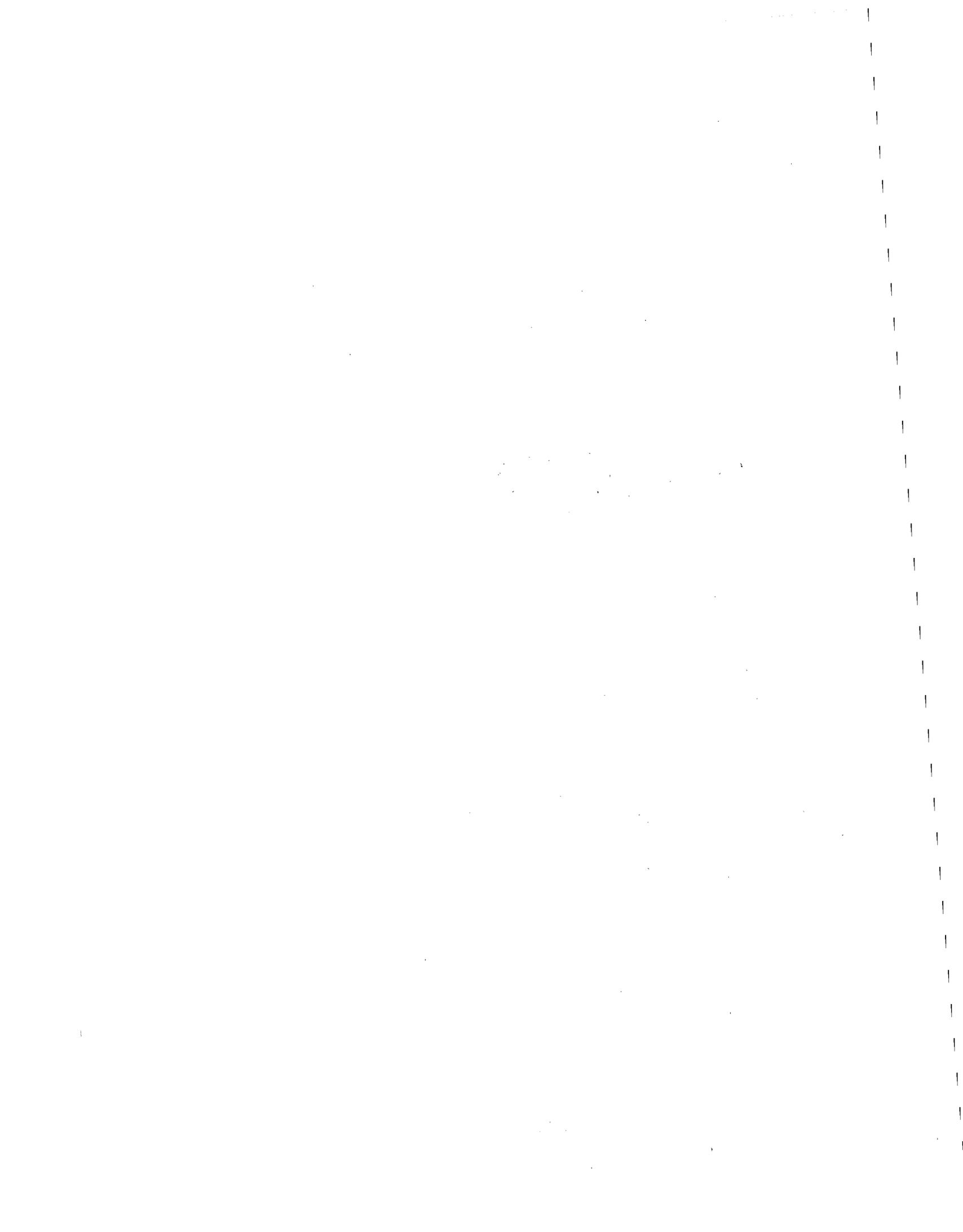
7. The data indicate that TCDD is toxic to wildlife at low levels. This will drive any future ecological risk assessments. Additional research is needed to insure the

accuracy of the data and to validate effects in the field. For the models, parameters should be measured, if possible, to reduce variability in the estimates. Endangered species also drive ecological risk assessments. While Bald Eagles are mentioned in the scenario, no special treatment of them is recommended. Endangered or threatened species receive special treatment because the individual must be protected versus protection of the population for other species.

Future ecological risk assessments will address Superfund sites contaminated with TCDD. More effort needs to be spent relating the ecological effects to an exposure source that can be remediated. Research should be conducted to establish sediment and water criteria. Increased effort should be made to use biomarkers and relate increased or decreased levels of them to significant TCDD exposure. This research should be applicable to a variety of aquatic systems. Additional data are required on the distribution of TCDD in the tissue of fish populations. These data will identify how large a problem elevated TCDD concentration are to aquatic system. A tiered approach in conducting ecological risk assessments should be put forward. Scientists will rarely, if ever, have all the data they want to assess risk to ecological systems. Tiers would be correlated to level of effort or level of concern. Tier 1 would be primarily a paper study using available data. Tier 2 would build on the result for Tier 1 and would address critical data gaps and reduce uncertainty. Tier 3 would use more complex methods and higher levels of effort to address data gaps and quantitate risk. The use of tiers would focus ecological risk assessments earlier in the process to address key information that impacts important assessment endpoints. It would also allow regulators and assessors to have an agreed upon level of effort in conducting ecological risk assessments and not result in never ending "financial black hole" assessments.

16. Certainly higher level ecological effects at the community, watershed or ecosystem level should be considered. However, it is doubtful that they would be more sensitive. Endangered and threatened species also are important to consider because they must be protected at the individual level.

17. The tools available are primarily models. However, the use of several layers of models can produce results that are not "real world". Models must be validated to field or laboratory data. Building in conservative assumptions in model parameters can result in useless results. The use of distributions instead of single value parameters can reduce this effect. When using models to support ecological risk assessment I believe it is best to apply protection levels (conservative estimates) at the end of the process and not to build them into models.



Nigel Blakley  
Washington State Department of Ecology



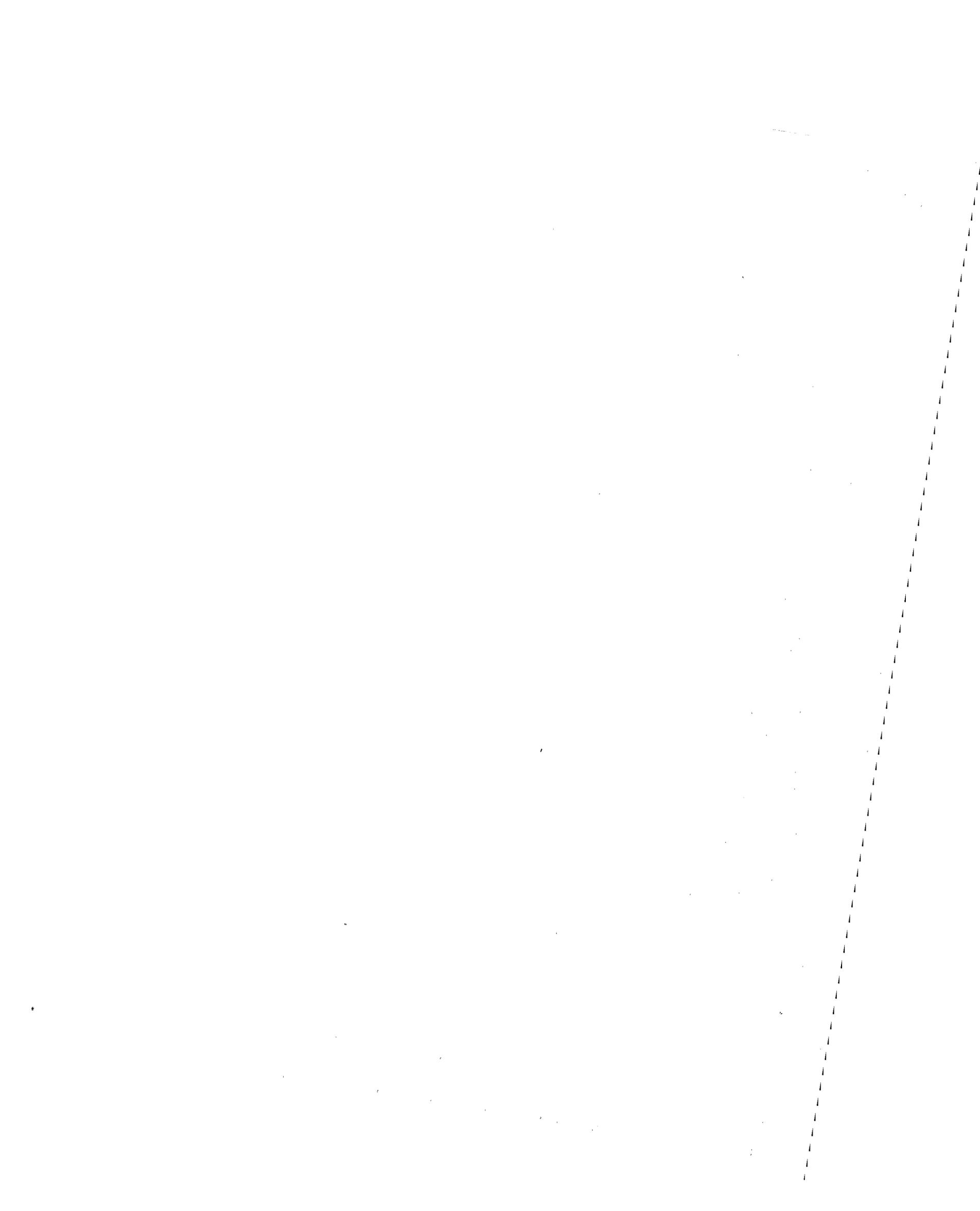
Comments on Preliminary Problem Formulation for a Dioxin Scenario - Nigel Blakley

1. Although it seems appropriate to expand the risk assessment from TCDD to TCDD-like compounds using TEFs, I wonder whether the assessment should be further expanded to include other chlorinated organics likely to occur in the effluent that may also effect fish reproduction in combination with TCDD-like compounds. The Interim Report concludes (p. 4-35) that "...the contribution of PCDDs and PCDFs in [chlorine bleached kraft pulp mill effluents] to observed biochemical and physiological changes in exposed fish populations is unclear at this time", citing (among others) Munkittrick et al. (1992), whose work suggests that other, possibly water soluble, chlorinated organics in the effluent may also have adverse effects on fish reproduction.

Although the Problem Formulation enclosure states (p. 1): "Other stressors and endpoints are subjects of other assessments and will not be considered here", it is not clear how additive or synergistic effects from multiple stressors in the effluent on the measurement or assessment endpoints will eventually be integrated.

2. Could TCDD sequestered in fat reserves begin circulating during periods when these reserves are mobilized? If so, there may be other sensitive stages in the life cycle (e.g., during migration or periods of food scarcity) which apparently have not been studied.

3. The assessment endpoint, "productivity" of various fish populations, seems somewhat vague and open to differing interpretations. A definition of productivity will be useful if a quantitative relationship between the measurement and assessment endpoint is needed. The conceptual model figures could also be improved (e.g., Fig. 2: Community structure and function is not proposed as an assessment endpoint in the scenario; Fig. 3: ingestion of sediment is not included as a pathway for direct accumulation of TCDD for fish, mammals or piscivorous birds.).



**Peter Chapman**  
**EVS Environmental Consultants**



## EXERCISE 1. ECOLOGICAL EFFECTS AND ENDPOINT SELECTION

### Issue 1. Is Focus on Fish Species Appropriate?

Fish, invertebrates and algae are typically used in toxicity testing, and are the focus of most historical and present methods development studies. Toxicity tests and species currently in use in the United States are reviewed by Adams (1993) and include the following for acute testing: salmonids (e.g., trout) and other fish, invertebrates (in particular daphnids, amphipods, midges, worms, mysids and other shrimp, mayflies and bivalves), and plants (e.g., algal cultures and duckweed). Chronic toxicity tests (Adams, 1993) include fish (e.g., minnows), daphnids, mysids, and algal cultures.

The use of fish species for testing is appropriate given their long history of usage. As regards focussing on fish species, this is appropriate at this time given that this group appears to be the most sensitive aquatic species to TCDD, and given the utility of "worst case" testing (or assessment) to assure environmental protection. Given our present state of knowledge, focus on fish species should result in adequate protection for the rest of the aquatic community in the reservoir from the direct or indirect effects of TCDD for reasons discussed below. However, note that the simple fact of fish having Ah receptors is not enough to say they are useful; the report notes that humans also have Ah receptors, yet as noted above (General Comments), humans appear to be relatively insensitive to TCDD.

"Worst case" testing is used to reduce uncertainty. It is impossible in science to "prove" that an event might not occur, under the right combination of (sometimes improbable) circumstances. Bioassay tests can never "prove" that an event might not occur in the real world, since they are by definition removed from that environment. However, if testing involves "worst case", e.g., most sensitive organisms and life-stages, most adverse testing conditions, and most likely exposure routes, and there are no effects, then it is reasonable to conclude that effects are unlikely. At the very least, such studies indicate low priority for regulatory or other action, which is an important finding given that there appear to be no lack of high priority issues presently requiring attention.

If, however, "worst case" testing indicates an effect, then there is a good likelihood that laboratory testing is overestimating what might be occurring or what is occurring in the real environment, and ideally providing early warning of potential problems before they become critical. Such early warning cannot be provided by field observational studies which, due to natural background "noise" in the distribution and abundance of natural populations, can only detect an effect after it has become severe or even catastrophic.

Issue 2.            Are TEF and BSAF Approaches Appropriate?

This question has major policy implications given that: (1) EPA has formally adopted the TEF procedure for use in all programs (Federal Register, November 07, 1989); (2) EPA did so as the lead in a six-nation project under the auspices of NATO. Technically, I have no problem with assuming additivity (this is a realistic "worst case", which is supported by various peer-reviewed studies, QSARs, etc.). I also have no problem with the concept of TEFs. Where I do have problems is with extrapolations that increase uncertainty. In other words, TEFs need to be directly related to what is being measured. In this regard, and given known inter-species differences, extrapolation from mammalian to non-mammalian systems is highly questionable and not presently supportable based on the evidence I have seen. This is not to say it is wrong, just that we cannot be sure at this point in time. Does this mean we should not use TEFs? No, because they are a useful tool. However, we should remember that they are only a tool whose utility may (or may not be) confirmed based on future research.

As regards BSAF approaches, these seem to be entirely appropriate, at this time, for assessing bioaccumulation. But (see later comments), bioaccumulation is a phenomenon, not an effect. Thus, these approaches should not be confused with determinations of effects.

Issue 3.            Should Tissue Levels be Used to Evaluate Effects?

A bioassay is an assay using a biological system. It involves exposing an organism to a test material and determining a response. There are two major types of bioassays differentiated by response: toxicity tests which measure an effect (e.g., acute, sublethal, chronic toxicity) and bioaccumulation tests which measure a phenomenon (e.g., the uptake of contaminants into tissues). Bioaccumulation can be from a variety of individual or combined routes, including respiration, ingestion, and direct contact. The responses from one of these two types of bioassays should never be used to predict the responses of another.

Bioaccumulation, which can result in bioconcentration, is a consequence of exposure, but cannot be considered a true response, since there are no data to provide direct correlations between tissue contaminant concentrations and adverse biological effects. Bioaccumulation is useful as an indicator of exposure to contaminants. For instance, the U.S. EPA/ACOE (1993) consider such testing to be an important evaluation of the potential of organisms to bioaccumulate contaminants of concern. Evaluation of the results of such testing are made by statistically comparing test and reference areas. In addition, bioaccumulation testing is conducted for human health reasons (are contaminants in

tissues at levels of concern if humans eat these tissues?), and can be done using both organisms eaten by humans and those which are not. In the latter case, the data are evaluated in light of the possibilities that certain contaminants can be transferred through food webs, and that tests with one species may indicate the potential for accumulation in other species.

Issue 4.            How far can Laboratory Test Data be Extrapolated?

Pragmatic environmental monitoring is ultimately directed towards pollution, which is defined as the presence of contaminants which result in an adverse biological effect. Chemistry measures contaminants, but provides no information on biological effects. Biological effects can only be determined directly, which is generally easiest, quickest and cheapest using bioassay tests in the laboratory. Such testing involves controlled experimentation, aimed at producing clear and reproducible answers. In contrast, studies of resident communities tend to be observational and cause-effect can never be directly determined, only inferred, by statistical and other methods which are presently much disputed (e.g., see Dauer, 1993; Smith et al., 1993). Further, resident community studies, as previously noted, cannot be predictive of effects, only record effects after they have become severe or even catastrophic.

Proactivity can be achieved by "worst case" testing (i.e., most sensitive species, most sensitive life-stages, most severe laboratory exposure conditions, likely most toxic and contaminated test samples), realizing that this is not necessarily "real case". Reactive testing evaluates whether present conditions can, or have the potential to, affect resident (or analogous) fauna.

For an approximation of "real case", appropriate species and end-points should be used (ideally test what you are trying to protect, in particular key taxa related to beneficial environmental uses). If appropriate individual species are protected (e.g., growth, reproduction, survival), it is inferred that the structure and function of the ecosystem will also be protected. The appropriate combination of laboratory and field data provides the best means presently available to assess whether effects will or are occurring, and their potential environmental significance.

There are two basic philosophies involved in the choice of bioassay test organisms. One philosophy is epitomized by the U.S. EPA (1986) who use standard species which they consider (after lengthy but not exhaustive testing) to represent the sensitive range of resident species of all ecosystems analyzed. Such "benchmark" species (U.S. EPA/ACOE, 1993) comprise a substantial data base, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. I (e.g., Chapman, 1991) and others (e.g., Cairns, 1993) consider that resident species

data (using species which have not adapted to the contaminant stress being tested for) are more directly relevant and avoid the major uncertainty of extrapolation between different species sensitivities. However, this is an area of some debate in the general scientific community.

The inclusion of field data is necessary because laboratory test data only reflect the test conditions, and conditions in the field can be very different due to, for instance, modifying factors. A **modifying factor** is "any characteristic of an organism or the surrounding water (or sediment) which affects toxicity" (Sprague, 1985). Modifying factors can act either to increase or decrease the concentration of a chemical required to produce a biological response, and their impact can vary dramatically between classes of chemicals and the organisms which are exposed. Modifying factors may affect the distribution, fate, concentration, chemical nature, bioavailability or toxicity of contaminants. They are divided into the following general, inclusive but not exclusive, abiotic and biotic groupings:

Abiotic Modifying Factors

climate  
temperature  
oceanography/limnology  
environmental quality

Biotic Modifying Factors

species/life stage  
sex/reproductive status  
nutritional/disease status  
competition/predation

A biotic factor not included above, but which can greatly modify sensitivity in bioassays, is the capability of organisms to adapt to toxic conditions. Such adaptation can take several forms, including the alteration of reproductive strategies or the development of **acclimation** (enhanced tolerance or resistance after first exposure). **Tolerance** or **resistance** are the ability of an organism to exhibit decreased response to a chemical relative to the response shown on the first occasion. Tolerance implies that the change is within the normal adaptive range of the organism and can be sustained indefinitely. Resistance implies that the magnitude of the factor lies outside of the normal range and that detrimental effects will eventually ensue.

Both tolerance and resistance may vary over an organism's life cycle and among organisms from different populations depending on their history of exposure. They also vary with a large number of biotic and abiotic modifying factors.

The advantages and disadvantages of the two types of bioassays, toxicity and bioaccumulation testing, can be broadly summarized as follows:

<u>Type of Bioassay</u>	<u>Advantage</u>	<u>Disadvantage</u>
Toxicity Test	holistic, measures toxicity of all stressors	does not indicate, without further testing, which stressor(s) are causing the observed effect(s)
	can be relatively simple and cost-effective	simple tests can give environmentally unrealistic answers
Bioaccumulation Test	indicates presence or absence of effect(s) on the organism(s) used, by the exposure tested	all possible organisms, exposures and end-points cannot be tested
	conducted under controlled laboratory conditions	field conditions are much different than the laboratory
Bioaccumulation Test	indicates whether the organism(s) used can accumulate the contaminants measured, by the exposure tested	all possible organisms and exposures cannot be tested; some toxic contaminants are transformed and not all can be or are measured
	provides quantitative data (levels accumulated), i.e., bioavailability	measures a phenomenon, not an effect

Laboratory bioassay data for specific contaminants provide information on their toxicity and/or potential to bioaccumulate, which can then be compared to likely organism exposures. Not all non-

target species can be tested in the laboratory, and extrapolation between species is highly uncertain. Similarly, prediction of effects under field conditions is not easy, since exposure is variable. However, laboratory data allow uncertainties about contaminant effects in the "real" environment to be reduced such that specific hypothesis can be formulated and tested, either through additional laboratory tests, or field studies, or a combination of the two.

The determination and prioritization of real environmental problems requires effective science which is relevant to and which will be realistically used in decision-making. In this regard, bioassays are an essential part of two critical assessments:

1. an after-the-fact evaluation (are environmental conditions better or worse? why or why not?) and,
2. a before-the-fact indication of whether environmental conditions may or will change (will environmental conditions become better or worse? why or why not?).

Issue 5.            Implications of Steep Concentration-Response Curve

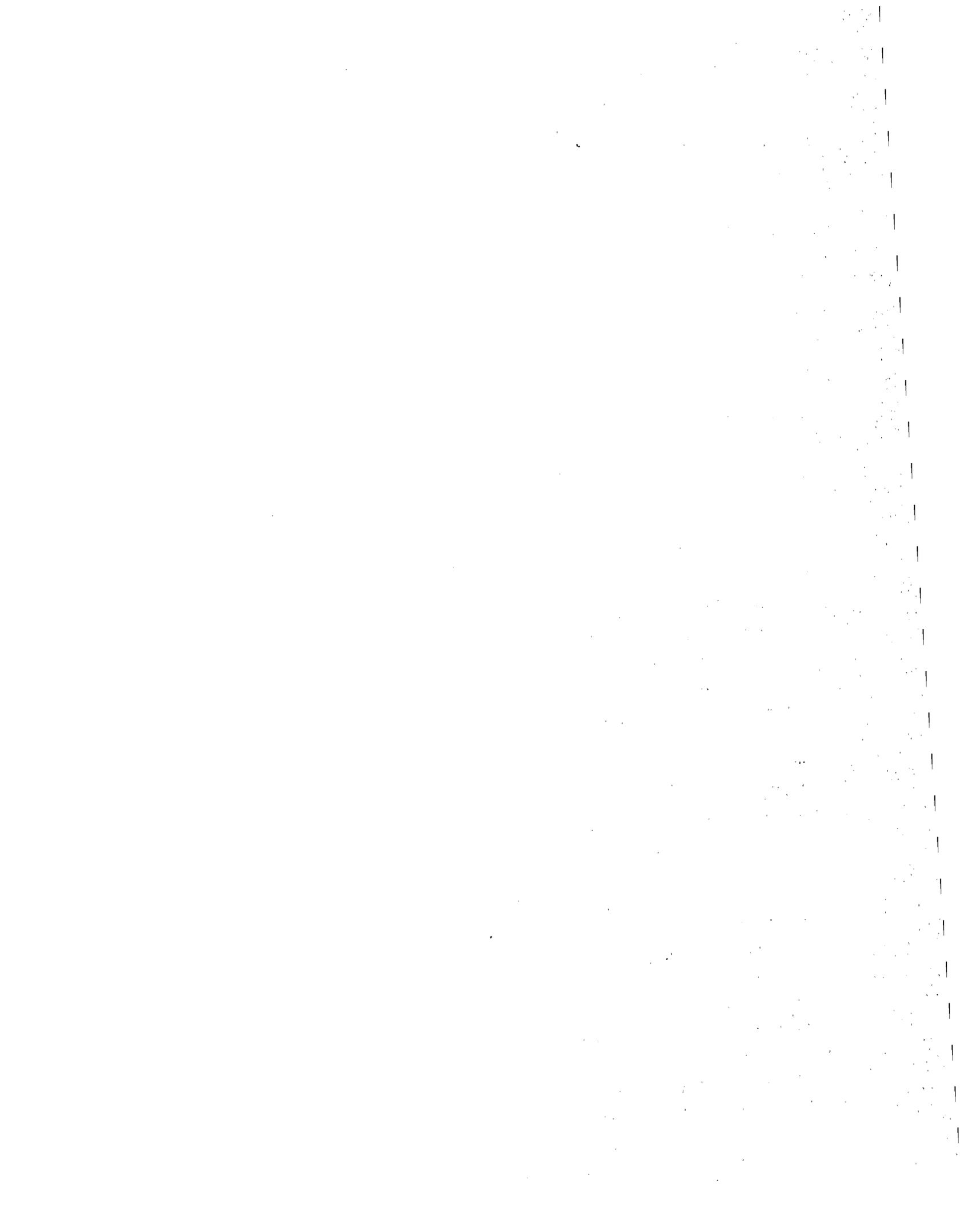
As noted by Paracelsus in the sixteenth century (Deichman et al., 1986) - "Only the dose determines that a thing is not a poison". Although debate is continuing regarding the possibility of some contaminants having effects at any level, this is not scientifically credible based on what we presently know. The fact that there is a concentration-response curve for TCDD clearly indicates that we can determine "safe" NOEL (no observed effect level) or, preferably, NEL (no effect level) concentrations. The steepness of the curve could indicate that there is less of a gradation of effects for TCDD than for other contaminants (in other words, it either does or does not have an effect), but may also be a function of the lack of intermediate concentrations.

Issue 6.            Data Utility and Uncertainties

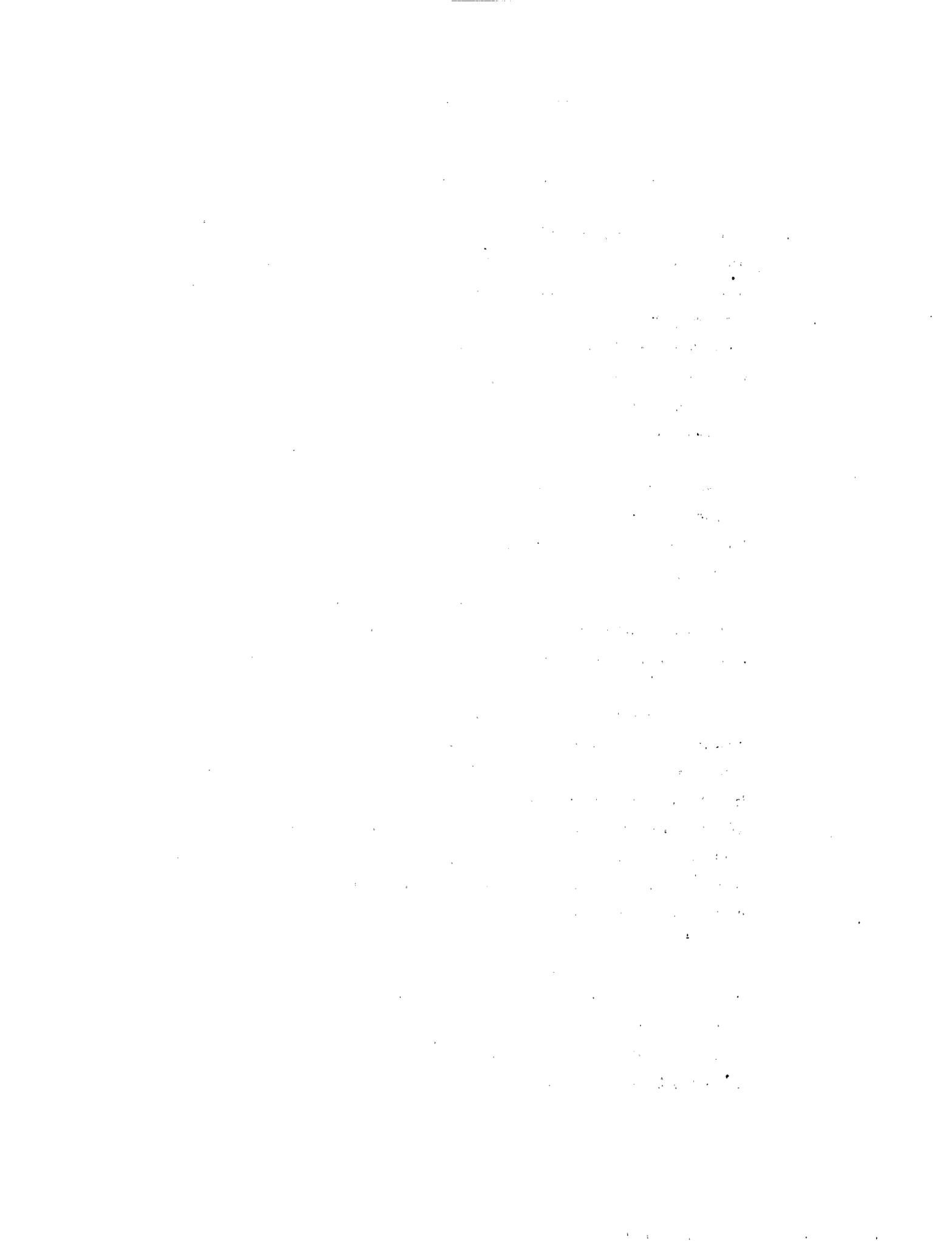
As noted in the report, fish early life stages (in particular salmonids) appear to be the most sensitive aquatic fauna to TCDD. As such they are, given our present knowledge base, appropriate to use in "worst case" testing and assessment as detailed previously. It is entirely possible that more sensitive species exist; it is also possible that no more sensitive species exist. However, science must proceed based on what is known, and based on what we presently know it appears to be entirely appropriate to use information regarding effects on fish early life-stages for decision-making. Clearly this will protect the majority of species, and possibly even all species.

Issue 7.            Other Effects Data/Important Scenario(s)

What is being asked here is not clear. If the first question is whether I am aware of other effects data not included in the report, then the answer is "I am not". The report appears to be quite complete. If the second question is asking what future information (e.g., research) would be useful, then clearly information on estuarine and marine organisms, in particular marine mammals would be desirable. However, direct testing of marine mammals, though scientifically desirable, is unlikely given animal-rights concerns and activism, and other considerations.



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## ECOLOGICAL EFFECTS AND ENDPOINT SELECTION

1. Based upon the currently-available data, focusing on the fish component of the aquatic community seems reasonable. The available data strongly suggest that fish are the most sensitive component of the aquatic community; therefore, if the fish are protected, presumably the other elements of the aquatic community (amphibians, plants, and invertebrates) will be adequately protected. This approach (using the available data for those species which have been studied through exposures to TCDD) would be the one most likely utilized for a "realistic" risk assessment, because such an assessment would be performed using existing information exclusively.

However, if an "ideal" risk assessment were to be performed, the individuals performing the risk assessment would more than likely want to have TCDD toxicological information for both the fish species to be protected (largemouth bass, catfish, crappie, and bluegill), and the vertebrate and invertebrate species that are the forage organisms for the species to be protected. Additionally, the risk manager may be interested in having TCDD toxicological data for other species that play an important role in the ecology of the reservoir, but that are not directly related to the food web of the fish species of interest.

2. A considerable amount of effort has been expended developing the TEF and BSAF concepts. However, there are still uncertainties associated with the application of these tools for evaluating the risks of TCDD-like compounds to aquatic life. However, because exposure effects data are currently not available for the majority of these types of compounds (particularly for the species to be protected in the reservoir), I feel it is reasonable to use the TEF and BSAF approaches in performing a "realistic" risk assessment for evaluating the effects and bioaccumulation of dibenzo-dioxins and dibenzofurans in the hypothetical paper mill effluent.

Under more "ideal" conditions, I feel that the risk manager would want to have actual data available for some (or all) of the species to be protected for those TCDD-like compounds that will be present in the effluent. However, it is probably unrealistic to think that exposure/effects data would be available for the fish species of interest for all of the TCDD-like compounds to be released. Perhaps a reasonable compromise would

be to perform the risk assessment using the TEF and BSAF approaches, and then to validate these approaches by performing laboratory studies exposing 1 or 2 of the species of concern to 1 or 2 of the compounds of concern.

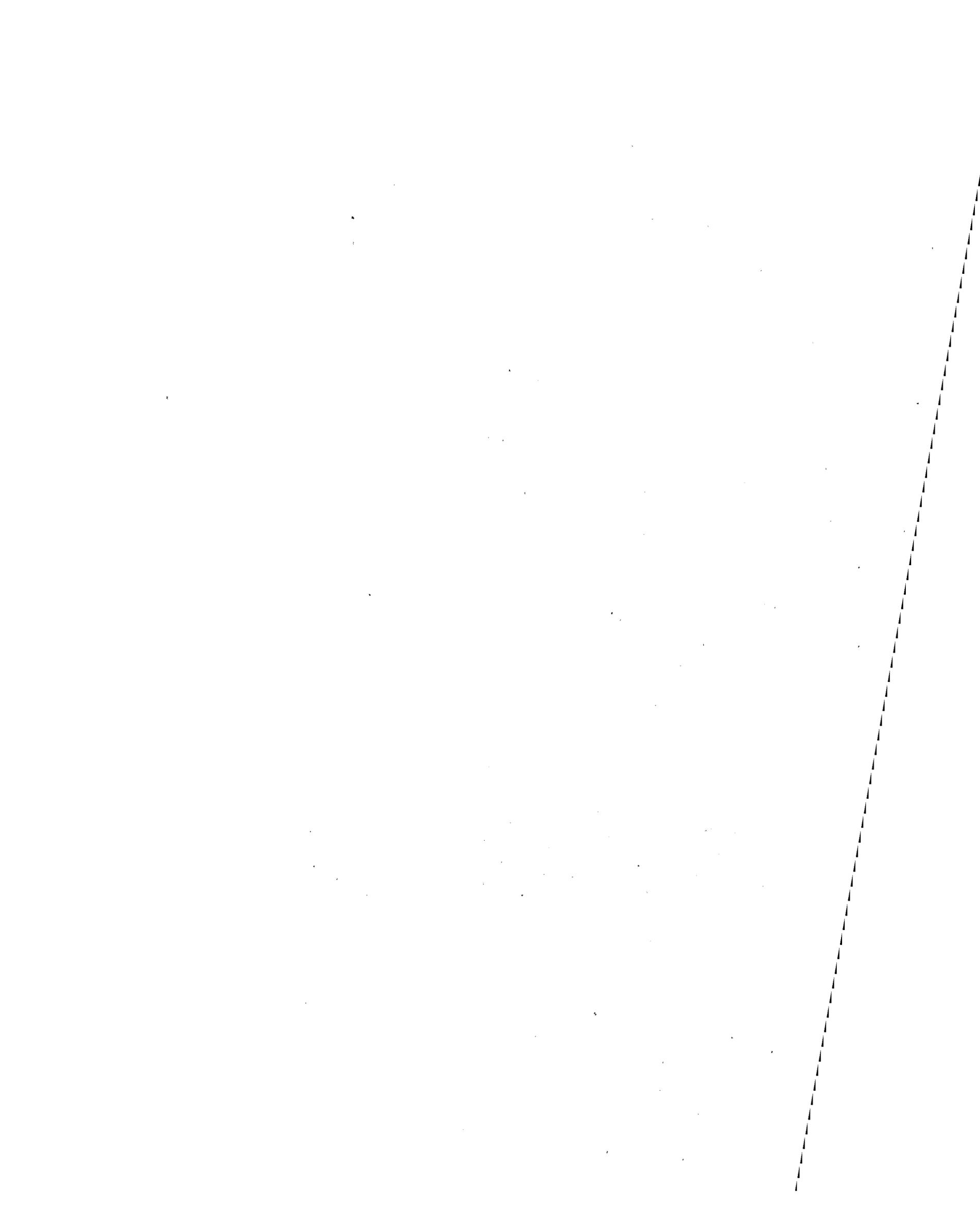
3. Utilizing TCDD tissue concentration levels (as opposed to anticipated exposure concentrations) to assess the risks associated with the discharge of the pulp mill effluent seems like the most effective approach. In order to accomplish this objective, the risk manager would need to utilize an accepted exposure model (like the one presented in Figure 4 of the Scenario) to determine the acceptable effluent TCDD concentration, i.e., that concentration below which there would not be a detrimental accumulation of TCDD in largemouth bass, crappie, catfish, and bluegill.
4. In situations where there are limited data to predict environmental consequences, the level of uncertainty is fairly high. The case for TCDD is no exception. Because there are TCDD effects data for relatively few individual species (and probably none of those that could potentially be affected by the pulp mill effluent), extrapolating the existing data to the pulp mill discharge scenario will result in uncertainties for evaluating the ecological effects. These uncertainties could be somewhat minimized if the risk manager could be sure that the existing data cover species that are more sensitive than (or at least as sensitive as) the species to be protected. However, the relative sensitivities cannot be determined for certain in this case, because we do not know if the species to be protected are more or less sensitive than those for which there are TCDD data in the literature.
5. The very steep concentration-response curve for TCDD indicates to me the importance of making very accurate predictions of the concentration of TCDD and TCDD-like compounds in the effluent. A factor of two error could result in a population or community being negatively affected, even though the risk assessment suggested that there would be no impact. Because of this steep concentration-response curve, it would probably be wise for the risk manager to apply a safety factor to the effluent discharge limit that would be protective even if the limit were intermittently exceeded. This type of situation (steep concentration-response curve) also emphasizes the importance of accurate TCDD effluent measurements at a low level of detection once the plant is in operation.
6. These data have limited utility for the pulp mill scenario because there is a limited amount of data for only a few of the species to be protected in the reservoir. As a

consequence, the associated degree of uncertainty is fairly high. The wildlife data are more uncertain than the aquatic life data, but in both cases a fairly substantial safety factor would be appropriate to compensate for the high level of uncertainty.

7. To the best of my knowledge, there is no information available on the bacterial component of the environment, which is very important in understanding the impact TCDD may have on decomposition in both the aquatic and terrestrial communities. Also, I am not aware of the existence of substantial data on the effects of TCDD on primary producers in the aquatic or terrestrial environments. And among the various fish and terrestrial species that have been evaluated, it is probably not safe to assume that the limited existing data are representative of the more sensitive species of primary producers or bacteria.



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**Comments on Workshop Exercises**

Exercise 1. Ecological Effects and Endpoint Selection

*1. The lack of Ah receptors in some species along with the results of a limited number of laboratory studies suggest that amphibians, invertebrates and plants are less sensitive to TCDD than fish birds and mammals. For fish, early life stages appear to be most sensitive. Because of this range in sensitivity, productivities of fish species were selected as assessment endpoints for the scenario. Comment on whether this focus on fish species will result in adequate protection for the rest of the aquatic community in the reservoir from the direct or indirect effects of TCDD.*

**Comment**

It is unlikely that a focus only on the fish species can provide an adequate protection for the rest of the aquatic community or even the fish. Most of the Interim Report deals with only the direct effects of TCDD upon the target organisms and little discussion of possible indirect effects occurs. In seasonal systems slight differences in the timing of reproduction can have severe repercussions. Several scenarios among many others are possible:

- 1) Chemicals tend to concentrate at the interfaces, especially organics and other lipophilic materials. Concentrations higher than in the water column interfere with the pupation or metamorphosis of the arthropods and other larval forms that important parts of the macrobenthic assemblage. The subsequent lack of reproduction alters the detrital processing and subsequently alters the resuspension of nutrients and algal production falls. Organisms higher in the food chain subsequently see a population reduction.
- 2) Slight alterations in reproductive success can dramatically alter competitive relationships and thereby change the structure of a community. Many of the sport fish described as the species of concern tend to specialize on certain components of the invertebrate assemblage. An alteration of this assemblage could alter the mix of fish, perhaps eliminating some species. As certain fish are eliminated the invertebrate and producer assemblages are again altered leading to another change of structure.
- 3) The myth of the most sensitive species (Cairns, 1986). As in most cases, only relatively few representatives are tested in the laboratory. Entire phyla are routinely omitted (I am as guilty as the next) while a great deal of concentration is focused on vertebrates of concern in Phylum Chordata. Given the lack of comparative data, extrapolation from a few invertebrates or algal species seems unwarranted.

The evolutionary biology of the Ah receptor seems unclear and without an understanding of the phylogeny of these molecule, generalizations as to sensitivity are difficult to be made. Is

the protein conserved, how many base pair changes are necessary to increase or decrease sensitivity, are the Ah receptors a family of proteins, can simple cDNA probes be synthesized for comparative purposes? Given the planar nature of the compound and the reactive halogen atoms I would suspect that there may be separate genotoxic (carcinogenic) and physiologic receptors.

4) Focusing on fish, particularly mobile sport fish, may actually underestimate the impact unless a clear understanding of the role that a particular location plays in the metapopulation dynamics of the species. Given that most fish produce an excess of fry, migration from population sources to sinks, sinks due to the elimination of the indigenous population, is highly likely. Two misleading measurements may occur: 1) body burdens below levels generally assumed to be of concern can be found in the tissues since the organisms are newcomers to the contaminated area, and 2) populations may seem to be plentiful due to migration from populations sources. As the habitat becomes more intolerable or the population source is eliminated through TCDD levels or habitat alteration, a drastic reduction in resident fish populations may appear and the manager may look for a proximate cause, however, the populations had been herded to the cliff for a number of years. Unless a mark recapture type of program is initiated, an accurate estimate of the population dynamics of the fish would be impossible, and likewise the true impact of the TCDD.

Likewise, organisms that are unable to range as widely as fish can be severely impacted. Macrophytes, mollusks, protists, and carnivorous invertebrates may subtly affected due to the continuous exposure to TCDD. Fish are not the only carnivores in a freshwater system. Mollusks are famous for their ability to accumulate organics to very high levels (Hugget, ASTM Symposium 1993).

5) The important variables that determine the status of an ecosystem also apparently change over time and circumstance. In the microcosm tests conducted at Western, we have consistently found that the variables important in identifying the treatment groups change over the time course of the experiment. This variability has been found for several jet fuel experiments and with several types of microcosm protocols (Landis et al, in press). Not only do the variables change in their ability to predict treatment, they change in a bounded stochastic manner. In other words, while the variable "Philodina" will likely be important during the latter stages of the experiment, it is difficult to pick the sampling day or relative ranking of the variable. In field studies Matthew's et al (1991a, 1991b) have found similar patterns, in which variables change in importance in the clustering of field results. A recent study by Dickson et al (1992) also tends to confirm this trend. While measured impacts on aquatic ecosystems were correlated with laboratory toxicity tests, the type of impact in the field was not predicted by the laboratory experiment. In fact, several types of impacts were correlated with the same basic suite of toxicity tests.

In summary, it is unlikely that a magic endpoint or measurement can adequately protect an ecological system, or be used as a vital diagnostic measurement as, lets say, cardiac enzymes in the bloodstream are indicative of heart attack. Search for such a measurement is probably a remnant of the ecosystem as super organism and ecosystem health as more than metaphor (see Suter 1993). Ecosystems are not organisms and do not have health in the sense that an organism does. It may prove difficult to find one variable that denotes a specific type of impact.

*2. Section 4.1 of the Interim Report describe the use of toxicity equivalency factors (TEFs) for TCDD compounds. Section 3.5 of the Interim Report discusses the use of TCDD biota-sediment accumulation factors for other related compounds. Comment on the use of these approaches for evaluating the effects and bioaccumulation of dibenzodioxins and dibenzofurans in the paper mill effluent.*

**Comment**

The TEFs are a problem until the exact nature and evolutionary history of the Ah receptor is understood. In essence, it seems that these compounds, that are reactive materials in some instances, are being treated as if they are narcotics. That is, the modes of action are similar enough that on a molar basis the materials are biologically equivalent. Are there a variety of Ah receptors, each slightly different so that different genes are transcribed to mRNA? Hormones and other types of initiator-receptor molecules tend to be very specific and I would not be surprised to find a family of Ah receptors.

The field data seem to come primarily from Lake Ontario, a quite atypical lake compared to a southern pond or a northwest glacial lake. How far can these data be extracted to these very different systems?

The lack of data by which to judge the efficacy of the BSAF methodology is troublesome. The potential to determine an equilibrium concentration appears to be there, although further validation is warranted. My major concern is the lack of data on rates of accumulation and the dynamics and heterogeneity of TCDD uptake. Equilibrium states may rarely be reached by an entire population and if it is the ecosystem is likely to be heavily contaminated. Given the importance of population dynamics and interactions in determining the structure of an aquatic community, information on the dynamics of the toxicant are vital. F. B. Taub (SETAC 92 presentation and personal communication) has demonstrated in a model of an aquatic microcosm the importance of timing and the subsequent outcome. Differences of a few days in the onset of mortality produce dramatic differences in outcomes for the algal and daphnid populations. I suspect that natural systems have even a larger variety of dynamics.

*3. Because of difficulties in extrapolating from various laboratory exposure conditions to observed effects, the Interim Report (section 4.2.3.1) emphasizes using tissue level of TCDD rather than exposure concentrations to evaluate effects. Comment of the applicability of this approach to evaluating the risks of TCDD from pulp mill effluent.*

**Comment**

Tissue levels are generally better indicators of exposure information than concentration. Compounds like TCDD and halogenated aromatics require a great deal of time to move into tissues. However, tissue concentrations in adult organisms would not as good as indicator of population level impacts as concentrations in yolk and fry.

While tissue concentrations are good indicators of exposure they are more an indication of total exposure for an organism that has survived in that environment. Hotspots and concentrations in crucial habitats are not effectively measured. Organisms that have been exposed to these areas, given the steep dose response curve of TCDD, likely do not survive.

It should not be surprising that the extrapolation of laboratory results to observed effects should be so difficult with a compound with an estimated Log P of 7.0. Such a material will likely migrate to interfaces containing high concentrations of organics, sediment, sediment-water column interface, the surface microlayer (Hardy et al 1982) and the awfuchs. These environments are difficult to accurately sample, yet they are important to many sensitive life stages and within reside highly productive aspects of the ecosystem. The chemistry at these interfaces is very dynamic and temporally and spatially heterogeneous.

Aquatic systems rarely seem to be approached in the same manner as terrestrial systems, that is seeing the lake or stream in the sense of a landscape. The landscapes in an aquatic sense would incorporate the spatial heterogeneity of the systems. These heterogeneities are crucial in maintaining the species richness of the planktonic community as hypothesized by Hutchinson (1961), Richerson et al (1970) and Tilman (1982). Given the biotic importance of the physical heterogeneity of aquatic systems, the nature of the distribution of toxicant must be equally important for estimating ecosystem level effects. Tissue concentrations of fish are generally moving averages of exposure, tissue burdens of sessile organism coupled with analytical measurements from interfaces would provide a more accurate picture of the exposure landscape.

*4. The Interim Report uses both laboratory and field information to predict levels of TCDD in fish and wildlife tissues that will cause adverse effects. The scenario proposed to use laboratory test data at the individual level of organization to predict population changes in fish and wildlife. Comment on the utility of available laboratory data to predict effects on field populations and discuss the associated uncertainties.*

**Comment**

Several books could be written on this topic (and have been). Using data at the individual level to predict population level effects has been done, but often with a probability of extinction as the measurement, a fairly dramatic outcome. Some of the uncertainties are:

1) Age Structure. Age structure of the recipient population can have a dramatic influence upon the subsequent dynamics. Populations may undergo dramatic cycles due solely to the influence of the initial age structure.

2) Genetic heterogeneity. A much greater diversity of genomes would be expected in a wild population, although many populations under bottlenecks that make local populations genetically distinct. The degree of diversity within a population, compared to that population used in the laboratory, is almost never measured.

3) *Intrinsic Dynamics of Population Growth*. Populations with high rates of reproduction can exhibit very different dynamics depending on the initial conditions. May and Oster (1978) discussed this at some length. Simple non-linear equations (such as  $N_{n+1} = N(1+r(1-N/K))$ ) can exhibit dynamics ranging from an asymptotic equilibrium to chaotic (in the mathematical sense) given appropriate  $r$  values. Initial conditions also dramatically alter the outcomes. In the graph below (Figure 2), the differences in the initial population sizes is only 2, yet when a stress occurs one population becomes extinct while the other oscillates around a new carrying capacity. Unless the initial conditions are precisely known, widely varying yet perfectly deterministic outcomes can occur. These types of dynamics can also become apparent in the spatial distributions of organisms, with the spatial distributions ranging from regular to contagious (Hassel et al 1991)

5) Stochastic influences. Along with the difficulties of non linear systems, certain aspects of population biology are stochastic. Chance events do occur, disease is largely unpredictable, along with storms and other events that have dramatic impacts.

6) Harvesting. Finally, the impacts of harvesting of game fish or commercially important species is always an important consideration. Even if only certain age-classes are chosen, a skewing of the age-classes of the population occurs. These dynamics, although the basis of catch quotas, have proven difficult if not impossible to comprehend so that accurate predictions can be made.

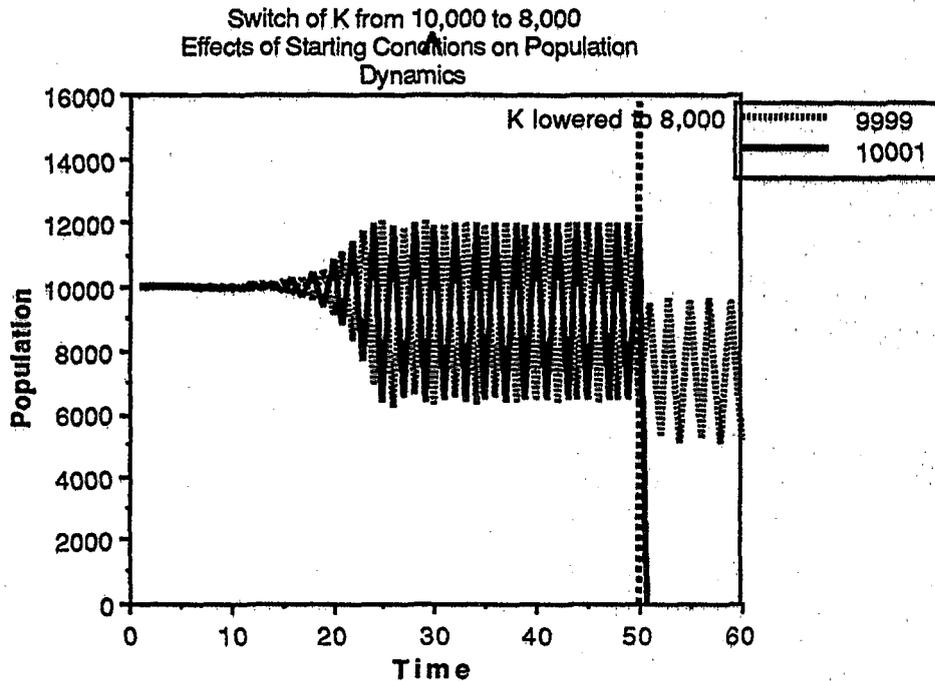


Figure 2. Different Outcomes from slightly different initial conditions.  $R$  is equal to 2.0,  $K$  is initially 10,000 and that is lowered at time 50 to 8,000. One population becomes extinct as the other cycles around 8,000.

5. The Interim Report sites data that indicate a very steep concentration-response curve for TCDD effects in fish and wildlife. Discuss the implications of this observation for evaluating ecological effects in the scenario.

**Comment**

The steepness of the dose-response curve is a problem. It appears to virtually a step with no effect to 100 percent effect. Essentially, indications at the individual level or even the population level may be too subtle to give any warning before impending doom. Localized extinctions or effects could also occur without warning, altering the landscape of that community a potential cascade of effects.

The advantage to a steep dose-response curve is that intrapopulation variance in regard to the toxicant should be relatively low. This should reduce the uncertainty associated with the impact of TCDD,

6. The general summary of effect levels for aquatic species and associated wildlife (Boxes 1 and 2, section 4) is based on extrapolations from a limited number of test species and from tests that

*do not span complete reproductive cycles. Associated uncertainties are summarized in section 5.1.3. Discuss the utility of these data and uncertainties for evaluating ecological effects.*

**Comment**

The database is one of the best for a non pesticide organic that I have seen. Most of the emphasis is on fish and laboratory animals, and I would have enjoyed more of a comparative molecular toxicological approach to ascertain the range of sensitivities. I would suspect that given the sensitivities of birds, mammals and fish that amphibians and reptiles would show similar sensitivities. Amphibians, with their early life stages comparable to fish, would be likely to see similar sensitivity. Reptiles are a difficult extrapolation, since they are a broad group of organisms in North America. Alligators, lizards and turtles are evolutionarily distinctive, and quite separate from the vertebrates of today. In fact, alligators may actually share more of an evolutionary linkage with birds than with the lizards.

A primary emphasis of this database are direct effects, the initial disturbance to the system that drives the follow on effects. Indirect effects are now being realized as more important and there is a considerable literature detailing these impacts for pesticides and other materials. Given the test methods, the type and magnitude of the indirect effects are virtually impossible to accurately predict.

Many of the questions about effects, extrapolation from laboratory tests and the examination of indirect effects could have been addressed by appropriately designed multispecies toxicity tests. Multispecies toxicity tests come in a variety of methods using bacteria, protozoa, a variety of metazoans, including fish, and can be used to test experimentally many of the assumptions and validate the models that are subsequently used in a risk assessment. Many of the methods are not experimental and several have histories of round robin testing and adoption by various laboratories. Examples with short summaries are presented in Appendix 1. Multispecies toxicity tests can also examine the dynamics of the entire community using a variety of tools developed over the last ten years. These methods range from normalized ecosystem strain developed by Kersting (1988), the state space of Johnson (1988a, 1988b) to the nonmetric clustering and projection techniques developed by Matthews et al (1991, Landis et al in press). Examining these relationships experimentally allows verification, validation and may turn up new relationships not easily derived from observational studies.

*7. As discussed in the Interim Report, few data on the effects of TCDD on estuarine and marine organisms have been reported (section 4.2.1.5), and no data were found in the literature for TCDD effects on reptiles or marine mammals. Although all the current wildlife toxicity data were reviewed, an analysis to establish an effects profile for terrestrial organisms was beyond the scope*

*of the report. Describe other effects data not identified in the Interim Report or the scenario that will be important for future ecological risk assessments.*

**Comment**

The lack of data on marine species is an important data gap, especially since pulp and paper mills are found along the Puget Sound and along the British Columbia coast. Marine systems, especially those of the Pacific Northwest, are highly productive and important commercially. Commercially important species include fish, crabs and shellfish. Each of these commercial species use a variety of different resources.

Comparative data on immune suppression or even enhancement are crucial in estimating the increased risk of individuals to disease. Immunologically suppressed populations may be more susceptible to devastating outbreaks of opportunistic infections.

Behavioral data on nesting patterns, parental care, sensory impediments, and other factors can also be important in the long term success of populations. These can be crucial with organisms that migrate to nesting or spawning grounds, rely of behavioral cues to establish mates, or are crucial in feeding offspring or providing other forms of parental care.

As typical, no data are forthcoming on additive or synergistic effects with other classes of toxicants. It is unlikely that TCDD and related compounds will be the only toxicant or even the one in the highest concentration. Are there synergisms when herbicides are added to the mix that increase the sensitivity of algae and macrophytes? As the various serene dependent proteins are inhibited by acetylcholinesterase inhibitors, does the presence of TCDD inhibit turnover and therefore make the organisms more sensitive to repeated dosing? What role do heavy metals play in concert with TCDD? Do the genotoxic aspects of TCDD make the organism more or less susceptible to other genotoxic materials, many of which like the aflatoxins, naturally occurring? It must be recognized that toxicants do not exist alone in the environment and hazard assessments should recognize the potential interactions.

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PRE-MEETING COMMENTS: RICHARD E. PETERSON

EXERCISE 1: ECOLOGICAL EFFECTS AND ENDPOINT SELECTION

1. If the risk characterization had to be done today, focusing on early life stage mortality of fish as the measurement endpoint to use for protection of fish and the rest of the aquatic community in the reservoir from direct or indirect effects of TCDD seems reasonable. This is because early life stage mortality in fish is the most sensitive endpoint reported for aquatic animals (or plants). Also it is an ecologically-relevant endpoint that can potentially be linked by cause-and-effect in population models to predict declines of fish populations in the environment.

Since literature findings show that aquatic invertebrates, amphibians and plants are less sensitive to TCDD toxicity than fish it would be inappropriate to focus on non-fish species in characterizing the risk to aquatic life in the pulp mill scenario. Nevertheless a more rigorous screen for presence of the Ah receptor and for Ah receptor-mediated toxicity is needed for representative species of invertebrates that inhabit the reservoir. This toxicity screen should include long term chronic toxicity tests that include measurements of TCDD accumulation in invertebrates. In separate studies expression of the Ah receptor in invertebrates should be assessed. Obtaining this information will reduce uncertainty as to whether the proposed focus on fish, will also protect invertebrates.

There is less concern about potential adverse effects of TCDD on aquatic plants, but determining if plants lack Ah receptors would be useful. Uncertainty about the toxicity of TCDD in amphibians at all life stages of development should also be determined by conducting studies in a prototype

amphibian species that is commonly exposed to TCDD-like compounds in inland waters of the United States. Such studies should focus on reproductive and developmental toxicity endpoints and include Ah receptor analyses and documentation of TCDD body burdens associated with toxic endpoints.

2. For predicting population declines in reservoir fish populations caused by complex mixtures of TCDD-like compounds in eggs, the fish-specific TEFs determined by Walker et al. (1991) offer the following advantages: (a) TEFs are based on a sensitive, ecologically-relevant, Ah receptor-mediated endpoint in fish - early life stage mortality, (b) TCDD-like PCDD, PCDF, and PCB congeners act as full agonists in producing the response, (c) dose response curves of individual congeners for producing early life stage mortality are parallel, (d) complex mixtures of TCDD-like congeners are assumed to interact in a near additive manner, and (e) fish-specific TEFs have the potential to be linked by cause-and-effect in population models that will be developed in the future to predict TCDD-induced declines in feral fish populations.

Another method for determining TEQs involves extracting all PCB, PCDD, and PCDF congeners from a biological sample and testing it's potency relative to that of TCDD in a fish or mammalian cell culture system. The endpoint used is generally induction of cytochrome P4501A1-mediated enzyme activity . However, TEF values obtained with this method tend to be greater than TEFs based on early life stage mortality in rainbow trout. In other words, cell culture systems tend to overestimate the potency of TCDD-like PCDDs, PCDFs, and PCBs in causing early life stage mortality in fish.

Another problem with using cell culture systems to determine TEFs is that all PCDD, PCDF and PCB congeners tested

in these systems do not act as full agonists. That is, the congeners do not all produce the same maximal level of cytochrome P4501A1 induction. Yet this is necessary for the proper determination of EC<sub>50</sub> values for TEF determination. If certain congeners are not able to cause the same maximal level of induction as TCDD the addition of TEQs contributed by these congeners is not valid for risk assessment.

For example, assume maximal induction achieved with TCDF in a cell culture system is 40% of that obtained with TCDD and an EC<sub>50</sub> was calculated for TCDF relative to its own maximal effect. If that EC<sub>50</sub> was used to determine a TEF for TCDF; TCDF's TEF would be artificially high relative to TCDD as would TEQs calculated for TCDF using that TEF. Thus, fish-specific TEFs based on a response where all congeners act as full agonists is preferred.

Fish-specific TEFs for PCDD and PCDF congeners and for coplanar PCBs have been published by Walker et al. (1991) and should be used in the risk characterization process for fish in the Omigoshie Reservoir. TEFs used by the US EPA for human health risk assessment of PCDDs and PCDFs are not specific for early life stage mortality in fish; it is suggested they not be used for this purpose. TEFs proposed by Safe (Crit. Rev. Toxicol., 1990) for PCBs also should not be used for fish because they overestimate the potency of PCBs in producing early life stage mortality. For example, mono-ortho chlorine substituted analogs of the coplanar PCBs (mono-ortho PCBs) are weak Ah receptor agonists in mammals but are completely inactive in causing early life stage mortality in rainbow trout.

TEFs used to predict adverse effects of TCDD-like compounds on piscivorous bird and mammal populations should be different than those used for fish. Ideally, one would like to

have TEFs based on developmental and reproductive toxicity endpoints in these wildlife species, but such data does not exist and will not be forthcoming in the near future. In the absence of such data, TEFs for PCDDs and PCDFs used by the EPA for human health risk assessment could be used for ecological risk characterization of avian and mammalian wildlife. For coplanar PCBs and mono-ortho PCBs TEFs proposed by Safe (1990) should be modified, based on findings of De Vito et al. (1993), to calculate TEQs.

3. A key determinant of whether TCDD-like congeners will produce toxicity is their concentration at their site of action in fish, birds and mammals. Exposure concentrations do not provide this type of information. Concentrations of TCDD in water, food, soil and sediment are too far removed from the site of action of TCDD in the body to be as useful for ecological risk characterization as tissue levels of TCDD. The most useful laboratory studies for ecological risk characterization are those that assess TCDD toxicity and TCDD accumulation in tissues of the same animals in the same investigation. Furthermore, expressing effects on the basis of tissue accumulation of TCDD allows for toxic effects observed in aquatic life and wildlife from TCDD exposures by different routes in laboratory and field studies to be directly compared and interpreted more meaningfully than would otherwise be possible.

4. Since the assessment endpoints proposed for risk characterization are productivity of fish, avian and mammalian populations the most meaningful measurement endpoints for TCDD are its known adverse effects on reproduction in birds and mammals and early life stage mortality in fish. All of these endpoints have the potential to be linked to decreases in the populations of fish, birds and mammals once species-specific population models are developed and validated in the future.

In the Interim Report results are presented showing how early life stage mortality of lake trout in Lake Ontario, caused by contamination of lake trout eggs by TCDD-like congeners, could be linked retrospectively to decreased productivity of lake trout. This example provides strong support for the use of early life stage mortality as a sensitive, ecologically-relevant, Ah receptor-mediated measurement endpoint for fish in the Omigoshsee Reservoir. For birds, the measurement endpoints are based on TCDD-induced decreases in egg production and embryo viability which might also be able to be linked to population declines in piscivorous birds once population models for these species become available. For mammals decreases in fertility and litter size also have the potential of being linked to changes in populations of mink and otter along the shores of the reservoir.

Nevertheless whenever laboratory data are used to predict effects on field populations of fish, birds and mammals uncertainty will exist. In particular there is uncertainty about the potential modulating influence of non-chemical stressors encountered by fish, birds and mammals in their natural environment on measurement endpoints. More specifically, all laboratory data relating to the measurement endpoints were obtained in lake trout fry, pheasants and rats under constant conditions of water temperature, water pH, room temperature and room humidity and standard laboratory diets and water were provided ad libitum. Compared to the rigors of the natural environment for aquatic life and wildlife these laboratory environment conditions under which the measurement endpoints were assessed were nonstressful. Thus, it is uncertain for each measurement endpoint and for each species of feral animal whether or in what direction naturally occurring non-chemical stressors that the species encounters in its natural environment will have in altering its sensitivity to the reproductive or developmental toxicity of

TCDD.

A non-chemical stressor that may or may not modulate early life stage mortality in fish is water temperature. The dose response assessment for TCDD-induced early life stage mortality in lake trout was conducted at a colder water temperature than fish would encounter in the Omigoshie Reservoir in the southern United States. However, until studies are conducted to determine if water temperature modulates TCDD-induced early life stage mortality in fish this type of uncertainty will be inherent in the risk characterization.

5. The main implication of the steep TCDD dose response curve for reproductive/developmental toxicity is that the response of fish, mammal or bird populations to TCDD-like congeners in the environment may be "all or none". Using fish as an example, until a threshold concentration of TCDD in the eggs is reached none of the embryos of a particular fish species in the reservoir would be expected to be adversely affected. However, a three-fold increase in the egg TCDD concentration (above the threshold for early life stage mortality) may cause 100% of the fish embryos of that species to die. This scenario assumes that the slope of the dose response curve for early life stage mortality for fish in the reservoir will be as steep as it is for lake trout where the NOAEL and LD<sub>100</sub>, 34 and 104 pg TCDD/g egg, are separated by a factor of three.

Another implication of the steep dose response curve is that if any of the fish or piscivorous mammal or bird species inhabiting the reservoir are more sensitive than the surrogate species used in laboratory studies, and an extrapolation factor for species differences in sensitivity to TCDD is not applied as part of the risk characterization process, then a

decline in the population of that highly sensitive species may occur. In this regard there is concern about the failure to incorporate an extrapolation factor in the risk characterization for fish. Uncertainty in the fish species sensitivity distribution for early life stage mortality raises the very real possibility that egg TCDD concentrations that provide adequate protection for lake trout (surrogate species) may not necessarily do the same for largemouth bass, catfish, crappie or bluegill that inhabit the Omigoshie Reservoir.

6. Reproductive and developmental toxicity of TCDD has been studied in few of the 10,000 to 15,000 freshwater fish species and in even less estuarine and marine fish species. Also it is well known that wide species differences exist among vertebrates, including fish, in sensitivity to TCDD toxicity. Given these facts and the uncertainty in the distribution of fish species sensitivity to TCDD-induced early life stage mortality it would be prudent to apply an extrapolation factor for species differences to the NOAEL for TCDD-induced early life stage mortality in lake trout. In support of this suggestion findings of Helder (1980; 1982a,b) suggest that northern pike may be as sensitive or more sensitive than lake trout to TCDD-induced early life stage mortality. Furthermore, future research may show adverse effects on reproduction of adult fish occurring at body burdens of TCDD that are lower than those that cause early life stage mortality in lake trout. Yet no extrapolation factor is applied to cover this possibility either.

Additional uncertainty for TCDD risk characterization in fish is in not knowing whether TEFs for early life stage mortality determined in rainbow trout are appropriate for other freshwater, estuarine and marine fish species. We assume this to be true, but it has not been verified experimentally. If bluegill were responsive to early life stage mortality

caused by the mono-ortho analogs of the coplanar PCBs the contribution of mono-ortho PCBs to TEQs in bluegill eggs would be greatly underestimated by using TEFs determined in rainbow trout (because mono-ortho PCBs do not cause early life stage mortality in rainbow trout).

If early life stage mortality is used as a measurement endpoint for ecologic risk characterizations in fish it would be prudent to document Ah receptor expression throughout early development in surrogate freshwater, estuarine and marine fish species. There is no information on Ah receptor expression at any time during fish early development in any fish species. Inasmuch as the risk characterization for TCDD-like congeners in fish is based on the premise that early life stage mortality is Ah receptor-mediated obtaining this type of information would help remove such uncertainty.

Given the limited TCDD database for reproductive toxicity studies in piscivorous mammals and birds, the laboratory studies selected (Murray et al., 1979 - rats; Nosek et al., 1992 - pheasants) to determine fish concentrations of TCDD associated with risk to mammalian and avian wildlife populations seem reasonable. Also the extrapolation factors applied to the results of each study to estimate risk to the most sensitive species seem appropriate. However, considerable uncertainty exists with each of these studies. It is due to several factors such as the one order of magnitude difference between TCDD treatment levels in each study. Also in extrapolating from laboratory animals to wildlife, species differences in the toxicokinetics and toxicodynamics of TCDD contribute further to the uncertainty. Lastly, it is assumed in the risk characterization that piscivorous avian and mammalian wildlife will consume only fish and aquatic invertebrates from the Omigoshie Reservoir. This seems unlikely. Certain species like mink will likely consume other

food items and birds such as bald eagles will undoubtedly forage over a wider range than the reservoir itself and will consume food other than aquatic animals obtained from the reservoir.

7. In the future other reproductive/developmental endpoints may be shown to be more sensitive to TCDD exposure than embryo mortality. If sublethal effects of TCDD on pups, hatchlings or fry (that decrease their ability to survive in the natural environment) are discovered in the future they may be more appropriate endpoints than embryo mortality. It is also possible that future research may reveal adverse effects of exposure to TCDD-like congeners during early development in fish and birds (i.e., during sexual differentiation) that cause adverse effects on reproductive function that are not manifested until adulthood. In support of this notion Mably and coworkers (1992) found that in utero and lactational exposure of male rats to TCDD decreased growth of androgen sensitive sex organs, inhibited spermatogenesis and feminized sexual behavior in adulthood. Lastly, there is a paucity of reproductive and developmental toxicity information on TCDD-like congeners in marine mammals and none in reptiles. Yet premature pupping in sea lions contaminated with PCBs (DeLong et al., 1973) suggests that marine mammals may be sensitive to TCDD-like congeners and should be included in future ecological risk characterizations.

Despite the possibility of more sensitive, ecologically-relevant effects of TCDD being discovered in the future, it is important to state, at this point in time, that the TCDD measurement endpoints proposed in the Interim Report for linkage to population effects in fish, birds and mammals in the pulp mill scenario are the most appropriate ones to use. The challenge now is develop and validate species-specific population dynamic models which will link these laboratory

effects of TCDD to shifts in the feral populations of fish, birds and mammals contaminated with TCDD-like congeners.

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... ..

**Thomas Sibley**  
**Fisheries Research Institute**  
**University of Washington**



Pre-meeting Comments for U.S. Environmental  
Protection Agency

Workshop on Ecological Risk Assessment Issues for  
2,3,7,8-Tetrachlorodibenzo-p-Dioxin  
14-15 September 1993

Thomas H. Sibley  
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Exercise 1. Ecological Effects and Endpoint Selection

1. In an ideal situation it would be desirable to have information on the toxicity or biological effects of TCDD to all the "important" species in the aquatic community being considered. However, the available data suggest that fish provide an appropriate focus to protect the rest of the aquatic resources. I question the use of productivity to assess TCDD effects. Productivity has a specific meaning to fisheries biologists and it is sometimes difficult to obtain sufficient data to calculate productivity. In addition there tends to be significant interannual variability that confounds any interpretation. It may be more useful to consider biomarkers or mortality of juvenile fish.

2. I am not in favor of TEFs. Relative toxicity is often not equivalent for different species so TEF values are species specific. Also, the bioavailability, and consequently the biological effects, of different chemicals will depend upon the physico-chemical properties of the environment and each chemical may be altered differently. Again, if the selected endpoint was a biomarker(s) the additive effects of different chemicals would be evaluated directly. In an ideal situation it would not be necessary to use BSAF factors because one could measure dissolved concentrations of TCDD. Realistically, this is a useful approach because the chemical concentrations can be measured accurately enough to compensate for errors introduced by the normalization procedures.

3. Again, because of the problems of measuring TCDD in solution, I believe this is an appropriate approach.

4. Obviously, laboratory test data at the individual level can be used to predict effects on field populations. It is necessary, however, to validate those predictions. Despite

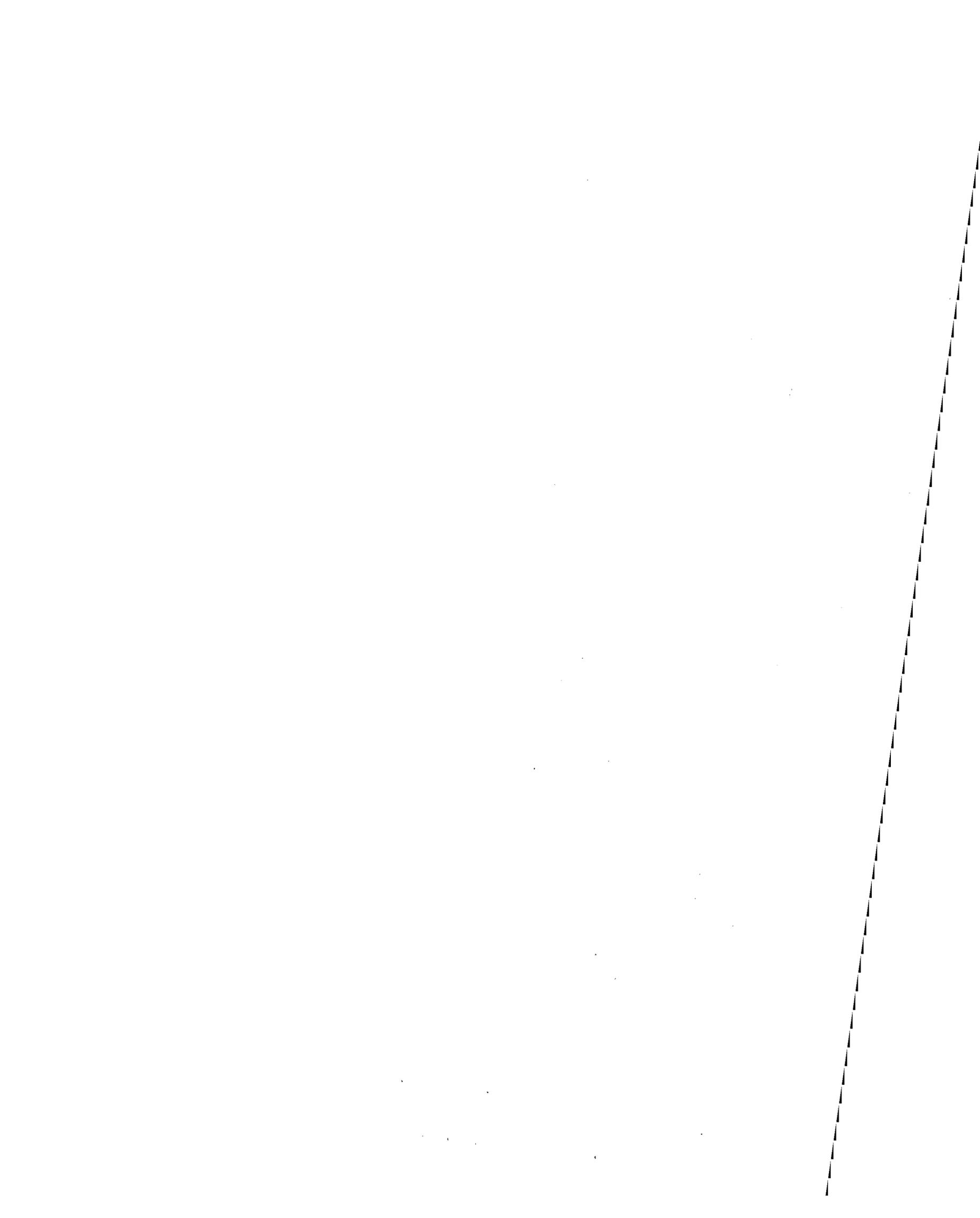
the data that are presented for Lake Ontario it is very difficult to obtain reliable validations. Since high mortality is normal for most populations in natural systems, the magnitude of increased mortality from toxicants is hard to quantify. Specifically, those individuals that are resistant to the toxicant have an increased probability of surviving if density-dependent processes are important.

5. Steep concentration-response curves allow one to make accurate predictions about the effect of a toxicant to a particular species. However, the concentration-response curve is species specific and cannot be extrapolated to other species. If we know the response of sensitive species, we can establish conservative standards. To establish appropriate standards for a particular aquatic community, it is necessary to have concentration-response curves for the species in that community.

6. It is always the case that we would like to have more data, and the uncertainty would be reduced somewhat if there was more data. It appears that the authors have provided an extensive review of the available information which includes information on sensitive species. Therefore, it is possible to establish reasonable limits for fish and wildlife.

7. There is very little information on plants and invertebrates. Only four publications are cited in Table 4.

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## Exercise 1. Ecological effects and Endpoint selection

John Stegeman, WHOI

### General Comment.

The Interim Report in some respects does not go far enough in the recommendations made to address important issues. The Issues for Consideration raised here, and drawn from or based on the Interim Report are important issues. These issues have a common feature: In each case, there is a deficiency in our basic understanding of the mechanisms underlying the toxicokinetics and the action of dioxin and other Ah-receptor agonists. The concerns regarding species extrapolation, dose extrapolation, and inducer structure-activity relationships will not be adequately nor defensibly resolved until there is an adequate mechanistic foundation for the effects of TCDD and its relatives. This requires more than simply identifying the presence or absence of an Ah receptor-like protein or system in selected taxonomic groups, but eventually establishing exactly how it is that agonists and antagonists interact with the receptor, which genes are regulated in addition to CYP1A, the mechanisms by which the genes regulated, and how they are involved in adversely affecting critical cellular processes.

### Issue 1.

If it can be shown that fish are indeed the most sensitive animal group, and that is true for all species, then the reliance on fish as a surrogate for other species in an ecosystem or for the ecosystem in general could be adequate. However, there is 1,000-fold plus range in sensitivity of mammalian species and strains to dioxin toxicity. Evidence suggests that there are equally extreme differences among fish species. Whether the same will be true of early life stages is not yet known. If there are species with resistant early stages, then fish productivity may not provide adequate protection for the system. It is also possible that other components of some ecosystems may be more sensitive than the resident fish, regardless of developmental stage. Until there is an additional foundation of knowledge to explain the basis underlying species differences and developmental differences in sensitivity, then the issue of species extrapolation will be unresolved.

Alternately, changes that presage more extensive effects might be monitored in a caged surrogate species. However, that too would require knowing the precise pathway or function to be examined. However, it is not clear what level of "protection" is desirable, or necessary, in a given setting, and how one coupled infer that level from responses in a surrogate species,

unless the sensitivity of that species was known to be greater than that of all resident species.

#### Issue 2.

Further information on species or group differences in inducer-SAR is required. These must be empirically established. Further, there is growing evidence that in some systems the most common basis for determining TEF, i.e., induction of cytochrome P4501A forms, can be negatively influenced at some doses. The utility of TEFs based on the action of compounds in mammalian cells we know to be inappropriate for evaluating the sensitivity of fish. The use of BASF can also be species dependent.

#### Issue 3.

Obviously, tissue concentrations (Internal dose) is most relevant in attempting to establish the dose-response relationship for a given effect, and for comparing the sensitivity of different species, etc. Not only the tissue concentration, but also the concentration in a given cell type, if specific target cells can be identified. However, once having established a relationship between environmental concentrations and those internal concentrations, and validated that relationship in the laboratory and the field, then one might use either tissue or environmental residue concentrations to evaluate risk. The reliance on environmental concentrations would require a knowledge of the bioavailability from the environmental matrix of greatest concern, at each site. It would be less arduous to measure the tissue concentrations.

#### Issue 4.

At present the laboratory data are not, to my knowledge sufficient to properly predict effects on field populations. One source of uncertainty is the lack of long term studies in the laboratory. The time of exposure in most environmental settings would be life-time. Laboratory studies simply have not provided sufficient information of that type.

A second problem is in the lack of suitable laboratory data on the interactive effects of various compounds. Both of these need to be addressed before any sound predictions can be made for effects on individuals, much less populations.

#### Issue 5.

The steep dose-response curve implies that a more sensitive site or endpoint than that examined would be very useful. Selection of such an endpoint would again depend on a mechanistic understanding of the processes involved in toxicity. It is also possible that the difficulties of dose extrapolation will be better resolved when the factors influencing the

toxicokinetics are better understood. For example, we have long known that the endothelium is a site of very strong induction of CYP1A, and hypothesize that the endothelial cell CYP1A could determine the nature of low dose effects and the penetration of highly efficacious but low mass ligands, like TCDD, into critical target cells. The role of the endothelium must be understood if we are to put dose extrapolations on a sound footing.

#### Issue 6.

Risky extrapolations.

#### Issue 7.

It is important to re-emphasize that a mechanistic basis for effects must be obtained. The types of information that are required but that are not in hand include: Identification of critical target cells; identification of critical target pathways; an understanding of the relationships between dioxin and the processes of growth and differentiation at the cellular level, for estimating the pathways to effect but also for developing more reliable *in vitro* approaches to determining TEFs. The basis for AhR involvement in toxic effects must be established, and the basis for any distinction between AhR and non-AhR mechanisms. The significance of CYP1A induction as a marker will need to be determined. In general, the significance of CYP1A induction will depend on 1) the catalytic function(s) of the protein(s), 2) the relative rates of activation and detoxification of the inducer and other compounds, 3) inducer avidity for Ah-receptors and hence efficacy in eliciting CYP1A induction or other gene regulatory changes, and 4) the sites where these events occur. Though most often studied in liver, induction and attendant changes in extrahepatic sites may determine the toxicity of inducers.



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I have attempted to address the listed items in order, but with my emphasis on avian and terrestrial wildlife, including some generic issues about food chain exposure assessment and overall concepts of chemical additivity and effects in the context of pulp mill scenarios. I have focused on those issue on which I feel I can provide the best commentary.

### Exercise 1. Ecological Effects and EndPoint Selection

#### Issues for Consideration

##### 1. Focus on life stages of fish in aquatic risk scenarios.

Amphibians, invertebrates, and plants are less sensitive to TCDD than fish, birds and mammals. For fish, early life stages appear to be most sensitive. Because of this range in sensitivity, productivity's of fish species were selected as assessment endpoints for the scenario. Comment on the whether this focus on fish species will result in adequate protection for the rest of the aquatic community in the reservoir from the direct or indirect effects of TCDD.

##### **Comment:**

Decreased sensitivity of amphibians and invertebrates to TCDD and other TCDD-like chemicals suggests that risk assessment approaches focused on fish early life-stages will provide a conservative protection of other aquatic species. It is not clear as to the physiological basis for decreased sensitivity in amphibians and invertebrates, but it is likely that target tissue (receptors fro TCDD) must be available for the toxic effects to be manifest. In simple systems, it may be that receptors do not exist for complex entities are not recognized to any extent, thus treated the same as inert molecules. I suggest that the rationale to use early life-stages provides a conservative risk assessment for the aquatic community.

##### 2. TEFs

Use of toxicity equivalency factors (TEFs) for TCDD-like compounds. Comment on the use of these approaches for evaluating the effects and bioaccumulation of dibenzodioxins and dibenzofurans in the paper mill effluent.

##### **Comment:**

Additive Nature of Organochlorine Chemicals, and TEF Concept.

Numerous articles support the contention that organochlorines probably do not act in a simple additive mode. There is no reported "generic effect" of organochlorines other than the fact that they are generally lipophilic and accumulate primarily in fatty tissue. In most studies using birds, toxic effects of organochlorines, TCDD for instance, organochlorines, in general, do not appear to thin egg shells or adversely affect reproduction. The effect of organochlorines on reproduction is still generally attributed specifically to individual chemicals. While numerous studies have attempted to produce an organochlorine additivity matrix for reproductive effects, many organochlorines show no demonstrable negative effects. It is clear that the organochlorines act in species-specific and chemical-specific ways. The effects of TCDD on reproduction has recently been evaluated specifically in birds and fish, and it is still unclear as to the interaction of these specific chemicals with other organochlorines. There appears to be a toxic effect of TCDD on early life stages in many birds, but much work is needed to delineate a clear dose response relationship. Although it is difficult, at best, to utilize a one-by-one evaluation of the toxicity of these chemicals, the database is still too slim to begin to build a complex matrix of combinations of these chemicals. It is clear that induction of AOX, EROD, and other enzyme induction is a natural response and clearly indicates exposure to one or more of these chemicals. It is further clear, however, that exposure is not an effect. The relationship between enzyme induction and effects must be the focus of continued research in this area. Some recent publications are attempting to provide these causal relationships, but with limited success. This further supports the contention that while the AOX and EROD measurement represents a measure of the organochlorine load, it is not necessarily an indication of the "total additive impact of organochlorines" in the medium.

Chemicals in the pulp mill effluent exhibit a range of biological responses; some being primarily time-dependent and some being primarily dose-dependent. Therefore it is inappropriate to consider the toxicity of the suite of chemicals as strictly additive. The correct interpretation is that the toxicity is weighted, and probably less than additive. Given the current stage of scientific understanding, there is no method that permits the accurate prediction of toxicity of such complex mixtures based on the individual chemical toxicity determinations. It is for this reason that regulatory entities (eg. EPA, state DEQs) have relied on bioassay toxicity tests that evaluate the site specific toxicity for the medium.

### 3. Using tissue levels of TCDD to evaluate effects-

Comment on the applicability of this approach to evaluating the risks of TCDD from the pulp mill effluent.

#### Comment:

Most toxicity data are collected from experimentations with single chemicals administered in different doses. Unfortunately, the exposure of aquatic and terrestrial wildlife outside the laboratory is not so clear-cut. It is difficult, at best, to develop the exposure component of a risk assessment using the environmental concentration (water or air or soil) since it is clear that environmental concentration is not necessarily exposure. Under perfect uptake and utilization, the aquatic concentration of a chemical may be the most representative of the possible exposure, but the actual uptake and sequestering of chemical must be defined in order to properly extrapolate effects. Many environmental toxicity tests (in the assessment process) are performed with unknown/undefined mixtures of potentially toxic chemicals. Attempts to predict toxicity of mixtures or to interpret causality of individual chemicals in complex mixtures are typically frustrating. Although tissue concentration may provide some indication of sequestered chemical, it also suffers from the fact that sequestered chemical is not necessarily producing toxic effects. It is the best indication of exposure, however, and provides a more firm foundation for the estimates of exposure. Some of the dialog regarding AOX or EROD measurements as indicators of potential toxicity is complicated by the knowledge gap prevailing over how to deal with complex mixtures. Attempts to correlate the tissue levels or AOX or EROD values with biological responses observed in various single-chemical toxicity tests or from effluent toxicity tests are confounded by the variability introduced by the fact that chemical interactions at the cell level (metabolic and physiological changes) produces measureable, observable effects, not just the exposure. The concept of appropriate cellular receptors of action to produce toxic effects is once again very attractive.

In recent publications, the US Fish and Wildlife Service (Eisler, US FWS) has recommended that the 2,3,7,8-TCDD limits necessary to "protect" wildlife should be set at:

- o 10-12 ppt in food items (prey) for birds and mammals. This level is considered protective of terrestrial wildlife.

Similarly, the US FWS has recommended that the 2,3,7,8-TCDD level in water that is still protective of aquatic wildlife (fish) should be:

- o 0.01 ppt = 10 ppq

The concentration of organochlorine residue in wildlife tissue is a constantly changing function determined primarily by the concentration of the chemical in the exposure route (water for aquatic animals and food for terrestrial animals). It is generally held that the rate of loss (depuration) is approximately the same as the rate of gain (uptake). Further, the rate of bioaccumulation/ bioconcentration varies greatly between species and chemical. The common practice of "back calculating" water or food concentration (exposure) using tissue concentration should be thought of as a means of developing an hypothetical value. The proof of the hypothesis is the actual measured environmental value. The use of an historic residue value of "a fish caught in 1985" etc., simply does not provide the needed validation of the relationship. To be valid, the proper approach is to show that the tissue concentrations are present in a randomly collected (but site-specific) sample. Any use of anecdotal information to demonstrate the back calculation hypothesis is neither scientifically nor statistically meaningful for 2,3,7,8-TCDD or for any other organochlorine.

Avians show little bioaccumulation of 2,3,7,8-TCDD, and low levels (<20-25x) of accumulation of other organochlorines. This is thought to be related to their ability to metabolize many chemicals, either altering their mode of action or inactivating them. Again, this phenomenon is generally species-specific.

Because of their high oil content, fish present the worst case for organochlorine uptake because the lipophilic characteristic of organochlorines results in their being sequestered in fat. However, it is also true that these deposits of chemicals are extremely variable and change in response to changes in the exposure level.

#### **4. Use of laboratory effects on individuals to predict effects on populations.**

Use laboratory test data at the individual level of organization to predict population changes in fish and wildlife. Comment on the utility of available laboratory data to predict effects on field populations and discuss the associated uncertainties.

##### **Comment:**

Attempts to utilize individual (laboratory and field) data to determine the probable impact of chemicals on populations of non-target wildlife are generally fraught with problems. Although it is not yet practical to conduct field studies that determine the effects of chemicals on entire

populations of wildlife, it is likely that efforts will be directed toward determining the impact of these chemicals on sub-populations or local populations of wildlife. The use of individual toxicity data for predictive models to simulate the effects of chemicals on terrestrial populations will be impeded by the enormous variety of species and special conditions associated with sites such as pulp mills and habitat associated with these sites. It simply is not possible to reliably extrapolate laboratory toxicity data to construct models that deal appropriately with all species and all circumstances of interest. Therefore, it would be useful, before building predictive population models from individual toxicity data, to determine which input (population) parameters are critical to the assessment endpoints. Once these assessment parameters are determined, they can be organized into hierarchical levels of importance vis a vis their relative impacts on population density or fitness. Use of such information will eventually enable the construction of simpler and more generic, predictive terrestrial population assessments. Important parameters that modify toxicity estimates that are based solely on tests of individuals include two, partly separable components: one comprising the purely mechanical descriptors of dynamics from given demographic parameter values, and the other describing the modulation of the demographic parameters by environmental factors such as changes in the physical environment, species interactions, pathogens, and xenobiotic chemicals. These problems will continue to confound the problems associated with extrapolations of individual data to populations in the terrestrial environment.

#### 5. Implications of Slope of Chemical Toxicity:

The Interim Report sites data that indicate a very steep concentration response curve for TCDD effects in fish and wildlife. Discuss the implications of this observation for evaluating ecological effects in the scenario.

#### Comment:

It is well known in classical toxicology that the toxicity of a chemical is altered in numerous ways, including temporal and spatial presentation. Additionally, many chemicals exhibit threshold levels that correlate with onset of effects and toxicity. A steep slope of toxicity suggests that the level of error allowed in the risk assessment is lessened and the assessment should be more conservative. This concept is chemical-specific, however, and merely suggests that good laboratory data is needed with more than a *trigger* number ( $LC_{50}$  or  $LD_{50}$ ). A receptor to TCDD that saturates at a specific level is consistent with the current information

about the toxicity of this chemical. Special consideration must be given to the threshold and/or receptor concept in risk assessments for TCDD.

#### **6. Uncertainty from incomplete data sets-**

The general summary of effect levels for aquatic species and associated wildlife is based on extrapolations from a limited number of test species and from tests that do not span complete reproductive cycles. Discuss the utility of these data and uncertainties for evaluating ecological effects.

#### **Comment:**

It is only appropriate to utilize the available data in a risk assessment, regardless of the gaps. In the data review process, it is critical to identify data gaps and uncertainty, in general. It is probable that data gaps will always exist during any real assessment, demanding the extrapolation from available information. Current extrapolation techniques in risk assessments add a safety factor (x10?) for each level of uncertainty. These safety factors can be used for data based on surrogates (sensitivity differences) and for any other extrapolation for which actual data are not available. Usually, these extrapolations are overly conservative and provide a satisfactory margin of safety by accounting in the negative sense, for these errors in such estimates.

#### **7.0. Few data on estuarine and marine organisms-**

No data were found in the literature for TCDD effects on reptiles or marine mammals. Describe other effects data not identified in the Interim Report or the scenario that will be important for future ecological risk assessments.

#### **Comment:**

It is likely that each ecologically realistic risk assessment will be missing data from numerous components of the system being evaluated. It is less important to cover each and every species and possible exposure scenario in a risk assessment than to estimate (measure) effects at some of the higher organizational levels. Use of top predators as flags to trigger concern about the total impact of a stress at lower levels. It is generally believed that the most appropriate sentries of environmental damage occur at higher levels of organization. Unfortunately, this approach is flawed in its inability to detect early, but possibly important, impacts on the understructure of the ecosystem. The most contentious issue in this approach is that there may be numerous dramatic impacts of chemicals on species and communities at lowest ecological

organizational levels that result in irreversible impacts to those animals affected.



Section 3

## **EXERCISE 2**

### ***Stressor Characterization***

**Workgroup Leader:**  
William Adams  
ABC Laboratories



**WORKSHOP ON ECOLOGICAL RISK ASSESSMENT ISSUES FOR 2,3,7,8- TCDD:  
PREMEETING COMMENTS**

Question #

**8. Difficulty in measuring  $K_{ow}$  and  $K_{oc}$  for TCDD and its implications for stressor characterization:**

Measurement of  $K_{ow}$  and  $K_{oc}$  for highly hydrophobic chemicals like TCDD is hindered by operational difficulties. The inability to separate colloids (dissolved organics) from the solute phase in  $K_{oc}$  measurements or to accurately account for the activity of TCDD in water saturated-octanol in  $K_{ow}$  measurements leads to operationally defined measurements that underestimate the chemical property of interest. The best approach is to measure and estimate the property of interest a number of different ways using extreme care and look for convergence of the data. A careful review of the  $K_{ow}$  data for TCDD suggests that the Log  $K_{ow}$  is 7.3-7.4. The inability to accurately measure key physical/chemical properties for hydrophobic compounds indicates a critical research need.

Approaches for characterizing exposure for TCDD risk assessment might include the following:

1. Assume the  $K_{ow}$  for TCDD is Log 7.3 since this appears to be realistic, is in good agreement with calculated Log P values, and agrees with the  $K_{oc}$  measurement of Jackson. Current models require the use of a number and this is as good as it is going to get for the present.
2. Existing theory on the use of  $K_{ow}$  for estimating bioconcentration in organism tissue, predicting  $K_{oc}$ , etc. is based on extensive data sets which have been developed with organic chemicals which have Log  $K_{ow}$  values in the range of <1-6. For this range of  $K_{ow}$ s the relationships are quite good. However, we cannot have the same confidence in this estimator ( $K_{ow}$ ) for values above a Log of 6.0. There are several reasons for this which include analytical difficulties in measuring extremely low concentrations, micelle formation with extremely hydrophobic compounds, binding to colloids, and kinetic hindrance of uptake across membranes of large molecules. The point here is that we have no guarantee that models which rely on  $K_{ow}/K_{oc}$  for estimators provide the same degree of reliability with extreme hydrophobes as they do for chemicals which are more soluble. These estimators are the building blocks of the bioaccumulation models we use, yet there is good reason to question their utility above a Log value of 6.0.
3.  $K_{ow}$  is a surrogate for estimating uptake and storage in fish lipid. A greater effort should be made to use laboratory and field data to corroborate the  $K_{ow}$  measurement. Let the fish be the octanol.
4. Focus research efforts on developing the approach for estimating TCDD transfer from sediment to organisms, ie, sediment bioaccumulation factors (sediment/organism transfer coefficients). A key to remember here is that the hydrophobicity of TCDD is so great that in natural surface waters there are sufficient solids and dissolved organics that the amount of TCDD in the free phase is inconsequential. Sediment concentrations are not always predictors of organism tissue levels, but when the tissue data are lipid normalized to sediment organic carbon levels, the sediment concentrations for extreme hydrophobes provide an upper bound estimate of tissue

sediment concentrations for extreme hydrophobes provide an upper bound estimate of tissue concentrations. With this in mind one can design risk assessment approaches based on residue effect levels.

**9. Implication of food as a primary exposure route for TCDD:**

- Exposure and uptake of freely dissolved TCDD from water as a route of exposure will be minimal. With a Log P of  $>7.0$  even small amounts of solids and organics will sorb TCDD: consider the ratio: 10,000,000 to 1.0 (carbon normalized).
- When the primary route of exposure is food-based the focus of the conceptual model has to shift to provide sufficient information to properly characterize stressors and effects. The models to assess stressors are different for food exposure than water and are not as well developed. Food-based residue effects are not well documented in the literature. Therefore, the amount of data needed to be gathered to characterize stressors and effects will be considerably greater than for a water based exposure assessment. Additionally, laboratory and field data will be needed to validate transport, uptake, and effects models. Sediment transport and binding as well as food/prey availability and mobility will be key considerations.

**10. Fate and transport models for TCDD:**

- My limited experience with fate and transport models suggest that there are adequate models available for conducting reasonable risk assessments for most chemicals. The key question I have is whether or not the models have been properly tested with chemicals with extreme hydrophobicity. Most models are based on a large number of assumptions. These assumptions should be challenged for chemicals like TCDD.
- The ability of transport models to predict the deposition of particulate bound TCDD will be very limited if generalized transport models are used. There are hydrogeological models that can be used, but a good deal of site specific information will be needed for the models to achieve a level of accuracy such that they are reliable for risk assessment purposes.

**11. Major exposure issues not presented in the paper mill scenario:**

- The conceptual model as it is presented in the scenario is fairly generic and as such is broad enough to assess many ecosystem types. A detailed assessment of the given river/reservoir would be more site specific in terms of the exposure characterization and the populations to be evaluated. Looking beyond the current river/reservoir scenario one would encounter many different issues in other types of ecosystems. For example, sea grasses and plant life would be important in estuaries, anadromous salmon fisheries would be important in the large rivers of the North West and North East. Marine fishes which move into estuaries to spawn would be important in many areas. Migrating waterfowl and other birds could be an important part of an assessment for some areas. In the river/reservoir system the reservoir provides a fairly good removal of TCDD due to deposition. In river systems without reservoirs stressor characterization would be different and the transport models used would have to characterize exposure over a much broader area. In estuaries the effects of tides on the transport of the stressor would have to be characterized.

**Keith Cooper**  
**Health Science Institute**  
**Rutgers University**



## K. Cooper Premeeting Comments Exercise 2 & 3

### Exercise 2. Stressor Characterization

8. The variable  $K_{ow}$  reported for these compounds is due to variation in temperature, water quality and the concentrations that were tested. This area needs to be examined in much greater detail and in a more systematic manner. The studies should be designed to answer questions which are applicable to real world situations. There needs to be a better understanding of the factors which modify the concentrations that are observed. Even though the scenario that are being discussed is freshwater the studies should include estuarine and oceanic salinities. There is a need to better understand the physical properties of the organic material that these compounds are associating with. There also needs to be studies on the particle/particle interaction and the sorption kinetics at varying concentrations. There also need to be studies carried out to explain why aged sediments appear to have less bioavailability than newer deposited sediment.

In the scenario of the pulp mill there would need to be studies examining the specific process and the different types of papers being made, as well as the different types of wood being used. There would also need to be a size distribution concerning the association of various compounds with different size particles. The treatment plant for the mill would also

K. Cooper Premeeting Comments Exercise 2 & 3

play a big role in how much if any of the fine suspended sediments would reach the river. The pulp mill facility should conduct studies to determine the bioavailability from the samples collected from the mill.

In the conceptual model the value of most interest for these compounds is not the  $K_{ow}$  but the  $K_{oc}$ . This is especially true in the fact that the paper mill treatment streams will have very high dissolved organic carbon.

9. The major route of exposure in almost all cases is through food consumption or through direct contact with either eggs deposited on contaminated sediment or animals living in or on the sediment. There needs to be information on the properties determining the amount of the compound that is biologically available (either from prey or from sediment). The prey species may also effect the amount of the compound that is available to be absorbed. Studies need to clarify if there is an effect of dose in a prey species and the percentage that is absorbed.

In the scenario set out the major route for bioaccumulation is through the food web for most of the aquatic animals. The animals which live in or on the sediments may have a very

K. Cooper Premeeting Comments Exercise 2 & 3

small amount taken up by direct contact, but this would be a minor route. Filter feeding organisms can remove small particulates from suspension, but just as with human lungs there are specific size sorting which will make some animals exposed to particles which may contain higher concentrations of the dioxins. Some of the bottom feeding fish species take in detritus and sediment at the water sediment layer, which is high in organic carbon. In the conceptual model there should also be routes of elimination from the system such as burial by cleaner sediments, photolyses and some reductive dehalogenation or oxidative metabolism of the furans.

10. Although I am not a fate and transport modeler I have dealt with several modelers. The difficulty with these models is that in many instances they can give you a worse case scenario in a completely mixed system, but if the system is very complicated then the models begin to break down. In order for the model to work there does need to be a large amount of data on flow, areas and other physical parameters. The models in the case for dioxin and compounds with similar high  $K_{oc}$  could assume association with specific sized particles and these could be modeled in the reservoir. The models could predict the sedimentation rates above the damn in the scenario with models that currently exist. Such a model could also

K. Cooper Premeeting Comments Exercise 2 & 3

predict worse case scenarios in the 50 and 100 year floods. The models once they have made a prediction should be tested to determine if the model adequately represents the real world situation (the model must be validated). This type of information was generated for Walters Lake, North Carolina for the Carolina Power and Light which is down stream from a large paper mill.

11. In the paper mill scenario you have a well defined boundary and a large reservoir, while in an estuarine environment this is a much more dynamic system. The effects of tidal movements could result in depositional areas not seen in the paper mill scenario. There could also be large areas that are nursery areas for anadromous fish that are only exposed for short periods of the year. Some fish species following spawning over winter in the near shore areas where these compounds could accumulate. Invertebrate species which serve as major food sources could accumulate the materials. The crustacea may be affected by these compounds because of the juvenile development and molting. Often the estuaries have large deltas or areas of deposition from upstream. The requirement for periodic dredging to maintain navigation poses the questions what to do with contaminated material. Storm events along the coast often will stir up large areas of bottom, which suspends more deeply buried contaminants. Many

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of the estuarine environments have multiple contaminants and sources for these compounds (atmospheric, chemical plants). There is also concern for the large mammals which may consume large quantities of contaminated fish for many years. There is little or no information on the effects on these animals. There is data on levels, but biological impact is unclear.

12. In my opinion there is very limited use for the BCF<sub>f</sub> values generated for real world scenarios. In any system the compounds will be associated with the suspended material and not free in the water. The figure 3-1 makes this point very well. In the case of the paper mill the average of 5% and a range from 2-15% would result in a very small portion free in the water if not zero. The best way to evaluate the potential BAF is to do it on a site by site basis including in the scenario food web and ecosystem characteristics. However, the Lake Ontario study and the comparison with other fish and areas demonstrates that there is fairly good agreement. The question about why different animals appear to accumulate at different levels needs to be better understood since it is not explained by any of the current models for sediment/water dissociation or the steady state based on lipid normalized data. The steady-state biota/sediment accumulation factors (BSAF) in both laboratory and field experiments are less than

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1. The variation (Table 3-3) between fish and invertebrates need to be examined further. The higher levels reported in the mesocosm work (Rubinstein et al. 1983) may be artificially high due to the exposure to contaminated sediments at a constant concentration. There is a large amount of data that has been generated by the paper mills in the United States and other industries that can give numbers for a number of the species listed in the paper mill scenario. In most cases there is not good data on sediment levels and organic carbon content. In some situations it might be important to know both the surface sediment and suspended sediment concentrations to evaluate the BSAF.

13. The major concern that might be raised is the fact that the amount of DOC and the matrix which the dioxins are associated with may result in very different concentrations dissolved in the water. Care should be exercised when using the Lake Ontario TCDD BAFs because of different sources of the material, which will effect its  $K_{oc}$  and greatly alter the BAF.

14. The BMFs for most of the dioxins and dibenzofurans when measured in the field are lower than what would be expected from computer models. The reasons for these differences are

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not known. In many cases it is stated that the differences are due to metabolism but in very few cases has this been demonstrated. The variability in the levels found in a lake area even when normalized to lipid can not be explained and this is an important area for future research. In the paper mill scenario there is no indication for levels in crawfish or other crustacea which may serve as a source of exposure to certain fish species. In many of these organisms there is a need for examination of the individual species in order to better understand the BMFs. In most of the contaminated sites the bottom feeding catfish and carp species are generally the highest. There is a need for experimentation to understand why this is the case. Similar types of studies need to be carried out on avian species as well.

15. The section 5.1.2 gives a fairly good summary of the problems with the uncertainties associated with bioaccumulation factors. Even with these uncertainties there can be a relatively good estimation for the Omigoshie Reservoir biota. After the estimated levels are determined than the field testing would either indicate that the model is reasonably estimating the levels or that for some reason the estimates are wrong. If the estimates are incorrect than the site specific approach should be adopted to determine why the estimates are not consistent with the real world scenario.



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*Comments on*  
"Interim Report on Data and Methods for Assessment of 2,3,7,8-  
Tetrachlorodibenzo-p-dioxin Risks to Aquatic Life and Associated Wildlife"  
[EPA/600/R-93-055]

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**Exercise 2. Stressor Characterization**

**8. Uncertainty in  $K_{oc}$  values**

Uncertainty in  $K_{oc}$  value used to assess fate, transport, and exposure of dioxin can have a major impact on the estimate of available dioxin (freely dissolved) in a suspension. For example, at 10 mg/l suspended solids a half-order of magnitude error in  $K_{oc}$  (e.g.,  $\log K_{oc} = 6.5$  versus 7.0) will manifest itself in a factor of **three** in the relative concentration of freely dissolved chemical, while the effect on the fraction in the particulate phase will be negligible. This uncertainty will lead to a large uncertainty in volatilization fluxes and the rate at which the chemical builds up in the sediments.

Ideally, for the pulp mill scenario a system-specific  $K_{oc}$  could be measured along with some understanding of how spatial or temporal variability in environmental conditions (e.g., sorbent type, characteristics, and concentration) will affect this parameter. Realistically, the uncertainty in this parameter inherent in using a non-site-specific value should be carried through the exposure calculation by employing something like a Monte Carlo modeling analysis similar to that conducted by Endicott, *et al.* (1992) in their Lake Ontario report.

It should also be noted, from studies of other hydrophobic organic chemicals (such as PCB congeners) in the Great Lakes, that in open lake water we are very unlikely to measure  $K_{oc} = K_{ow}$ . Studies such as Eadie's (1993) and

*J.V. DePinto*

analysis of data from the Green Bay Mass Balance Study suggest that the slope of a regression line for  $\log K_{oc}$  versus  $\log K_{ow}$  has a slope much less than one and an intercept much larger than zero. Although the exact mechanism is not known for sure, it has been suggested that sorbents in open lake water, dominated by algae and algal detritus, behaves differently than soils and tributary suspended sediments because of the different amount and character of organic carbon in the solids. Also, some work (Swackhamer, 1993) has suggested that sorption and desorption kinetics are more significant for algae than for low  $f_{oc}$  tributary solids and that this phenomenon is the cause of the open lake observations.

Another observation with PCB congener-specific studies (Bierman, *et al.* 1992) is that binding to operationally-defined DOC is much weaker than to POC. It seems that  $K_{DOC} \approx 10^{-2} K_{POC}$  for the Green Bay system. Does this happen with dioxin? Or does dioxin behave more like mirex, which does not show as big a difference (studies of Hassett at SUNY-CESF)? Again, this uncertainty may have a very large impact on both exposure and effects assessments, and it should be carried through all calculations until it is resolved.

#### 9. Implications of food chain exposure dominance

There are many sources of uncertainty in computing fish exposure through the food chain, even assuming that the water column and sediment bioavailable concentration is well known. First, these calculations assume that the food chain is static (*i.e.*, non-variable forcing function) and that each trophic level component (like fish of a certain age class or zooplankton) is assumed to behave according to average metabolic and growth and chemical assimilation properties. We know that in actual systems, these parameters are highly variable from site to site and exhibit large spatial and temporal distributions within a given site. The effect of this variance in food chain bioaccumulation parameterization remains to be adequately assessed.

***J.V. DePinto***

A second question is the observation that the relationship between log BAF and log  $K_{ow}$  deviates (decrease in slope, less dependence on  $K_{ow}$ ) from a straight line for HOCs with  $\log K_{ow} \geq 6.5$ , which places dioxin in this category. Any computation of BAF on the basis of  $K_{ow}$  should consider that effect.

Another observation, related to the  $K_{oc}$  for algae in lakes, is that computation of food chain bioaccumulation in fish is very sensitive to the chemical partitioning to the base of the food chain (the algae). This value has been shown to be spatially and temporally variable for PCB congeners in Green Bay; therefore, its uncertainty in a given site-specific scenario such as the pulp mill problem must be considered.

#### 10. Availability of fate and transport models

GBTOX, which is the fate and transport mass balance model developed for the Green Bay Mass Balance Study (GBMBS), is the current "state-of-the-science" for modeling HOCs in surface waters. This model, which was developed for PCB congeners would be quite suitable for TCDD, assuming the chemical-specific properties of TCDD are known. It has been our experience that accurate sorbent dynamics is the most crucial aspect of a toxic chemical fate and transport calculation. In that regard GBTOX models both transport and transformation of three organic carbon based state variables: biotic particulate carbon (*i.e.*, viable algae), particulate detrital carbon (all particulate matter that is not viable algae), and "dissolved" organic carbon (passes a filter). Each of these sorbents has different source, transport, transformation, and fate pathways, each of which have important ramifications for organic contaminant exposure pathways and fate. I would recommend that this type of model become the "state-of-the-art" for aquatic risk assessment.

#### 11. Major issues not covered in paper mill scenario

Following is a list of issues that I feel were not dealt with adequately in the paper mill scenario:

*J.V. DePinto*

- Depending on the hydraulic and sediment-water interaction characteristics of the system of interest, achieving steady-state after start up may take many decades. In particular, building-up dioxin to steady-state levels in the sediments will be the long-term process. In fact, parts of the reservoir sediments may essentially never reach steady state.
- The description of sediment dynamics in the reservoir and the main river channel is not described in nearly enough detail to be able to characterize the dioxin exposure regime for this system, especially in the sediments. No deposition and flow-driven resuspension in the river is unrealistic. We need to specify how much of the reservoir SS is due to autochthonous algal production.
- We cannot estimate exposure without knowledge of the hydrologic and morphometric characteristics of the system: flows from each tributary and plant discharge; hydraulic retention time; volume, mean depth, and surface area of whole reservoir and each arm.
- We need wind data to estimate air-water exchange rate and we need solar radiation data to estimate photolysis.

#### 14. Biomagnification Pathway

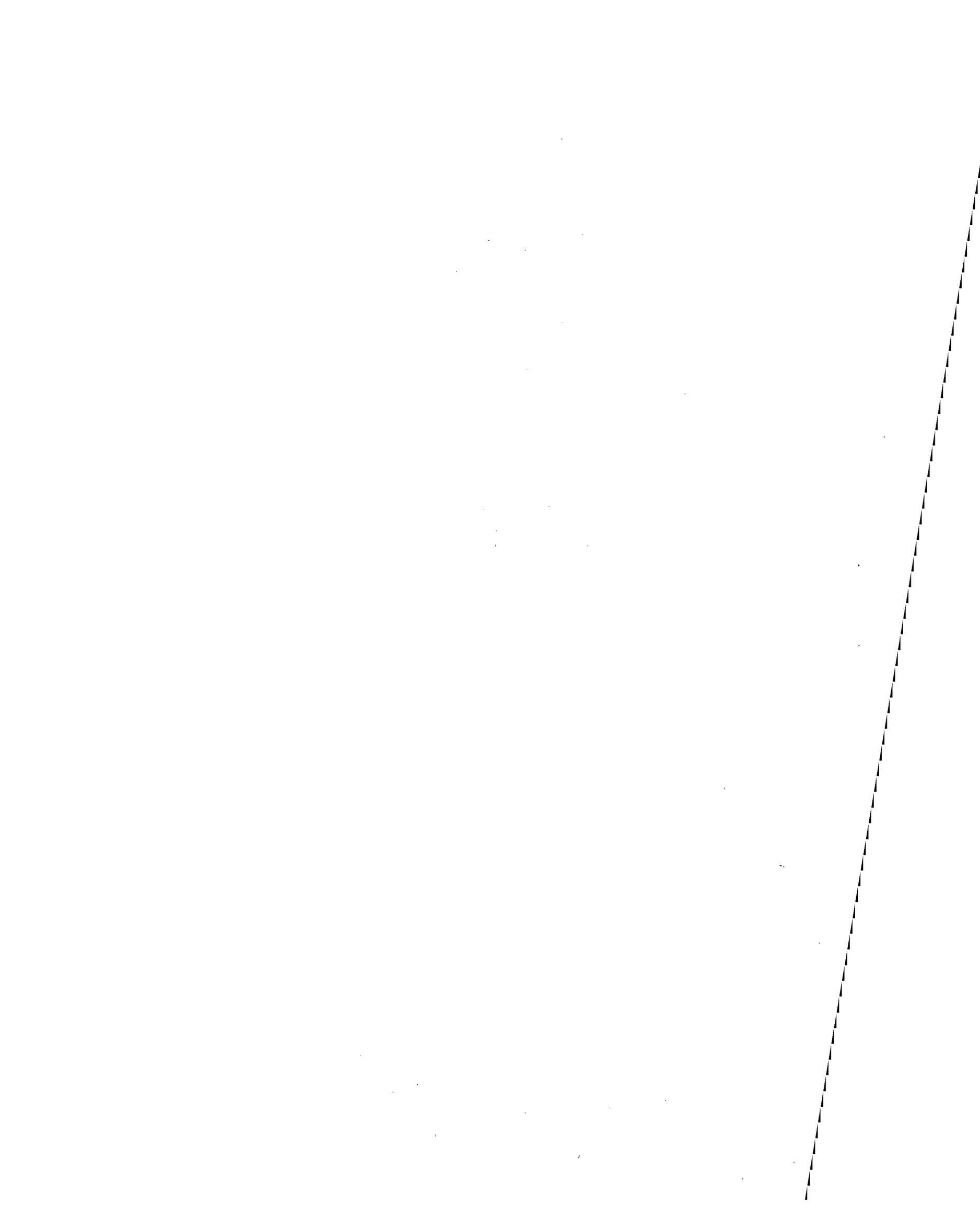
The low lipid-normalized BMF between lake trout and forage fish may not only be the result of dioxin metabolism in the lake trout. It may be the result of differences in chemical assimilation efficiencies for fish versus fish-eating birds. This parameter is difficult to assess and a source of uncertainty in exposure models. Also, one should ask the question of what age class of lake trout were used to determine the value; there is approximately a three year lag time between initial exposure and achieving some level of steady-state.

*J.V. DePinto*

### 15. Uncertainties in exposure prediction

Bioaccumulation in aquatic systems is driven by dissolved dioxin. Among the major uncertainties in governing dissolved dioxin in Lake Ontario are solids settling velocity, dioxin volatilization rate, and  $\log K_{oc}$ . There is no particular reason to believe that this result will be particularly different for the case study. Of course, there are a variety of uncertainties associated with computation of BAF that should be added to the list.

*J.V. DePinto*



**Robert Huggett**  
**Virginia Institute of Marine Science**  
**College of William and Mary**



STRESS CHARACTERIZATION

Question 8: Probably the weakest links in the Interim Report's treatment of the fate, transport and effects of TCDD in aquatic systems, center around the inability to analytically determine "dissolved" environmental concentrations. Due to this limitation, dissolved levels are derived by via calculations from media with higher concentrations. Obviously errors are involved with each calculation due to uncertainties associated with the coefficients. To be conservative, the assessor may want to take "worst case " scenarios. However, it should be kept in mind that due to disequilibria, different types of sediment organic carbon, different types of dissolved organic matter and different suspended sediments, the actual concentrations could be considerably different.

Question 9: The Interim Report's predicted routes of TCDD exposure as being mainly through food is supported by its very high Kow. A major implication of this when assessing exposure routes in the conceptual model is that to have accurate estimates, one must be able to accurately predict, "who eats what." Many warm and cold water species have terrestrial organisms as a major part of their diets. As well, many species shift their diet as various foods become more abundant. Again, "worst case" scenarios can be used but with the same caveats as given in Question 8.

Question 10: Both parts "a" and "b" appear to be essentially the same for very hydrophobic chemicals. The crucial question centers on how good the models predict deposition and resuspension of very low density (eg. high organic carbon) particulates and at what temporal and spacial scales. Flocculation, bioturbation, fecal pellet deposition and

physical disturbances (eg. periodic scouring as current velocities change and/or wave action) are all site specific and most mathematical models do not handle them well.

Question 11: In estuaries, a TCDD risk assessment would have to consider the "Turbidity Maximum", if one occurs, as well as the fact that many of its fish and crustaceans are migratory, thus spending only part of the year in the system.

In terrestrial systems, one must consider many of the same issues raised in Question 9. The fact that many terrestrial mammals and birds are omnivorous and migratory, complicates the situation even more.

Question 12: As previously mentioned, the inability to analytically quantify TCDD in solution is a serious limitation in the paper mill scenario (see comments for Question 8). As discussed in Question 9, diets will not only be different in different locations but also they will change with time. These points need to be kept in mind.

Question 13: This question appears to be redundant with parts of questions 8 & 12 and those answers are appropriate here. It is apparent that research is needed to lower the detection limit for dissolved TCDD.

Question 14: The biomagnification pathway in the Stressor Characterization and the Conceptual Model appear realistic.

Question 15: The Interim Report clearly states that there are uncertainties associated with TCDD bioaccumulation factors (BCF's). Steady state BCF's, derived in the laboratory vary over an order of magnitude. What would the variance be in the environment where steady state is probably rare? The reasons for non-steady state have been previously given. Therefore, residue predictions should be considered tentative at this time.

**Charles Menzie  
Menzie-Cura & Associates, Inc.**



## PREMEETING COMMENTS

Charles A. Menzie  
Workshop on Ecological Risk Assessment Issues For TCDD

### Comments on Exercise 2. Stressor Characterization

8. Uncertainties associated with estimates of Kow and Koc may lead to uncertain predictions of TCDD partitioning and exposure. How should this be handled in stressor characterization and conceptual model development for this scenario?

#### **Comment**

The implications of the uncertainties associated with Kow and Koc estimates will depend upon the method employed to represent the exposure field. I suggest that the distribution of TCDD and related compounds in the reservoir be characterized by a suspended particulate/sediment transport model which estimates TCDD distribution based on estimated suspended solids and particulate organic carbon concentrations, and movement and settling of particulates within the system. Regardless of the uncertainty associated with the Kow and Koc estimates, most of the TCDD will track with the particulates and will be distributed in accordance with sedimentation patterns. Because of the uncertainty associated with the forms in which TCDD may be present within the water column (with POC & DOC) and the uncertainty associated with the Kow and Koc estimates, it may be most useful to relate exposure to fish to resultant sediment levels of TCDD.

If models based on Kow and Koc are employed to estimate exposure, it would be useful to conduct a sensitivity analysis. It appears the best estimate of the log of these values is approximately 7.0. Alternative values that might be considered include 6.0 and 8.0. A sensitivity analysis would reveal the possible importance of these variations on the estimate of exposure. As an enhancement, it may be possible to derive site-specific partitioning and uptake coefficients using a combination of chemical analytical and biological methods. This would require obtaining a representative sample of effluent either from a similar plant or from a pilot plant.

9. Address the implications that most TCDD exposures will arise from food consumption and contact with sediments.

#### **Comment**

I think the Interim Report makes a good case for the importance of these exposure routes. The major implication is that exposure to fish should be related to either sediment and/or food concentrations of TCDD and related

compounds. As indicated in Section 3.1 of the Interim Report, since the BSAF and BSSAF do not vary significantly with Kow, the great uncertainty existing for the Kow of TCDD is not incorporated into these bioaccumulation factors. Limited available information presented in the Interim Report suggests that the BSAF for warmwater fish species varies over a relatively small range given all the possible sources of uncertainty in making estimates. This suggests, that for predictive purposes, the assessment should be based on a model that yields an exposure field for sediments. It may be more difficult to predict the levels of TCDD in prey organisms and thus difficult to estimate exposure to fish based on accumulations in their prey.

10. Comment on the availability of fate and transport data/models suitable for use with TCDD. Discuss the applicability of available transport models for predicting the deposition of particulate-bound TCDD in the reservoir.

**Comment**

This is not my area of expertise. However, recognizing that all models are wrong but that some may be helpful, I think it would make sense to consider a range of models from very simple partitioning or box models to more complex models in the following order: 1) simple box model for reservoir as a whole with TCDD partitioned between suspended sediments, water, sediment, and biota under steady state conditions with a constant source, losses from the system through advection, and possibly losses through sedimentation/bioturbation; this could be accomplished with available simple models including the Level III Fugacity Model; 2) box model that treats the reservoir as above but recognizes the different regions of the reservoir; these regions include the trunk near the proposed mill, the two arms into which two other streams discharge, and the broad basin near the dam; and 3) finite element models such as Toxiwasp which divide the system into many discrete cells and track mass flux of suspended sediment and associated TCDD et al. between them. Such a model would need to incorporate sedimentation so that exposure concentrations in the surface sediments could be estimated for the various cells. Mixing of sediments through bioturbation and sedimentation would need to be taken into account in order to derive a surface sediment concentration. Such models are available but require estimates of bioturbation and sedimentation rates. If this is an important issue, it may be possible to estimate these processes within the reservoir using a suitable tracer (e.g., lead 210).

It is my understanding that models do exist for estimating the movement and deposition of particulates in lakes and reservoirs. These models are applicable to estimating the distribution of particulate-bound TCDD. A key consideration in all these models is the degree of sophistication needed

to reach an "acceptable answer" for risk assessment and associated risk management purposes. The more sophisticated models require more information about the system. If an acceptable answer can be reached using simple models this is superior to gathering a great deal more information for use in more sophisticated models.

There are several methods available for assessing transport of water and particulates. Field measurements should be considered for assessing the factors that affect these processes within the system. The kinds of information sought include: contribution of the three tributaries to the solids loads to the reservoir, importance of events (e.g., storms, spring floods) with regard to loadings and resuspension, variations in freshwater flows to the reservoir, circulation and residence times within the various arms of the reservoir. It would be helpful if there was a tracer that could be used to estimate the contribution of solids for the tributary on which the mill is located; it is unlikely that this is the case unless it is draining a region that is geologically different from the other tributaries. Measurements should also be obtained on particulate formation (primary production) and deposition within the system. A sediment survey of the reservoir would provide useful information on deposition patterns and the overall characteristics of the sediments.

11. List some of the major exposure issues not present in the paper mill scenario that may be encountered in future ecological risk assessments.

**Comment**

There are two major factors to be considered in future ecological risk assessments regardless of the system:

1. the physical characteristics of the system will dictate the manner in which the chemical is distributed and the nature of exposure; these physical characteristics are unique to different kinds of aquatic systems (e.g. large lakes vs small lakes and reservoirs, estuaries vs rivers, estuary and coastal systems vs offshore areas, shallow basins vs deep waters, wetlands vs forrests)
2. the ecology of the ecological receptors will dictate how they might be exposed; in the present case study we are examining warm water fish species that tend to inhabit shallow waters and nest on or in the sediments; the species all are typified by limited home ranges during much of the year; in contrast, anadromous fish species, species that have planktonic eggs and larvae, or migratory birds would experience a very different

exposure regime.

12. Discuss the applicability of bioconcentration, bioaccumulation, biomagnification, and biota-sediment accumulation factors to stressor characterization for the paper mill scenario.

**Comment**

These are all processes that are recognized to exist. However, there is considerable uncertainty associated with estimating the relative importance of the processes. I think it would be most useful to identify the most straightforward method for relating exposure to body burdens for the fish. Based on my review of the Interim Report, this would probably involve the use of a range of BSAF values applied to estimated sediment concentrations. This is appropriate for this case study because of the ecology of the target fish species. These species tend to have limited home ranges and also tend to be closely associated with sediments both in terms of where they occur in the water column and also with regard to their food.

13. Comment on applicability of Lake Ontario BAF<sup>dl</sup> to the paper mill stressor characterization.

**Comment**

The big "if" appears to be if  $C^{hd}$  can be estimated accurately. I do not know if this is possible and it seems somewhat hypothetical at this time. I do know that sediment contact and ingestion of prey items associated with the sediments may be important and may not be captured by a  $C^{hd}$  derived for a physically different system, one in which the water volume to sediment area is probably much greater than in the reservoir.

14. Comment on the biomagnification pathway.

**Comment**

The biomagnification pathway would be important to consider if the effects information for birds (and mammals) were related to contaminant burdens in specific target organs. Based on the Interim Report, data are not adequate for this purpose and the effects data are based on administered dose. Thus, at present, the issue of biomagnification pathway is moot. As data are developed on relationships between body burdens and effects for birds and mammals it would be important to consider the role of biomagnification. However, when that time comes it might be most useful to relate directly body burdens to dietary doses rather than to residues in the wildlife. I suggest this because the information that would be generated in future risk assessments would initially be body burdens in the fish. Thus, ultimately, we will be relating effects on wildlife to body burdens in fish. Focusing on biomagnification does not

appear to help in this regard. Such information would be useful as part of developing an effects data base that could be used to support epidemiological studie.

15. Discuss the relevance of uncertainties in bioaccumulation factors to prediction of TCDD residues.

**Comment**

In the face of uncertainty, I think it would be prudent to conduct a sensitivity analysis using the ranges and best estimates on bioaccumulation factors. Because the agency is in the position of reaching decisions that should be protective of the environment, I assume that greater weight would be given to those estimates based on higher BAFs. If the results of such an analyses were either clearly good or bad (depending on your point of view) there should be little argument about the results. However, where results are equivocal, this should indicate where additional information could be gathered to reduce the uncertainty in the estimates.

Consideration could be given to the development of these factors in laboratory bioassays or mesocosms in which fish are exposed to representative effluent that has been allowed to partition among water, sediments, and suspended sediments.



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## PRE-MEETING COMMENTS FOR THE PEER PANEL WORKSHOP ON 2,3,7,8-TCDD

### Comments on Exercise 2. Stressor characterization

**8. Problems associated with TCDD phys/chem properties and measurements of partitioning:** There is definitely considerable uncertainty in the estimates of WS and Kow of TCDD and other 2,3,7,8-substituted congeners. This uncertainty makes application of chemical fate and food chain models to the pulp and paper scenario quite difficult unless a lot of site specific measurements are obtained.

**1. Water solubility.** Although there are a wide range of WS values for TCDD, values in the range of 200-480 ng/L (Lodge 1989) are the most consistent with results reported for other PCDD and PCDF congeners (Friesen et al. 1990; Shiu et al. 1988). Most of the latter results were obtained with a generator column which is the best available technique for WS determination of hydrophobic compounds. The results of Marples et al. and Adams and Blaine did not use the generator column methodology and therefore should be used with caution. For the paper mill/reservoir scenario, WS determined at 20°C would seem most appropriate because water temperatures are presumably in the 20-30°C range at this site much of the year. Water temperature information would be required for the conceptual model along with other basic water chemistry parameters (TSS, DOC, POC).

**2. Octanol-water partition coefficient.** The results of Sijm et al. and Marples et al. are probably as close as we will get to direct measurement of  $K_{ow}$  values for TCDD. All direct methods (including generator columns with octanol phases) appear to be prone to emulsion formation. Perhaps an approach involving different ratios of octanol to water (both presaturated), similar to Lodge's for  $K_{oc}$  determination, would address the emulsion issue. I agree with the interim report (p. 2-3) that WS is more reliably measured than Kow. Log Kow can be calculated from Yalkowsky et al.'s relationship. It is interesting that using a WS of 200 ng/L ( $0.625 \times 10^{-9}$  mol/L) the original estimate of TCDD solubility (Crummett and Stehl, 1975), gives a log Kow of 6.9 using Equation 2.2 (p.2-3) which is close to most measured Kows and to the value of 7 (from Burkhard and Kuehl) used in the interim report.

**3. Koc values:** Koc is a key parameter in defining the distribution and exposure concentrations of hydrophobic contaminants. As noted in the interim report there are uncertainties in Koc values for TCDD due to difficulties in measuring dissolved concentrations. Broman's data are probably the best field measurements but his "dissolved" phase includes a colloidal DOC fraction. The method used by Lodge and Cook (1989) is perhaps the most elegant approach to the problem of a non-settling phase in Koc measurements i.e. extrapolation to zero solids. Although sparging techniques (Resendes et al. 1992) offer some promise of direct measurement of freely dissolved concentrations they are also prone to interferences from stripping of bacteria and other suspended particles from the water column by bubble action (Friesen et al. 1993 in press). Setting  $Koc=Kow=10^7$  for 2,3,7,8-TCDD and other 2,3,7,8-substituted congeners seems appropriate for generic risk modelling purposes.

In the case of the pulp and paper scenario consideration should be given to actual site specific measurements of Koc of TCDD using suspended solids from mill effluent, river water and bed sediments, to confirm the above assumption. Both field and lab based procedures could be used. Prior to startup of the proposed mill Koc values could be determined in the laboratory with sediment and water obtained from the depositional zone in the reservoir, using the method of Lodge and Cook. During operation of the mill, field monitoring using methods such as *in situ* sparging (Resendes et al. 1992) and high volume sampling/filtration (Broman et al.) could be employed, with HRGC-MS analysis to determine TCDD in "dissolved" and particulate phases.

Chemical fate models such as WASP and EXAMS also address pore water diffusion which may be a significant source or sink of hydrophobic organics in the water column. For buried TCDD diffusion may be the only process of movement. The effective diffusivity  $D_{eff}$  can be calculated if the molecular diffusivity in water, Koc and sediment porosity are available (Formica et al. 1988).

**9. Exposure routes in the conceptual model:** There is good evidence from field and laboratory studies that TCDD exposure of fish arises mainly from food consumption. This implies that the conceptual model needs to include detailed consideration of the predator-prey relationships in the river and the reservoir. The schematics (Fig. 3 and 4) representing the Thomann model with links to fish-eating birds and mammals are an appropriate first step. However to make accurate predictions of concentrations in each species will require knowledge of what species are present, their prey, their age distribution, growth rates, feeding rates, and pharmacokinetic data for TCDD uptake and depuration. For modelling over an entire year consideration has to be given to temporal changes in density of organisms, especially benthic organisms, and resulting diet shifts. Benthic insects may form a major part of the diet of bottom feeding and pelagic fish for brief periods in the spring as they emerge as adults. Emerging insects have been shown to transfer TCDD to the water column and surrounding terrestrial environment (Fairchild et al. 1992). In riverine environments, filter feeding insects may trap suspended solids originating from secondary treatment ponds of pulp mills, which they subsequently transfer to fish (Birkholtz et al. 1992). The stable isotope technique used by Broman et al. to characterize the predator-prey relationships in the Baltic pelagic food chain is a useful technique to apply to any detailed site specific study of the proposed pulp mill.

**10. Fate and transport models.** Available models include WASP and EXAMS (USEPA) and RIVER/FISH (from NCASI; Hinton 1991). These three models have been used to predict fate and distribution of TCDD and related hydrophobic in lakes and rivers. RIVER/FISH is less sophisticated in that its current version does not include sedimentation - it takes a dilution partitioning approach to estimate exposure concentrations in the water column. Nevertheless the model has been successful in predicting TCDD levels in fish near pulp mills. WASP was used by Endicott et al. (1990) to predict TCDD fate in Lake Ontario. It has also been used along with a colorful graphics output to predict TCDD/F concentrations in

sediments of Howe Sound (BC) (Holloran 1993). WASP4 would definitely be the most appropriate model for initial chemical fate modelling the river-reservoir pulp mill scenario because of its flexibility. Several Canadian modelling efforts for TCDD near pulp mills have concluded that more sophisticated hydrodynamic and sediment transport models, than are available in WASP4 should be used for large river systems (Marmorek et al. 1992). The model MOBED (Krishnappan 1981) was recommended for sediment transport in the Fraser River.

#### **12. Applicability of BCF, BAF, BSAF to paper mill scenario:**

**BCF<sub>1</sub>:** BCFs are strictly lab-based measurements because they involve exposure via water only. For chemicals with long equilibration times BCFs must be estimated from the ratio of first order uptake ( $k_1$ ) and elimination ( $k_2$ ) rate constants. These constants can be used in food chain accumulation models.

As noted on p. 3-10 there is a wide range of BCF<sub>1</sub>'s reported. The large variation is due to difficulties in measuring freely available TCDD in the water and to underestimation of  $k_1$ . Given this variation it is impossible to recommend a single BCF value to use in the pulp and paper scenario. But  $k_2$ 's for TCDD are known with greater certainty because they are less influenced by initial exposure conditions. Fish size and lipid content effect  $k_2$  values although precise allometric relationships have not been reported for TCDD or other hydrophobic organics. A range of  $k_2$  values could be recommended for food chain modelling of TCDD and related PCDD/Fs by selecting appropriate values based on organism size and lipid content. It would be difficult to recommend a range of  $k_1$ 's for TCDD from the existing literature.

**BAF<sub>1</sub>:** These values can be calculated from field measurements of TCDD in biota if TCDD concentrations in water can be estimated. The EPA has done this in initial assessments of TCDD from pulp mills (US EPA 1990) and Muir et al. (1992a) have calculated BAF<sub>1</sub>'s for fish near Canadian bleached kraft mills. But as with BCF values, BAFs are dependent on water concentrations (i.e.  $C_w^d$  or  $C_w^t$ ) for which few measurements are available. BAFs are also dependent on food chain relationships. Lipid normalized BAFs for TCDD in mountain whitefish, and in white and longnose suckers, sampled near pulp mills in Alberta and BC differed consistently by 3 to 4-fold. This has been explained by feeding differences (Birkholtz et al. 1992; Muir et al. 1992a). The whitefish feed on emerging insects and filter-feeding insects in the water column while suckers are bottom feeders. The filter-feeding insects may accumulate high concentrations of TCDD/F by trapping biosolids from the secondary treatment ponds of the mills which are subsequently accumulated by whitefish.

**BSAFs:** Unlike BCFs and BAFs, BSAFs can generally be calculated for field and lab studies because concentrations in sediment and biota are usually measurable. But there are problems of selecting and sampling the representative sediment particles that benthic organisms may be feeding on. BSAFs may also vary from site to site because of disequilibrium between sediment and water - this effect is especially important in laboratory and field microcosms (Servos et al. 1992) that are not at steady state and in lakes, such as Lake Ontario, where past TCDD emissions are buried and not exchanged readily with the water column. Despite these problems

BSAFs are perhaps the most consistent and well documented bioaccumulation parameter available for TCDD. Cook et al. (1990) found less than a factor of 2 variation in BASFs for pelagic fish in Lake Ontario. BASFs have also been reported for bottom feeding marine organisms (Harding and Pomeroy 1990), oligochaetes (Rubinstein et al. 1990) and for fish near pulp mills (Muir et al. 1992). BASFs for fish near 17 Canadian pulp mills were generally greater than those reported by Cook et al. (1990) (geometric mean 0.47, range, 0.14-0.96) but were nevertheless less variable than BAFs or BCFs calculated for the same sites. Site specific differences in food chains (e.g. whitefish vs suckers) and sediments (rivers vs lakes) appear to account for much of the variation in BASFs. If some of these differences could be documented it should be possible to select BASFs for various species in the paper mill/reservoir scenario and to derive tissue concentrations from TCDD concentrations estimated for sediments.

**13. Application of the Lake Ontario BAF<sup>d</sup>:** In principle this BAF (i.e.  $1.9 \times 10^6$ ) could be widely applied to estimate tissue concentrations of TCDD assuming that POC and DOC are known because  $C_w^d$  could be calculated (i.e. equation 3-6 or Fig. 3-1) from estimates of  $C_w^t$ . But Lake Ontario, an oligotrophic, cold water lake, may not be the most representative environment from which to derive BAFs for a mesotrophic, warm water, southern reservoir. Food chain relationships, species differences in age and lipid, levels of suspended solids and bed sediment characteristics (10% vs 2% OC) may simply be too different. Among the species studied by Cook et al. in Lake Ontario, white perch, had 3-fold higher BASFs (and BAFs also) than lake trout because of age differences. Prediction of bioaccumulation using BASFs or BSAFs or food chain models offers a more robust approach because  $C_w^d$  does not have to be estimated.

**14. Significance of biomagnification pathway:** BMFs for TCDD in fish and their food measured in the field and in the lab are usually between 1 and 2; far less than for PCBs of similar Kow. As noted in Sect. 3.4 this is probably due to biotransformation of TCDD by fish. But invertebrates do not seem to be able to biotransform PCDD/Fs - hence the wide range of PCDD/Fs (including non-2,3,7,8-substituted congeners) found in crab hepatopancreas (Harding and Pomeroy; Norstrom et al. 1991) and filter feeding caddisflies (Birkholtz et al. 1992). Thus BMFs at the lower end of the food chain may be >1 although data to support this are limited. If this is correct then information would have to be available on bioaccumulation by invertebrates to successfully implement a food chain bioaccumulation model, as part of the risk assessment for the pulp mill scenario. This information would could either be  $k_1$  and  $k_2$  values (rare for invertebrates) or BASFs derived from field and lab studies.

**15. Uncertainties in BAFs:** In the paper mill/reservoir scenario, TCDD concentrations in dissolved and suspended particulate phases will be calculated using chemical transport/fate models. The models will presumably give concentrations in surficial sediments assuming that sedimentation and resuspension rates are known. BAFs and/or BSAFs, or a food chain model (Fig. 4) could then be employed to

calculated tissue concentrations. As noted on p. 5-3 there are uncertainties in BAFs because  $C_w^d$  and  $C_w^t$  are based on estimates and are not verified by field studies (except by Broman et al.). Even if  $C_w^d$  could be measured in the Omigoshie Reservoir using large volume samples there would still be debate about what was truly dissolved because there are problems with all available techniques (i.e. filtration, sparging). BSAFs also have uncertainties due to site specific differences in food chains, sediment-water disequilibrium etc. But BSAFs have the advantage of being verifiable with existing techniques via sampling and analysis of biota and sediments. Using BSAFs to estimate tissue concentrations in lower food chain organisms combined with food chain modelling (application of pharmacokinetic parameters, growth rates, age and feeding preferences) to estimate concentrations in forage fish and piscivorous fish is probably the best approach for the present scenario.



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## RESPONSE TO EXERCISE 2. Stressor Characterization (questions 8-15)

8. In principle, the TCDD concentration in fish can be calculated by assuming equilibrium among particles, water, and fish. In fact, that is an extreme assumption and even having certain values for  $K_{ow}$  and  $K_{oc}$  would not make the predicted body-burdens less tenuous. I would assume that all the TCDD from the plant adsorbs onto particles passing the plant and use empirically defined ratios of body-burdens to TCDD on particles to predict the body-burdens. The need is for more empirical observations than for better physical-chemical constants.

If I had to predict body burdens with only the information in hand I would calculate the steady-state concentration of TCDD on suspended particles and, using all the attendant adjustments for lipid and TOC, apply the BSAF of 0.3 from the Interim Report. This is the upper end of the BSAF range and using it errs on the side of overestimating body-burdens. There are no empirical data for BSSAF (bioaccumulation relative to suspended solids) so I would be letting the BSAF values substitute for them. It would not be valid to calculate body-burdens relative to deposited solids because the TCDD concentration on the floor of the reservoir will not reach steady state until the upper mixed layer consists entirely of particles that entered the system after the paper mill began operating. (Prior to that settled particles will be mixing with and be diluted by preexisting particles)

9. The conceptual model has all the arrows for transfer of TCDD, but the great advantage of the equilibrium assumption is that pathways are irrelevant. Once the thermodynamic activity in one phase of the system is known, it is known everywhere else. This assumption has not been demonstrated to hold but I would still be more confident relating TCDD body-burdens in fish to TCDD on suspended solids than I would be trying to define all the partitioning and rate constants needed to apply the conceptual model.

10. At steady state the flux of dioxin into the system equals the flux out. The flux out is the discharge of TCDD-laden suspended solids through the dam plus the flux of TCDD-laden particles to the floor of the reservoir. This latter efflux does not really remove dioxin. At first it causes exponentially increasing concentration of dioxin on the sediment bed and eventually that concentration becomes constant and the deposited layer just becomes thicker. The rate of change of TCDD on the floor of the reservoir depend on rates of deposition and particle mixing by bioturbation. Steady-state for TCDD concentration will not be achieved for years.

On the other hand and ignoring resuspension of deposited particles, the TCDD concentration on suspended particles can be modeled by assuming that all the particles passing the plant achieve the same TCDD concentration (per g of organic carbon) and that *in toto* that is all the TCDD. In terms of a mass balance the aqueous and biotic TCDD can be ignored. So downstream of the plant the suspended solid TCDD is known immediately. Now we need to know how much settling occurs before the TCDD-particles mix with and are diluted by particles from

other areas. The simple calculation assumes no particle settling upstream of the reservoir and uniform settling in the reservoir. That leads quickly to a steady-state TCDD concentration on suspended particles as simply the suspended solid TCDD concentration at the plant ( i.e. mass of dioxin per second/mass of suspended solids per second) times the fraction of all suspended particles in the reservoir that come from the river with the paper mill.

The simple assumption is probably good enough because I see no alternative to assuming that all the fish in the reservoir are uniformly exposed to TCDD. If what they really have to do is swim up near the plant to get their TCDD dose, the mean exposure of the mean fish is still going to be what is calculated by assuming a uniform distribution of suspended TCDD

11. The largest difference between the reservoir scenario and coastal or estuarine scenarios is that entire populations of organisms will not be exposed to TCDD. In the reservoir, each individual fish experiences the same TCDD exposure. If there is a reproductive loss associated with that exposure the effect on the population, in subsequent years, is calculable given huge assumptions about compensatory mechanisms. In open systems, all individuals are not exposed and the population effects of TCDD will diminish in proportion to exposed versus unexposed individuals

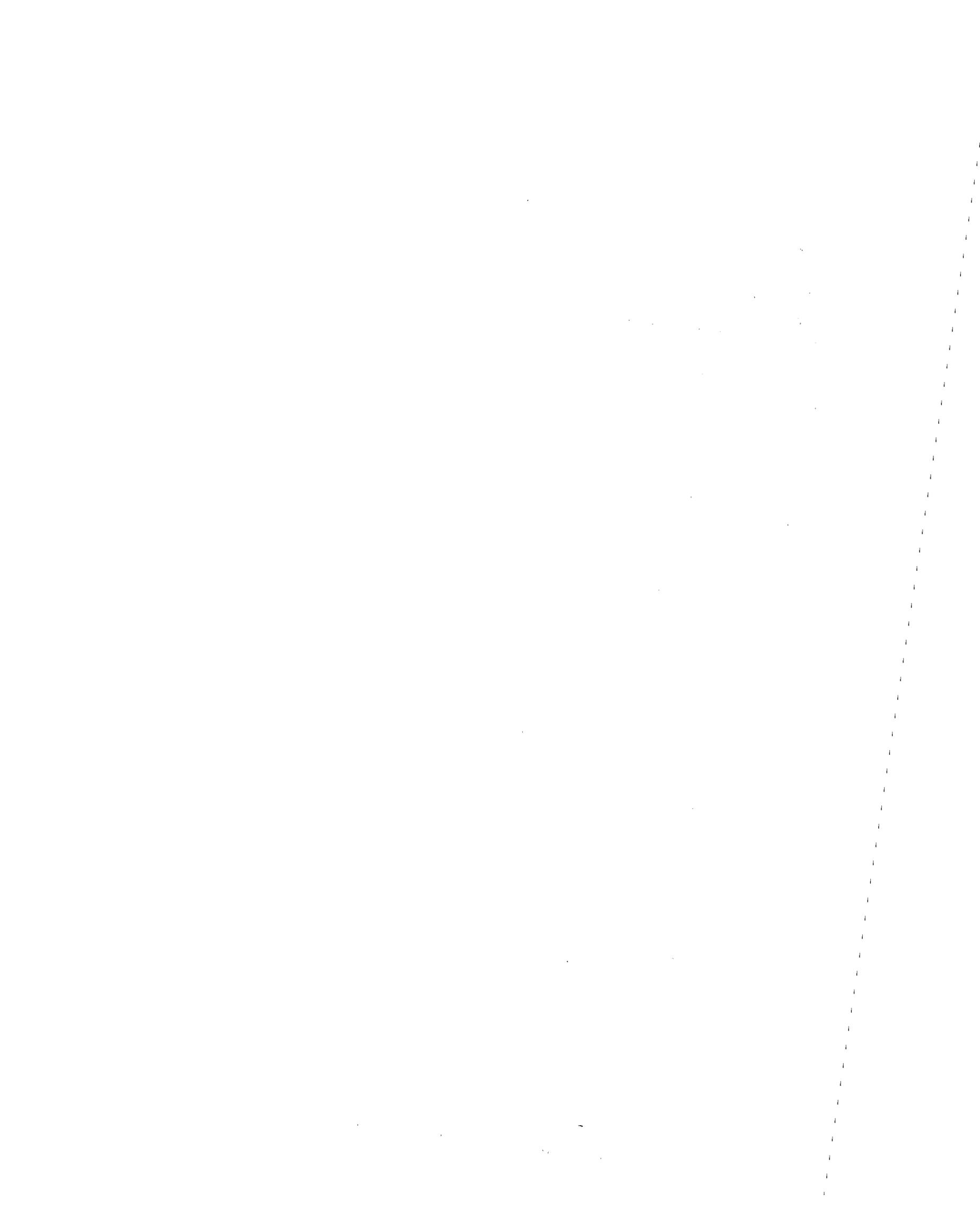
12. I think I have done this. My obvious penchant for empirical information has led me to use BSAFs. Their use requires fewer assumptions than alternative methods for guessing TCDD body-burdens.

13. Use of BAFs assumes equilibrium which is one big assumption but, to compound this excursion into the nether world,  $C_w^d$  is strictly a calculated number never blemished by empirical verification. The BAFs in the Interim Report were calculated on the basis of measured body-burdens, choices of  $K_{ow}$  and  $K_{oc}$  and assumptions of equilibrium. The BASFs in the report have the advantage of at least being based on measured concentrations in fish and in surface sediment.

14. The conceptual model seems to assume equilibrium among all aqueous phases and therefore excludes biomagnification between trophic levels. Birds are not exposed to the same abiotic phases as fish so there is no basis for assuming equilibrium and lipid-adjusted body-burdens in birds cannot be assumed equal to their counterparts in fish. Calculation of their body burdens requires kinetic data. Even without rate constants, however, empirical data on body-burdens in birds and in fish they eat are invaluable.

15. The uncertain assumptions and constants required to convert this proposed TCDD discharge to TCDD body-burdens are too large to make a worthwhile prediction. The empirical BSAFs for Lake Ontario can be used in a pinch but it would be much more preferable to spend the resources needed to get more empirical relationships on TCDD concentrations in fish, suspended sediment, and deposited sediment in the vicinity of paper mills.

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PTI Environmental Services**



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**PREWORKSHOP COMMENTS**  
**Workshop on Ecological Risk Assessment Issues**  
**for 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)**

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**INTRODUCTION**

These technical comments address issues on the assessment of ecological risks associated with chlorinated dioxins and related compounds. The comments were developed for use at the U.S. Environmental Protection Agency's Workshop on Ecological Risk Assessment Issues for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) to be held in Minneapolis, Minnesota, on September 14-15, 1993. The comments address the issues of stressor characterization and development of a conceptual model described in materials distributed prior to the workshop. The issues were based on a hypothetical case study of a pulp mill wastewater discharge into a river that enters the Omigoshsee Reservoir. The stated risk management goal is to develop final permit conditions and treatment standards that will maintain chlorinated dioxins and related compounds in discharges at concentrations below those expected to have detrimental effects on fish and wildlife of the reservoir. In developing these comments, Figure 1 was prepared to provide an overall framework for achieving the risk management goal for the Omigoshsee Reservoir scenario.

Throughout these comments, the term PCDDs will be used to refer to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its congeners. The term PCDFs will be used to refer to 2,3,7,8-tetrachlorodibenzofuran and its congeners. Other abbreviations for terms used in risk assessment models are defined in the preworkshop materials or in the *Interim*

*Report on Data and Methods for Assessment of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Risks to Aquatic Life and Associated Wildlife (U.S. EPA 1993).*

In the following discussions, each comment is preceded by the statement of the issue shown in italics. Issues are numbered as in the preworkshop materials. Because issues concerning ecological effects and endpoint selection are not addressed herein, the numbering begins with Issue 8.

## **STRESSOR CHARACTERIZATION ISSUES**

### ***Exposure Issues***

*Issue 8: The Interim Report (Sections 2.1 and 2.2) indicates that there is considerable uncertainty in the estimates of parameters including  $K_{ow}$  and  $K_{oc}$  and the partitioning of TCDD onto organic matter. This uncertainty results in part from difficulties in analytical measurements of various fractions of TCDD in water. Since these limitations may affect predictions of TCDD partitioning and exposure, please address how they should be handled in stressor characterization and conceptual model development for this scenario.*

Uncertainties in exposure assessment should be addressed through quantitative analysis of the sources of variability and error in models and their input parameters. The following procedures are recommended for uncertainty analysis:

- Use of a distributional approach (e.g., Monte Carlo) if possible
- Use of an average exposure case and a plausible maximum exposure case as an alternative to the distributional approach if the final risk models are deterministic

- Evaluation of sources of uncertainty to determine data collection and research needs (e.g., collect data for those variables important in the risk model when the high uncertainty can be substantially reduced by obtaining new data)
- Replicate measurements of key input variables, with simultaneous quantification of field and analytical variability.

The use of a distributional approach to uncertainty analysis (e.g., Monte Carlo analysis) in deriving sediment criteria and effluent discharge limits represents a state-of-the-art approach. Difficulties will likely be encountered in assigning probability distributions for all key variables in a risk model. Nevertheless, this approach is preferable to past approaches that rely on “safety factors” or conservative point estimates for each variable in the risk model. For example, distributional analysis should be used in estimating NOAELs in place of the “safety factor” approach. Use of point estimates involves more policy decisions during risk calculations than use of a distributional analysis approach. The choice of a mean and shape of the distribution for variables with high uncertainty may involve some assumptions, but no more so than the choice of conservative point estimates. For those variables where the uncertainty is high, sensitivity analysis may be used to evaluate the effect of the form of the distribution on model outcomes.

*Issue 9: The Interim Report (Section 2.4) indicates that most TCDD exposures will arise from food consumption and contact with sediments or suspended solids, with the water pathway being less important. Address the implications of this information relative to the exposure routes in the conceptual model.*

The conceptual model for exposure pathways (Figures 3 and 4 of the preworkshop materials) is relatively complete; however, there are some omissions as well as some inconsistencies. For example:

- Although the interim report acknowledges the importance of sediment ingestion as an exposure pathway for some fish species, this pathway is not included in the conceptual model. Sediment ingestion should also be included for some predatory mammals and birds.
- The conceptual model currently does not address mammals that feed on freshwater mussels. This could be a serious omission for some reservoir systems.
- The role of amphibians as food for predatory fish and birds should be considered.
- Each of the wildlife receptors may receive a portion of their diet from outside the Omigoshée Reservoir. The “other diet” pathway is indicated for only mammals in the proposed conceptual model.

Finally, the graphics for the model (Figures 2 and 3 of the preworkshop materials) could be improved by using bolder arrows to indicate the major exposure pathways of food ingestion, sediment contact, and sediment ingestion.

The development of a food web exposure model for this risk assessment could benefit from a more detailed consideration of species dietary patterns and life histories. An example of a procedure to guide development of the trophic model is shown in Figure 2. It should be emphasized that “trophic species” need to be considered, not taxonomic species.

*Issue 10: Fate and transport models are beyond the scope of the Interim Report, but are clearly critical for risk assessment. In stressor characterization and the conceptual model, they will be necessary for linking TCDD source loads to concentrations in different compartments of the reservoir.*

- *Comment on the availability of fate and transport data/models suitable for use with TCDD.*
- *Discuss the applicability of available transport models for predicting the deposition of particulate-bound TCDD in the reservoir.*

Fate and transport models for chlorinated dioxins and related compounds have been reviewed by U.S. EPA (1992). Based on their review and work by the U.S. Army Corp of Engineers for modeling suspended solids and sediments in reservoirs, available models should be adequate for linking the source load to compartments in the reservoir system.

*Issue 11: List some of the major exposure issues not present in the paper mill scenario that may be encountered in future ecological risk assessments (e.g., marine/estuarine, terrestrial, etc.).*

Major issues that may be encountered in other risk assessment scenarios include:

- Model assumptions or algorithms needed to address sources other than the one for which a permit is being developed. For example, regional background concentrations, cumulative impacts, and wasteload allocation may need to be addressed. Also, different sources of PCDDs and PCDFs will result in different mixtures of congeners, which may have slightly different requirements for model development.
- Effects of PCDDs and PCDFs on other species groups for which few data are available. For example, other assessments may need to address marine mammals (marine/estuarine systems) or soil biota (terrestrial systems).

- Different sediment types that may be difficult to model (e.g., very low or very high organic carbon content) or impractical to sample (e.g., flocculent sediments).
- Mixing zones in marine and estuarine systems.
- High spatial heterogeneity in terrestrial systems.
- Confounding effects of complex mixtures (e.g., presence of petroleum hydrocarbons or metals).

### ***Bioaccumulation Issues***

*Issue 12: The Interim Report (Sections 3.2–3.5) summarizes available data on TCDD bioconcentration, bioaccumulation, biomagnification, and biota/sediment accumulation factors from laboratory experiments and field measurements. Discuss the applicability of these factors to stressor characterization for the paper mill scenario.*

The information on BCFs, BAFs, BMFs, and BSAFs for 2,3,7,8-TCDD summarized in the interim report is useful for the general stressor characterization and exposure assessment. For the stated risk management objective related to development of permit specifications, however, the empirically derived BSAFs may be the primary factors of interest for estimating bioaccumulation (see comments on Issue 17). BCFs will likely be less useful than the other factors because of the importance of food-chain accumulation of PCDDs and PCDFs. Evaluation of BAFs and BMFs may aid in finalizing a list of receptors.

*Issue 13: The Lake Ontario BAF<sub>1</sub><sup>d</sup> may be useful as a predictor of residue levels in other systems if C<sub>w</sub><sup>d</sup> can be estimated accurately (Interim Report, Section 3.3). Comment on the applicability of this BAF for the paper mill stressor characterization.*

The immediate use of BAFs for PCDDs and PCDFs is questionable because of the problem of quantifying the concentration in lake water (C<sub>w</sub><sup>d</sup>). Aside from this potential problem, the use of the Lake Ontario trout BAF is tenuous for the following reasons:

- The Lake Ontario trout BAF was estimated from a model that has not been validated
- The Lake Ontario trout BAF was estimated for lake trout, which is not a receptor in the reservoir
- The configuration of the reservoir may lead to significant spatial heterogeneities in sediment concentrations of PCDDs and PCDFs that are not reflected in the concentrations in the water column. Because the sediment exposure route is important, any disequilibrium between sediment and water would invalidate the use of a BAF.

*Issue 14: The Interim Report (Section 3.4) indicates that biomagnification is significant between fish and fish-eating birds but not between fish and their food. Comment on the biomagnification pathway relative to stressor characterization and the conceptual model.*

The biomagnification pathway is especially important for any risk assessment for hydrophobic chemicals such as TCDD and related compounds. The conceptual model should therefore address biomagnification for key receptors representative of all groups of higher trophic level species (e.g., bass, mink, heron). It should not be concluded that biomagnification is not significant between fish and their food items based on the available data for several reasons. First, the available data are limited in coverage of

species, selected TCDD and TCDF congeners, and specific exposure conditions (e.g., kinds of compounds, duration and magnitude of exposure). The interim report acknowledged that the apparent low biomagnification factor for 2,3,7,8-TCDD in fish could be due to biotransformation; however, the metabolic products will retain some residual potency, which may be significant when passed to the next step of a food chain. Second, the significance of biomagnification will vary substantially among fish species, depending on their trophic position. Third, the significance of biomagnification at a given step in a food chain can only be judged relative to the length and species composition of the entire chain.

The description of this issue did not mention fish-eating mammals, but they are included in the conceptual model (e.g., Figure 3 of the preworkshop materials). Biomagnification could also be important in the link between freshwater mussels and mammals, such as mink and raccoon. Biomagnification could play an important role in the exposure of all of these trophic groups to PCDDs and PCDFs.

Because of the importance of biomagnification, more information on the trophic structure of the reservoir food web would have to be developed for this risk assessment. Characteristics of trophic compartments that are most susceptible to high TCDD exposures need to be more fully defined. Then, the list of key receptors can be finalized, including identifying life stages, age classes, and ecotypes of most concern.

*Issue 15: Uncertainties associated with bioaccumulation factors are discussed in Section 5.1.2 of the Interim Report. Discuss the relevance of these uncertainties to the prediction of TCDD residues in Omigoshée Reservoir biota.*

The uncertainties in BAFs appear to be greater than those in BSAFs. The issues of analytical difficulties in quantifying  $C_w^d$ , relative bioavailability in laboratory and field systems, and interspecies extrapolation of the Lake Ontario trout BAF would be responsible for substantial uncertainties in the use of BAFs for the reservoir.

Section 4

**EXERCISE 3**

***Conceptual Model Development***

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Comments on Exercise 3. Conceptual Model

16. Comment on whether the focus on fish and wildlife captures the full range of potential ecological effects for this scenario.

Comment

It probably does not capture the full range but probably captures the most important ones from a risk assessment and risk management standpoint. Obviously, there could be effects on the benthic invertebrates, plankton, and aquatic plants. However, limited data suggest that these are less sensitive receptors. It would probably be useful to have a thorough discussion of this. It would also be useful to review available field data on the presence of benthic invertebrates and other organisms in TCDD contaminated sediments. I think there is probably a considerable amount of field data that could have been relied upon in the Interim Report to consider if TCDD contaminated sediments or water have affected benthic and planktonic plants and invertebrates.

17. Discuss the utility of available risk assessment tools for accomplishing the goal of linking tissue residues to loadings.

Comment

I do not think this falls into the category of "risk assessment tools" per se. I think the best approach is to: 1) estimate surface sediment concentrations of TCDD et al. in different areas of the reservoir associated with steady state loadings from the paper mill; this would be done using an appropriate model or models; and, 2) relate these sediment levels to fish body burdens using a best estimate and range of BSAF values. I think it would be helpful to examine other similar systems (paper mills on reservoirs or slow flowing rivers) that exist to develop a more robust set of estimates for BSAFs. It may also be useful to derive a BSAF for the complex mixture of dioxins/furans in the effluent (from a similar plant or pilot plant) in an experiment bioaccumulation bioassay. This would involve exposing representative fish species (probably adults) to sediments which have been exposed to the complex effluent at various concentrations.

18. Discuss the applicability of the limited field data available for estimating BAFs and BSAFs.

**Comment**

Sediment, water, and food chain models are all subject to considerable uncertainty. I think the use of BSAFs provides a simple model which probably has less uncertainty than models that attempt to represent the complexities of interactions. I think these simple models are applicable and perhaps more desirable than the more complex approaches. Application of these simple models should be accompanied by a sensitivity analysis. It would be useful to compare the characteristics of the sediments and water column in the reservoir to those sites for which BAF and BSAF values have already been developed. Key pieces of information include: suspended sediment, DOC and POC for the water column and TOC and grain size for the sediments. In the case of the sediments, information on the benthic biota would be helpful for comparing the degree to which the sediments may differ in bioturbation rates.

19. Comment on how temporal dynamics and disequilibrium situations should be considered in establishing (1) the time course for build-up and/or decrease of TCDD levels.

**Comment**

To some degree, the relevant time course depends on the area under consideration (i.e., localized areas around the mill, the trunk of the reservoir near the mill, the reservoir as a whole). The time course for build-up and decrease will differ for these different spatial scales. For each spatial scale, the time course selected for build-up should be based on steady state conditions at which surface sediments are no longer significantly increasing. A temporal constraint on this analysis is the operational lifespan of the facility. It may be useful to consider conditions after several

selected time periods: e.g., 1, 5, 10, and 20 years. I agree with the Interim Report with regard to using long averaging times for estimating body burdens in response to exposure conditions. The report suggests 1 year. However, the location and exposure of species could vary seasonally within the reservoir and if there are pronounced spatial gradients in exposure concentrations, such seasonal movements should be taken into account.

20. **Additional Comment**

Exposure and possible risks of TCDD to fish, birds, and mammals in and around the reservoir can be evaluated at various spatial scales. At the localized level, there might be effects on individuals but if these are limited to a small area, they may not translate to population-level risks. Because TCDD will not be homogeneously distributed throughout the reservoir, it may be useful to estimate the fraction(s) of the reservoir habitat (aquatic and terrestrial) that fall within various risk levels. For species with limited home range, this would provide an initial estimate of the fraction of the "local population" that is at risk. This approach would require estimates of the distribution of TCDD throughout the reservoir, identification of habitats within and along the reservoir that would be utilized by selected receptor fish, bird, and mammal species, information on the general biology of these species with regard to foraging range and how this range might vary during the year. A technical and philosophical issue that arises with this approach is the definition of what constitutes the "local population".



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### EXERCISE 3. CONCEPTUAL MODEL DEVELOPMENT

Comments were invited, not required, on this Exercise. Given the time constraints, I have provided only brief comments to the questions.

I preface these comments by noting that risk assessment is not science, it is simply a technique by which scientific information can be converted into regulatory action. Mathematical calculations result in quantitative risk assessment, which provides an appearance of rigour, since numbers are involved, but which is not science. Rather, this is best described as "trans-science" (Weinberg, 1972), whose primary function is to attempt to bring order and consistency out of the chaos resulting from public and other pressures to act, often based on perception rather than reality. Risk assessment is basically "...an instrument of social compromise, providing numerical answers in the face of vast scientific uncertainties" (Gots, 1992).

Conceptual models are an extremely useful way to delimit concerns and provide testable hypotheses for scientific study. In this regard, bioassays (and other environmental assessment tools) can provide information regarding three different scenarios: cases where there is clearly an effect, cases where there is clearly not an effect, and the intermediate (and, unfortunately, predominant) case where there may or may not be an effect. There are four possible responses when "worst case" testing indicates a potential problem: (1) if clearly required, control/regulate; (2) if the results are unclear and a delay will not be catastrophic, test and verify; (3) if the issue has low priority compared to other problems, put available resources to dealing with the highest priority problems, and deal with this issue when and if it is appropriate to do so; (4) if there is no environmental problem, take no action.

#### Issue 16.      Is the Approach Broad Enough?

This approach does not (and cannot, since we do not know everything there is to know) necessarily capture the full range of potential ecological effects. However, based on what we do know, it is a reasonable attempt to ensure environmental protection by focusing on the "worst case", i.e., fish and wildlife that consume fish. Worst case exposures would, from the model, appear to be in the main arm of the reservoir.

Issue 17.            Utility of Available Risk Assessment Tools

General comments on risk assessments are provided above. Comments on the incompatibility of tissue residue levels and adverse effects have been provided previously. I am not sure what is actually being asked here.

Issue 18.            Comments on the Omigoshsee Reservoir Conceptual Model

Again, I am not sure what is being asked. The model appears to be reasonable conceptually, and as a basis for assessing the system. But, clearly, data need to be collected. For example, baseline data on concentrations of bioaccumulative organic chemicals, which are noted as missing, are required. Such and other information would, I would expect, be collected as part of the EIA.

Issue 19.            Consideration of Temporal Dynamics and Disequilibrium

Presumably what is being asked here relates to either or both of: (1) predictions as to "worst case" accumulation and purging of TCDD from the system, and (2) the timing of environmental monitoring should the mill proceed. Predictions are only as good as the data they are based on, and in this case additional site- and situation-specific data are needed. In particular, some experimentation is required, in the laboratory, using plant bench-scale processes to determine rates of accumulation of TCDD in sediments and biota. As regards timing of monitoring, this should be based on what is known (e.g., present data, the EIA, these experimental results), and should initially be fairly conservative (e.g., frequent, "worst case" philosophy), then amended depending on what is found.

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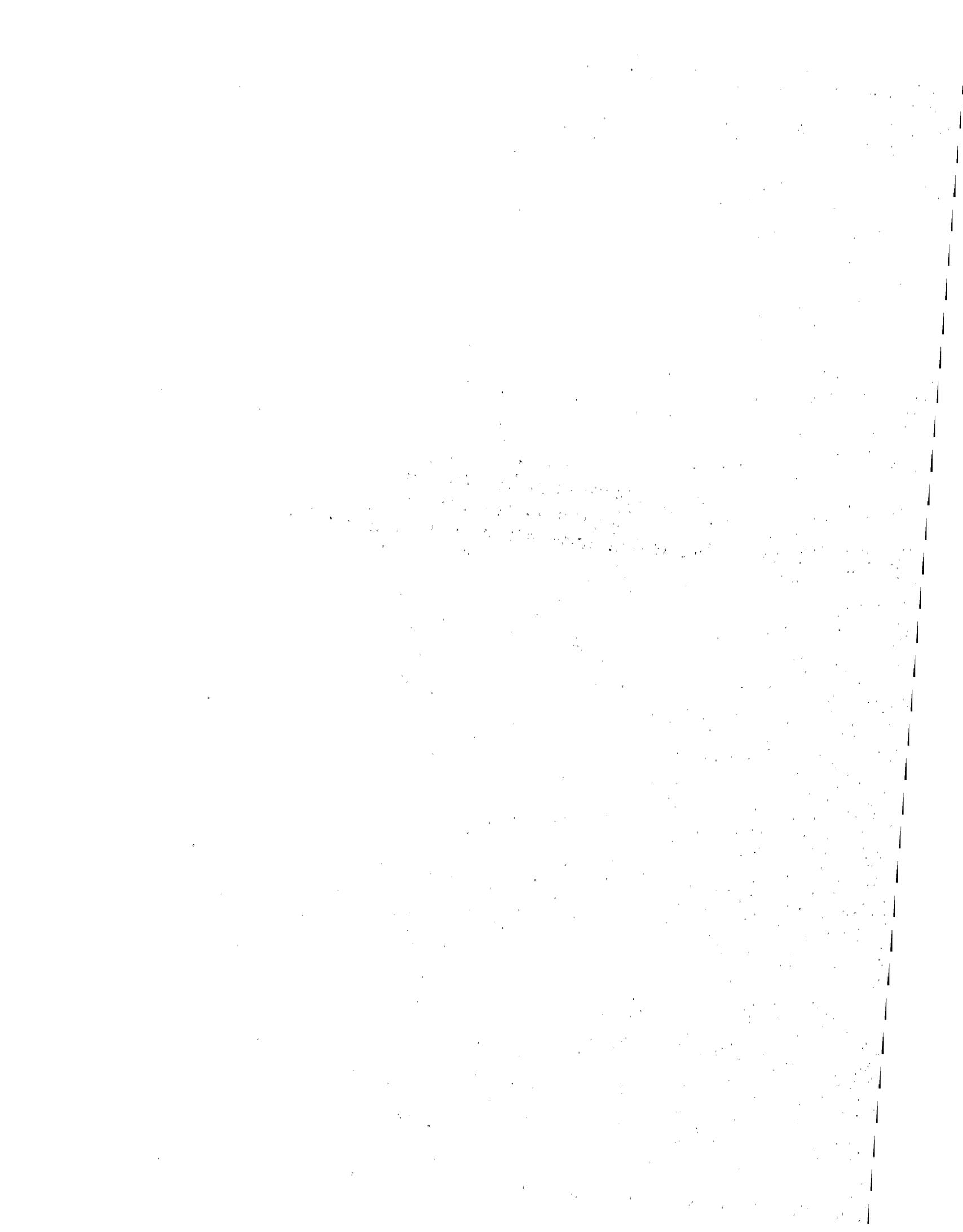


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## CONCEPTUAL MODEL

16. Given that fish and fish-eating birds and wildlife are at the top of the food chain in the area of this reservoir, and these are among the most sensitive of those organisms that have been evaluated to date, at this time the suggested approach is probably the most reasonable. However, because we do not fully understand the wide range of potential effects of TCDD and TCDD-like compounds (bacteria, primary producers, for example), it is not reasonable to assume that the full range of ecological functions will be protected by protecting fish and wildlife. The degree of protection for the non-target components of the aquatic and terrestrial communities will only be understood by long-term monitoring programs after the mill is in operation.
17. I feel that we have the necessary tools to accomplish this objective. We either know (or we can determine) the lipid concentrations in the fish and wildlife species to be protected, and we can determine the organic carbon content of the sediments in the reservoir. With this information, an acceptable TCDD effluent discharge calculation can be made for NPDES permitting purposes.
18. Although there are only limited existing field data that are available for estimating BAFs and BSAFs, it is probably true that the utility of these approaches is controlled to a large degree by the accuracy of the sediment organic carbon measurements and the lipid determinations. I feel that as long as these measurements are accurately performed for the reservoir, BAF and BSAF approaches can be utilized for the conceptual model.
19. Because of the rather constant TCDD discharge anticipated in the mill effluent, it seems reasonable to expect that the water, sediments, and tissues will reach steady-state after a period of time; that period of time should be predictable based upon the volume of effluent being discharged per day, the volume of the reservoir, the organic carbon content of the sediments, and the lipid concentrations in the fish. Similarly, when the source of TCDD has been eliminated, the decreases in water, sediment, and tissue TCDD concentrations are predictable based upon the reservoir flushing rate, the sediment/water equilibrium kinetics, and the TCDD tissue depuration rate(s).



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**Exercise 3. Conceptual Model**

16. *Consistent with the Interim Report, the conceptual model focuses on effects on fish and wildlife that consume fish. Comment on whether this approach captures the full range of potential ecological effects for this scenario.*

**Comment**

Of course not, as delineated in many of my previous comments. The conceptual model treats community structure and function as a risk and not as a hazard. In fact, given any alteration at the population level, a subsequent alteration in the structure of the ecosystem occurs, with indirect effects likely. Perhaps the risk should be stated as the probability that the alterations in structure and function of the community exceed that able to support game fish and associated wildlife.

Given the lack of data on the alterations in community structure due to TCDD and related compounds, the fact that invertebrates are reported to not degrade these compounds, the lack of data on invertebrate toxicity, and the lack of information of synergisms and antagonisms with other compounds, a wide range of effects are likely to occur given the southern lake scenario. Pesticides and herbicides are likely to occur in small amounts, dioxin and related chlorinated aromatics are likely to occur in small amounts due to forest burning and other sources. All of these may facilitate impacts at other structural levels within the ecosystem.

Another factor in the assessment of the overall impact of the proposed mill is its location. Apparently the TCDD will have the opportunity to dose most of the lake, potentially restricting migration from population sources to area affected by the dioxin. The location will probably accent the impact due to the release of the toxicant.

I also have major concerns with the Assessment Endpoints and the Measurement Endpoints.

1) Assessment Endpoints concentrate on the productivity of the bass, catfish, crappie and bluegill. Driving the system to maximize productivity of each of these species will likely cause important alterations to the remainder of the system. How are normal population cycles incorporated into the assessment endpoint description? I would redefine the Assessment Endpoints as design parameters using the habitat and resource requirements of these species as the design set. Such a set would incorporate resource availability, habitat, and predator-prey relationships. Of course, productivity needs to be clearly defined. If one species declines, others may temporarily increase, altering the top down control on the system. As the structure changes other resource populations are likely to change, eventually altering long term the fish assemblage of the lake.

2) Measurement Endpoints are defined as the effects of TCDD on the reproductive success as defined as egg production and larval survival. Measured or estimated concentrations in tissues are taken as the best means of estimating these effects. In both instances there are

large uncertainties related with both measurement endpoints. Perhaps a more in-depth look at the lake, assessing the structure and energy flow may be more appropriate. Given the dose response curves of dioxins and related materials and the variability in tissue concentration reported in the Interim Report, by the time tissue concentrations have accumulated and are measured in some individuals, large parts of the affected population have ceased to reproduce. More sensitive measures that can provide some warning may be more appropriate. Binding of the Ah receptor, behavioral measurements, or alterations to structural components of the community may provide better measurement endpoints.

Finally, in regards to the lake system, how much data are really available for this ecosystem. From the initial conceptual framework, apparently little is known about the current inputs of toxicants, population dynamics of the fish and invertebrates, and the historical ranges of these factors. As Katz et al (1987) have recently demonstrated, the past history of a lake is the best predictor of the future. In fact, for most species other lacks are particularly bad at predicting future dynamics even when the lakes are as similar as those in the Wisconsin lakes district. You might never know, but an in-depth understanding of the current status and trends within the lake may change dramatically the assessment and measurement endpoints.

*17. The Interim Report emphasizes using tissue residue levels to estimate the adverse effects of TCDD. However, to do the risk assessment outlined by the conceptual model, it will be necessary to link predicted loadings of TCDD in the paper mill effluent to residues in the organisms identified in the assessment endpoints. Discuss the utility of available risk assessment tools for accomplishing this goal.*

**Comment**

There are various models, such as FIGETS and EXAMS that calculate transport and bioaccumulation. These and other simulation models may prove very useful but should be looked upon with caution. Often the virtual can be looked upon as reality and the system an expert because of the convenience and lack of validation. Roofs have collapsed and aircraft crashed because of overlooked aspects in simulation modeling. Experimental determinations would likely improve the predictability of the models and serve as validation.

Toxicokinetics models may be very useful, especially those developed for anesthetics and other lipid soluble materials.

*18. The Interim Report describes the limited field data that are available for estimating BAFs and BSAFs. Discuss the applicability of these factors to the Omigoshess Reservoir conceptual model.*

**Comment**

These BAFs and BSAFs are probably the best shots for estimating eventual tissue concentrations. As currently designed, however, the lake is assumed to be rather homogenous, and it is not. Without bathymetry data it is hard to judge, but it is unlikely that the accumulation of TCDDs will be similar in the four principal basins. Basins 2 and 3 are likely to be shallow, with a great deal of input from the surrounding watershed. Sediment loading is reported to be high with a great deal of shoreline complexity. Of course, the complexity of the shorelines make this an excellent habitat for sport fish and wildlife. The complexity of the system means that dioxin concentrations are likely to be spatially and temporally heterogeneous. Variation in flow rates from the various tributaries will also alter the relative TCDD inputs.

I would suggest strongly a adoption of these models with a breakdown into smaller sections to get a feel for the heterogeneity of the bioaccumulation over space and with different flow and input rates. From these data a probability distribution may be derived to more accurately describe these factors for lake.

*19. The temporal dynamics and the disequilibrium situations commonly associated with TCDD are mentioned in the Interim Report (section 2.3). Comment on how these aspects should be considered in establishing (1) the time course for the build up of TCDD levels following initiation of the paper mill discharge and (2) the time course for the decrease of TCDD levels and recovery of biota should the paper mill cease operation.*

**Comment**

Temporal dynamics and disequilibria determine the structure and resulting function of ecosystems, they are often the stuff of dramatic evolutionary changes, including speciation. Given the toxicity, long half life and the characteristics of the lake, the time courses for TCDD buildup and subsequent decrease will complex. The mean trend will likely be well behaved, but the numbers surrounding the increase and decline could appear stochastic.

In all likelihood, the dynamics will be typical of non-linear systems. Good examples are trends in weather and in disease. Both exhibit yearly vagaries that are essentially bounded chaotic systems. In the case of disease, the application of a vaccination program may not show a decreasing trend in the prevalence of the disease because of the chaotic dynamics, temporally and spatially. Outbreaks of the disease may still occur, not because of the lack of efficacy of the vaccine, but because of the intrinsic mathematics of the spread of the agent. The act vaccination actually puts new bounds of the chaotic dynamics, resulting in an overall decreasing trend. Persistent materials, coupled with a heterogeneous habitat are also likely to exhibit similar dynamics as the pulp mill effluent is eliminated.

In contrast, the addition of TCDD will be in two steps, first the contamination of the media, followed by the bioaccumulation. The first will be more straightforward, rather simple input-output

with variability due to the morphology of the lake. The tissue concentrations will more likely follow the bounded chaotic dynamics since tissue concentrations depend heavily upon prey population productivities, algal dynamics and meteorological inputs.

Lastly, decline in the concentration of TCDD in the fish populations does not mean a return to the original conditions found in the lake. In fact the contrary is probably likely. I now believe that recovery to the original state of an ecosystem is not possible. As in the terminology of the theory of complex systems, ecosystems including their constituent populations retain a "memory" past events (Nicolis and Prigogine 1989). Because of this memory complex systems are irreversible, the opposite of classical Newtonian mechanics. This is not to say that viable fish populations will not occur, but the structure of the populations and the structure of the supporting ecosystem will be different. The importance of this in the long term is that the response of the system to additional stressor events will likely be quite different because of the history of TCDD contamination. Even another TCDD event will likely have different outcomes. **The almost automatic assumption that stability exists and some sort of recovery to an original state is unwarranted and perhaps dangerous in that it may lead to an underestimation of long-term effects.**

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### **Appendix 1: Examples of Test Systems for the Examination of Impacts at the Community Level**

#### *Effects of Atrazine on Freshwater Microbial Communities*

<b>Organisms</b>	Multiple species
Test community collection:	On polyurethane foam (PF) substrates (6.0 x 5.0 x 4.0 cm)
PF substrate exposure:	14 days
<b>Experimental design</b>	
Test type:	Multispecies toxicity test
Test vessel size and type:	High density polyethylene tubs (35 x 28 x 15 cm) with an inlet tube to deliver a mixture of diluent water and toxicant at one end and three drain holes at the other end
Test volume:	7 L
Toxicant stocks:	Primary stock was made by dissolving 200.84 mg of atrazine in 100 mL of methanol and
diluting	n distilled water to 1 L

	Secondary stocks were made by diluting the appropriate amount of primary stock with diluent water
	Both stocks were made once at the start of the test and again on day 10
Number of replicates:	Triplicates of five concentrations of atrazine, a diluent control, and a solvent (methanol) control (total of 21 chambers)
Toxicant concentrations:	3, 10, 30, 100, and 300 µg/L (measured at the start of the test and after 10 and 21 days)
Test duration:	21 days
<b>Physical and chemical parameters</b>	
Temperature:	Uncontrolled; ranged from 13.5°C to 15.0°C
Light Intensity:	5000 lux (bulbs located 30 cm above the test systems)
Photoperiod:	16 h light / 8 h dark
Type of dilution water:	Tap water dechlorinated by passage through activated charcoal
Hardness:	70 ppm CaCO <sub>3</sub>
Alkalinity:	45 ppm CaCO <sub>3</sub>
pH:	8.4
Flow rate:	Approximately 7 turnovers of the 7
Clinical Examinations/Endpoints:	Species richness, total biomass, protein concentrations, chlorophyll <i>a</i> concentration

Source: Pratt, J.R., N.J. Bowers, B.R. Niederlehner and J. Cairns, Jr. 1988. Effects of Atrazine on Freshwater Microbial Communities. *Archives of Environmental Contamination and Toxicology* 17:449-457.

*Summary of Test Conditions for the Outdoor Aquatic Microcosm Tests to Support Pesticide Registrations*

<b>Organisms</b>	Bluegill sunfish ( <i>Lepomis macrochirus</i> ), fathead minnow ( <i>Pimephales promelas</i> ), channel catfish ( <i>Ictalurus punctatus</i> ), or others may be present: Phytoplankton, periphyton, zooplankton, emergent insects, and benthic macroinvertebrates
Size of organism:	Biomass of fish added to the microcosms should not exceed 2 grams per cubic meter of water
<b>Experimental design</b>	
Test type:	Microcosm

Test vessel size and type:	Tanks with a surface area of at least 5 m <sup>2</sup> , a depth of at least 1.25 m, and a volume of at least 6 m <sup>3</sup> made of fiberglass or some other inert material; smaller tanks could be used for special purposes in studies without fish
Addition of test material:	Allow microcosms to age for approximately 6 to 8 weeks before adding test material Apply by spraying across water surface, apply the test material in a soil/water slurry, or apply test material in a water based stock solution
Sampling:	Begins approximately two weeks after the microcosms are constructed and continues for two or three months after the last treatment with test material; frequency depends characteristics of test substance and on treatment regime Dosage levels, frequency of test material addition, and number of replicates per dosage level are determined based on the objectives of the study
<b>Physical and chemical parameters</b>	
Temperature:	Maintained by partially burying tanks in the ground or immersing in a flat-bottomed pond
Sediment:	Obtained from existing pond, containing a natural benthic community is added to each microcosm directly on the bottom, in trays or other containers; sediment should be 5 cm thick
Water:	Obtained from healthy, ecologically active pond; Water level should be set in the beginning and not allowed to vary more than $\pm 10\%$ throughout study; if water level falls more than 10%, add pond water, fresh well water, or rain water; if water level rises more than 10 %, surplus should be released and retained
Meteorology:	Should be recorded at the study site or records obtained from a nearby weather station; data should include air temperature, solar radiation, precipitation, wind speed and direction, and relative humidity or evaporation

*Photosynthetic Carbon Metabolism of Size-Fractionated Phytoplankton during an Experimental Bloom in Marine Microcosms*

<b>Organisms</b>	Phytoplankton
<b>Experimental design</b>	
Test type:	Marine microcosm
Test vessel size and type:	12 L acid-washed polycarbonate bottles
Size and number of replicates	

per sample:	60 mL subsamples removed 4 times during the experiment to measure <sup>14</sup> C incorporation into macromolecules
Number of test organisms per chamber:	Phytoplankton samples are fixed in Lugol's iodine then identified and counted under an inverted light microscope
Test duration:	9 days
Clinical examinations:	Concentrations determined: Nitrate, nitrite, soluble reactive phosphorus (SRP); determined four times during the experiment. Samples for total dissolved phosphorus (TP) and total dissolved nitrogen (TN) are maintained deep-frozen and subsequently analyzed after UV-induced oxidation. Size fractionated chlorophyll <i>a</i> is measured twice a day

### Physical and chemical parameters

Temperature:	15°C
Light intensity:	Photon flux density (PFD) of 100 μE/m <sup>2</sup> s from north-light fluorescent tubes
Photoperiod:	14 h light /10 h dark
Endpoint:	Growth

Source: de Madariaga, I. and E. Fernandez. 1990. Photosynthetic Carbon Metabolism of Size-Fractionated Phytoplankton During an Experimental Bloom in Marine Microcosms. *J. Mar. Biol. Ass. U.K.* 70:531-543.

### *Summary Of Test Conditions for the Standardized Aquatic Microcosm: Fresh Water*

#### Organisms

Type and number of test organisms per chamber:

Algae (added on Day 0 at initial concentration of 10<sup>3</sup> cells for each algae species):  
*Anabaena cylindrica*,  
*Ankistrodesmus* sp.,  
*Chlamydomonas reinhardi* 90,  
*Chlorella vulgaris*,  
*Lyngbya* sp.,  
*Nitzschia kutzigiana* (Diatom 216),  
*Scenedesmus obliquus*,  
*Selenastrum capricornutum*,  
*Stigeoclonium* sp., and  
*Ulothrix* sp.

Animals (added on Day 4 at the initial numbers indicated in parentheses):  
*Daphnia magna* (16/microcosm),  
*Hyalella azteca* (12/microcosm),  
*Cypridopsis* sp. or *Cyprinotus* sp.  
(ostracod) (6/microcosm),

Hypotrichs [protozoa] (0.1/mL) (optional),  
and *Philodina* sp. (rotifer) (0.03/mL)

### Experimental design

Test type:	Multi-species
Test vessel type and size:	One-gallon (3.8 L) glass jars are recommended soft glass is satisfactory if new containers are used; measurements should be 16.0 cm wide at the shoulder, 25 cm tall with 10.6 cm openings.
Medium volume:	500 mL added to each container
Number of replicates:	6
Number of concentrations:	4
Reinoculation:	Once per week add one drop (circa 0.05 mL) to each microcosm from a mix of the ten species = $5 \times 10^2$ cells of each alga added per microcosm
Addition of test materials:	Add material on Day 7; test material may be added biweekly or weekly after sampling
Sampling frequency:	2 times each week until end of test
Test duration:	63 days
<b>Physical and chemical parameters</b>	
Temperature:	Incubator or temperature controlled room is required providing an environment 20 to 25°C with minimal dimensions of 2.6 by 0.85 by 0.8 m high
Work surface:	Table at least 2.6 by 0.85 m and having a white or light colored top or covering
Light quality:	Warm white light
Light intensity:	80 $\mu\text{E m}^{-2}$ photosynthetically active radiation $\text{s}^{-1}$ (850 to 1000 fc)
Photoperiod:	12 h light / 12 h dark
Microcosm medium:	Medium T82MV
Sediment:	Composed of silica sand (200 g), ground, crude chitin (0.5g), and cellulose powder (0.5 g) added to each container.
pH level:	Adjust to pH 7

Endpoint: DO, pH, enumeration of species, diversity, P/R ratio, nutrients, available algae, total algae, total Daphnia etc.

ASTM E 1366-91 (1991) Standard Practice for the standardized aquatic microcosm: fresh water, Vol 11.04. pp 1017-1051. American Society for Testing and Materials, Philadelphia.

Taub, F.B. (1989) Standardized aquatic microcosms. *Environm. Sci. Technol.* **23**, 1064-1066.

**Richard Peterson  
School of Pharmacy  
University of Wisconsin**

## QUESTION 17

1. The following table shows the number of employees in each of the departments of a company. The company is considering a restructuring plan that will involve the following changes:

Department	Current Number of Employees	Proposed Number of Employees
Department A	120	100
Department B	150	130
Department C	180	160
Department D	200	180
Department E	220	200

2. The company is also considering a restructuring plan that will involve the following changes:

Department	Current Number of Employees	Proposed Number of Employees
Department A	120	100
Department B	150	130
Department C	180	160
Department D	200	180
Department E	220	200

3. The company is also considering a restructuring plan that will involve the following changes:

Department	Current Number of Employees	Proposed Number of Employees
Department A	120	100
Department B	150	130
Department C	180	160
Department D	200	180
Department E	220	200

4. The company is also considering a restructuring plan that will involve the following changes:

Department	Current Number of Employees	Proposed Number of Employees
Department A	120	100
Department B	150	130
Department C	180	160
Department D	200	180
Department E	220	200

5. The company is also considering a restructuring plan that will involve the following changes:

Department	Current Number of Employees	Proposed Number of Employees
Department A	120	100
Department B	150	130
Department C	180	160
Department D	200	180
Department E	220	200

**PRE-MEETING COMMENTS: RICHARD E. PETERSON**

**EXERCISE 3. CONCEPTUAL MODEL**

16. If TCDD-like congeners reduce the populations of fish upon which birds and mammals forage in the reservoir it may indirectly affect their populations. It is not clear how such an indirect effect of TCDD on bird and mammal populations will be estimated in the conceptual model.

It is possible that the most sensitive fish species in the reservoir to TCDD-induced reproductive toxicity or early life stage mortality is a forage fish such as a species of minnow. The conceptual model does not consider this possibility from either a direct effect (TCDD decreasing the population of minnows) or indirect effect (decrease in the minnow population decreasing the population of piscivorous fish) point of view.

For river otters the main source of TCDD-contaminated food from the reservoir may be crayfish rather than fish. However, the conceptual model does not consider this possibility in assessing the exposure of river otters to these chemical contaminants.



Thomas Sibley  
Fisheries Research Institute  
University of Washington



Pre-meeting Comments for U.S. Environmental  
Protection Agency

Workshop on Ecological Risk Assessment Issues for  
2,3,7,8-Tetrachlorodibenzo-p-Dioxin  
14-15 September 1993

Thomas H. Sibley  
Fisheries Research Institute (WH-10)  
University of Washington  
Seattle, WA 98195

Exercise 3. Conceptual Model

16. No, focusing on fish and wildlife that consume fish will not capture the full range of potential ecological effects. However, it is the most reasonable approach given the available data and should identify those species that will be affected first.

17. I think the available tools are more advanced than the data. The risk is to believe the results of the model and forget the questionable nature of the data that are input for the model.

18. I think it is necessary to use these factors because analytically they are among the more reliable measurements. However, the appropriate values will depend upon the organic content of the sediments and the biological species being considered. It would be more desirable to make predictions based upon measured concentrations of dissolved TCDD but that is not feasible for most environments.

19. There are limited data available for these assessments and most of those data assume equilibrium. It is likely that few, if any, systems are at equilibrium but the necessary reaction rates to consider kinetic reactions have not been obtained yet. In my opinion, attempting to introduce the kinetics would introduce so much uncertainty that it is better to utilize the equilibrium models.



**Bill Williams**  
**Ecological Planning Toxicology, Inc.**



### Exercise 3. Conceptual Model

#### Issues for Consideration

##### 16. Fish-eating birds and mammals-

Consistent with the Interim Report, the conceptual model focuses on effects on fish and wildlife that consume fish. Comment on the whether this approach captures the full range of potential ecological effects for this scenario.

##### Comment:

The point source of TCDD and other chemicals in the example is manifest in the water column and sediment, ultimately finding its way up the food chain to the fish and predators of fish. This is a good approximation of the potential exposure and effects of these chemicals.

##### 17. Linkage of environmental concentration, residues and effects-

The Interim Report emphasizes using tissue residue levels to estimate the adverse effects of TCDD. However, to do the risk assessment outlined by the conceptual model, it will be necessary to link predicted loading of TCDD in the paper mill effluent to residues in the organisms identified in the assessment endpoints. Discuss the utility of available risk assessment tools for accomplishing this goal.

##### Comment:

Tissue levels of TCDD must be linked to exposure levels (water column and sediment) in order to utilize the information in future assessments. Without the link, each successive risk assessment, or the evaluation of the success of any mitigation efforts will be lessened by lack of these important data. It is critical to tie environmental concentration, exposure level, tissue level, and specific effects to the scenario being evaluated.

18. The Interim Report describes the limited field data that are available for estimating BAF's and BSAF's. Discuss the applicability of these factors to the Omigoshie Reservoir conceptual model.

##### Comment:

The concentration of organochlorine residue in wildlife tissue is a constantly changing function determined primarily by the concentration

of the chemical in the exposure route (water for aquatic animals and food for terrestrial animals). It is generally held that the rate of loss (depuration) is approximately the same as the rate of gain (uptake). Further, the rate of bioaccumulation/ bioconcentration varies greatly between species and chemical. The common practice of "back calculating" water or food concentration (exposure) using tissue concentration should be thought of as a means of developing an hypothetical value. The proof of the hypothesis is the actual measured environmental value. The use of an historic residue value of "a fish caught in 1985" etc., simply does not provide the needed validation of the relationship. To be valid, the proper approach is to show that the tissue concentrations are present in a randomly collected (but site-specific) sample. Any use of anecdotal information to demonstrate the back calculation hypothesis is neither scientifically nor statistically meaningful for 2,3,7,8-TCDD or for any other organochlorine.

Avians show little bioaccumulation of 2,3,7,8-TCDD, and low levels (<20-25x) of accumulation of other organochlorines. This is thought to be related to their ability to metabolize many chemicals, either altering their mode of action or inactivating them. Again, this phenomenon is generally species-specific.

Because of their high oil content, fish present the worst case for organochlorine uptake because the lipophilic characteristic of organochlorines results in their being sequestered in fat. However, it is also true that these deposits of chemicals are extremely variable and change in response to changes in the exposure level.

#### 19. Accumulation and depuration of TCDD-

The temporal dynamics and disequilibrium situations commonly associated with TCDD are mentioned in the Interim Report (section 2.3). Comment on how these aspects should be considered in establishing (1) the time course for the build-up of TCDD levels following initiation of the paper mill discharge and (2) the time course for the decrease of TCDD levels and recovery of biota should the paper mill cease operation.

#### Comment:

The important consideration in this effort would be to provide a rate-relationship for the bioaccumulation measured in actual exposure studies and then to track (in spatially and temporally random fish samples) the reliability of these projections when compared to actual measurements. The metrics in this example must be pre-determined in clearly defined data

limit objectives (e.g. if actual data do not fall outside the predicted natural variation in these measurements, it must be assumed that no definitive exposure or effect has occurred). The concentration of organochlorine residue in wildlife tissue is a constantly changing function determined primarily by the concentration of the chemical in the exposure route (water for aquatic animals and food for terrestrial animals). It is generally held that the rate of loss (depuration) is approximately the same as the rate of gain (uptake). Further, the rate of bioaccumulation/ bioconcentration varies greatly between species and chemical. The common practice of "back calculating" water or food concentration (exposure) using tissue concentration should be thought of as a means of developing an hypothetical value. The proof of the hypothesis is the actual measured environmental value.



**Robert Huggett**  
**Virginia Institute of Marine Science**  
**College of William and Mary**



### CONCEPTUAL MODEL

GENERAL: My first thought is that if a conceptual model (pages 8 & 9) does not need to be any more detailed than this, one could develop generic conceptual models based on Kow's since fish, bird or mammal species are not mentioned nor is much of anything else except that the assumed TCDD mode of action is via the embryo. Should not more about the biotic and abiotic components and their interactions relative to TCDD in the southern reservoir be given? I think it would be helpful if for no other reason than to help the uninformed reader who may only see this part.

Question 16: To categorically state that the impacts of TCDD will only be on fish and fish eating wildlife is risky. While I think that it is a good assumption, we really do not know much about benthic worms, etc. relative to TCDD.

Question 17: The discussions given in my comments on stressor characterization are appropriate here. In effect there will be a "House of Cards" built of various partitioning coefficients. It is the best we can do at this time, but it is still shaky.

Question 18: The same discussions given for the stressor characterization and question 17 are appropriate here.

Question 19: Please see the answers to questions 9, 10, 12 and 15. In addition, I expect the time course for decrease in TCDD residues, should the paper mill cease operation, would mainly be affected by the sedimentation rates. This has been shown to be the case for other hydrophobic chemicals such as

kepone in Virginia and DDT and PCB in coastal waters of California.

Keith Cooper  
Health Science Institute  
Rutgers University

the fact that the  $\text{C}_{60}$  molecule is a truncated icosahedron, a polyhedron with 32 faces, 60 vertices, and 90 edges. The faces are composed of 12 regular pentagons and 20 regular hexagons. The structure is highly symmetric, with icosahedral symmetry.

The  $\text{C}_{60}$  molecule is a truncated icosahedron, a polyhedron with 32 faces, 60 vertices, and 90 edges. The faces are composed of 12 regular pentagons and 20 regular hexagons. The structure is highly symmetric, with icosahedral symmetry. The molecule is often referred to as a "buckyball" due to its resemblance to a soccer ball.

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K. Cooper Premeeting Comments Exercise 2 & 3

Exercise 3. Conceptual Model

16. Although most of the data would suggest that invertebrates respond at much higher concentrations (or dosages) the studies have mainly dealt with high dose short term exposures and have not examined life stages that may be more sensitive. As with the adults from other species the sensitive stages are those which are undergoing rapid development and are sensitive to enzymatic alterations. The assumption that because an animal does not possess an Ah receptor does not preclude effects being observed. This is an area of research that needs to be examined.

17. The only way that a good correlation can be made is by relating the effects to tissue dose. By establishing threshold levels for tissue dose then an estimation can be made for potential effects on the organism. In most of the studies there has been little work done to examine the concentrations into other tissues other than the edible muscle (because of human health concerns). There is a need to relate the levels in the various tissues to effects in the animal. In some of the laboratory studies toxicokinetics can give some indication as to relative levels in the other organs of some of the fish described in the scenario. There needs to be evaluations of distribution at different concentrations and

K. Cooper Premeeting Comments Exercise 2 & 3

for different size organisms. In the scenario there needs to be an estimation of the loading into the reservoir and the estimated concentrations in the sediment and suspended sediment. The estimated levels must then be confirmed by field sampling. There might also be a way to sample fish, and birds examining the levels obtained from blood samples in a similar manner that humans are currently examined. These same animals could be tagged and then monitored at later dates to establish any change in body burdens. This assumes that there is a good relationship between serum fat levels and general body burdens.

18. As stated above there can be a tiered approach using best estimates and then validation by field sampling.

19. The amount of dioxins which accumulate can be monitored at specified areas down stream from the plant. The plant prior to operation normally carry out environmental assessments which should include background surveys of both fish and sediment. The establishment of information concerning the populations of fish, invertebrates and birds should be characterized. This characterization should continue after the opening to the plant. The levels which are entering the river will be determined by the facilities treatment of the effluent prior to discharge. Part of the problem in early

K. Cooper Premeeting Comments Exercise 2 & 3

detection of levels is the analytical sensitivities. These can be overcome with high volume sampling and the most up to date cleanup procedures and detection.

There is very good data from a number of paper mills and state agencies which have undergone modifications to their process to eliminate TCDD to a large extent. This information can be obtained for a number of species that inhabit downstream locations. The  $t_{1/2}$  of elimination for a number of fish can be obtained from laboratory studies and field information. It would appear for bivalve mollusks that the rate of elimination is related to the amount of water that is filtered. There is little information on crustacea for the rate of elimination of these compounds.

The temporal dynamics and disequilibrium situations will result in lower expected levels in the tissues if a lake wide average is used. The movement of fish and other animals into less or more contaminated areas will effect the amount estimated in the tissues. The physiological status of lower vertebrates, and invertebrates varies over the year and with these variations the amount of material taken up and eliminated will vary. Because of these temporal changes sampling should be carried out during different times of the year to examine the different physiological states.



**Joseph DePinto**  
**Department of Civil Engineering**  
**State University of New York at Buffalo**



### Exercise 3. Conceptual Model

#### 16. Adequacy of focusing on fish and wildlife

There is no way that this approach captures the full range of ecological effects. I am sure that there are subtle effects that effect trophic structure and function that are not captured by the conceptual model. The important thing is that we have focused in on an endpoint that is as sensitive to the stressor as we can assess within our current sphere of available data and knowledge base. I think that focusing on fish and fish-eating wildlife has accomplished that objective.

#### 17. Linking loadings to tissue residues

Most of the previous discussion has focused on this problem, having indicated the current capabilities in this area and the major uncertainties. The only additional comment here is that a complete risk assessment, quantitatively linking material input to effects, **must put more emphasis** on physical-chemical fate and transport modeling than was indicated in the scenario description. This is essential if it is desired to manage risk by imposing loading restrictions.

#### 18. Using BAFs and BSAFs

These concepts can provide a reasonable estimate, but the analyst must recognize that these numbers will be highly site-specific as well as temporally and spatially variable within a given system. The only way to narrow the variances (uncertainty) in these values is to continue to measure them in a variety of systems and for a variety of environmental conditions.

#### 19. Dynamics and disequilibrium conditions

The report recognizes that there may be a time lag in biotic response (bioaccumulation) to a given exposure. Dynamic bioaccumulation models such as Connolly's and Thomann's have demonstrated a typical two to three year lag

*J.V. DePinto*

between the manifestation of a given exposure concentration in a system and the response in the top predator fish. However, as discussed above the most significant time response is associated with the approach to steady-state following initiation of a loading and the time course for washout following cessation of the load. Not having the appropriate data for the Omigoshie River and Reservoir, I cannot give a good estimate of the time scale of these responses. However, in systems like this there are typically two characteristic responses: a relatively rapid response related to the response time of the water column alone, and a typically much slower response related to the slow response time of the sediments and the associated sediment-water interactions driven by diffusion and resuspension. In systems like this the rapid response for an HOC will be on the order of the hydraulic retention time of the water body, whereas the significance and speed of the slower sediment response will depend on the degree to which sediment-water interactions are important in the system. A good estimate of the significance of sediment-water interactions is the value of the overflow rate (ratio of mean depth to hydraulic retention time) of a system; the smaller the overflow rate, the more significant a given interfacial transport process becomes in terms of its impact on the water column. For a system like the Omigoshie the sediment response time may be on the order of 10-20 hydraulic retention times.

If there are management questions related to system response time, then a time-variable (not steady-state) chemical fate, transport, and food chain bioaccumulation model **must** be used for this portion of the analysis. Then the analysis of effects could either use the time profile of exposure or a time average of the calculated chemical concentrations.

### **Summary**

I recommend that the full risk assessment for dioxin could benefit greatly from what has been learned about the fate, transport, and bioaccumulation of PCB congeners from the Green Bay Mass Balance Study.

***J.V. DePinto***

**Derek Muir**  
**Department of Fisheries and Oceans**



## PRE-MEETING COMMENTS FOR THE PEER PANEL WORKSHOP ON 2,3,7,8-TCDD

### Comments on Exercise 3. Conceptual model

#### **17. The availability of tools for estimating tissue residues:**

Chemical transport and fate models such as WASP4 and RIVER/FISH (combined with food chain models) are available and have already been used to link TCDD concentrations in pulp and paper mill effluent. Results of these simulations have shown reasonable agreement between predicted and observed TCDD concentrations in fish (Hinton 1991; Holloran 1993). These models will calculate TCDD concentrations in dissolved and suspended particulate phases in the water column as well as levels in surficial sediments. These concentrations could be determined within segments of the aquatic environment i.e. immediately downstream of the mill, the reservoir and its tributaries. The models can be run in steady state, i.e. no change of flow, sedimentation rates etc or in a dynamic mode in which case detailed hydrodynamics and sediment transport are required. This should not be a problem for the paper mill/reservoir scenario. A key parameter is Koc value (or Kow from which Koc is calculated) which determines the extent of partitioning between water and suspended particles in effluent, river water and bed sediments. There is much uncertainty as to the best Koc value for TCDD but  $10^7$  could be used as a value for initial modelling purposes.

Accurate estimates of Koc would best be obtained by lab measurements using suspended solids from mill effluent, river water and bed sediments spiked with TCDD (and suitably aged), using the method of Lodge and Cook. During operation of the mill, field monitoring using methods such as *in situ* sparging (Resendes et al. 1992) and high volume sampling/filtration could be employed, with HRGC-MS analysis to determine TCDD in "dissolved" and particulate phases.

Tissue concentrations can be estimated with the Thomann food chain model using WASP outputs for concentrations in various compartments and segments of the aquatic system. In this model assimilation efficiencies (AE) from food for organisms at each trophic level are key parameters (Thomann 1989) along with  $k_1$  and  $k_2$  values. AE values for TCDD are available for a limited number of fish species. AE's for TCDF were independent of concentration over a 100-fold concentration range (Muir et al. 1992) but may be dependent on feeding rate (Clark et al. 1992). AE's have been derived for accumulation of a few hydrophobic organics by benthic invertebrates but information on TCDD assimilation is limited. To overcome the lack of data for lower food chain organisms the food chain model of Gobas (1992) assumes equilibrium between sediment/water and biota (i.e. BSAF or BAF values). This model has been successfully applied to predict concentrations of TCDD in fish near pulp mills on the Fraser River (Gobas 1993).

**18. Applicability of BAFs and BSAFs.** BAFs and/or BSAFs, or a food chain model (Fig. 4) could be employed to calculate TCDD tissue concentrations in the paper mill/reservoir scenario, assuming that levels in dissolved, suspended particulate, and sediments are calculated using chemical transport/fate models. There are uncertainties in BAFs for TCDD because  $C_w^d$  and  $C_w^t$  are based on

estimates and have not verified by field studies. Even if  $C_w^d$  could be measured in the Omigoshie Reservoir using large volume samples there would still be debate about what was truly dissolved because there are problems with all available techniques (i.e. filtration, sparging). BSAFs also have uncertainties due to site specific differences in food chains, sediment-water disequilibrium etc. But BSAFs have the advantage of being verifiable with existing techniques via sampling and analysis of biota and sediments. There is also a reasonably large dataset of BSAFs although there is a need for a broader selection of data. Laboratory and field derived BSAFs for oligochaetes are similar for PCBs (Ankley et al. 1992) - thus lab studies could be used to develop more BSAFs for different sediment types, and species.

Using BSAFs to estimate tissue concentrations in lower food chain organisms combined with food chain modelling (application of pharmacokinetic parameters, growth rates, age and feeding preferences) to estimate concentrations in forage fish and piscivorous fish is probably the best approach for the present scenario.

*19. Temporal dynamics and disequilibrium.* Using WASP or similar transport models, with information on the hydrodynamics and sediment transport in the river and reservoir, it should be possible to simulate the dispersion of the paper mill effluent and the deposition of solids on the river bed and in the reservoir. Although the river is reported to have no depositional zones, processes such as flocculation of suspended organic particles from the mill treatment ponds may result in locally high concentrations of TCDD contaminated particles in the river bed (Krishnappan 1993). Flushing of bed load in the river immediately downstream of the mill during high flow events could result in a pulsed rather than continuous deposition in the deep zones of the reservoir. During the initial deposition of TCDD surface concentrations will not be at steady state, disequilibrium between sediments and water ( $R_{ws}$ ) will be  $>1$ . This situation has been simulated in lake enclosures spiked with radiolabelled 1,3,6,8-TCDD (Servos et al. 1992) and with 2,3,7,8-TCDF (Muir et al. 1992a). In both cases, TCDD and TCDF were sorbed on particles and BSAFs for benthic invertebrates were temporality elevated. Disequilibrium between organisms and sediment ( $R_{as}$ ) will also be  $>1$ , especially for filter feeding organisms. During a shutdown phase, or switch to chlorine-free technology, TCDD in sediments will be buried by less contaminated material. This is similar to the situation in Lake Ontario studied by Cook et al. (1990). In this case  $R_{ws}$  and  $R_{as} < 1$  because transfer of TCDD to the water column or to biota from sediment will be limited kinetically although the fugacity of TCDD is higher in sediment than water. BSAFs from Lake Ontario would be most applicable to this scenario.

The time course for the buildup of TCDD levels will be very much dependent on the hydrodynamics and especially on sediment transport dynamics. Assuming Koc values of 7 or more, almost all TCDD would be expected to be deposited in deep sediments. Also critical to understanding the bioavailability of the deposited TCDD is the depth of mixing zone of benthic invertebrates (this can be determined with  $^{210}\text{Pb}$  and other isotopes) because freshly deposited and buried sediments could be remobilized.

## References not cited in the interim report

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**Robert Pastorok  
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**PREWORKSHOP COMMENTS**  
***Workshop on Ecological Risk Assessment Issues  
for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)***

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***CONCEPTUAL MODEL ISSUES***

*Issue 16: Consistent with the Interim Report, the conceptual model focuses on effects on fish and wildlife that consume fish. Comment on whether this approach captures the full range of potential ecological effects for this scenario.*

The focus on fish and wildlife for the quantitative risk assessment is appropriate for development of criteria. However, effects on plankton, macrophytes, and benthic macroinvertebrates should be addressed at least qualitatively, if not quantitatively. The conclusion that these groups are less sensitive to chlorinated dioxins than are fish and wildlife may be warranted based on the interim report, but this does not imply that these groups are not themselves at significant risk. Therefore, secondary effects on fish and wildlife could be realized through changes in species composition or community structure of plankton, macrophytes, and macroinvertebrates. The interaction of plankton, macrophytes, and suspended solids is especially important in reservoirs. The water-level fluctuation in the reservoir must also be considered because of its potential effect on the abundance and species composition of macrophyte beds.

The focus on sport fishes alone may not be warranted. Effects on other fish species could result in indirect effects on the sport fishes of interest.

Before certain organism groups are dismissed from the conceptual model, they should be ranked relative to their exposure susceptibility, sensitivity to PCDDs and PCDFs, and their importance as food for key receptors, such as predatory fish, mammals, and birds.

*Issue 17: The Interim Report emphasizes using tissue residue levels to estimate the adverse effects of TCDD. However, to do the risk assessment outlined by the conceptual model, it will be necessary to link predicted loadings of TCDD in the paper mill effluent to residues in the organisms identified in the assessment endpoints. Discuss the utility of available risk assessment tools for accomplishing this goal.*

The most important tools needed to link tissue residue levels with discharge rates for PCDDs and PCDFs are:

- Sediment BSAFs developed from empirical data or a bioaccumulation model similar to that of Thomann et al. (1992) to link sediment concentrations of PCDDs and PCDFs to tissue residue levels (This model represents a state-of-the-art approach to estimating BSAFs.)
- A model such as WASP4 to link discharge rates of PCDDs and PCDFs to allowable sediment concentrations in the reservoir.

The bioaccumulation model of Thomann et al. (1992) could be applied to develop permit specifications, which is the stated risk management objective for the scenario. Allowable tissue residue levels in selected fish species would be specified from available information on NOAELs for selected receptors and endpoints (e.g., fish embryo toxicity, fish food in the diet of mink). However, several important questions remain about application of the approach, including:

- Which data sets should be used to develop NOAELs?
- Should a quantitative uncertainty analysis (e.g., Monte Carlo analysis) be applied to the bioaccumulation model to derive probability distributions for allowable tissue residue levels (i.e., the selected NOAELs), BSAF values, and allowable sediment concentrations of PCDDs and PCDFs?
- How will the model be validated?
- How will spatial variability in parameters of the model be taken into account during validation and application of the model?

The issue of spatial variability and spatial analysis of data in the development of the approach and its application is especially important. Using literature data, realistic assumptions need to be developed for home ranges and diets of mammalian and avian wildlife to be addressed in the model. The exposure factors handbook for ecological receptors that is being developed by EPA's exposure assessment group may be useful in assigning parameter values.

Proper design of a sampling and analysis program to validate the model for fish is essential. The model should be validated using data from areas where the water column contamination is primarily influenced by the sediment compartment rather than direct discharges from multiple sources. During model validation, the sampling program will need to consider explicitly the fish home range and the spatial heterogeneity of sediment contamination.

Finally, the model as specified by Thomann et al. (1992) and the conceptual model for the reservoir scenario appear to ignore direct ingestion of sediment by fish. This exposure pathway could be especially important for forage fish such as English sole that feed on benthic invertebrates and incidentally ingest

sediment. Ecological risk assessments for terrestrial wildlife species have demonstrated the relative importance of direct soil ingestion compared with other exposure pathways for persistent contaminants. Similarly, sediment ingestion may be an important pathway for exposure of fish to contaminants compared with the water and food routes. If data to quantify sediment ingestion by fish are not available in the literature, then a focused laboratory or field study may be needed to support development of the model.

WASP4 is capable of modeling all of the conditions and processes potentially affecting the reservoir, including multiple types of solids and chemicals, lateral transport of particulate and dissolved phases, vertical settling of particles, particle resuspension, horizontal bed load transport, adsorption/desorption in both the water column and sediment, pore water diffusion, spatially variable sediment mixing depths, and spatial and temporal variability in both conditions (e.g., initial chemical concentrations) and processes (e.g., sorption equilibria).

WASP4 can be configured to use a network of compartments (or boxes) that accurately depicts physical site conditions, and can be applied in one, two, or three dimensions. Site-specific data on current velocities, particle composition and settling velocities, sediment accumulation rates, and sediment contaminant concentrations can be collected during field investigations to support use of the WASP4 model in a detailed risk assessment. Additional site-specific information that would improve model accuracy includes dispersion coefficients.

Because the WASP4 model is essentially a forward-mode fate and transport model, it does not have the capability to back-calculate allowable source loadings based on allowable sediment levels determined from the bioaccumulation model and the selected NOAELs for tissue residues in fish. Therefore, the WASP4 model would have to be run in an iterative fashion to find a convergent solution.

***Issue 18: The Interim Report describes the limited field data that are available for estimating BAFs and BSAFs. Discuss the applicability of these factors to the Omigoshie Reservoir conceptual model.***

Application of BAFs for PCDDs and PCDFs is limited by the current inability to measure PCDDs and PCDFs at low concentrations in water. Thus, the key issue in applying the model to develop effluent permit specifications is the use of BSAFs. The BSAFs for various PCDDs and PCDFs will need to be developed before complete ecological risk assessments can be conducted. BSAFs could be developed for each primary food item of the key receptors used to derive permit specifications. If the apparent constant relationship between the BSAF for PCBs and the BSAF for 2,3,7,8-TCDD mentioned in the interim report can be confirmed through additional research, then an empirical BSAF for PCBs could be determined for the case study reservoir, and the BSAF for TCDD could be estimated based on a wider database on the ratio of PCB-BSAF to TCDD-BSAF from systems with both groups of chemicals. BSAFs for PCDDs and PCDFs other than 2,3,7,8-TCDD could be derived in a similar manner.

Limitations of the BSAF approach include the inability to evaluate the residues for any receptor groups that are absent due to TCDD effects and the inability to measure BSAFs in the system of interest before the discharge is initiated.

***Issue 19: The temporal dynamics and disequilibrium situations commonly associated with TCDD are mentioned in the Interim Report (Section 2.3). Comment on how these aspects should be considered in establishing 1) the time course for the buildup of TCDD levels following initiation of the paper mill discharge and 2) the time course for the decrease of TCDD levels and recovery of biota should the paper mill cease operation.***

It is not clear why the issue of temporary disequilibrium under field conditions is relevant to the stated risk management objectives for the reservoir scenario.

The permit specifications for discharge limits on PCDDs and PCDFs should be developed from steady-state models (or empirical determinations of BSAFs from data on other systems). These approaches will not apply to the buildup of PCDDs or natural recovery.

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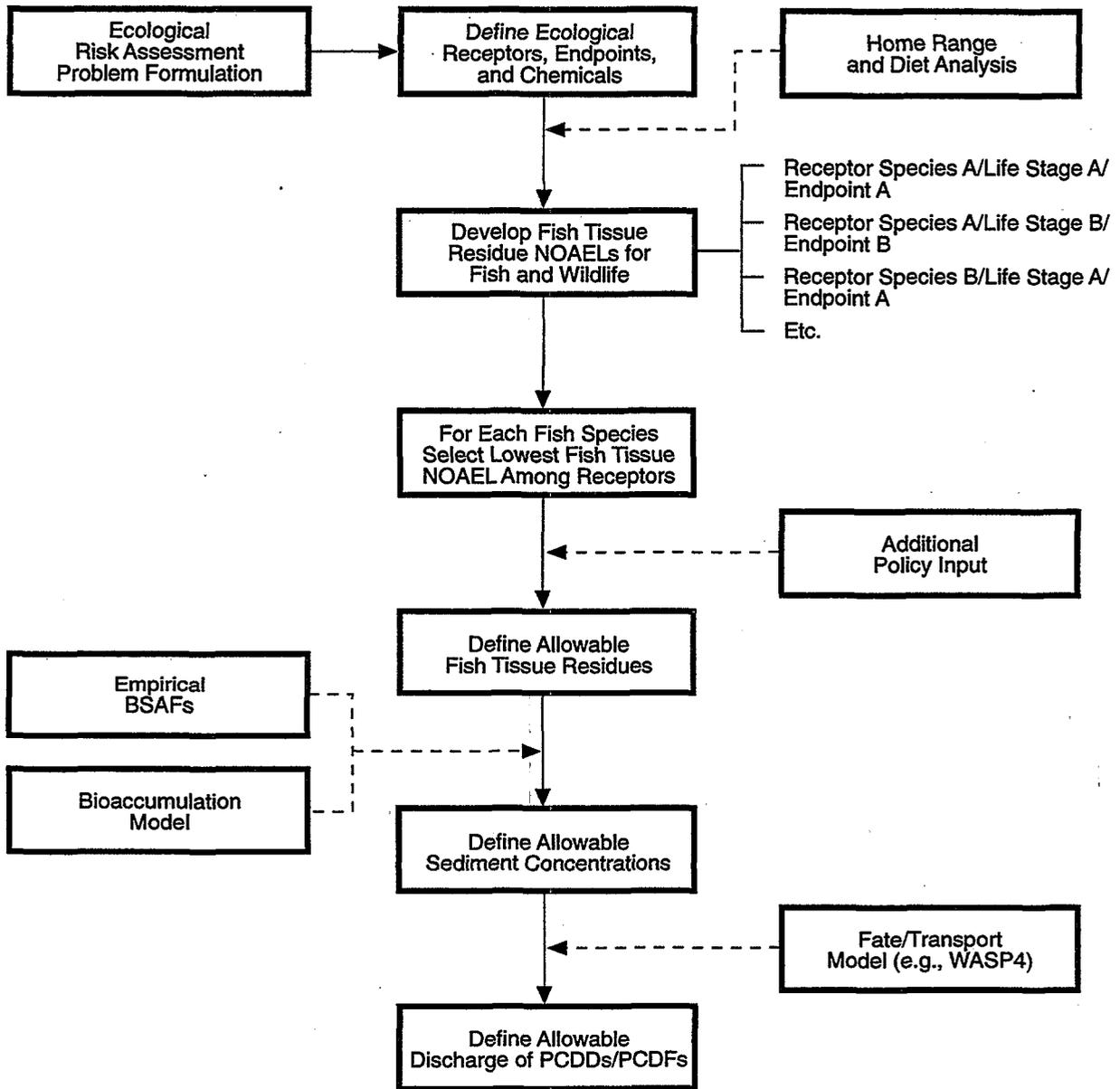


Figure 1. Development of discharge permit limits for PCDDs/PCDFs based on ecological risk assessment.

**RECEPTORS**

**MODELING**

**CONTAMINANTS**

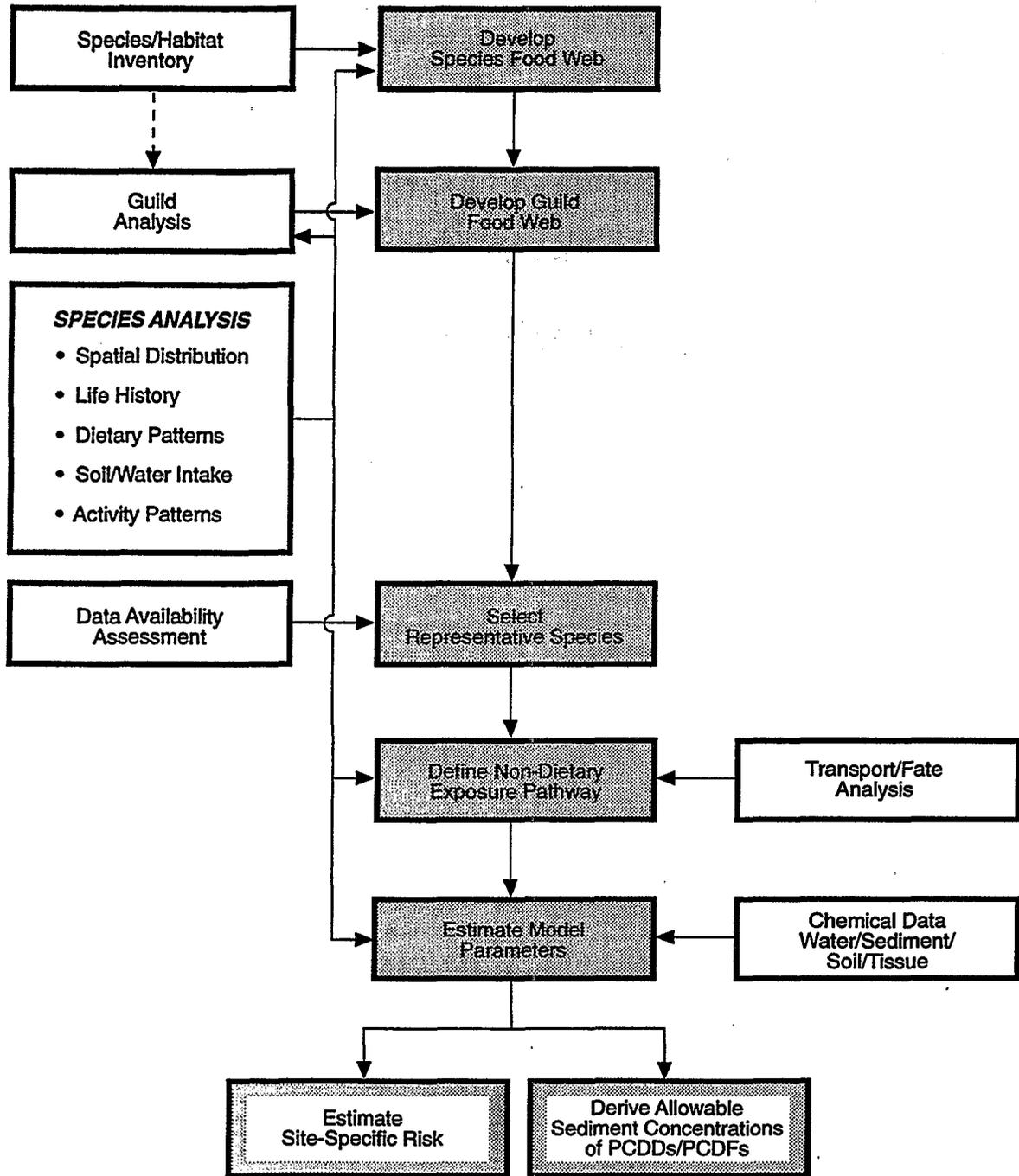


Figure 2. Application of proposed terrestrial food web exposure model.

**Section 5**

**J.P. GIESY**



**Workshop on the Ecological Risks of  
2,3,7,8-Tetrachlorodibenzo-p-dioxin**

**Minneapolis, Minnesota  
Sept. 14-15, 1993**

**Preconference Comments**

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I have reviewed the document entitled *Interim Report on Data and Methods for Assessment of 2,3,7,8-Tetrachlordibenzo-p-dioxin Risks to Aquatic Life and Associated Wildlife*. I found this document to be comprehensive and very well organized and written. I have compared the results of the model calculation of environmental concentrations associated with ecological risk. While similar, my determinations of safe concentrations are different from those proposed by the authors of the document. I have included a discussion of my criteria and calculations and compared those to both the literature and to the EPA document.

I have also reviewed the document containing the three Workshop exercises and my comments are also enclosed. I have provided answers to each of the questions posed by the organizers for this working group.

### ***Issues for Consideration***

#### **Question 1.**

It is true that plants, invertebrate and amphibians should, based on current models of the mode of action of TCDD be less sensitive than fish, birds and fur bearers. Focusing on fish will adequately provide protection of other species if the accumulation of TCDD into organisms that eat the fish is considered. Based on information currently available, I believe that the risk assessment should focus on fish, fish-eating birds (such as eagles) and furbearers (such as mink).

It is not appropriate to assume that the use of survival of fish eggs and fry will be protective of other organisms. Our most recent research has indicated that reproduction is not necessarily the most sensitive endpoint for the effects of TCDD to fish. In fact our work with rainbow trout has demonstrated that the adults were much more sensitive than effects on fry. Thus, it is important to include long-term studies which use dietary exposure to determine effects.

Our research has indicated that there is uncertainty in determining concentrations which can be related to ecological risk and that the concentrations proposed by the Interim document would not be sufficiently protective of fish and wildlife from ecological risk.

Here, we describe the current concentrations of TCDD-EQ in tissues of birds of the Great Lakes Region and discuss their relationship to egg lethality and birth defects. We also discuss the interactions among some of these *Ah-r*-active compounds, their relative toxic potencies, the relative importance of the various congeners and other possible contributors to the total toxicity of complex mixtures. We also describe and compare several methods of assessing the toxic potency of complex mixtures to birds and compare the results of hazard assessments for wildlife with those to protect human health.

## Question 2

### Chlorinated Hydrocarbons in the Environment

Many persistent, synthetic, PCH have been released to and are widely distributed in the environment.<sup>(35-40)</sup> Some of the PCH are very persistent,<sup>(35,41)</sup> and are bioconcentrated and biomagnified.<sup>(35,39,41-44)</sup> Of these, some classes cause adverse effects at minute concentrations in biota.<sup>(45-48)</sup> Some of the major groups which are of environmental significance are the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs).<sup>(47,48)</sup> Together the PCBs, PCDDs and PCDFs represent 419 individual congeners. Within this overall class are several subclasses defined by the pattern of substitution of the chlorine atoms.<sup>(39,43,48,49)</sup> One subset of these groups of compounds, those that are both laterally substituted and either non- or mono-ortho substituted can attain a planar configuration and are referred to as *planar or co-planar congeners* (p-PCH) of the.<sup>(48)</sup> Our discussion will consider only these p-PCH.

### Complex Mixtures

It is difficult to understand or predict potential effects of complex environmental mixtures of PCH on biota because, not only are there a great number of compounds, but because their concentrations of the individual components change as a function of space and time. Thus, the mixture to which organisms are exposed at one time or at one location may be very different from that to which they are exposed at other times or locations.<sup>(22,50,51)</sup> Furthermore, the relative concentrations of the various p-PCH congeners is different from trophic level to trophic level.<sup>(52)</sup> These differences are caused by environmental "weathering" and the sorting of compounds, based on their solubilities, volatilities and rates of degradation. The result is mixtures in the environment which not only change spatially and temporally, but which are different from the technical mixtures which were released into the environment. Thus, at this time, it is impossible to use the results of studies, which have determined the dose-response relationships of technical mixtures under laboratory conditions to predict effects in real world wildlife. The study of effects of p-PCH on fish and wildlife was limited for two decades by the fact that it was impossible to assess the toxicological implications of constantly changing mixtures and need to monitor for total concentrations of p-PCH. Recently, greater understanding of the mechanisms of toxic action of the p-PCHs has made it possible to express the potency of mixtures of p-PCH to elicit adverse effects relative to one prototype p-PCH. When this approach has been used, it has been possible to obtain better correlations between observed effects and 2,3,7,8-tetrachlor-dibenzo-*p*-dioxin equivalents (TCDD-EQ, sometimes

referred to as TEQ) than could be obtained for single p-PCH congeners of classes of p-PCH.<sup>(48)</sup>

### Toxicity of p-PCH

While the suite of effects caused by TCDD may seem diverse and unrelated, they are thought to all be caused through a common mode of action.<sup>(52)</sup> The mechanism is receptor mediated and involves the binding of particular intracellular receptors which lead to a host of common cellular and subcellular responses and subsequently effects on the whole animal. The similarities of molecular structure and conformation of the p-PCH compounds result in similar toxic effects.<sup>(40,46-48)</sup> Their primary toxic effects are thought to be exerted through a common mode of action.<sup>(53-55)</sup> The most widely accepted proposed mechanism of action for p-PCHs involves the expression of their biological potency through a specific cytosolic receptor, the *Ah* receptor (*Ah-r*).<sup>(55-57)</sup> This receptor binds p-PCHs, which are approximately  $3 \times 10^5 \text{ \AA}$  in size, such as planar PCBs (p-PCBs), PCDDs and PCDFs, with differing affinities.<sup>(48)</sup> The resulting receptor-ligand complex is then translocated to the cell nucleus where it elicits specific changes in gene expression.<sup>(48,56,58,59)</sup> The transformed receptor binds to sequences of DNA called the dioxin responsive enhancers (DRE). There are a number of DRE in the genome of most animals, thus a number of genes can be affected.<sup>(60)</sup> Many of the observed toxic effects of the p-PCHs are attributable to specific alterations in gene expression.<sup>(48,53,55,61-64)</sup> The relative toxicity of individual p-PCH is directly proportional to the strength of binding to the *Ah-r* and the potential to induce cytochrome P4501A isozyme activity.<sup>(46-48,57,65,66)</sup> The p-PCH are generally not acutely toxic, but cause chronic toxic responses,<sup>(48,59,67,68)</sup> including impaired reproductive potential of fish-eating, water birds. Because the p-PCH are not generally acutely toxic and accumulate into top predators, such as birds, colonial water birds have been suggested as useful biological monitors for the accumulation and effects of p-PCHs in the Great Lakes ecosystem.<sup>(24,66,69,70)</sup>

The most characteristic of these responses include measurement of mixed function oxidases. These enzymes are part of the general detoxification response animals to toxic substances. These enzymatic responses are generalized and lead to, or are associated with side-effects on many critical substrates used in routine metabolism. Levels of hormones, vitamins, and by-products of normal cellular activities are often altered enough to produce a characteristic set of responses now recognized as symptoms of subchronic or chronic exposures to p-PCH (Table 2).<sup>(8)</sup>

There are a number of pleiotropic effects of TCDD and p-PCBs on organisms. These effects can be direct or secondary responses to gene regulation. Some of these responses can be directly related to the adverse effects observed, while some are useful as biomarkers, but their relationships to observed adverse effects are less well characterized. The most subtle and important biological effects of TCDD and the dioxin-like p-PCB congeners on wildlife are their effects on endocrine hormones and

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vitamin homeostasis.<sup>(71)</sup> TCDD also mimics the effects of thyroxine as a key metamorphosis signal during maturation.<sup>(62)</sup> TCDD has also been shown to down-regulate the epidermal growth factor receptor,<sup>(72)</sup> which may result in disruption of the patterns of embryonic development at critical stages.

Altered concentrations of thyroid and steroid hormones and vitamin A are frequently reported to co-occur with embryonic abnormalities in wildlife populations exposed to p-PCHs.<sup>(8)</sup> Individuals from these exposed populations have been observed to have altered sexual development,<sup>(71,73)</sup> sexual dysfunction as adults and immune system suppression.<sup>(56,74)</sup> The observations on adult sexual dysfunction are especially significant since young, which appear to be normal while raised by intoxicated parents may become reproductively dysfunctional when they mature.<sup>(75-79)</sup>

Poor reproductive efficiencies and adventive, opportunistic diseases are characteristic of the wild animals in these exposed populations of the Great Lakes region.<sup>(80)</sup> Because of these conserved biochemical mechanisms concentrations of TCDD-EQ correlated with egg lethality or birth defects in populations of colonial, fish-eating, water birds while concentrations of the total concentrations of PCBs, PCDF and PCDD do not.<sup>(22,81,82)</sup>

Vitamin A (retinol) is important in many functions in animals, such as embryonic development, vision, maintenance of the dermally derived tissues, immune competence, hemopoiesis and reproductive functions.<sup>(83-85)</sup> Vitamin A is necessary for normal embryonic development<sup>(86)</sup> and, thus, changes in the status of vitamin A in the plasma or liver may be responsible for the birth defects observed in birds, which have been exposed to p-PCH. Laboratory studies have determined that both vitamin A and its storage form in the liver (retinal palmitate) were depleted in birds exposed to sublethal doses of the dioxin-like, p-PCB congener 77.<sup>(87)</sup> We have observed an inverse correlation between the concentration of vitamin A in serum and concentrations of p-PCHs in tissues of birds from the upper Great Lakes.<sup>(24,69)</sup>

p-PCH affect concentrations of vitamin A in both the blood and liver of exposed organisms. These effects are thought to be due to least two processes. In blood, some of the hydroxylated metabolites of PCBs to the carrier protein transthyretin.<sup>(84)</sup> In the liver, induction of hepatic enzymes such as acyl-CoA:retinol acyltransferase and uridine diphosphate glucuronyl transferase (UDPGT) is thought to alter the metabolic pathways involved in the storage and mobilization of vitamin A, and results in the observed depletion of retinols in the liver.<sup>(83,85)</sup>

TCDD is known to have effects on both male and female steroid hormones.<sup>(87-89)</sup> For instance, TCDD is both estrogenic and antiestrogenic effects, in different tissues, depending on timing of exposures during development.<sup>(89)</sup> Furthermore, 2,3,7,8-TCDD is a potent thyroxine agonist which may account for its capability to cause wasting syndrome in homeotherms.<sup>(67,85)</sup> The induction of the mixed function monooxygenase system can also reduce the concentrations of circulating steroid hormones, which can have adverse effects on the reproduction of wildlife.<sup>(25)</sup>

Thyroid hormone, which is an important regulator of development and

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metabolism can be influenced by exposure to p-PCH.<sup>(67,86-89)</sup> There are several possible mechanisms for the observed effects on circulating T<sub>3</sub> and T<sub>4</sub>. First hydroxy-substituted PCB congeners have been observed to displace thyroxin (T<sub>4</sub>) from its carrier protein, Transthyretin (TTR; prealbumin), which results in effects similar to thyroxin deficiency.<sup>(87-89)</sup> p-PCHs can induce UDPGT activity in the liver, which then decreases the concentration of TTR in the blood. Concentrations of TTR are not determined directly in the plasma, but rather, T<sub>4</sub> binding capacity is measured. Therefore, it is not possible to distinguish which of the two mechanisms may be causing the observed effects.

There is also evidence that exposure to TCDD can shift normal carbohydrate dominated metabolism to a fat metabolism, causing afflicted individuals to be unable to utilize a major source of energy. This effect is thought to be due to inhibition of the synthesis of the glucose carrier protein by p-PCH.<sup>(24,90)</sup>

Methyl sulfone metabolites of p-PCH have also been observed to cause adverse effects. Methyl sulfones accumulate in lung tissue of some marine mammals and are thought to be responsible for some toxic effects.<sup>(91)</sup>

In addition to the effects of the pPCBs it is known that the di-ortho-substituted PCBs, which are not very toxic, due to effects, which are mediated by the Ah-r can cause adverse effects in wildlife. Specifically, these congeners can inhibit dopamine synthesis in the brain, which results in behavioral differences in mammals.<sup>(92-95)</sup> We have observed behavioral effects in colonial water birds, which may be caused by these types of effects,<sup>(96)</sup> but to date, little information is available on this phenomenon. We feel that it is unlikely that the effects of the di-ortho substituted PCBs are responsible for the observed birth defects, but may be important in subtle behavioral shifts.

### QSAR

The most potent of the p-PCHs, identified thus far, is TCDD. The relative potency of other p-PCH congeners to cause biochemical or toxicological effects mediated through the Ah-r mechanism, is determined by the pattern of substitution of the chlorine atoms on p-PCH.<sup>(48)</sup> The most potent congeners are those that have at least four chlorine atoms in at least two of the lateral (*meta* and *para*) positions of both of the phenyl rings.<sup>(48)</sup> The relative potency can be expressed as proportions relative to TCDD and are referred to as Toxic Equivalency Factors (TEF).<sup>(47,48,92,96)</sup> The concentrations of individual p-PCH congeners can therefore be measured for Ah-r-mediated potency and reported as TCDD-Equivalents (TCDD-EQ).<sup>(48,49)</sup> The potency of complex mixtures, which can include several of the more Ah-active congeners in addition to many less active congeners, can also be expressed relative to TCDD as TCDD-EQ.<sup>(93,94)</sup> One method involves the quantification of each p-PCH congener in a complex mixture from a biological sample. The potency of the mixture is then

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calculated by multiplying the concentration of each congener by its TEF value and summing the products. <sup>(10,50,42,48,90,93-95)</sup>

### Biochemical Response Assays

There are a number of limitations associated with the determination of TCDD-EQ in complex mixtures from instrumental analysis and application of TEFs. The large number of p-PCHs in environmental samples make the chemical analysis time consuming and expensive. In addition, the most important congeners occur at small, but toxicologically relevant concentrations that are sometimes difficult to quantify by routine procedures. The wide range of biological potencies for p-PCHs<sup>(48)</sup> and potential synergistic or antagonistic interactions among p-PCH congeners and other chemicals <sup>(46,48,97-100)</sup> suggest that an additive model of toxicity is generally adequate but may not always be appropriate.<sup>(101)</sup> Interactions between or among p-PCH congeners and other compounds in the mixture may further complicate interpretation of the toxicological significance of these mixtures to wildlife. In addition, assessment of possible toxic effects of mixtures on wildlife are complicated by the wide range of reported TEF values for different species and various toxic endpoints used to set TEF values.<sup>(48)</sup> Depending on the test species and chosen endpoints the use of different TEF values will result in different calculated TCDD-EQ concentrations.<sup>(102,103)</sup> Furthermore, TEFs are not available for all biological endpoints and most have been derived for those species amenable to laboratory studies.

The concentrations of TCDD-EQ in complex mixtures of p-PCHs may also be determined by use of *in vitro* cell systems in a manner analogous to a chemical detector.<sup>(82)</sup> One method which uses H4IIE rat hepatoma cells relies on the fact that p-PCHs induce specific cytochrome P450-mediated monooxygenase (MO) enzymes through the Ah-r-mediated mechanism.<sup>(82,93,94,104,105)</sup> Furthermore, relative induction of MO activity by individual p-PCHs, as well as by mixtures of these compounds, are correlated with their toxicity to certain species [Safe, 1986, 1987, 1990], particularly birds.<sup>(46-48,75-79)</sup> Therefore, induction of cytochrome P450-mediated MO enzymes *integrates* the concentration and potency of all of the p-PCH congeners present in complex, environmental mixtures. This induction of enzymes has been well characterized *in vitro* with rat hepatoma cells (H4IIE cells) upon their exposure to p-PCH-containing extracts of environmental samples.<sup>(104-106)</sup> This induction measures the potency of an extract,<sup>(22,82,93,94,103,107-109)</sup> which can be expressed as TCDD-EQ by comparing the dose-response of an unknown extract to a standard curve generated with TCDD.<sup>(82)</sup>

The H4IIE bioassay method of TCDD-EQ determination has both advantages and disadvantages relative to the use of chemical analysis and TEF values. The bioassay is more rapid and less costly than congener-specific chemical analysis. Since the bioassay is a mechanistically-based determination of an integrated biochemical

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response, the results can be expected to be more biologically relevant. The bioassay integrates possible interactions between p-PCH congeners and compounds of other chemical classes thereby providing an integrated measure of potency measured at a cellular site proximate to the site of action. One limitation to the use of bioassay systems is that culture conditions and the use of various carrier solvents or end-points may make comparison of results among different research groups difficult.<sup>(48,82)</sup> Also, results obtained using the H4IIE bioassay have only been calibrated against controlled laboratory studies with rodents. This makes it difficult to interpret the toxicological implications of concentrations of TCDD-EQ observed in wildlife species. Only limited correlations are demonstrated in field studies of wildlife species<sup>(22)</sup> and only a few controlled laboratory studies with individual p-PCH congeners have been conducted.

Until recently only limited comparative data between the instrumental and bioassay approaches have been available for complex mixtures of p-PCHs in samples collected from the environment.<sup>(74)</sup> More recently we have determined the TCDD-EQ by both instrumental and H4IIE bioassay analysis of a set of p-PCH-containing extracts from birds and their eggs collected at Green Bay, Wisconsin, U.S.A.<sup>(102,109)</sup> It was found that the TCDD-EQ determined by the instrumental and bioassay techniques were positively correlated, but that the use of the results of the instrumental analyses and application of TEFs in an additive model underestimated the TCDD-EQ measured in the H4IIE bioassay (Fig. 2). The two possible reasons for the observed differences were: 1) The mixtures were interacting synergistically; or 2) There were compounds present that were not quantified yet contributed to the response of the H4IIE cells. It is likely that compounds other than the PCBs, PCDDs, and PCDFs, were responsible for a greater concentration of TCDD-EQ in the H4IIE assay than could be accounted for by an additive model, which considered the compounds measured. This discrepancy was not caused by the use of inappropriate TEFs or exposure systems, since the TEFs used were derived by using the same techniques in the H4IIE system. This difference is thought to be due to the fact that they assay responds to all of the p-PCH compounds, while we only quantified the PCDD, PCDF and planar PCB congeners. Concentrations of TCDD-EQ determined by calculation using TEFs or H4IIE bioassay have been correlated with adverse effects in birds.<sup>(10,22,67,107)</sup>

### Relative Contributions of Individual p-PCH

The proportion of TCDD-EQ contributed by PCDD and PCDF congeners in environmental samples from the Great Lakes region is generally small, frequently less than 5% in the fish-eating bird species (Figs. 3 & 4). A similarly great relative importance of pPCBs has been reported in certain marine mammals.<sup>(41)</sup> This indicates that marine ecosystems are also strongly influenced by the planar PCBs with toxic responses mediated through the *Ah*-receptor mechanism. In contrast to the fish-

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eating birds in the Great Lakes, the 'terrestrial' avian species examined in a Green Bay studied contained lesser concentrations of TCDD-EQ<sup>(102)</sup> and the relative proportion of TCDD-EQ, which was contributed by PCDDs and PCDFs in these species ranged from 3.2% to 71% and was greater than the fish-eating species (Fig. 4). However, the *absolute* concentrations of TCDD-EQ contributed by PCDDs and PCDFs were similar to the piscivorous species.<sup>(102,109)</sup> This suggests that contamination with PCDD and PCDF can be widespread in all species. In the fish-eating water birds the accumulation of TCDD-EQs from PCB congeners due to water and food by forage fish is an additional trophic level transfer and thus provides greater bioaccumulation potential than the terrestrial species. Also, there are known local sources of PCBs, whereas the sources of PCDD and PCDF may be more distant and due to atmospheric deposition.

The majority (> 90%) of the TCDD-EQ in the eggs of cormorants and terns in the Great Lakes was due to p-PCBs,<sup>(102)</sup> rather than PCDD or PCDF, which accounted for between 2 and 9% (12-22 ppt) of TCDD-EQ measured in water bird eggs in Lakes Superior, Huron and Michigan. The primary contributions to the TCDD-EQ were due to the *dioxin-like* p-PCBs, especially non-ortho-chlorinated PCB congeners 126 (3,4,5,3'4'-PeCB), 77 (3,4,3',4'-TCB), 169 (3,4,5,3',4',5'-HCB) and mono-ortho-chlorinated congeners 105 (2,3,4,3',4'-PeCB) and 118 (2,3',4,4',5-PnCB). The understanding that dioxin-like bioeffects in fish-eating, colonial water birds are due largely to pPCBs is an emerging consensus worldwide, except near TCDD point sources.<sup>(41,50)</sup>

Currently, much of the discussion of the safety of consuming fish flesh from the Great Lakes is centered on the concentration of TCDD-EQ contributed by the PCDD and PCDF.<sup>(84)</sup> However, the concentrations of TCDD-EQ from both PCDD and PCDF is generally in the range of 5 to 10 pptr, wet weight, while concentrations of TCDD-EQ contributed by the PCB congeners can be as great as 250 pptr.<sup>(110)</sup>

### Interactions among p-PCH and between p-PCH and Other Compounds

There has been much discussion about the possible interaction between and among individual congeners of p-PCH and between p-PCH classes and other synthetic, halogenated compounds in extracts of environmental matrices, which contain complex mixtures of p-PCH.<sup>(111)</sup> An additive model for the prediction of TCDD-EQ is plausible and it is unlikely that the use of this model will result in a great deal of error in predicting the concentrations of TCDD-EQ due to synergisms. While such non-additive responses could be either greater or less than additive, it seems that the biochemical effects of p-PCH congeners are simply additive.<sup>(48)</sup> However, there are reports of both *infra-* and *supra-*additivity between and among individual p-PCH congeners, complex mixtures of p-PCH and other halogenated hydrocarbons.<sup>(48,57,62,64,112)</sup>

When nonadditive responses between individual p-PCHs and complex mixtures

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have been observed, they have generally been antagonistic.<sup>(75,100,113-115)</sup> This interaction is probably due to the fact that the less *Ah-r*-active congeners still have receptor binding affinities, which when combined with the relatively great concentrations of these less toxic congeners, make them effective competitors for binding sites.<sup>(114,115)</sup> This reduces the probability of the more toxic p-PCH binding to the *Ah-r*. However, these less active congeners do not seem to bind with great enough affinity to be effective inducers of EROD induction or cause any of the other adverse effects, which are caused by the *Ah-r*-active congeners.<sup>(48)</sup> The di-ortho-substituted PCB congener, 2,2',4,4',5,5' has, under some conditions, been found to be an effective antagonist for 2,3,7,8-TCDD, but some of the results of the studies of interactions among congeners are equivocal. For instance, PCB congener 77 and TCDD caused greater than additive induction of AHH activity in the liver of rainbow trout at doses which corresponded to 0.13 and 0.5 toxic unit (proportion of dose required to elicit a given endpoint). However, at greater doses the mixture was less than additive.<sup>(116)</sup> Similarly, there was no effect of PCB congener # 153 (2,2',4,4',5,5') on the induction of EROD activity by TCDD.<sup>(117)</sup> PCB congener 153 was found by the same researcheres to have an antagonistic effect on the potential of TCDD to induce PROD activity.<sup>(117)</sup> Pretreatment of mammalian cells results in an increase in the concentration of *Ah-r*, which could cause a greater response to TCDD, but this is only observed at submaximal exposures to TCDD.

The evidence seems to support the conclusion that complex mixtures of halogenated p-PCH congeners in the extracts from the wildlife would be less than additive (antagonistic) rather than more than additive (synergistic). A number of compounds, which can bind to the *Ah-r*, but do not effectively induce the same monooxygenase (P450IA1) enzyme activity as 2,3,7,8-TCDD, are partial antagonists.<sup>(97-100)</sup> The potency of simple combinations and complex mixtures of PCDD and PCDF to induce EROD activity in in hepatocytes or H4IIE cells indicated that the effects were due to the simple additive responses to the 2,3,7,8-substituted congeners.<sup>(118)</sup> Therefore, it is not likely that non-additive interactions between or among *Ah-r*-active or inactive congeners would result in differences between the concentrations of TCDD-EQ derived by instrumental or bioassay methods. In fact, if there were strong antagonisms between the p-PCH and other components of these complex mixtures one would expect the TCDD-EQ determined in the bioassay to be less, not more than those calculated from the additive model, which was the case when the results of the two methods were compared.<sup>(102)</sup>

### Selective Enrichment of p-PCH

When studying a complex mixture, such as p-PCH, if the relative proportions of the different congeners changes during the bioaccumulation process this change must be accounted for in the hazard assessment. It is difficult to determine safe exposures if concentrations observed in the field can not be compared to the results of controlled

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laboratory studies, which have been conducted with the original technical mixtures of the compounds. Chemical weathering due to differential solubilities, volatilities, adsorption constants and degradation rates, can result in patterns or relative concentrations of p-PCH congeners which are different from the technical mixtures and also different from one location to another.<sup>(119)</sup> Furthermore, these patterns can change over time<sup>(120)</sup> such that the pattern of congeners observed is significantly different than that in the original technical mixtures, which were released to the environment.<sup>(119)</sup> Also, there are changes in the relative pattern of accumulation in the ecosystem as trophic biomagnification occurs.<sup>(102,121,122)</sup>

In the Great Lakes system, one way to estimate the relative toxic potency of mixtures is to calculate TCDD-EQ, which measures the total concentration of congeners from PCDD, PCDF and PCBs and divide this by the total concentration of PCBs.<sup>(22,52,102)</sup> This relative potency ratio will account for different contributions from the three major classes of congeners and relate it to that fraction, which is thought to account for most of the toxic potency, the PCBs.

Selective accumulation of the more toxic PCB congeners can result in a mixture in the tissues of target animals, which is more toxic than would be predicted from an estimate of the original Aroclor® mixture.<sup>(22)</sup> This enrichment of the more toxic, non-ortho-substituted PCB congeners results in a relative toxic potency of the mixture which is from four to six times greater than the original technical mixture.<sup>(22)</sup> The toxic potencies determined as the ratio of TCDD-EQ determined by the H4IIE assay to total concentration of PCB of extracts of double-crested cormorant eggs were 2.5 to 5.24 (mean = 3.77) times greater than technical mixtures of aroclors®, which were also measured in the eggs (Fig. 5).<sup>(29,81,82)</sup>

When a potency ratio was calculated for several of the locations in the Great Lakes it is found that indeed the ratio of toxic potency varies among locations (Fig. 6). Furthermore, the greatest ratio was observed to occur with the least total concentration of PCBs. This relationship is most likely for the greater correlation between adverse effects and TCDD-EQ than with total concentrations of PCBs (see below). Since the greatest proportion of the TCDD-EQ in birds of the Great Lakes is contributed by the PCBs there is a general correlation between the concentrations, but there is sufficient variation in the relative potency that a measure of TCDD-EQ gives better prediction of the effects of complex mixtures. The relative potencies (EC-50 for EROD induction) of extracts from Green Bay ranged from 6 to 56 pg TCDD-EQ/ $\mu$ g PCB, which indicates that total PCB content of a sample is a poor indicator of the biological potency of the toxicity mediated through the Ah-receptor, even though the measured concentrations of TCDD-EQ were correlated with the total concentration of PCBs.<sup>(102)</sup> If all of the TCDD-EQ could be attributed to PCB congeners and these congeners were sorted equally in the environment and assimilated and metabolized equally there would be no significant difference in relative potency among samples, except for the contributions of other compounds.

The smallest PCB-normalized potencies observed in these samples were

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approximately 10  $\mu\text{g}$  TCDD-EQ/g PCB.<sup>(52)</sup> This value is similar to the potencies observed for technical grade PCB preparations in the H4IIE bioassay system.<sup>(22,105)</sup> However, most of the samples in this study had PCB-normalized potencies which were considerably greater than those of technical grade PCB preparations.<sup>(22)</sup>

The greatest enrichment of TCDD-EQ, relative to technical mixtures of Aroclors is due to trophic transfers.<sup>(52)</sup> This is due not only to the presence of non-PCB congeners, but to the enrichment of Ah-r-active congeners, relative to the total concentrations of PCBs. The enrichment of specific p-PCH congeners has previously been demonstrated in waterbirds.<sup>(123-125)</sup> This enrichment was greatest for the 2,3,7,8-substituted congeners which have a relatively great biomagnification potential and are poorly metabolized by most species.<sup>(126)</sup>

To assess the enrichment of individual PCB congeners within the Great Lakes ecosystem, the relative proportion of the congeners to total concentration of PCBs in bird tissues was compared to that of technical Aroclor<sup>R</sup> mixtures and to samples of fish tissue from lake Michigan (Table 3;<sup>(95,96,110,127)</sup> The relative concentrations of PCB congener 77 (IUPAC) in extracts of bird tissues was the same or less than in the technical mixtures of Aroclors<sup>R</sup> 1242 and 1248 but greater than that in Aroclors<sup>R</sup> 1260. Thus, depending on the relative proportions of these Aroclors<sup>R</sup> in the original mixture released to the environment, this congener may have been enriched, diminished or stayed the same. Similarly, PCB congener 105 could have been enriched or diminished in the samples, relative to the original technical mixtures depending on the relative proportions of different Aroclors<sup>R</sup> making up the complex mixture. PCB congener 126 was enriched in the extracts of bird tissue regardless of which of the Aroclor mixtures to which they were exposed. The relative contributions of these three congeners to the total mass of PCBs were also greater than those in chinook salmon from Lake Michigan. It is uncertain whether PCB congener 169 was enriched. Values for the relative contribution by weight in Aroclor<sup>R</sup> 1254 ranged from 0.00005 to 0.08. Thus, it is difficult to determine if the value of 0.00141, observed for bird tissues in our study is an enrichment or diminution (Table 3). The enrichment of these PCB congeners, however, does not explain the discrepancy between the measured and predicted TCDD-EQ, based on the H4IIE TEF values, in the tissue samples. The combined mass contribution of the PCDD and PCDF congeners to the total concentrations of TCDD-EQ was less than 0.5% of the mass of total PCBs present in all samples (Fig. 4).

The relative potencies are different among the original technical Aroclor<sup>R</sup> mixtures (Table 3; Fig. 5). Aroclor<sup>R</sup>-1016, which is Aroclor<sup>R</sup>-1242 with the PCDD, PCDF and p-PCBs removed contains essentially no detectable TCDD-EQ. The greatest relative potency is observed in Aroclor<sup>R</sup>-1248 and the least potency is observed in Aroclor<sup>R</sup>-1260 (Fig. 5; Table 3). The relative potency in cormorant eggs from the Great Lakes was more than twice as great as any of the Aroclor<sup>R</sup> mixtures. Thus, the observed enrichment could not be caused by any combination of the original technical Aroclor<sup>R</sup> mixtures. Furthermore, contributions of PCDD and PCDF were not included

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in these extracts of eggs of fish-eating colonial water birds. Thus, we feel that the greater than predicted potency is primarily due selective enrichment of the *Ah*-r-active PCB congeners, but some contribution may be due to the presence of unidentified *Ah*-r-active compounds as well as PCDD, and PCDF congeners.<sup>(102)</sup>

Biotransformation is the key first step in the elimination of P-p-PCH congeners and differential biotransformation of PCDD and PCDF congeners by cytochrome P450-mediated pathways has been demonstrated in some species.<sup>(128)</sup> Both fish and birds have enzymes that are capable of metabolizing PCB congeners.<sup>(123,129)</sup> However, the activities of cytochrome P450-requiring oxygenase enzymes in fish-eating birds are greater than those in most fishes, but less than those in most mammals.<sup>(124,125)</sup> Therefore, birds can be expected to eliminate p-PCHs, but possibly more slowly than mammals. In general the more substituted congeners tend to be less rapidly metabolized.<sup>(123)</sup> Also, those congeners, which are laterally substituted, such that they do not have two adjacent, un-substituted carbon atoms are more slowly metabolized<sup>(123)</sup> and tend to accumulate in animals.<sup>(41)</sup> PCB congeners with vicinal hydrogen atoms in the *ortho* and *meta* positions with more than one *ortho*-chlorine atom are also resistant to metabolism.<sup>(123)</sup> The hexa-chloro biphenyl (2,2',4,4',5,5') was not metabolized by pigeons, rats or brook trout.<sup>(130)</sup> More chlorinated congeners can be metabolized if they have adjacent, unsubstituted carbon atoms, whereas those with no vicinal unchlorinated carbons were not metabolized and only slowly excreted.<sup>(131-133)</sup> This indicates that congeners, which were poorly metabolized would not be as readily excreted and would tend to be accumulated selectively in tissues, relative to other congeners, such as the 4,4'-di-chloro and 2,2',5,5'-tetrachloro, congeners, which were metabolized to more polar hydroxy-metabolites, which could be excreted.

In addition to the effects of metabolism, position in the food chain can affect the relative concentrations of PCB congeners in the tissues and eggs of birds.<sup>(134)</sup> This is due to many factors, but is primarily due to the relative quantities of different prey items taken by different species of the same species in different locations.

The greater PCB-normalized potency of the p-PCH extracts of avian species from Green Bay, relative to that of Aroclor<sup>R</sup> indicates that mechanisms of trophic selection or the presence of unidentified *Ah*-r-active compounds in the extracts could be the cause of the elevated relative potency of the p-PCH mixture. There are two principal ways to correct for changes in potency during a risk assessment: First, each of the active congeners could be quantified and their individual concentrations corrected for relative potencies. Alternatively, an application factor or enrichment factor could be applied to the total concentrations. Since the individual PCB congeners which express the greatest toxic potency have not been measured traditionally and are still seldom considered in regulations, if a constant correction factor can be justified, it could be applied to total concentrations of PCB and correct for the effects of weathering and enrichment through biomagnification. To investigate the assumption that an enrichment factor could be applied to total concentrations of

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PCBs to correct for different potencies, we calculated water quality criteria (see section below) then calculated exceedance values for current conditions and compared the exceedances based on total concentrations of PCBs and those based on TCDD-EQ the ratio of the water quality criterion to protect double-crested cormorants at seven locations, based on total concentrations of PCB and TCDD-EQ (Table 3; Fig. 7).

### p-PCHs Other than TCDD TCDF and p-PCBs.

A number of compounds, structurally similar to pPCBs, PCDDs and PCDFs, and thus, should act similarly to these p-PCHs have been observed at concentrations in the environment that, if they have similar toxicological properties, could be of toxicological significance (Table 4). Unfortunately, for many of these compounds, there is currently little known about their toxicological or environmental fate properties. Thus, even if their concentrations in environmental samples were determined instrumentally, it would still be difficult to assess their potential to cause significant adverse effects. It is likely that these compounds do contribute a significant quantity of TCDD-EQ in environmental samples. When TCDD-EQ are measured in the H4IIE bioassay and compared to the TCDD-EQ calculated from instrumental analyses for PCDDs, PCDFs and PCBs and their TEF values measured in the H4IIE bioassay we are unable to account for 30 to 50% of the TCDD-EQ measured in the bioassay, especially in samples taken from urbanized and industrialized areas (Fig. 4).

The potency of extracts of peat were found to cause greater induction than could be accounted for by the concentrations of PCDD and PCDF in the extracts.<sup>(135)</sup> This suggests that there are compounds other than the traditionally measured planar molecules which can be Ah-r-active and thus measured in bioassays of P4501A activity.

Compounds that could contribute to the total concentrations of TCDD-EQ measured in the bioassay also include any or all of the following classes of polychlorinated compounds (Table 4): naphthalenes (PCNs), diphenyl ethers (PCDEs), diphenyl toluenes (PCDPT), phenoxy anisoles (PCPAs), biphenyl anisoles (PCBAs), xanthenes (PCXE), xanthenes (PCXO), anthracenes (PCAn), fluorenes (PCFIs), dihydroanthracenes (PCDHAs), diphenyl methanes (PCBMs), phenylxylylethanes (PCPXEs), dibenzothiophenes (PCDT), quaterphenyls (PCQs), quaterphenyl ethers (PCQEs) and biphenylenes (PCBE). In addition to the chlorinated compounds, brominated and chloro/bromo-substituted analogues of PCDD and PCDF have been found in the environment<sup>(136)</sup> and are known to induce ethoxyreurefin-o-deethylase (EROD) activity *in vivo* and *in vitro*.<sup>(48)</sup> In addition to the above mentioned compounds, there are a number of polychlorinated compounds, which are the alkylated forms of these same classes. These include polychlorinated-alkylbiphenyls (PCAB), alkyl naphthalenes (PCAN), alkylphenanthrenes (PCAP) and alkyl dibenzothiophenes (PCADTh).<sup>(137)</sup> Alkylated analogs of all of these compounds,

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including PCDDs and PCDFs are especially prevalent sludges and sediments near paper mills that use chlorine in the bleaching process.<sup>(138)</sup> Polychlorobiphenyls (PCBB) have also been observed in the vicinity of pulp mills.<sup>(139)</sup>

The PCNs are known to occur in the environment at great enough concentrations sufficiently great in some locations that, coupled with their surprisingly high TEF values, could be of toxicological significance similar to that of the PCDD, PCDF and pPCBs.<sup>(101,140)</sup> The TEF values reported by Hanberg (1988) for PCN ranged from 0.000007 to 0.002. Total concentrations of PCN in fishes from the Great Lakes have been reported to be as great as 5 mg/kg, wet weight. The TEF for the most prevalent PCN were 0.002. Thus, PCN could contribute significantly to the total TCDD-EQ. Chlorinated PCNs have been found in great concentrations in the effluents and sludge of pulp and paper processes.<sup>(137)</sup>

The PCDEs are byproducts in the manufacture of chlorinated phenols.<sup>(129)</sup> Significant concentrations of these compounds have been found in the tissues of humans, fish and wildlife.<sup>(141)</sup> PCDEs can be accumulated by fish<sup>(142)</sup> and induce MFO activity.<sup>(143)</sup> Furthermore, an unidentified tetrachlorinated PCDE isomer was found at a concentration of approximately 0.9 mg/kg, wet wt. in the eggs of common terns in Michigan.<sup>(144)</sup> Concentrations of tri- and tetra-chloro PCDE as great as 3 mg/kg have been found in the tissues of common tern chicks.<sup>(145)</sup> The PCDEs are similar to PCBs in their dynamics and persistence in animal tissues and induce P-450 type monooxygenase activities. The ED<sub>50</sub> values for several PCDEs are in the range of 15 to 110  $\mu\text{mol/kg}$ .<sup>(141)</sup> Unlike PCBs the mono- and dichloro-substituted PCDE are also potent inducers of EROD activity.<sup>(146)</sup> It has been postulated that the additional distance between the phenyl moieties reduces the strong effect of ortho-substitution, which is observed in the PCBs. Thus, PCDEs in the environment may exert a greater effect on the EROD activity than predicted from QSAR relationships developed for PCBs. The PCDEs are rarely monitored in environmental samples due to the paucity of authentic standards and because they occur in the same fraction as the PCDF and several of the congeners result in the same mass fragments as some of the PCDF.<sup>(141)</sup> Thus, since their potencies to induce MFO activities are similar to those for PCBs, PCDEs could account for a significant amount of the induction measured in the H4IIE assay.

The PCDPT are not used in North America, but are manufactured and widely used in Europe as substitutes for PCBs, especially as hydraulic fluids in mining and have been found in the tissues of aquatic organisms.<sup>(147,148)</sup> The PCDPT, which are also called diphenyl-methanes, are structurally similar to PCBs and cause similar effects.<sup>(149)</sup> PCDPT are potent inducers of EROD activity in mammals and fishes.<sup>(148)</sup> It is not likely that there are sufficient concentrations of PCDPTs in the tissues of North American wildlife to contribute significantly to the TCDD-EQ measured in samples. However, the PCDPT could currently make contributors to the total TCDD-EQ in samples from some locations in Europe<sup>(150)</sup> and may contribute to the TCDD-EQ measured in North America at some time in the future.

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The PCPXEs are known to be potent inducers of EROD activity in the livers of fishes,<sup>(151)</sup> but we are aware of no reports of these compounds occurring in the tissues of fish or birds taken from the environment. Thus, neither the hazard nor the risk presented by these compounds to wildlife can be assessed at this time.

A complete assessment of the possible environmental hazard posed by PCDT is impossible because little is known about their persistence, bioconcentration, or toxicological properties. The PCDT are formed during incineration of organic compounds which contain sulfur and chloro-compounds, such as tires.<sup>(152,153)</sup> The PCDT have a relative potency, as measured in the H4IIE assay, similar to that of the PCDD.<sup>(155)</sup> There are few reports of the occurrence of PCDT in environmental samples: however, concentrations of the 2,4,6,8-tetra-CDT as great as 8,300 and 1,000 pg/g, wet weight were observed in the tissues of crab and lobster respectively, from the Elizabeth river, New Jersey. Concentrations of other tetra-chlorinated congeners were as great as 500 pg/g in the crabs from the same location. Therefore, it is possible that PCDT could be present locally in sufficiently great concentrations to contribute to TCDD-EQ in environmental samples, including the tissues and eggs of birds from the Great Lakes region of North America. The TEF for a synthetic mixture of PCDTs (2.4 % Cl<sub>2</sub>, 74.6% Cl<sub>3</sub>, 22.4% Cl<sub>4</sub> and 6% Cl<sub>5</sub>) was found to be 0.000425 in the H4IIE assay.<sup>(108)</sup>

PCBE have been little studied, but are structurally similar to the PCDD.<sup>(156)</sup> PCBE have been found to occur in the environment, generally due to pyrolysis of PCBs.<sup>(51)</sup> Few authentic standards are available for the PCBE and the concentrations in the environment are generally quite small, compared to those of PCBs. In addition to the chlorinated diphenyl ethers, brominated analogues of these compounds have also been found to occur in the aquatic environment at concentrations, which could be toxicologically relevant.<sup>(157)</sup>

Both PCQs and PCQEs have been found in environmental samples and have been demonstrated to be toxic to animals.<sup>(158)</sup> However, they are reported to be much less toxic than PCDD or PCDF. Both of these classes of compounds were found in patients who consumed Yusho oil,<sup>(158)</sup> but it is unlikely that either of these classes of compounds occur in wildlife of the Great Lakes region at concentrations great enough to be of toxicological significance.

In addition to the diaromatic-type compounds, single ring compounds are known to induce P450-type monooxygenase activity. For instance hexachlorobenzene (HCB) induces several monooxygenase activities,<sup>(53,159)</sup> but it is unlikely that it contributes significantly to the concentrations of TCDD-EQ in wildlife since it does not induce the P450IA1-type isozyme which is measured as EROD activity. HCB has been detected in the eggs and tissues of birds in the Great Lakes,<sup>(160)</sup> but the significance of the concentrations is unknown at this time.

Polybrominated compounds such as PBBs and PBDEs which are used in flame retardants such as Bromkal<sup>R</sup>, which contains polybrominated diphenyl ethers (PBDEs).<sup>(48)</sup> PBBs are known to occur in the environment and can be accumulated

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into biota.<sup>(157)</sup> The Bromkal<sup>R</sup> mixture is known to induce EROD activity *in vitro*, but the potency for induction is much less than that of the PCDD, PCDF or pPCBs.<sup>(101)</sup> Since concentrations of these brominated compounds are not generally measured, it is impossible to assess the contribution that these compounds may contribute to the concentrations of TCDD-EQ measured in extracts from biota.

There are a number of polycyclic, aromatic hydrocarbons (PAH) compounds which are known to bind to the Ah-r. These compounds may contribute to the toxicity observed in some species. However, it is unlikely that these compounds would be responsible for the effects observed in eggs, since they are rapidly metabolized by vertebrates and thus would not be biomagnified into eggs. Also, since the polar metabolites of these compounds would not be expected to occur in the extracts which are used in the H4IIE assay, it is unlikely that they are responsible for the EROD induction which can not be accounted for in comparisons of bioassay and instrumental analyses. Because a sufficiently great number of persistent compounds can interact with the Ah-r, it would be useful to use techniques, such as the H4IIE bioassay to determine if all of the potential hazardous compounds in complex mixtures have been accounted for.

When the environmental contamination with Ah-r-active compounds is discussed, their impacts are often dismissed because the manufacture of PCBs which contribute the greatest proportion of the known TCDD-EQ in biological samples, has been discontinued.

It is often concluded that nothing can be done about these compounds that have already been released to the environment and that the concentrations of these compounds will eventually decrease to insignificance. While this is partially true, we believe it is important to remember that a number of other compounds, with similar ecotoxicological properties are still manufactured, used and released into the environment at concentrations that could be of ecological or toxicological significance. Therefore, the use of these compounds should be as tightly regulated as the other p-PCHs and their continuing uses reevaluated as more information on their distribution is gained through monitoring. This is especially important if these other classes of compounds are introduced to commerce as substitutes for other members of the more pernicious p-PCH classes.

An implication of these observations for regulators is that discharge limits for a single p-PCH projecting its toxic effects at a given level of discharge must account for the additivity with other p-PCHs in the environment to ensure protection of wildlife as well as humans. We know of no case where this policy has been used in the regulatory community which has avoided the issues raised by mixtures and interactions. However, the Great Lakes Initiative, developed by the US EPA will take these factors into account.

The use of BSAF values does not seem to be supported by anything other than a few empirical ratios. It is difficult to believe that these values will be transportable from one location to another. If the information necessary to evaluate these ratios on

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a site-specific basis is available, they will be unnecessary.

In conclusion, it is my opinion that the complex nature of the exposure of animals to compounds which work through the same mode of action we must use toxic equivalency approaches, but the endpoints selected and the way in which they are integrated is very important. The use of these techniques must include a calibration step, which accounts for the activity at the receptor and for pharmacological aspects.

### **Question 3**

I think that it is appropriate to use tissue levels for conducting risk assessments for compounds which are already in the environment. This approach will obviously not work for situations where risk assessments are to be conducted for compounds which have not yet been released to the environment. When possible, it would be useful to have information on the tissue specific concentrations of TCDD-EQ. We have developed such values for birds (based on chickens and field observations) fish (based on a long-term feeding study with rainbow trout) and mink (based on a long-term feeding study with carp from the Great Lakes).

### **Question 4**

There is little information on the effects of TCDD on wildlife species. Studies are difficult to reconcile one with the other because animals were exposed in different ways via different routes, for different periods of time. Here I give a little information on the effects of TCDD on birds of the Great Lakes region. We have also conducted studies with fish and mink. This information will soon be published, but for now is given as briefing documents in Appendix I and Appendix II.

### **p-PCH Effects on Wildlife in the Great Lakes Region**

The greatest weakness of all ecotoxicological wildlife studies when considering cause and effect relationships is that all such work is necessarily correlational and non-experimental. No single chemical cause can be isolated in wildlife and tested for effects, as it is done in laboratory situations that can control variables or test each variable singly or in combination. Ideally, one would apply Koch's postulates to wildlife contamination problems in the search for cause-effect relationships (Table 5). However, for studies of wildlife under field conditions this would be difficult. This is true because it is difficult to conduct controlled laboratory studies with wildlife species and it is difficult to conduct studies with sample sizes which are large enough to allow sufficient statistical power to test hypotheses about effects, such as deformities. Also, it is difficult to conduct laboratory studies of known exposures to the same complex mixtures to which wildlife is exposed under

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field conditions. We have conducted several studies with animal models, which have been fed fishes from the Great Lakes, in an attempt to simulate more natural exposures. However, the logistics for such studies are difficult. Thus, the task is to correlate and then compare to other similarly and differently contaminated populations and species. The best that can be achieved is to consider the weight of evidence and reconcile effects observed in wild populations with controlled laboratory studies done with animal models. These studies can be made more powerful by using both *in vivo* and *in vitro* studies of complex mixtures or fractions into which certain p-PCH have been isolated or enriched. Fox<sup>(161)</sup> has formalized the six criteria to be used in determination of the validity of ascribing chemical causes to effects in epidemiological studies of wildlife populations. These include consistency of observations, strength of the association, specificity of the association, time sequence, coherence, and the predictive power of the relationship. On these bases, an informed judgment can be made that p-PCHs have an influence on populations of wildlife species with a great degree of certainty.<sup>(8,24,69)</sup> Here we present what we feel is the strongest information which supports the hypothesis that the p-PCBs are the most likely cause of the observed effects on colonial water birds in the Great Lakes region of North America.

All of the symptoms observed in colonial birds of the Great Lakes region are known to be caused by the p-PCH. Thus, even though some of the details of the mechanisms of action remain undescribed, the widespread common effects of the p-PCH on wildlife worldwide, and especially in areas of greatest exposure to p-PCH, suggest largely additive effects of these substances on exposed wild populations. While these phenomena are not yet studied at the ecosystem or community level, it seems likely that at least some of the recent decreases in sizes of populations, extinction's, or shifts in species dominance are at least influenced, if not caused in their entirety, by p-PCHs. For example, in the Great Lakes region, the smaller tern species, such as the common (*Sterna hirundo*), Forster's (*S. Forsterii*), and black (*Chilodoniast niger*) were once more abundant in the region. However, in the last 30 years populations of all three species have decreased and now each is listed as a threatened or endangered species in one or more states. Gulls and cormorants which have larger body sizes and thus lesser energy requirements per unit body weight are more tolerant to dioxin-like planar contaminants and their populations have continued to increase since the concentrations of DDE have decreased to below critical levels.<sup>(67,107)</sup> It is possible that the interspecies dynamics and wildlife populations balance on the Great Lakes are affected as much by contaminants as other traditional habitat parameters. The p-PCHs are prime suspects in these phenomena. These selective effects are subtle and may be difficult to separate from other dynamics of the ecosystem.

**GLEMEDS**

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Of all of the adverse effects observed to occur in colonial water birds of the North American Great Lakes region, the most obvious and that which can be most directly related to survival of individuals and population-level effects are embryo lethality and developmental deformities. Most of the embryos or chicks, which die during early development have been observed to also have developmental deformities,<sup>(8,23,24,107)</sup> particularly abnormalities which are of ectodermal origin.<sup>(162)</sup> One of the best documented abnormalities which has been correlated with concentrations of p-PCH in bird eggs is the crossed-bill syndrome (Fig. 8) in North American cormorants.<sup>(24)</sup> This suite of conditions found in Great Lakes wildlife has been named the GLEMEDs syndrome (Great Lakes Embryo Mortality, Edema and Deformity Syndrome)<sup>(8)</sup>; it mimics chick edema disease caused in offspring of hens exposed to PCDD and PCDF in their feed.<sup>(27)</sup> The effects observed in birds are similar to those observed in mammals which were exposed to these chemicals.

The few available case studies illustrating the effects of these chemicals are the reports of the Canadian Wildlife Service on studies performed on herring gulls living on Lake Ontario during the 1970s, particularly between 1974 and 1977. In the late 1960s, anecdotal evidence circulated among field biologists of poor egg hatchability of Lake Ontario herring gull eggs.<sup>(12)</sup> Official Canadian Wildlife Service surveys began in 1971. Hatchabilities of less than 20% were found at some colonies in Lake Ontario. Productivity was reduced to less than one fledged young per ten nests. Maintenance of a stable population requires fledging rates in the range of 5-6 chicks per ten nests per year for this species. Initial examination of herring gull eggs and eggs of other species in Lake Ontario documented the presence of DDT and PCBs. However, analytical techniques at the time were insufficient to discriminate among dioxin, furan and p-PCB congeners or to quantify some of the more toxic p-PCB congeners. Reliable congener-specific chemistry was a decade away. The characteristic symptoms in surviving chicks were similar to those of chick edema disease, which is caused by PCDDs in poultry and had been previously described.<sup>(27)</sup> Subsequent reanalysis in the late 1980s of a variety of eggs which had been collected from herring gull colonies in Lake Ontario in the 1971-1976 period and archived contained 1-3 ppb of actual TCDD. This TCDD is thought to have been discharged into the Niagara River as a result of herbicide manufacture.<sup>(163)</sup> This chemically-caused epizootic in Lake Ontario is probably the best documented example of *dioxin-caused* effects on wildlife. During the last decade, the symptoms of chick edema disease and GLEMEDs have decreased significantly in the herring gull population of Lake Ontario,<sup>(8)</sup> but more subtle, biochemical effects persist all species of fish-eating colonial water birds of Lake Ontario and the other Great Lakes.<sup>(24,69)</sup>

Similar studies of nesting Forsters terns, conducted in Green Bay from 1983 to 1988,<sup>(10,23,164)</sup> and double-crested cormorants and Caspian terns<sup>(107)</sup> have revealed a similar suite of biological effects, but implicate different p-PCHs than TCDD as probable causes. In the Green Bay experience, Kubiak *et al.*<sup>(10)</sup> found a variety of developmental deformities in the embryos and chicks of Forster's terns including

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growth deficiencies, deformities and behavioral differences in parental care of eggs compared to an inland control colony where exposures were significantly less than on Green Bay. Extrinsic adult behavioral abnormalities of inconsistent incubation led to a four day longer incubation period than the reference colony. Reciprocal transplant studies of eggs a similar time delay ascribed to toxic substances in the eggs. That study suggested widespread, complex contaminant effects on the reproductive cycle, such as longer incubation times, smaller individuals, and wasting syndrome in those that did hatch. Similar, but less acute problems, have been observed in double-crested cormorants and Caspian terns in the upper Green Bay area, where TCDD-EQ calculated from concentrations of p-PCH and TEFs, from 175 up to over 440 ppt have been measured.<sup>(23,107)</sup>

A study of Caspian terns in Saginaw Bay following the disturbance of sediments in a flood incident has documented concentrations of p-PCB in eggs which was similar to those observed in Green Bay. In this case between 96 and 98% of the TCDD-EQ was due to the presence of only four p-PCB congeners. These were, in order of relative contribution to the total TCDD-EQ, congeners 77, 126, 169, and 105.<sup>(81,107)</sup> The developmental effects observed in Caspian terns at Saginaw Bay were severe. Caspian tern eggs contained doses equal to the lethal concentration for 95% of white leghorn chicken eggs for p-PCB congeners 77 and 126, plus 10% of the LD-50 dose of TCDD. The concentrations of five individual p-PCB congeners reported as TCDD-EQ were more than an order of magnitude greater than that which has been found to cause heart defects in developing chickens.<sup>(165)</sup> Thus, it is likely that the observed concentrations of TCDD-EQ were sufficient to cause the adverse effects which were observed. Even though the dioxin-like p-PCB congeners have relative potencies which are less than that of TCDD, they are hundreds to as much as thousand-fold more abundant in the environment and thus can have toxic effects on the wildlife species.

Egg mortality from locations in 5 geographic regions on and one off of the Great Lakes were found to be directly proportional to the concentration of TCDD-EQ (Fig. 9).<sup>(22)</sup> The current concentrations of both PCBs and TCDD-EQ in cormorant eggs are greater than the estimated no-effect concentrations for this species. The concentrations of TCDD-EQ were determined by the H4IIE assay in double-crested cormorant eggs from several locations in 1986-88.<sup>(22)</sup> The total concentrations of TCDD-EQ, as well as the relative potencies, varied among locations (Table 6, Fig. 6), but was fairly consistent across all of these regions. The death of eggs was found to be directly proportional to the concentration of TCDD-EQ in the eggs (Fig. 9)<sup>(22)</sup> with a significant positive correlation ( $R^2 = 0.703$ ;  $p < 0.0003$ ). These estimates are based on actual doses in the eggs, thus there would be less error in predicting these exceedences than would be expected for ratios, calculated from the WQC, which include more assumptions. The exceedance range was less than when several species were compared, but there was an almost 10-fold difference among locations (Table 6).

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We investigated the relationships between birth defects observed in both Caspian terns and double-crested cormorants at a number of locations in the Great Lakes. For this study we classified and enumerated the number of various types of deformities in both double-crested cormorants and Caspian terns (Table 7; Fig. 10). We found rates of abnormalities that ranged from two to 12 per thousand chicks embryos and chicks examined (Fig. 11). We observed the greatest rate of deformities in double-crested cormorants to occur in Green Bay (Fig. 11). The greatest percentage of deformities of Caspian terns occurred in the colony on the contained disposal facility (CDF) in Saginaw Bay. When the rates of deformities were correlated with the concentrations of TCDD-EQ, we found very strong, statistically significant correlations (Fig. 12). While this observation alone does not make a cause and effect relationship, this correlation is better than that with any of the other contaminants. Concentrations of TCDD-EQ and the relative potencies of the p-PCH mixtures in cormorant eggs varied among locations in the Great Lakes (Figs. 13 and 6), but the greatest concentrations were measured in eggs collected from Green Bay,<sup>(81)</sup> where the greatest rates of deformities and poorest survival of eggs were observed.<sup>(24)</sup>

### Wildlife Hazard Assessments for PCBs

Here we provide a proposed method for conducting wildlife hazard assessments, by using results of field and laboratory studies on target and surrogate domestic species, along with environmental monitoring and chemical analyses, to determine the appropriate water quality criteria (WQC) to protect sensitive wildlife species from the adverse effects of PCBs. We then compare the results of this assessment to an assessment, which used human cancer and reproductive effects as endpoints. We based our hazard assessment on PCBs instead of 2,3,7,8-TCDD, because the PCBs have been found to be the primary source of most of the TCDD-EQ for which concentrations in biota have been determined. We conducted wildlife hazard assessments by comparing the threshold for effect to the concentrations of key contaminants which are currently observed in tissues of fishes that are eaten by domestic species and eggs of wild birds in the Great Lakes basin. The thresholds for effects, termed the lowest observable adverse effects level (LOAEL) were derived from field observations and correlations with concentrations of key toxicants or from controlled laboratory studies where domestic species of interest were fed known quantities of contaminants in Great Lakes fishes. We studied four species of wild birds: herring gulls (*Larus argentatus*), bald eagles (*Haliaeetus leucocephalus*), Caspian terns (*Sterna caspia*), double-crested cormorants (*Phalacrocorax auritus*), and a domesticated surrogate species, white leghorn chickens (*Gallus gallus*)<sup>(173)</sup> (Tables 8 & 9). The no observable adverse effects level (NOAEL) values were defined to be 10% of the LOAEL (safety factor of 10; equation 1) to correct for the uncertainty in determining the NOAEL from the LOAEL. The LOAEL can be reported (Equation 1).

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$$\text{NOAEL} = \text{LOAEL} \times 10^{-1} \quad (\text{Eq. 1})$$

as the reference dose ( $\text{Ref}_t$  = dose in the target tissue), which is defined as a threshold concentration (mg/kg, wet weight or lipid weight) in a tissue such as that of egg, which causes a defined adverse effect, such as egg lethality or birth defects (Table 10). Alternatively, the dose can be given as the daily intake or by making the appropriate assumptions about food consumption, as the concentration in food ( $\text{Ref}_f$  = dose in food). Here, LOAEL values are reported as either the concentration in bird eggs or in the food of chickens (Table 10).

The bioconcentration factor (BCF) was used to predict the concentration of p-PCH in water, which would result in the reference dose in whole fish tissues, which these animals eat (Table 10). The biomagnification factor (BMF) was used to predict the concentration in water that would result in the reference dose in eggs (Table 10).

Water quality criteria to protect wildlife were derived by dividing the NOAEL by the bioconcentration or biomagnification factor (BCF or BMF) for the species of interest (Table 10).<sup>(67)</sup> For piscivorous fishes we did not attempt to separate the proportion of the contaminants observed in the tissues which was derived directly from the water and that which was obtained from the food. We reasoned that in a food chain exposed to chemicals in water, a ratio which related the concentration of the chemical in the fish to that in the water could be derived. This included both vectors of accumulation, because the prey consumed by the predatory fish would also be in steady state conditions with the concentrations in the waters. We note that, in addition to fish, the food web includes other fish-eating wildlife. This fact is usually ignored in setting WQC and thus the BMF of toxic chemicals can often be underestimated. We have calculated the reference dose for water (Table 10) by Equation 2.

$$R_w = R_f / (\text{BAF}_{w-t}) \quad (\text{Eq. 2})$$

Where:

$R_w$  = Reference dose in water  
 $\text{BAF}_{w-t}$  = Bioconcentration factor from water to fish tissue corrected for relative potency and biomagnification from one fish to another.

WQC were then developed by applying the appropriate application factors. The only application factors used in these studies were a 10x factor to estimate the NOAEL from the LOAEL. This factor was applied for the WQC for each species. The final WQC for PCBs was corrected for uncertainty in among-species sensitivities by the application of an additional 10X uncertainty factor. The final WQC for TCDD-EQ was estimated, based on accumulation by eagles, but not corrected for any uncertainty factors.

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It is difficult to determine WQC because of the uncertainty associated with estimates both exposure and the dose-response relationships. Estimates of the factors required to predict the probable accumulation of PCBs or TCDD-EQ from water are particularly uncertain. First of all, the relative proportion of these compounds that are freely dissolved in the water or bioavailable, is impossible to know. Furthermore, the forms of these compounds is continuously changing and never at steady state, which are the conditions under which most predictions are made. We did not use a correction factor for the bioavailable fraction of PCBs or TCDD-EQ. We made the conservative assumption that all of the PCB or TCDD-EQ were bioavailable. In reality, the bioavailable fraction is probably from 1 to 10% of the total. For this reason, there is probably at least a 10 to 100-fold safety factor in our estimates of NOAEL. Since it is difficult to know what portion of the PCB or TCDD is available at any given time and that when the equilibrium between readily available and bound PCB or TCDD-EQ such that there is a continuous source of these compounds in the bioavailable fraction, we chose to not correct for this factor. The concentrations of PCBs measured in the dissolved and particulate fractions of the Great Lakes (Table 11) were positively correlated with the total concentrations of PCBs in the eggs of double-crested cormorants. This indicates that regardless of the relative available fraction the concentrations in the eggs of fish-eating birds are proportional to the concentrations of PCBs in the water.

Furthermore, since the BCF and BMF values used in our assessment were derived from field observations, they are more likely to be apparent BCF and BMF values, which take into account the bioavailable fraction.

The values reported for bioaccumulation factors are influenced by bioavailable fraction as well as the physiology and food habits of the species for which bioconcentration or biomagnification factors are to be predicted. Laboratory studies with fishes, generally do not include the accumulation of p-PCH from food and thus must be corrected for what would be expected to be accumulated from food, if water exposures are used to predict the BCF.<sup>(179)</sup> Better estimates of biomagnification factors (BMF) are available, based on field observations. For an accurate estimate of the exposure from consumption of contaminated food, in the absence of observations of the actual concentrations in food, site-specific estimates of accumulation potentials are needed. Ideally, the exposure dose, expressed as mass of toxicant, per unit mass of organisms per unit time should be known. For instance pg of TCDD-EQ/g, bw/day. For these estimates, in addition to knowledge of the concentrations of the contaminant of interest in each of the dietary items, one would need to have an estimate of the proportion of each item taken in the diet and a conversion value for accumulation efficiency for each compound from each food item. At this point in time this level of resolution can not be attained. Therefore, we used average values for accumulation and assumed that the efficiency was the same from all types of food and that the concentrations in food were uniform. Furthermore, we have assumed that the diet consists solely of food items with the specified concentration of toxicant

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of interest. These assumptions can clearly add uncertainty to the estimates. When possible, it is more accurate to estimate hazard potential from assessments based on concentrations of contaminants in the tissues of prey. We have taken this approach in our hazard assessment, where possible.

The reference doses of complex mixtures to wildlife are difficult to relate to exposures in food or water. The information on toxicity, reported in the literature is often based on single exposures and may be via injection. Thus, it is difficult to determine the effects of longer-term, continuous exposures in food. For this reason, we have, where possible used the results of feeding studies with great lakes fishes. These exposures are confounded by the fact that there are complex mixtures of multiple toxicants in the fishes which could influence the response to PCBs or TCDD-EQ. We have chosen to base the hazard assessments on reproductive endpoints, since it is thought that these should be as or more sensitive than effects on adults. For birds we have, thus, based the hazard assessments on the concentrations of PCBs or TCDD-EQ in eggs, which have been associated with observable adverse effects. Alternatively, we have related the effects levels to doses in food, which was fed during longer-term exposures. The hazard assessment for current conditions are reported as exceedance values, which are the ratio of current water concentrations to the WQC (Equation 3).

$$\text{Exceedance} = [\text{Concentration in water}]/(\text{WQC}) \quad (\text{Eq. 3})$$

Subsequent to completing the hazard assessment for TCDD-EQ, the concentrations of PCBs in fish tissues and bird eggs were corrected for the change in relative potency, due to a greater concentration of TCDD-EQ per unit PCB in the tissues of fishes and bird eggs than technical PCB mixtures (see section on selective enrichment). In addition to the total concentration of PCBs, we conducted a hazard assessment with the concentration of TCDD-EQ.

### WQC for PCBs

We calculated the water quality criterion to protect sensitive species of birds from the adverse effects of PCBs to be approximately 1.0 pg PCB/l (part per quadrillion) (Table 8). In 1986, the year for which the most recent data are available, the total concentrations of PCBs in all of the Great Lakes ranged from 1 to 10 ng/l (pptr) with the greatest concentrations occurring in Lakes Michigan, Ontario and Erie (Table 11). The concentrations of PCBs in the water of all five of the Great Lakes in 1986 exceeded our proposed WQC for all five species studied (Table 12). The degree to which the WQC were exceeded varied from a minimum of 11 for herring gulls living on Lake Superior to a maximum of 1800 for bald eagles living on Lake Michigan (Table 12). The exceedances were the least for all species for Lake Superior and tended to

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be the greatest on Lake Michigan, followed by Lakes Erie and Ontario (Table 12). The exceedence values were calculated for both total PCBs and TCDD-EQ in eggs of double-crested cormorants from seven locations in the Great Lakes (Table 13). In general, the available concentrations of PCB corrected for enrichment by use of the H4IIE enzyme induction bioassay, predicted to be in water were approximately three orders of magnitude greater than the NOAEL. The proposed WQC to protect wildlife from the adverse effect of PCBs range over 5 orders of magnitude (Table 14). The value of 17 pg/l proposed by the Great Lakes Initiative<sup>(180)</sup> is approximately a factor of 10 greater than what we have proposed as a protective value.

### WQC for TCDD

There is a range of sensitivity to TCDD and TCDD-like compounds among different species of birds.<sup>(77)</sup> Effect concentrations of TCDD-EQ in birds range from approximately 10 to 500 pg/g, bw.<sup>(75,76,179)</sup> Clearly, the chicken is a very sensitive species. In fact, when concentrations of TCDD-EQ in the eggs of wild fish-eating birds are compared to the effect concentrations for birds it would be predicted that none of the wildlife species would be able to successfully reproduce in the Great Lakes and that for some species the LC-99 would be exceeded. At the other end of the spectrum, the pheasant.<sup>(26)</sup> Since no direct studies of the toxicity of TCDD on wild, fish-eating birds were available, we used the information on the toxicity of TCDD to chickens to derive a WQC, which should be sufficient to protect all wildlife species (Table 15). We also used estimates of the no effect concentrations in birds from our field monitoring studies (Table 13 and 15).

The WQC was calculated from the bioaccumulation factor and the NOAEL for several species, including the chicken. The product of the BAF used in our hazard assessment was  $4.15 \times 10^4$ . There is a great degree of uncertainty in estimating the accumulation of TCDD from water. In their comprehensive assessment of TCDD Cook *et al.*<sup>(179)</sup> were able to remove some of the uncertainty in estimates of accumulation of TCDD from water to fish by correcting for organic carbon content of the water and lipid content of the animal to which the TCDD was to be accumulated. Unfortunately, there are few estimates of TCDD concentrations in water or in fish tissue, which provide the necessary information to make these corrections. The concentration of TCDD-EQ in forage fish from Lake Huron is approximately 10 pg TCDD-EQ/g, ww, while that in large lake trout is approximately 350 pg TCDD-EQ/g, ww.<sup>(52)</sup> Thus, the BAF from forage fish to predators is approximately 10X. This is also approximately the biomagnification factor (within a factor of 2) for persistent, neutral organic compounds with a molecular weight similar to TCDD. Since fish-eating birds would be more likely to eat forage fish than the large lake trout and the BAF for lake trout was used in the calculations, no correction was made for biomagnification from fish to bird.

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The WQC for TCDD-EQ, based on the white leghorn chicken would be 0.04 pg TCDD-I (Table 15). That for the herring gull, which is known to be more tolerant of the effects of TCDD, was estimated to be 0.3 pg TCDD/l. This level of protection takes into account the potential accumulation of TCDD-EQ into higher trophic levels. For instance, birds which eat larger fish, which have greater concentrations of TCDD-EQ or other birds would not be adequately protected. Specifically, concentrations of TCDD-EQ in eagle eggs from Thunder Bay, Lake Huron have been found to be as great as 2,000 pg TCDD-EQ/g. Since the concentrations of TCDD-EQ in forage fish from this location contained an average concentration of 10 pg TCDD-EQ/g, ww, the BMF from fish to eagle egg would be approximately 200. Thus, the WQC would need to be approximately 200 time less to protect eagles as colonial fish-eating water birds, if the eagles are approximately as sensitive to the effects of dioxins as are the colonial birds. To be sure of the protection of eagles we have selected the chicken as a surrogate. If the WQC to protect chickens is used and a 200 fold BMF is applied, the WQC to protect eagles would be approximately 0.0002 pg TCDD-EQ/l.

The values determined to represent a "low" risk to avian species by Cook et al.,<sup>(179)</sup> were based on the effects on pheasants and made different assumptions about the degree of availability of the TCDD in water. Even though our assessment used different assumptions, the results of our analysis of colonial fish-eating water birds were similar to those predicted by the US EPA (Table 16).

Because of the potential uncertainties associated with determining WQC and because birds accumulate essentially all of their exposure to TCDD-EQ from their food<sup>(187)</sup> to assess current conditions we compared the current concentrations of TCDD-EQ in forage fish from Lake Huron<sup>(52)</sup> to the dietary NOAEL for white leghorn chickens.<sup>(173)</sup> When this was done it was found that the current exceedance value is approximately 17 (Table 17). This is a conservative estimate of the current situation, since larger fish, which could also be taken in the diet contain greater concentrations of TCDD-EQ.

This exercise seems to support the use of the NOAEL calculated from the chicken since the exceedance is in the range that one would expect to see subtle effects on the colonial water birds and more severe effects on birds, such as eagles, which are of a higher trophic level. This is, in fact what is observed: There are subtle effects on survival of embryos and deformities observed in the colonial birds, while eagles, which feed on fish from this location fail to reproduce.

Based on our field observations, the value of 6 pg TCDD-EQ/g ww of fish, which is given as "low risk" by the US EPA hazard assessment<sup>(179)</sup> is probably appropriate for the protection of double-crested cormorants, but would not be sufficient to protect higher trophic levels such as eagles and may not be protective of some of the smaller, more sensitive species of colonial fish-eating birds. We have observed a concentration of approximately 10 pg TCDD-EQ/g, ww of forage fish which is similar to the concentration indicated by the EPA<sup>(179)</sup> to be a small risk to birds. In these areas we have observed adverse effects, such as deformities and

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embryo lethality, but these effects do not seem to be limiting populations of double-crested cormorants. In these areas wasting syndrome has been observed in Caspian terns and the Caspian terns can not reproduce normally in some of these areas.<sup>(52)</sup> Thus, based on our field observations and the hazard assessment, we do not feel that this value would be sufficiently protective of some of the more sensitive species or those, such as eagles, which are a higher level of the food web.

### Water Quality Criteria: Human vs Wildlife Health PCBs

It is difficult to compare WQC developed for humans and wildlife because humans can restrict consumption of contaminated food items while wildlife cannot. For this reason fish consumption advisories that set an allowable quantity of fish that may be eaten from a particular water body are appropriate for protection of human health but not for the protection of wildlife.<sup>(188-190)</sup> Similarly, because individuals consume different quantities of fish establishment of a WQC to protect all human's health is not appropriate for everyone. The WQC to protect fish and wildlife from the effects of TCDD-EQ, which was proposed by the US FWS is 5 pg/l.<sup>(6)</sup>

The WQC to protect humans from the adverse effects of PCBs, proposed by the Great Lakes Initiative is 17- and 3- fold greater (Tables 10 and 14) than that proposed by Swain<sup>(191,192)</sup> to protect humans from the non-cancerous developmental, behavioral and cognitive effects of PCBs. The WQC calculated by Swain<sup>(191,192)</sup> range from 0.6 to 6.0, with a median of 1.0 pg PCB/l (based on the McCarthy visual cognition scale) depending on assumptions of exposure.<sup>(162,192-199)</sup> However, the WQC proposed to protect human health<sup>(180)</sup> assumed that the hazard of cancer was less than that of other non-cancer end points, which can occur in a dose-dependent fashion at very small intrauterine or intra-egg exposures. For instance, concentrations of PCBs between 1.5 and 2.5 ug PCB/kg, wet weight in blood of human umbilical cords has been correlated with subsequent adverse cognitive effects in human infants.<sup>(195)</sup> Greater concentrations of PCBs (7.9-12.9 ug PCB/kg) have been observed in the blood of women who ate an average of 23.5 lb (10.6 kg) of Lake Michigan fish per year and gave birth. The WQC proposed to protect humans<sup>(180)</sup> are similar to the values estimated for the protection of wildlife by Ludwig *et al.*<sup>(67)</sup> These results indicate that if the most sensitive wildlife species are protected, then humans will also be protected from the most subtle effects. Thus, the adverse effects of p-PCH-type compounds on wild species can serve as an early warning system for potential effects in the human population, but only if WQC for wildlife based on real effects in wild populations are included in the regulatory process.

The WQC that we propose to protect wildlife is approximately 40-fold less than that proposed by the Great Lakes Initiative.<sup>(180)</sup> Interestingly, the WQC based on both cancer and non-cancer endpoints for the protection of human health from the effects

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of TCDD are greater than those proposed to protect wildlife (Table 18). This is probably justified, since humans eat less fish in their diet. We feel that the lesser value should be adopted since this would protect both wildlife and humans. Thus, in the case of TCDD, it appears that if wildlife are protected so will humans. Thus, wildlife would be a good sentinel species.

There is on-going controversy over the relative sensitivity of humans to the effects of p-PCH<sup>(200)</sup> including both PCBs<sup>(207)</sup> and PCDDs.<sup>(201-204)</sup> There is even some controversy over the potency of TCDD as a mammalian carcinogen.<sup>(205,206)</sup> This uncertainty is leading to a reassessment of the reference doses and uncertainty factors used in human health hazard and risk assessments *based on cancer* for these types of compounds. It is difficult to conduct controlled studies on the effects of chemicals on humans. For that reason, the chronic effects of chemicals on humans are estimated from short-term, high-level exposures of animal models, which may be more or less sensitive to the carcinogenicity of p-PCH than humans. A safety factor of 10 fold is generally added to the assessment process to correct for among-species differences in sensitivity. This assumes that humans are more sensitive to chronic effects than are shorter-lived animals.<sup>(188,189,207)</sup>

Recent epidemiological evidence and the results of studies of the mechanism and modes of action of p-PCH indicate that humans may be much less sensitive to the *Ah-r*-mediated toxic effects of p-PCH than other species<sup>(200-202,204,207)</sup> This is particularly true for carcinogenesis.<sup>(202-204)</sup> The US EPA has stated that, there is inadequate evidence of carcinogenicity of PCBs in humans".<sup>(208)</sup> We agree. The reason for this greater resistance or tolerance is unknown, but may be related to the interaction of the *Ah-r* with the p-PCH or with the DNA.<sup>(200)</sup> Long-term studies on the effects on humans exposed to p-PCH from accidental or industrial exposure for over 30 years generally have failed to exhibit rates of cancer greater than expected for the population.<sup>(200)</sup> Exposure to p-PCH, such as PCDD, at Seveso, Italy or PCB in electrical workers have not resulted in measurable increases in the rates of cancers in these exposed groups. These observations have stimulated a reassessment of the risk of cancer posed by these compounds to humans<sup>(200)</sup> and a possible change in the proposed reference doses for cancer that could lead to a relaxation of proposed WQC.<sup>(202)</sup> Currently, the standards for environmental concentrations of PCDD in Europe and Japan are 170 and 1,700 times less stringent than those in the United States.<sup>(204)</sup>

If the environmental standards for p-PCH, based on human exposure and the cancer endpoint are relaxed, then the WQC will not be sufficient to protect either wildlife or humans from the non-cancer adverse effects of these compounds. Many subtle effects in humans will be ignored. Currently, the Science advisory Board of the US EPA is reexamining the model that will be used to set environmental standards for p-PCH.<sup>(204)</sup> The first phase of the reassessment will be to develop a better model of human health effects, which is based on the current state of knowledge about the *Ah-r*-mediated mechanism of action of the p-PCH.<sup>(200)</sup> Subsequently, an assessment

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of aquatic ecological risk of p-PCH will be conducted. The results of our analysis indicate that the protection of wildlife species will require more stringent regulations than for the protection of the humans from cancer. We suggest that the protection of wildlife populations and humans from subtle non-cancer effects should be given equal priority to that of protecting human populations from cancer. We have derived water quality criteria, based on the responses of wildlife rather than cancer in humans.<sup>(67)</sup> Our analysis indicates that WQC based on human cancer effects are this is not adequate to protect sensitive, wildlife species. Additionally, the method for deriving WQC has profound implications for remediation, litigation and damage assessments. If wildlife are protected for the most sensitive endpoints, the most relevant of which seems to be reproduction, all components of the ecosystem including human health should be protected adequately.

The proposed WQC are very protective and if these criteria are met we would not expect to see any adverse effects due to these compounds in wildlife. This does not mean that if the proposed criteria are exceeded that adverse effects would be expected to be observed. Also, since the proposed criteria do not consider bioavailability, it would be expected that the actual safe concentration in water could be as much as 10 to 100 times as great without causing adverse effects. Also, the proposed criteria assume that the species of interest consume only the identified prey with a specified BCF or BMF. This too provides some degree of safety. The 14 ng PCB/l of total PCBs, which was suggested in the 1980 WQC document<sup>(209)</sup>, might protect some species of wildlife since we have documented effects at a concentration of less than 2 ng PCB/l in areas of Lake Michigan.

There is a great deal of uncertainty in the risk assessment process (Table 19). WQC developed as we did here are probably no better than  $\pm 10$  to 100X. For this reason it does not seem very worthwhile to argue about the exact WQC, especially, when it will be impossible to directly validate models of concentrations in the water. For compounds, which are already in the environment such as PCBs and TCDD-EQ, we advocate monitoring of wildlife species or their diet. For new compounds not already released into the environment, we advocate a conservative approach to allowed released of bioaccumulatable compounds.

Water quality criteria (WQC) which are used to establish water pollution standards and permissible loadings of substances to public waters can be derived by several methods. These techniques generally involve hazard and risk assessment procedures. For non-persistent, non-biomagnified compounds and elements, WQC are derived from the acute and chronic toxicity to aquatic organisms. For persistent organic compounds which are bioaccumulated and biomagnified, the effects on organisms higher in the food web must be considered. In the Great Lakes region of North America, the primary emphasis has been on the potential for adverse effects to humans who eat fish. The primary endpoint considered in hazard and risk assessments has been cancer. Other endpoints such as teratogenicity, intellectual

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performance, and immune-suppression and reproductive impairment are seldom considered. By hazard and risk assessments, regulators have endeavored to predict safe concentrations of potentially toxic chemicals which could be allowed to enter the environment. Once hazardous materials have entered the environment, these same criteria can be used as target values to determine if environmental damage has occurred, and the degree of remediation required to restore an ecosystem. For aquatic environments, these take the form of water quality criteria (WQC). These WQC consider many environmental processes, such as dissipation, bioaccumulation, biomagnification, degradation and dilution. For the most widespread and hazardous, synthetic, organic chemicals, these regulatory decisions are preoccupied by potential effects on the health of humans, particularly the risk for additional cancers in the population. Regulatory actions assume that ecosystems have assimilative capacities for persistent chemicals<sup>(8)</sup> and that risk of human cancer is the most relevant and sensitive endpoint. For that reason, long-term effects of persistent, organic chemicals, that have the potential to biomagnify and cause *chronic, population-level* effects on wildlife have received scant attention. Humans have the option of restricting consumption of contaminated food. In fact, most fish consumption advisories are established to recommend a safe quantity of fish to be consumed in some specified time period by a person of average size, age, and sensitivity. On the other hand, wildlife cannot avoid contaminated food supplies and thus are at greater risk.

Cancer in humans may not be the most sensitive endpoint to measure.<sup>(200,210,211)</sup> There are physiological and developmental effects in a number of species, including humans, that are more sensitive, immediate and demonstrative endpoints than cancer.<sup>(8,211)</sup> Subtle effects such as growth retardation<sup>(162)</sup> or altered development,<sup>(193-195)</sup> immune system suppression and elevated rates of disease,<sup>(162)</sup> wasting syndromes,<sup>(212-213)</sup> and behavioral changes in both adults and juveniles<sup>(10)</sup> have been observed in human and wildlife populations exposed to synthetic, p-PCHs such as PCBs. Furthermore, the epidemiological evidence supports the contention that human in the Great Lakes basin are expressing subtle, chronic effects due to the exposure to PCBs and similar compounds.<sup>(191-192)</sup>

Not only are there endpoints in humans that are more sensitive than cancer, but humans may not be the most sensitive species to the effects of p-PCHs such as PCBs,<sup>(214,211)</sup> PCDDs, and PCDFs.<sup>(205,48)</sup> There are many wild species that may be inherently more sensitive to some classes of contaminants or receive greater exposures because they do not have the varied diets of humans. When wildlife species have been considered in the derivation of WQC, generally only direct, acute effects on aquatic organisms such as death have been considered. Some states in the Great Lakes region have even based their criteria for wildlife only on the water they consume, ignoring the much more important route of exposure for p-PCH represented by consumption of fish. Even though some of the methods for deriving WQC are very complex and include mechanisms to predict chronic effects and differences in

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sensitivities among species, they generally do not include the wildlife species, such as mink, eagles and other fish-eating water birds, that may eat contaminated aquatic organisms exclusively. The currently used risk assessment procedures do not account for exposures to complex mixtures of potentially hazardous compounds, that can cause subtle, long-term, chronic effects on the dynamics of wildlife populations. Typically, these agents cause effects on the offspring of contaminated adults through decreased fecundity or even reproductive failure. These phenomena can affect wildlife populations directly over longer periods of time.<sup>(8)</sup> Documented adverse effects in wildlife populations are consistent with the types and frequencies of effects observed in laboratory studies of the effects of p-PCH. These effects have been observed in fish,<sup>(215)</sup> birds,<sup>(8,22,210)</sup> turtles,<sup>(216)</sup> and mink<sup>(217)</sup> and humans.<sup>(191-192)</sup>

The use of non-conventional *real-world* bioeffects and reproductive endpoints to establish LOAEL values and NOAEL for persistent, lipophilic, toxic contaminants will require new testing protocols and data on the reproductive outcomes of serial exposed generations of human and wildlife populations. Although recommended by several authors,<sup>(65,66)</sup> the effectiveness of regulations to protect wildlife, such as birds, have rarely been assessed by conducting field studies. Regulations have generally been set based on modelled expected results. The longitudinal studies of behavioral effects of contaminants on children of parents who have been exposed to PCBs through eating contaminated Great Lakes fishes is providing some of these data for humans.<sup>(193-195)</sup> Long-term studies of herring gulls in the Great Lakes region have produced a twenty-year long record of bioeffects which have been linked to chemicals.<sup>(169)</sup> Similar studies conducted for shorter periods of time have demonstrated the same types of effects in other species of colonial, fish-eating water birds.<sup>(22,68,161)</sup>

### Question 5

Our research indicates that this assumption is correct and that there is a fairly steep dose-response curve for TCDD in the species which we have worked. This makes it difficult to relate tissue concentrations of TCDD to responses (adverse outcomes) at a particular geographic region. However, as was described above, when these relationships are examined over greater geographic regions, then relationships can be observed.

### Question 6

As discussed above, we need to use the best estimates available. Our research has indicated that there are observed adverse effects which can be related to concentrations of TCDD-EQ in tissues that are currently occurring at exposures which the Interim document indicates would be of little risk. The use of the pheasant as a

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model species to protect birds at the top of the food web is probably inappropriate.

### **Question 7**

It is true that there is limited information on marine organisms, but there is some limited information of the effects of TCDD and similar compounds on seals. This area could certainly use more research. See reviews in *Ambio*, Vol. 21 Number 8 1992 and The *Journal of Toxicology and Environmental Health*, Vol 33, Number 4. See information discussed above.

### **Question 8**

It is true that there is a great deal of uncertainty in the estimation of exposure to TCDD. This is due to several factors. First, it appears that can be adverse effects form exposure to water at concentrations, which are currently below detection limits. The available fraction is an important parameter, which has a great impact on the results of risk assessments and is difficult to predict or measure empirically. Furthermore, the forms in which TCDD occurs can change over time. Simply because a small fraction of the total is not available, does not mean that it can not be accumulated into target tissues to concentrations which are toxicologically relevant. The fact that the uncertainties are multiplicative results in a great deal of uncertainty in the hazard assessments. It is our opinion that the uncertainty caused by estimations of the exposure are greater than those due to estimates of the dose-response relationships.

### **Question 9**

This statement is misleading. At the lower trophic levels the greatest exposure is from water. At trophic levels above 3 or 4 the proportion obtained from the diet is the greater fraction. For those species, which will have the greatest exposure and thus are exposed to the greatest risk, the primary route of exposure is through the diet.

### **Question 10**

There is nothing special about TCDD, which would preclude modeling it's environmental fate with existing simulation models. Thus there are models available. It is the parameter estimate to be used in the models, which are lacking. There is little

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information on the forms of TCDD in various compartments, which could be used to parameterize models or verify model output.

### Question 11

This issue has been discussed above

### Question 12

There is definitely a lack of information on bioaccumulation and this results in the greatest uncertainty in the risk assessment procedure. For TCDD we propose that wildlife can serve as sentinals and tha tmore effort should be given to assessing the use of integrative models of toxic potency.

#### Wildlife as Environmental Sentinels

The field of *Ecotoxicology* and especially *Wildlife Toxicology* are relatively new fields of endeavor.<sup>(218)</sup> However, even in ancient times humans exhibited an awareness of the condition of birds: Hence the Greek maxim *a bad crow lays a bad egg*.<sup>(218)</sup> Even though there are records of human-caused episodes of toxicological effects in populations of wildlife species from ancient times, only with the wide-spread use of synthetic organic chemicals since World War II have large-scale chemically-induced wildlife epizootics occurred. The ability to describe these effects and to understand the role of toxic chemicals has required developments in a number of scientific fields, including environmental, analytical chemistry and wildlife biochemical toxicology. Limited knowledge of the basic biochemistry, physiology and natural histories of wildlife species has also limited the ability to document and understand the effects of contaminants on wildlife populations. The analytical tools of these multidisciplinary fields are providing a comprehensive picture of the effects of trace concentrations of toxic, synthetic hydrocarbons in wildlife species.<sup>(8,89,161)</sup> The study of effects of toxic chemicals on wildlife populations is limited by the complexity of a large number of species interacting with each other as well as their natural habitat and human-caused physical changes to their environment along with the effects of synthetic organic chemicals. However, through the efforts of multidisciplinary research teams of experts in environmental chemistry, chemodynamics, toxicology, biochemistry, pathology and ecology rapid progress is being made. The ability to establish the cause-effect relationships between concentrations of chemicals in complex mixtures with adverse effects and to be able to predict the fates and effects of these chemicals in the ecosystem is developing rapidly.<sup>(161)</sup>

In our efforts to investigate the linkages between certain, synthetic halogenated

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chemicals in the Great Lakes and effects on populations of wild birds we have followed Koch's postulates (Table 5). To date, we have observed adverse effects, including egg mortality and deformities in chicks. These effects have been correlated with the concentrations of several compounds, but the strongest correlations are with the concentrations of TCDD-EQ. The types of effects observed are the same as those that can be caused by these compounds in laboratory studies of animal models. Furthermore, the concentrations required to elicit the responses are in the same range that would be expected from laboratory studies. Thus, we feel as if we have completed the first four of the postulates. It is likely that the effects observed are due to the TCDD-EQ contributed by PCDD, PCDF and p-PCBs. This conclusion is further supported by the fact that when the p-PCH fraction is removed by selective carbon column chromatography we were able to remove the fraction that caused the induction in the H4IIE bioassay.

We have not completed the fifth postulate *in vivo* because it is difficult to conduct studies of this type in the laboratory with the same species that occur in the wild. This is because of many logistical problems, such as the fact that an uncontaminated source of organisms. Even if uncontaminated wildlife species were available, our techniques to rear them in the laboratory are not sufficiently well developed to allow valid laboratory comparisons. Instead, we have fed fish from the Great Lakes to chickens.<sup>(173)</sup> The results of these studies indicated that the same types of effects observed under field conditions could be induced in these species under laboratory conditions. Finally, relative to studies of the potential for these compounds to cause deformities, it is difficult to demonstrate statistically significant effects due to the small rates of deformities observed and the small sample sizes that are possible in laboratory studies.

A method for conducting wildlife hazard assessments that uses results of field and laboratory studies on target and surrogate domestic species, along with environmental monitoring and chemical analyses, to determine the appropriate WQC to protect sensitive wildlife species from the adverse effects of p-PCHs has been developed.<sup>(67)</sup> When compared to an assessment, which used human cancer risk assessments and visual cognition and memory effects as endpoints, the WQC to protect humans and wildlife were found to be similar in magnitude.<sup>(192)</sup>

When possible it is best to use actual concentrations of PCBs or TCDD-EQ in tissues of fish or birds or their eggs, as the most proximate measure of exposure. If this is not possible to make measurements directly on the target organisms, the next best estimate of exposure is to measure the concentrations of compounds in their food. Use of concentrations in water is the least accurate estimate of exposure and thus includes more safety factors to assure protection of all species from any adverse effects.

Here, we have established that the types of effects observed in wildlife populations are similar to those observed in mammals, which are used as models of the potential effects on humans and that environmental criteria to protect wildlife are

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similar to those predicted to protect human health. The allowable effects in wildlife are greater than those in humans, but their exposure is greater, since they can not restrict their intake. The result is that if wildlife species, such as birds, which are at the top of the food chain are protected, it is likely that human health in the same ecosystem will also be protected. We do not imply that if effects are observed in wildlife, there will necessarily be similar effects in humans. Conversely, if wildlife are protected from adverse effects due to environmental exposures to contaminants, it is likely that humans will be protected from the same sources of exposure. However, it must be remembered that there may be other significant exposures to toxicants, such as household and occupational exposures. Thus, while wildlife biomonitoring can not protect humans from all exposures, monitoring their responses can be a useful procedure. For this reason, wildlife, especially colonial water birds of the Great Lakes region have been advocated as sentinels of environmental exposures.<sup>(2,15,69,213,215)</sup>

### **Question 13**

I do not think that the use of the BAF from Lake Ontario is appropriate unless there is no other information on which to base the risk assessment. It can be used as a starting point and the uncertainty introduced by using this value assessed with sensitivity analyses.

### **Question 14**

Our results (see briefing package on the effects of TCDD on rainbow trout) indicates that the BMF between fish and their food was relatively small (see table on BMF values in Appendix I). Values for colonial fish-eating water birds seems to be approximately 30X from food (fish) to egg<sup>(52)</sup>. The apparent accumulation factor for TCDD-EQ from forage fish into eagle eggs is approximately 200. However, this is probably due, in part to the fact that eagles take birds as well as fish in their diet.

### **Question 15**

The greatest uncertainties in the risk assessment procedure are involved in the estimation of accumulation from water or sediment. We estimate this uncertainty to be approximately 1000X while that from within and among-species sensitivity is only approximately 10X to 100X. Even the biomagnification factors from fish to birds is only a factor of approximately 2X.

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### **Question 16**

**Yes, this would be an adequate method to assure protection of other species. See the discussion above of the relationship between WQC based on different species and endpoints. Based on our research, the WQC to protect rainbow trout from any adverse effects would be approximately 0.02 ppq. Thus, if eagles were used in the hazard assessment rainbow trout would also be protected, since the value to be protective of eagles is approximately 10X less. It is difficult to compare the results of our studies with rainbow trout to those with lake trout, which seem to be more sensitive, but by comparing the concentrations in eggs that cause effects in these species, it is probably safe to say that the lake trout are approximately 10X more sensitive than rainbow trout. Thus, the value to protect eagle would also be protective of lake trout.**

### **Question 17**

**As discussed above, the models to predict concentrations of TCDD in fish and their predators are limited and uncertain. One method to increase the predictability would be to use semi-permeable monitoring devices (SPMDs) to predict concentrations in fish before operation of the mill. Subsequent monitoring should focus on fish and/or SPMDs or a calibrated SPMD.**

### **Question 18**

**They seem to be the best available, but as is indicated in the briefing document, they are of limited utility and will result in exposure estimates, which have an uncertainty of at least 100X to as much as 1000X.**

### **Question 19**

**The best that could be done would be to use a loading simulation model coupled to sediment dynamics models. This type of modeling has been used to understand the sources and sinks of PCBs in the lower Fox River in Wisconsin, but the information required for the model has required six years and some \$10,000,000 to obtain. These type of modeling efforts are very site-specific. Thus the techniques can be used, but the data is missing.**

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Table 1. Potential causes of deformities observed in embryos and chicks of the Great Lakes region.

Nutritional deficiencies due to changes to prey base
New generation pesticides
Continued effects of traditional contaminants
Disease, such as viral infections
Genetic inbreeding
Old, persistent Pesticides
Unidentified chemicals, pulp and paper industry

**Table 2. Effects observed in birds of the Great Lakes region.**

<b>Eggshell thinning</b>
<b>Deformities</b>
<b>Tumors</b>
<b>Behavioral Changes</b>
<b>Immune suppression</b>
<b>Edema</b>
<b>Cardiovascular hemorrhage</b>
<b>Hormonal changes</b>
<b>Enzyme induction P4501A1 and P4502B1</b>
<b>Metabolic changes, Wasting syndrome</b>
<b>Depletion of vitamin A</b>
<b>Porphyria</b>

Table 3. Mean, relative proportions of four planar PCB congeners in technical Aroclor mixtures and bird and fish tissues. Reprinted with permission from Jones et al.<sup>(102)</sup>

Sample	Total PCB (mg/kg)	Relative Proportion of Congener(%)			
		77	105	126	169
Bird Tissues <sup>102</sup>	4.58	2.28	3.62	0.03	0.00141
Chinook Salmon Lake Michigan <sup>110</sup>	1.0	0.22	2.42	0.08	---
Aroclor 1242 <sup>95</sup>	---	0.52	---	0.0020	---
Aroclor 1248 <sup>95</sup>	---	0.61	---	0.0062	---
Aroclor 1254 <sup>95</sup>	---	0.06	---	0.0046	0.08-0.00005
Aroclor 1260 <sup>95</sup>	---	0.03	---	0.00080	0.05
Aroclor 1242 <sup>127</sup>	---	0.30	0.42	0.003	---
Aroclor 1254 <sup>127</sup>	---	0.02	5.49	0.003	---
Aroclor 1260 <sup>127</sup>	---	0.001	0.03	0.000	---

**Table 4. Compounds, which may, based on experimental evidence or structure could be expected to have the potential to cause adverse effects through the Ah-r mediated mechanism of action.**

Polycyclic Aromatic Hydrocarbons
Polychlorinated Biphenyls
Polychlorinated Dibenzo-p-Dioxins
Polychlorinated Dibenzo Furans
Polychlorinated Napthalenes
Polychlorinated Dipnenyltoluenes
Polychlorinated Diphenylethers
Polychlorinated Anisoles
Polychlorinated Phenoxy Anisoles
Polychlorinated Xanthenes
Polychlorinated Xanthenes
Polychlorinated Anthracenes
Polychlorinated Fluorenes
Polychlorinated DihydroAnthracenes
Polychlorinated DephenylMethanes
Polychlorinated Phenylxylylethanes
Polychlorinated Dibenzothiophenes
Polychlorinated Quaterphenyls
Polychlorinated Quaterphenyl Ethers
Polychlorinated Biphenylenes
Polybrominated Diphenyl Ethers
Polychlorinated Azoanthracenes

**Table 5. Koch's Postulates.**

1) Observe Effect(s)*
2) Correlate to Cofactor*
3) Isolate Suspected Causative Agent*
4) Identify Suspected Causative Agent*
5) Introduce Suspected Causative Agent and Elicit Effect

\*Indicates that this postulate has been completed.

Table 6. Concentrations of PCBs (total), TCDD-EQ, relative toxic potencies and egg lethality for eggs of double-crested cormorants in the Great Lakes region.

Location	[Egg] Total PCB (ng/kg)	[Egg] TCDD-EQ (ng/kg)	Relative potency <u>ng/kg TCDD-EQ</u> mg/kg PCB (ng/mg; ppm)	Potency ratio to aroclor 1242	Egg lethality % at 23 days
Pigeon Is. Lk. Ontario	5.5 <sup>(166)</sup>	217 <sup>(168)</sup>	39.5	3.99:1	28 <sup>(169)</sup>
Spider Is. Green Bay	6.5 <sup>(166)</sup>	337 <sup>(168)</sup>	51.9	5.34:1	37 <sup>(170)</sup>
Little Gull Is. Green Bay	7.3 <sup>(167)</sup>	277 <sup>(168)</sup>	37.9	3.83:1	34 <sup>(171)</sup>
Gull Is. N. Lk. Michigan	6.7 <sup>(167)</sup>	175 <sup>(168)</sup>	26.1	2.64:1	27 <sup>(171)</sup>
St. Martins Lk. Huron	5.7 <sup>(167)</sup>	145 <sup>(168)</sup>	25.4	2.57:1	20 <sup>(171)</sup>
Taquamenon Is. Lk. Superior	3.5 <sup>(167)</sup>	146 <sup>(168)</sup>	41.7	4.21:1	19 <sup>(171)</sup>
Lk. Winnepegosis Manitoba	0.9 <sup>(166)</sup>	35 <sup>(168)</sup>	38.5	3.89:1	8 <sup>(171)</sup>
Mean potency	---	---	37.7:1	3.77:1	---

**Table 7. Common congenital deformities observed in colonial fish-eating, water birds of the Great Lakes.**

<b>Crossed Bill</b>
<b>Clubed Foot</b>
<b>Hip Displasia</b>
<b>Dwarf Appendages</b>
<b>Ascites/Edema</b>
<b>Eye Deformities</b>
<b>Brain deformities</b>
<b>Skull Bones</b>
<b>Gastroschisis</b>

**Table 8. Lowest observable adverse effect levels (concentrations) of polychlorinated biphenyls (PCBs) in bird eggs or fish tissue for wildlife species inhabiting the Great Lakes region.**

Species/Location	Adverse Effect	PCB-LOAEL (mg/kg) (wet wt.)	Reference
bald eagles Great Lakes Territories	egg lethality	4.0	(172)
herring gulls Great Lakes	embryonic deformities egg lethality	5.0	(169)
Caspian terns Saginaw Bay, 1988	egg lethality 21% embryonic deformities	4.2	(23)
double-crested cormorants Lake Superior, 1986-1991	egg lethality twice as great as control	3.5	(22,23)
White leghorn chickens MSU Lab. Feeding study	embryonic deformities 42% greater than control	0.294	(173)

Table 9. Concentrations of 2,3,7,8-TCDD-EQ to cause effects in wildlife species.

Species	Endpoint	TCDD-EQ Concentration (pg/g, pptr)
Herring gull-Field <sup>(174)</sup>	LD <sub>50</sub> Egg	2,000 <> 1,000
Double-crested cormorant-Field <sup>(171)</sup>	LD <sub>100</sub> Egg	1,029
White leghorn chicken-Lab <sup>(30,76)</sup>	LD <sub>100</sub> Adult	1,000
Caspian tern-Field <sup>(171)</sup>	LD <sub>50</sub> Egg	750
Herring gull-Field <sup>(168)</sup>	LD <sub>19</sub> Egg	557
Double-crested cormorant-Field <sup>(22)</sup>	LD <sub>50</sub> Egg	460
Caspian tern-Field <sup>(171)</sup>	LD <sub>35</sub> Egg	416
Double-crested cormorant-Field <sup>(166)</sup>	LD <sub>37</sub> Egg	344
Double-crested cormorant-Field <sup>(171)</sup>	LD <sub>27</sub> Egg	217
White leghorn chicken-Lab <sup>(165)</sup>	LD <sub>50</sub> Adult	140
White leghorn chicken-Lab <sup>(169)</sup>	200% increase in heart defects	65
Double-crested cormorant-Field <sup>(165)</sup>	LD <sub>8</sub> Egg	35
White leghorn chicken-Lab <sup>1</sup>	LOEL for heart defects	6.4

Table 10. Reference doses and bioconcentration factors used to calculate WQC for PCBs to protect Great Lakes wildlife species.

Species (Tissue or diet)	Reference dose (NOAEL or LOAEL $\times 10^{-1}$ ) (mg PCB/kg wet wt.)	BCF <sub>c</sub> <sup>1</sup> or BMF <sub>c</sub> <sup>2</sup>	Water Quality Criterion (pg/l, ppq)
bald eagle (Egg)	4.0 $\times 10^{-1}$	4.0 $\times 10^8$ (BMF <sub>c</sub> )	1.0
herring gull (Egg)	5.0 $\times 10^{-1}$	1.6 $\times 10^7$ (BMF <sub>c</sub> )	31.0
Caspian tern (Egg)	4.2 $\times 10^{-1}$	2.4 $\times 10^7$ (BMF <sub>c</sub> )	17.0
double-crested cormorant (Egg)	3.5 $\times 10^{-1}$	1.5 $\times 10^7$ (BMF <sub>c</sub> )	23.0
white leghorn chicken (Diet)	2.9 $\times 10^{-2}$	1.6 $\times 10^7$ (BCF <sub>c</sub> )	2.0

<sup>1</sup>BCF<sub>c</sub>=BCF from water to fish corrected for selective enrichment of non-ortho-substituted PCB congeners.

<sup>2</sup>BMF<sub>c</sub>=Biomagnification factor from fish to bird egg corrected for selective enrichment.

Table 11. Concentrations of PCBs in waters of Great Lakes in 1986.

Location	Dissolved (ng/l, ppt)	Particulate (ng/l, ppt)	Unfiltered Water (ng/l, ppt)
Lk. Superior	0.5 <sup>(175)</sup>	---	0.337 <sup>(176)</sup>
Lk. Michigan	1.4 <sup>(177)</sup>	0.6 <sup>(177)</sup>	1.8 <sup>(178)</sup>
Lk. Huron	0.7 <sup>(177)</sup>	0.3 <sup>(177)</sup>	0.631 <sup>(175)</sup>
Lk. Erie	0.7 <sup>(177)</sup>	0.3 <sup>(177)</sup>	1.378 <sup>(175)</sup>
Lk. Ontario	0.6 <sup>(177)</sup>	0.3 <sup>(177)</sup>	1.41 <sup>(175)</sup>

Table 12. Exceedance<sup>1</sup> values for total concentrations of PCB for four species in the Great Lakes region if they consumed fishes of average PCB content from each of the five Great Lakes.

Lake	Eagle	HG <sup>2</sup>	CT <sup>2</sup>	DCC <sup>2</sup>
Superior	340	11	20	15
Michigan	1800	58	110	78
Huron	630	20	37	27
Erie	1400	45	81	60
Ontario	1400	45	83	61

<sup>1</sup>Exceedance = (PCB in water)/WQC: Exceedance values are based on total concentrations of PCBs for each lake (Table 9) and average concentrations in fish resulting from exposure to this concentration.

<sup>2</sup>HG = Herring gull

CT = Caspian tern

DCC = Double-crested cormorant

Table 13. Exceedance values for total PCBs and TCDD-EQ in double-crested cormorant eggs.

Location	PCB Exceedance	TCDD-EQ Exceedance
Pigeon Is. Lk. Ontario	15.7	62.0
Spider Is. Green Bay	18.6	96.3
Little Gull Is. Green Bay	20.9	79.1
Gull Is. N. Lk. Michigan	19.1	50.0
St. Martins Lk. Michigan	16.3	41.4
Taquamenon Is. Lk. Superior	10.0	41.7
Lk. Winnipegosis Manitoba	2.6	10.0

Exceedance = [concentration of PCB or TCDD-EQ in egg tissue]/(Ref.)

Table 14. Water quality criteria for PCBs recommended by the Great Lakes Initiative<sup>(180)</sup> based on three different endpoints.

Endpoint	Water Quality Criterion (pg/l, ppq)
Humans Cancer-Drinking Water	3.0
Humans Cancer-Nondrinking water	3.0
Human Health-Fish Consumption	79
Human Health	20
Human Health-Drinking Water	$5 \times 10^5$
Wildlife	$1.7 \times 10^6$
Wildlife	$1.0 \times 10^3$
Wildlife	17

**Table 15. Water quality criteria for TCDD-EQ recommended for the Great Lakes. Concentrations of TCDD-EQ in fishes to protect birds and wildlife are also given.**

<b>Species (Tissue or diet)</b>	<b>Reference dose (NOEL) (ng/kg, wet wt.)</b>	<b><sup>1</sup>BAF</b>	<b>Water Quality Criterion (pg/l, ppq)</b>
<b>herring gull (Egg)</b>	<b>10<sup>4</sup>(168)</b>	<b>4.2x10<sup>4</sup></b>	<b>0.3</b>
<b>Caspian tern (Egg)</b>	<b>7.5<sup>4</sup>(168)</b>	<b>"</b>	<b>0.2</b>
<b>double-crested cormorant (Egg)</b>	<b>4.6<sup>4</sup>(168)</b>	<b>"</b>	<b>0.1</b>
<b>Bald Eagle (Egg)</b>	<b>1.5<sup>(173)</sup></b>	<b>8.4x10<sup>6</sup></b>	<b>0.0002</b>
<b>white leghorn chicken (Egg)</b>	<b>1.5<sup>5</sup>(173)</b>	<b>4.2x10<sup>4</sup></b>	<b>0.04</b>

<sup>1</sup>BAF=Bioaccumulation factor (BCFxBMF)

<sup>2</sup>BCF<sub>f</sub>=BCF from water to fish for TCDD-EQ

<sup>3</sup>BMF<sub>f</sub>=Biomagnification factor from fish to bird egg for TCDD-EQ

<sup>4</sup>LC-50/100

<sup>5</sup>LOEL/10

Table 16. Water quality criteria and fish quality proposed by Cook et al.<sup>(179)</sup> to protect humans and wildlife from adverse effects of TCDD-EQ in the Great Lakes.

Risk	Fish (pg TCDD/g, ww)	Water (pg TCDD/l)	
		POC=0.2 mg/l	POC=1.0 mg/l
High <sup>1</sup>	6	0.07	0.35
Low	60	0.7	3.5

<sup>1</sup>POC= organic carbon content of water

Table 17. Dietary NOAEL, based on TCDD-EQ in fish fed to white, leghorn chickens<sup>(173)</sup>.

Dietary NOAEL (pg/g, ww)	Concentrations of TCDD-EQ in Forage Fish	Exceedance
0.6	10	16.7

**Table 18. Water quality criteria proposed by several governmental agencies for the protection of wildlife and humans.**

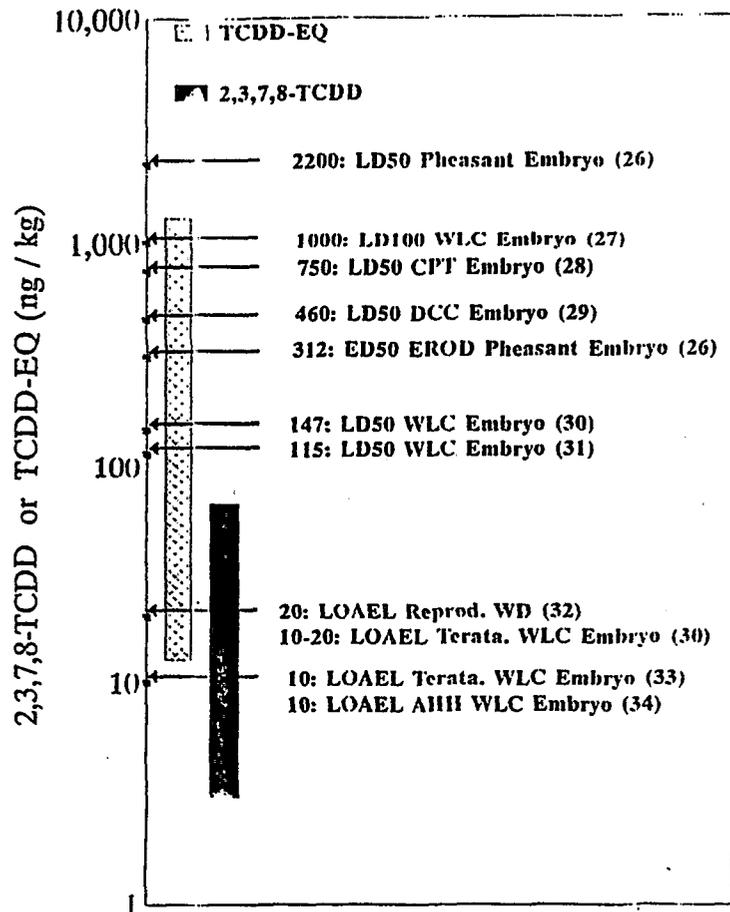
Endpoint	Water Quality Criterion (pg TCDD/l, ppq)	Reference
Human Health-Cancer:Drinking Water	$1.0 \times 10^{-2}$	180
Human Health-Noncancer:Drinking water	$1.0 \times 10^{-1}$	180
Human Health-Cancer:Nondrinking water	$1.0 \times 10^{-2}$	180
Human Health-Noncancer:Nondrinking water	$1.0 \times 10^{-1}$	180
Human Health	$1.3 \times 10^{-2}$	186
Human Health	$1.4 \times 10^{-2}$	186
Wildlife	$8.5 \times 10^{-3}$	180

**Table 19. Uncertainties associated with parameters used in hazard assessment.**

<b>Factor</b>	<b>Range of Uncertainty</b>
NOAEL for a Species	10X
Species Sensitivity	10X
Lipid Content	5X
Trophic Level	2X
Available Fraction	10 - 100X
BMF	3X
BCF	10 - 100X

**Figure 1. 2,3,7,8-TCDD Avian Toxicological Effects Thresholds.** The hatched and solid bars represent the relative range of concentrations (pg/g or ng/Kg) of TCDD-EQ and 2,3,7,8-TCDD, respectively, that are currently found in the eggs of colonial fish-eating water birds of the Great Lakes. The arrows indicate various toxicity thresholds for avian species which have been tested in laboratory or field studies. The references are given by number.

## 2,3,7,8-TCDD Avian Toxicological Effects Thresholds



**Figure 1**

**Figure 2.** Concentrations of calculated TCDD-EQ as a function of H4IIE bioassay-derived TCDD-EQ. TCDD-EQ were calculated using TEF values using either Tillitt<sup>(108)</sup> (Squares) or Tillitt<sup>(108)</sup> and Safe<sup>(48)</sup> TEF values (stars). Bioassay TCDD-EQ values were determined using the H4IIE bioassay as described in the text. The dashed line indicates equality between calculated and bioassay equivalents. The upper darker solid line is the linear regression for TCDD-EQ derived from Tillit-Safe TEFs while the lower solid line represents the linear regression for the TCDD-EQ derived with Tillitt TEFs (Reprinted from Jones *et al.*<sup>(102)</sup> with permission).

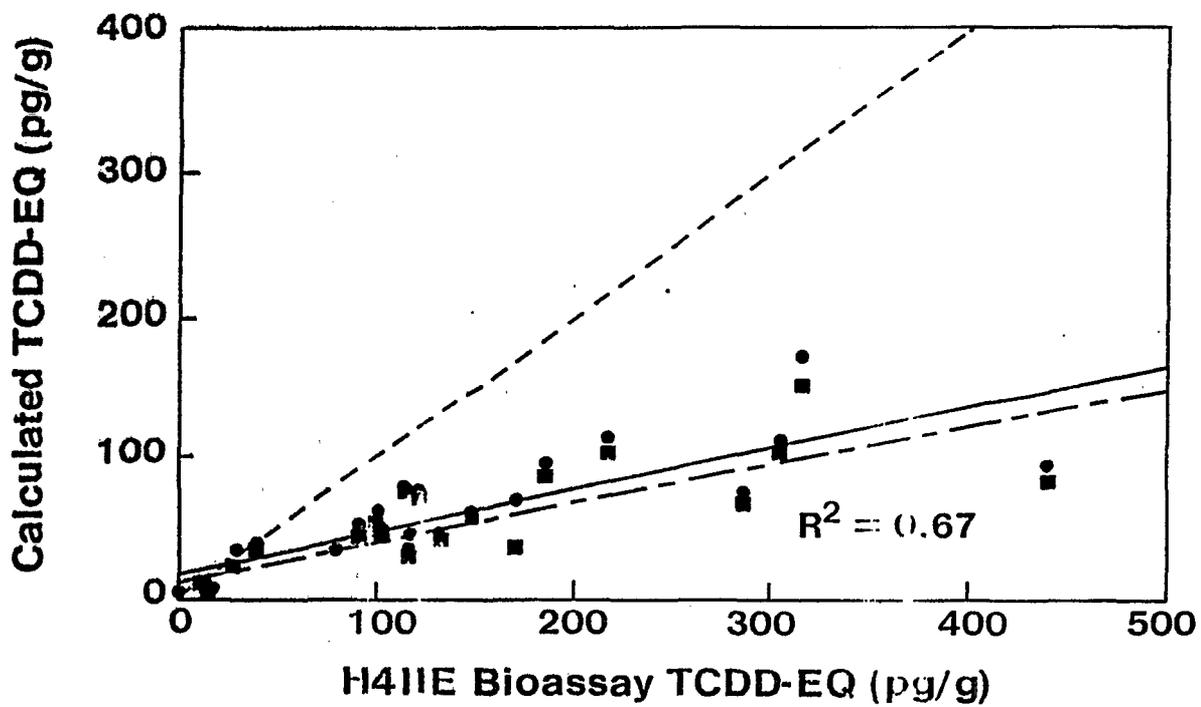
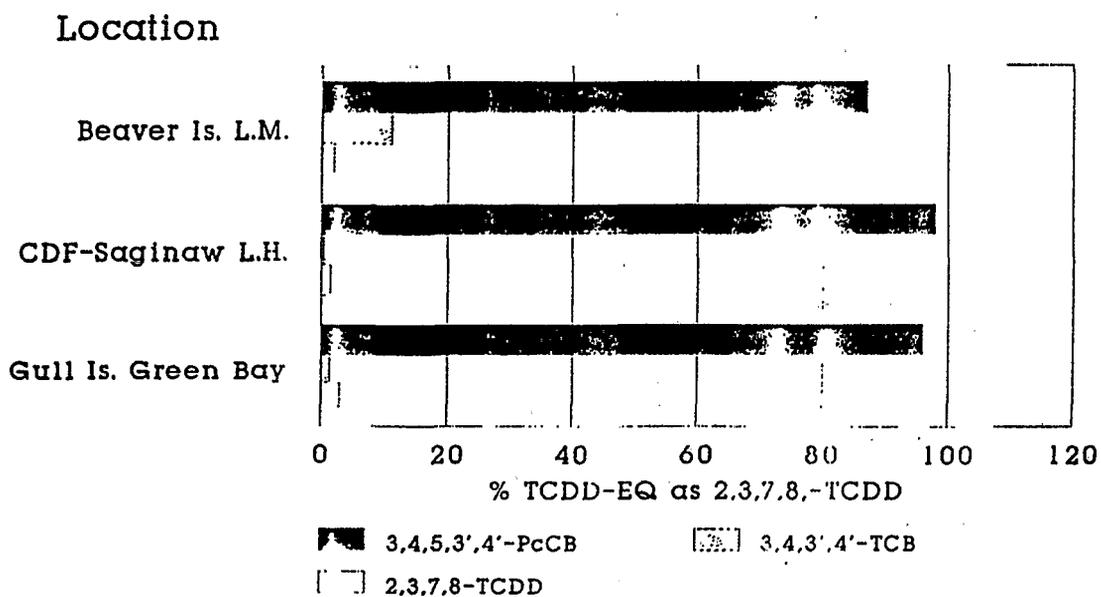


Figure 2

**Figure 3. Relative proportions of total TCDD-EQ predicted from instrumental analyses and TEF (EROD induction) values, which are contributed by the pPCB and 2,3,7,8-TCDD. Samples are of 23-day old Caspian tern eggs. TEFs used were those of Safe<sup>(48)</sup>.**

TCDD-EQ  
23-Day Caspian Tern Eggs-1988



Additive Potency from GC-MS Analyses  
Based on EROD Relative Potency

**Figure 3**

**Figure 4. Relative contribution of specific p-PCH congeners to total concentrations of TCDD-EQ. TCDD-EQs were calculated, using Safe<sup>(48)</sup> TEFs, for individual p-PCH congeners. The contribution of each congener to the total concentration of TCDD-EQ is expressed as a percentage of total calculated TCDD-EQ concentration. Due to the small contributions of equivalents by PCDD and PCDF congeners the TCDD-EQ contributions of all dioxin and furan congeners were combined (D&F). Values represent species means. Abbreviations are; F. Tern; Forster's tern; C. Tern, common tern; R.W.B.Bird, red winged black bird; T. Swallow, tree swallow. The segments for each bar graph are in the same order from left to right as shown in the legend (Reprinted from Jones *et al.*<sup>(102)</sup> with permission).**

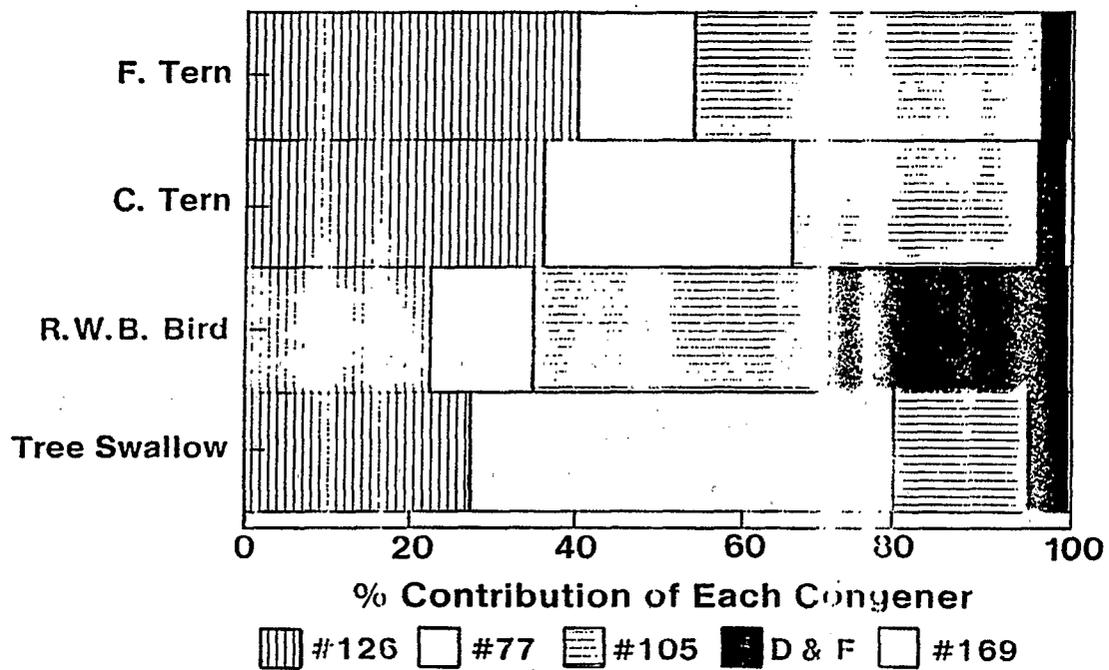
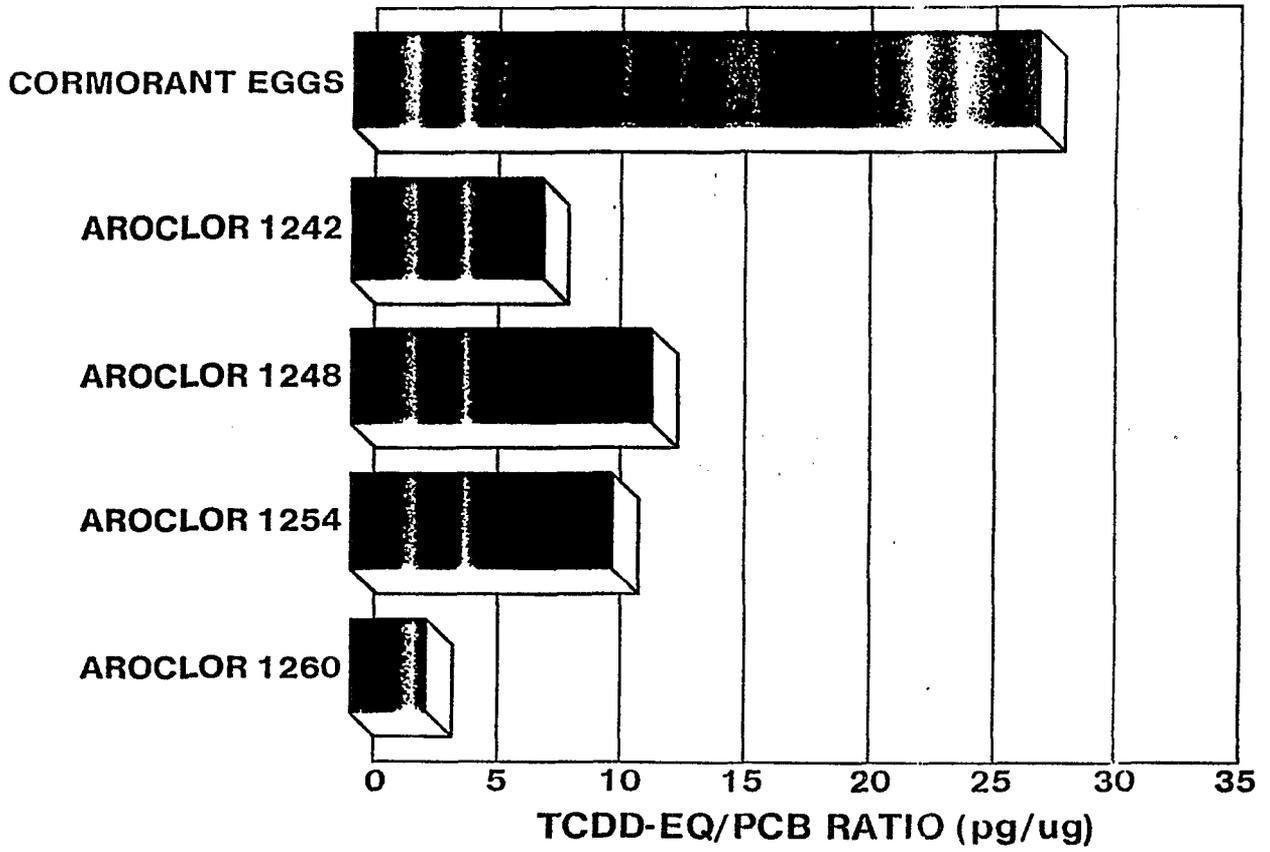


Figure 4

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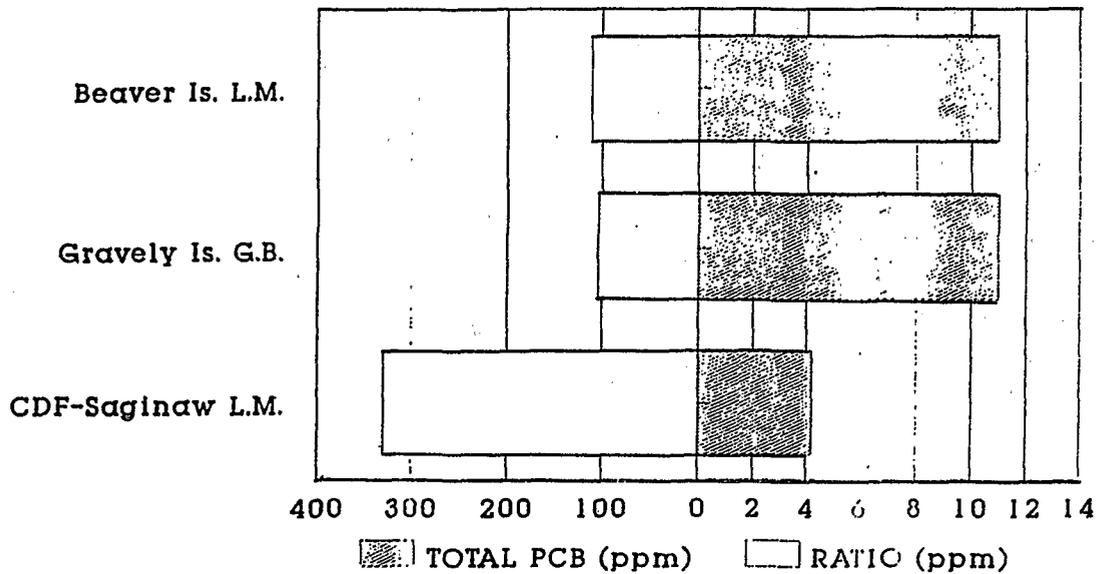
**Figure 5. Relative potencies of technical Aroclor<sup>®</sup> mixtures and extracts of double-crested cormorant eggs. The relative potency is the ratio of the concentration of bioassay-derived TCDD-EQ (ng/kg) to total concentration of PCBs (mg/kg). The resulting ratio has units of mg/kg (ppm) (Reprinted from Tillitt *et al.*,<sup>(22)</sup>).**



**Figure 5**

**Figure 6. Relative potencies and total concentrations of PCBs in Caspian tern eggs from three locations on the Great Lakes.**

# RELATIONSHIP BETWEEN TCDD-EQ AND TOTAL PCBS IN CASPIAN TERN EGGS-1988



TCDD-EQ calculated from GC-MS and EROD induction potencies. Additive model.

Figure 6

## Giesy Briefing Document, Minneapolis, MN Sept 14-15, 1993

**Figure 7. Exceedance values for the effects of total PCBs as a function of the exceedance values in bioassay-derived TCDD-EQ for double-crested cormorant eggs from seven locations in 1988 (reprinted from Ludwig *et al.*<sup>(67)</sup>)**

# PCB-Exc Vs. TCDD-EQ-Exc Double-crested cormorants

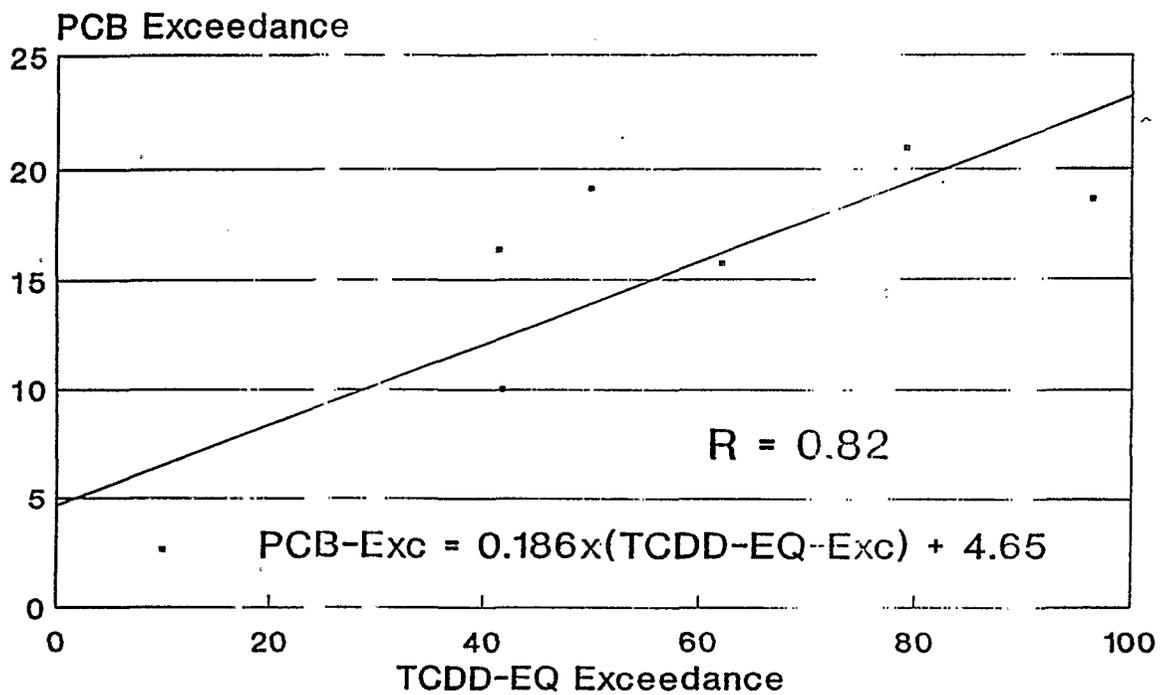
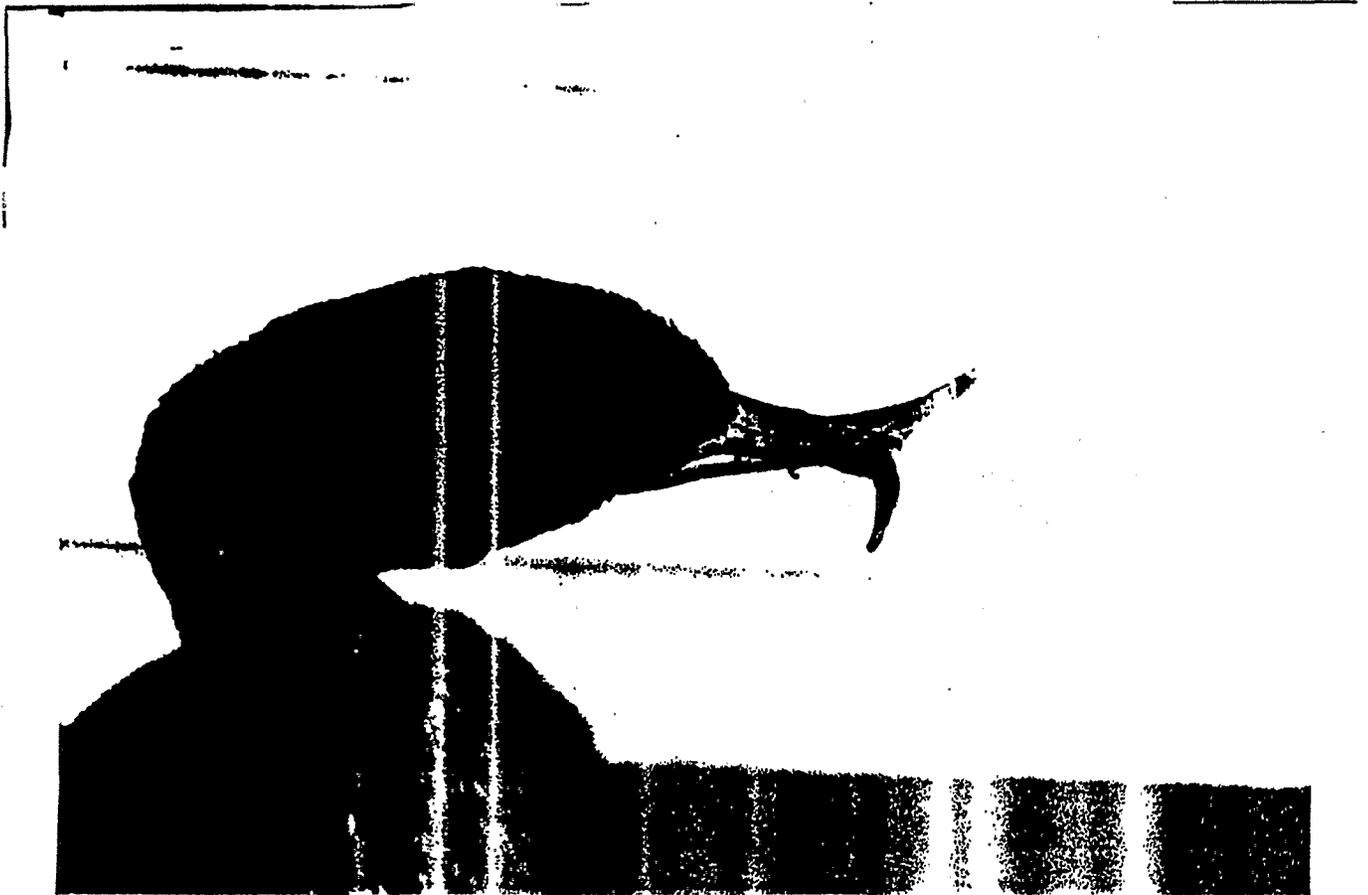


Figure 7

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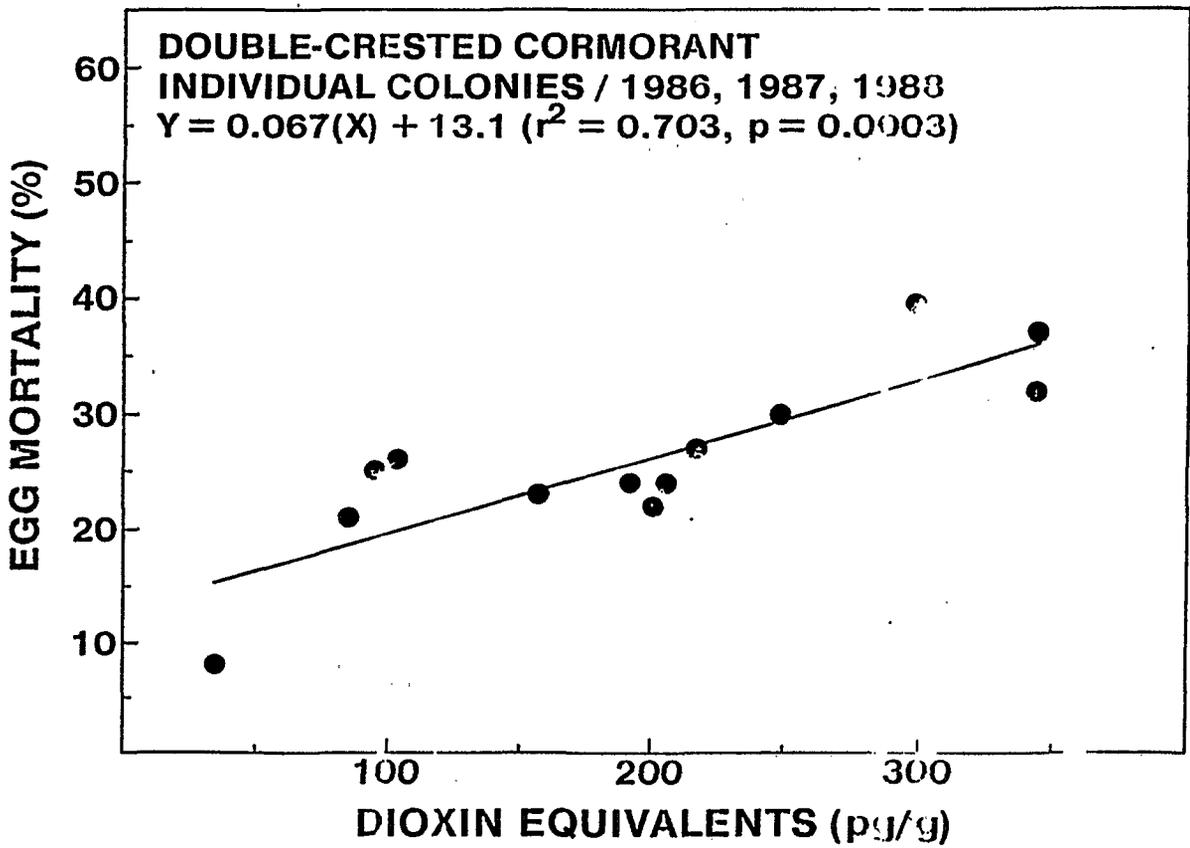
**Figure 8. Deformed bill of double-crested cormorant from Green Bay Wisconsin (Photo by J.P. Giesy, 1991).**



**Figure 8**

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**Figure 9. Egg mortality of double-crested cormorants from 12 locations on and one location off of the Great Lakes as a function of TCDD-EQ (Reprinted with permission from Tillitt *et al.*<sup>(22)</sup>)**

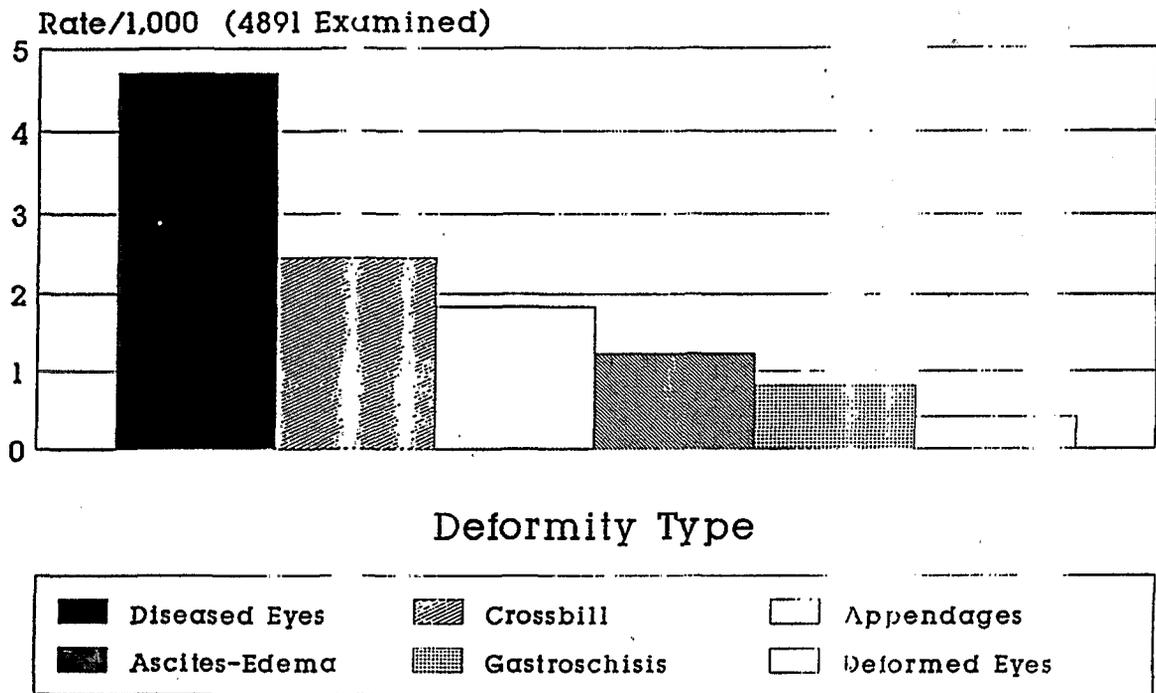


**Figure 9**

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**Figure 10. Occurances (per 1,000 chicks examined) of different types of deformities in the embryos and chicks of double-crested cormorants in Green Bay, Lake Michigan.**

# DEFORMITIES IN CORMORANTS GREEN BAY-1986-1989

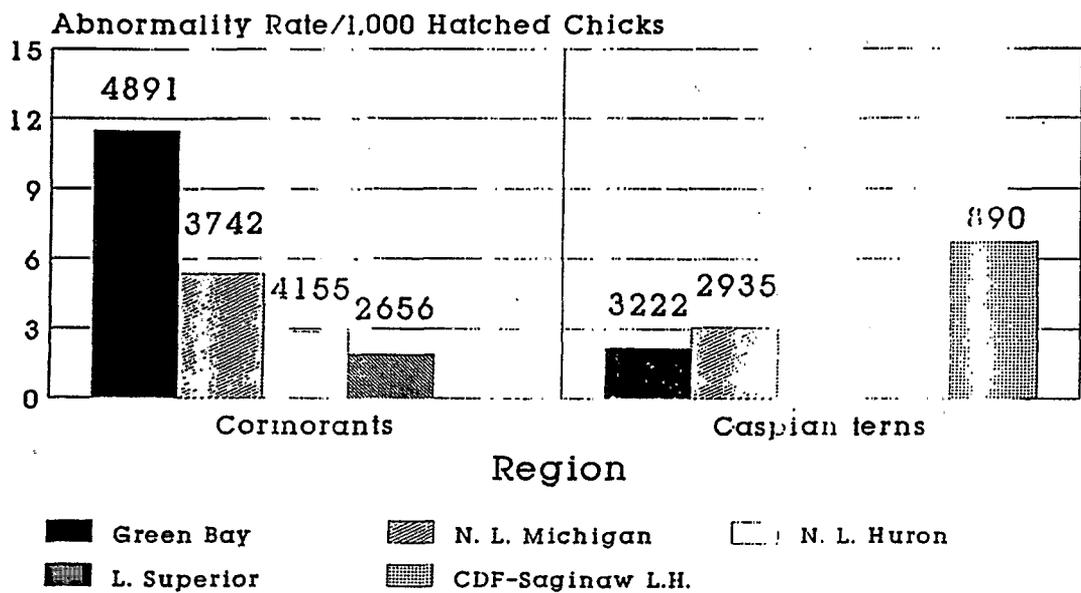


**Figure 10**

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**Figure 11. Rates of deformities in double-crested cormorants and Caspian terns from five regions of the Great Lakes. The number of embryos and chicks examined at each location is given above each bar.**

# RATES OF DEFORMITIES CORMORANTS 1986-1989



Number of Chicks Examined Above Bar

Figure 11

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**Figure 12. Rates of deformities of double-crested cormorants and Caspian terns in the Great Lakes, as a function of TCDD-EQ.**

# Deformities Vs. Concentrations Cormorants and Caspian Terns-1988

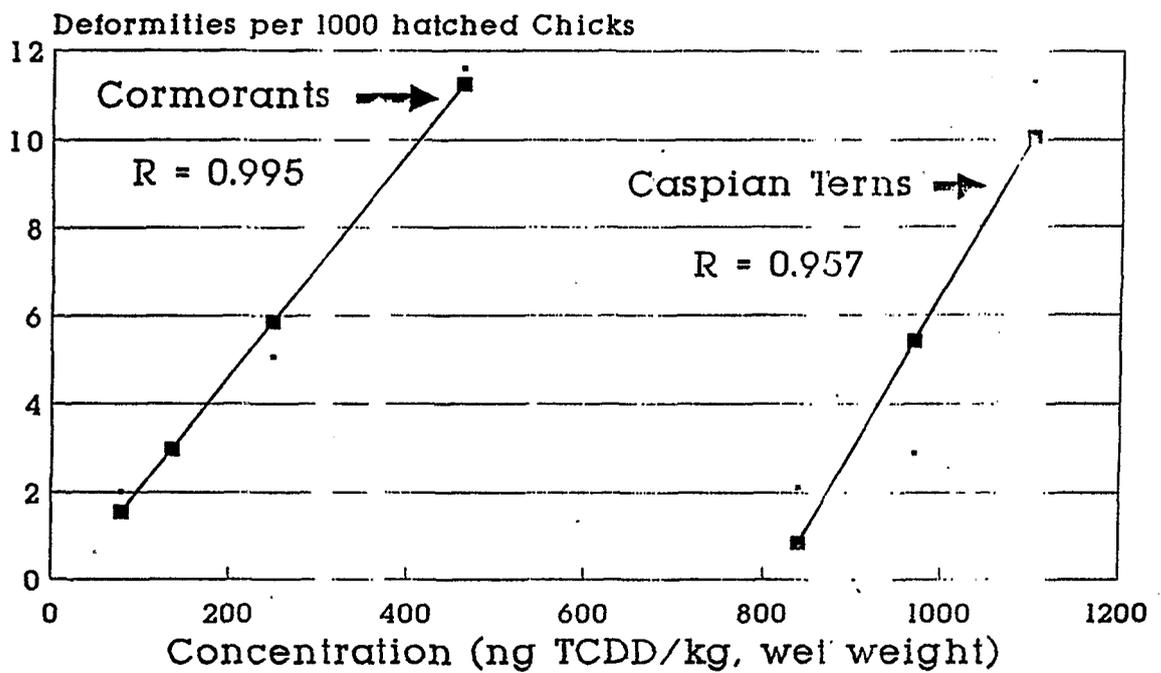


Figure 12

**Figure 13. Concentrations of TCDD-EQ in the eggs of double-crested cormorants from eight locations in the Great Lakes.**

# TCDD-EQ in Cormorants Means of 1986, 1988, 1989 by Location

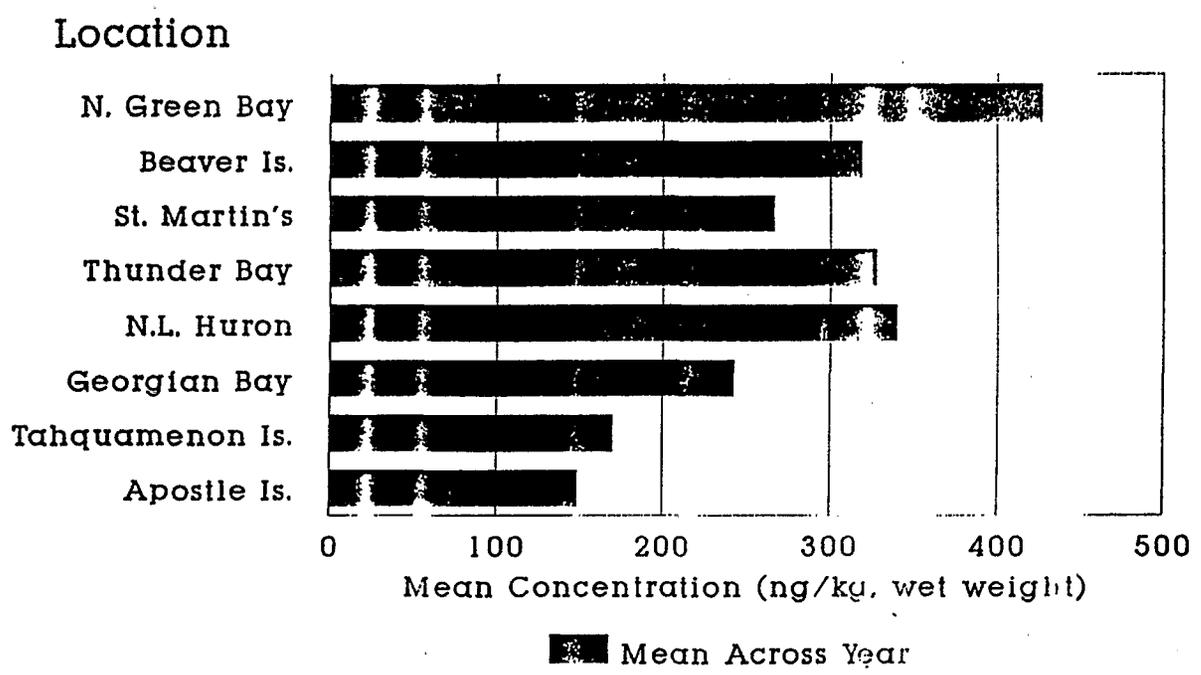


Figure 13

**Giesy Briefing Document, Minneapolis, MN Sept 14-15, 1993**

**Appendix I**

**Briefing Documents on the Effects of TCDD on Rainbow Trout**

# **OBJECTIVES**

## **To Determine:**

---

- 1. Accumulation of Dietary 2 , 3 , 7 , 8 -TCDD**
- 2. Disposition in Tissues**
- 3. Survival & Growth of Adults**
- 4. Effects on Hematology of Adults**
- 5. Effects on Histology of Adults**
- 6. Effects on Reproductive Performance**

# **METHODS**

## **Organisms & Sampling**

---

**Rainbow Trout ( *Onchorhynchus mykiss* )**

**350 g - Age Class II - Females**

**35 Fish Exposed for 350 Days**

**4 fish from each treatment after:**

**50 , 100 , 150 , 200 Days**

**Fish collected upon death or at spawning**

**Eggs spawned with composite milt of control males**

**Eggs incubated until 28 days post-swim-up**

C-329

# **METHODS**

## **Exposure Conditions**

---

- **Fish Were Held in 1700 L , Covered Flow - Through Tanks**
- **Flow Rate : 71 . 5 L / hr**
- **Turnovers : 2 / day**
- **Loading : 2 g / l of Flow / day**
- **Temperature : 12 Deg-C**
- **Lighting: Changed Weekly to Match Outside**
- **Control Fish Held in A Separate Room**

C-330

# **EXPOSURE**

## **2 , 3 , 4 , 7 , 8 - Tetrachlorodibenzo-p-dioxin**

---

- **Tritium - labelled TCDD Synthesized at MSU**
- **Purity & specific Activity checked by GC/MS  
>99 . 9 % Pure**
- **Specific Activity Varied to Keep Exposure to  
3-H *B* Radiation Equivalent in Each Treatment**
- **TCDD Incorporated into Trout Chow**
- **Fish Fed TCDD in Food every Third Day  
Exposure Expressed As Daily Average**
- **Fish Fed at 1 . 5 % Body Wight per Day**

C-331

# METHODS

## Diets

<b>CONTROL: 0.0 PG/G</b>
<b>0.0 Bq/g</b>

<b>LOW: 1.8 PG/G</b>
<b>541 Bq/g</b>

<b>MEDIUM: 18 PG/G</b>
<b>57 Bq gb</b>

<b>HIGH: 90 PG/G</b>
<b>10.5 Bq g</b>

# **HISTOLOGY**

## **Results**

---

### **Gross Morphology**

**Scoliosis**

**Jaw Deformities**

**Fin Asymmetry**

**Eyes**

**Snout**

### **Histology**

**No Effects That Could Be Attributed to  
Dose-specific Exposure to TCDD**

C-333

# **SURVIVAL OF ADULTS**

---

- 1. Little Mortality was Observed for the First 150 Days and There was no Significant Difference Among Treatments Until That Time**
- 2. The Greatest Dose Caused the Greatest Rate of Mortality and Fish Exposed to This Treatment Died Sooner.**
- 3. At the End of the Experiment the Number of Fish that Died in the Two Greatest Doses was the Same ( 30 % )- Note Fish Were Removed For Sampling**
- 4. The Greatest Dose (1.8 pg / g in Diet Caused Significant Lethality of Adult Fish.**

C-334

# **REPRODUCTION**

## **Parameters Examined**

---

- **Numbers of Eggs Produced**
- **Size of Eggs Produced**
- **Density of Eggs**
- **Caloric Content of Eggs**
- **Lipid Content of Eggs**
- **Fertility**
- **Survival of Fry to 28 D Post-hatch**
- **Teratogenicity**

# **LIVER P4501A (EROD)**

---

- 1. Dose-Dependent Induction After 50 days**
- 2. 3 X Induction Over Background  
20 pm / min / mg prot**
- 3. 200 Days Maximum Induction at  
18 pg / g in Diet; 1.7 pg / g, ww in Liver**
- 4. All Activities Were Less at 100 Days**
- 5. After 200 Days EROD was not different  
from control. Greatest Induction only 2X  
at 1.8 pg / g in Diet**

C336

# BIOMAGNIFICATION FACTORS - I

## BMF - Day 200

Diet ( pg / g )	Adipose	Muscle	Liver	Ovary
1.8	1.1	0.22	----	0.61
18	0.88	0.22	0.16	0.29
90	1.0	0.089	0.18	0.52
Mean	0.99	0.18	0.17	0.47
SD	( 0.11 )	( 0.07 )	( 0.01 )	( 0.17 )

C-337

# **BIOMAGNIFICATION FACTORS - II**

## **BMF - Day 200**

---

- 1. BMF For Adipose Tissue Was Approximately 1.0**
- 2. BMF For Muscle was 25 % of That For Adipose ( Belly Flap )**
- 3. BMF For Ovary ( Egg ) Was About Half of That For Adipose and Five Times That in Muscle**
- 4. BMF in Liver Was Approximately 25 % of That in Adipose**

C-338

# BEHAVIORAL EFFECTS

---

**Fish Which Were Fed TCDD at All Concentrations Tended to Be Listless and did not Feed Actively or Respond to Stimulation**

**Fish Did Not Struggle When Transferred from one Tank to Another**

C339

# LOWEST OBSERVABLE EFFECTS CONCENTRATIONS

## LOEC - Lethality - I

Dose	Value
<b>Dietary:</b>	
<b>Concentration-</b>	<b>1 . 8 pg TCDD / g food</b>
<b>Rate ( Initial )-</b>	<b>0 . 027 pg TCDD / d / g , bw</b>
<b>*Organismal:</b>	
<b>Adipose</b>	<b>2 . 0 pg TCDD / g , ww</b>
<b>Muscle-</b>	
<b>wet wt.-</b>	<b>0 . 043 pg TCDD / g , ww</b>
<b>lipid-</b>	<b>14. 0 pg TCDD / g , lip wt</b>
<b>Liver-</b>	<b>0 . 43 pg TCDD / g , ww</b>

C-340

# COMPARISON TO LITERATURE INFORMATION

Spitsbergen et al. 1988

J. Toxicol. Environ. Health 23:333-358

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**IP Injections into Adults**

**LD-50 = 10,000 pg / g**

**LD-20 = 5,000 pg / g**

**Significant Hematological Changes = 1,000 pg / g**

**LOAEL in Feeding Study = 1 . 8 pg / g**

**200-day LD-33 = 18 pg / g**

**Few Significant Long-term Hematological Effects**

C-341

**Giesy, 1993**

# **COMPARISON TO LITERATURE LOAEL & NOAEL**

**Mehrle et al. 1988**

***Environ. Toxicol. Chem. 7:47-62***

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**Swim-up Fry Exposed in Water**

**LOAEL ( 28-day LD-45 ) = 765 pg / g**

**NOAEL ( 28 day ) = 25 pg / g**

**In the feeding Study We did not Observe  
Significant Effects in Grown From Eggs  
Which Contained 90 pg / g**

C-342

# **COMPARISON TO LITERATURE LOAEL & NOAEL**

**Walker & Peterson, 1991**

***Aquatic Toxicol.* 21:219-238**

---

## **Egg Microinjection-Studies: Rainbow Trout**

**LD-50 for survival to Swim-up When Eggs Were Injected**

**LD50 = 230 to 488, Depending on Strain Tested**

**No Significant Effects Were Observed  
in Fry from Eggs Which Contained 90  
pg / g During The Feeding Study.**

**This Was Less Than The Predicted LC-50  
Steep Dose-Response**

C-343

**Giesy, 1993**

# CONCLUSIONS-I

---

1. **Significant Effects Observed on Survival Growth and Behavior at a Dietary Concentration of 1 . 8 pg TCDD / g**
2. **Eggs May not be the Most Sensitive Life Stage**
3. **LOAEL ( Dose ) = 0 . 027 pg TCDD / d / g , bw**
4. **LOAEL Occurred at a Concentration Which Resulted in Little Induction of EROD Activity**
5. **Long-term Feeding More Sensitive Than IP-Injection by Approximately 5,000 X**
6. **The LOAEL is Estimated to be Approximately 1 pg / g , ww in the whole body**

C344

# **CALCULATION OF WATER QUALITY CRITERION**

**Based on LOAEL in Rainbow Trout**

---

**Assumptions: \*BAF = 1.9 e 6 ( lipid Basis )**

**Available Fraction = 0 . 4**

**Koc = 1 e 7**

**lipid content of fish = 3 %**

**WQC = 2.7 e -2 pg / L ( 0 .027 ppq )**

**\*Assumes 40 % in dissolved fraction**

**\*Cook et al., 1993**

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**Appendix II**

**Briefing Materials on Effects of TCDD-EQ on Mink**

**REPRODUCTIVE PERFORMANCE OF MINK  
FED SAGINAW BAY CARP: DOSE-  
RESPONSE RELATIONSHIPS FOR TOTAL  
PCBs, DIOXIN-LIKE CONGENERS AND  
DIOXIN EQUIVALENTS**

C-347

**Giesy & Kubiak, 1993**

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C-348

# **OBJECTIVES-I**

**Conduct Wildlife Risk Assessment:**

**Mink Exposed to Current Concentrations of Complex Mixtures of planar, halogenated Hydrocarbons in Fish from Saginaw Bay, Michigan**

C-349 **Determine Whether Current or Historical Conditions Would be Expected to Restrict Populations of Mink**

**Determine Target Concentrations of 2,3,7,8-TCDD-EQ Which Would not Have Adverse Effects on Populations of Mink**

# OBJECTIVES-II

**Determine:**

**Dose-Response Relationships ( LOAEL & NOAEL )**

**Dietary Exposure ( ng TCDD-EQ / Kg )**

**Dietary Dose ( pg TCDD-EQ / Kg BW / day )**

**Tissue Dose ( ng TCDD / Kg tissue)**

**Allowable Daily consumption**

**Allowable Exposure Concentration**

**Hazard Ratios for Different Species of Fish**

# OBJECTIVES-III

**Determine Biomagnification factor ( BMF ) for TCDD-EQ from Fish to Mink Liver**

**Determine Changes in Patterns of Relative Proportions of PCDD, PCDF and co-planar PCB Congeners from Fish Tissue to Mink Liver**

**Compare Concentrations of TCDD-EQ as Determined by TEFs and a Simple Additive Model and as Determined by the H4IIE bioassay**

**Site-specific Hazard Assessment For Saginaw River / Bay**

# **METHODS**

**Feed Ranch Mink Various Proportions of  
Carp from Saginaw Bay in the Diet**

**Determine Concentrations of Individual  
PCDD, PCDF, planar PCBs and TCDD-EQ  
in the Diet and Liver of Mink**

**Determine Survival and Growth of Adult Mink**

**Determine Reproductive Outcomes of Mink**

**Calculate Hazard Indices as the Ratio of  
Concentrations or Dose of TCDD-EQ in Fish  
to LOAEL or NOAEL for TCDD-EQ to Mink**

# TOTAL PCBs SAGINAW BAY FISHES

FISH	Total PCBs ( mg / kg )
Walleye 1	0 . 97
Walleye 2	0 . 87
Walleye 3	1 . 8
Walleye 4	2 . 8
Walleye 5	1 . 2
Walleye 6	2 . 9
Alewife	1 . 4
Shad	0 . 5
Carp	7 . 2
Mean-WC	2 . 2
Mean-WOC	1 . 5

# PCBs

## SAGINAW BAY FISH

	<b>Fish</b>	<b>Total PCBs ( ppm )</b>
<b>Mean</b>	<b>All</b>	<b>2.2</b>
<b>SD</b>	<b>All</b>	<b>2.1</b>
<b>Max</b>	<b>Carp</b>	<b>7.2</b>
<b>Min</b>	<b>Shad</b>	<b>0.5</b>

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# CALCULATED TCDD-EQs IN SAGINAW BAY FISHES

FISH	Total PCBs ( mg / kg )	TEQ-A ( ng / kg )	TEQ-C ( ng / kg )
Walleye 1	1 . 0	4	9
Walleye 2	0 . 9	13	13
Walleye 3	1 . 8	14	16
Walleye 4	2 . 8	87	34
Walleye 5	1 . 2	14	13
Walleye 6	2 . 9	11	41
Alewife	1 . 4	31	9
Shad	0 . 5	11	11
Perch	ND	4	11
Carp	7 . 2	194	50
Mean-WC	2 . 2	38 . 3	21 . 0
Mean-WOC	1 . 5	21 . 0	17 . 3

TEQ-C Calculated from H4IIE TEFs & Additive Model  
TEQ-A Determined by H4IIE Assay

Giesy & Kubiak, 1993

# CALCULATED TCDD-EQ<sub>s</sub> IN SAGINAW BAY FISHES

<b>FISH</b>	<b>TEQ-A ( ng / kg )</b>	<b>TEQ-C ( ng / kg )</b>
Walleye 1	4	9
Walleye 2	13	13
Walleye 3	14	16
Walleye 4	87	34
Walleye 5	14	13
Walleye 6	11	41
Alewife	31	9
Shad	11	11
Perch	4	11
Carp	194	50
Mean-WC	38 . 3	21 . 0
Mean-WOC	21 . 0	17 . 3

TEQ-C Calculated from H4IIE TEFs & Additive Model  
TEQ-A Determined by H4IIE Assay

# CALCULATED TCDD-EQs IN SAGINAW BAY FISHES

<b>Parameter</b>	<b>PCDD ( ng / kg )</b>	<b>PCDF ( ng / kg )</b>	<b>PCB ( ng / kg )</b>
<b>Mean</b>	<b>7 . 4</b>	<b>1 . 4</b>	<b>17 . 2</b>
<b>SD</b>	<b>6 . 2</b>	<b>1 . 2</b>	<b>12 . 0</b>
<b>Min</b>	<b>2 . 6</b>	<b>0 . 33</b>	<b>5 . 3</b>
<b>Max</b>	<b>16 . 3</b>	<b>4 . 1</b>	<b>41 . 1</b>

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Calculated from H4IIE TEFs & Additive Model

Giesy & Kubiak, 1993

# TCDD-EQ H4IIE ASSAY SAGINAW BAY FISH

	<b>Fish</b>	<b>* TCDD-EQ ( ng / kg )</b>
<b>Mean</b>	<b>All</b>	<b>40</b>
<b>SD</b>	<b>All</b>	<b>62</b>
<b>Max</b>	<b>Carp</b>	<b>194</b>
<b>Min</b>	<b>Walleye Fry</b>	<b>4</b>

\* TCDD-EQ determined by H4IIE assay

# PCBs & TCDD-EQ

## Summary : Saginaw Bay Fish

Tissue or Source	PCBs ( mg / kg )	TEQ ( Assay ) ( ng / kg )	TEQ ( Calc. ) ( ng / kg )
<b>Mean ( All Fish )</b>	<b>2 . 2</b>	<b>38 . 3</b>	<b>20 . 6</b>
<b>Mean ( Without Carp )</b>	<b>1 . 5</b>	<b>21</b>	<b>17 . 3</b>
<b>Max ( Carp )</b>	<b>7 . 2</b>	<b>194</b>	<b>50</b>
<b>Min</b>	<b>0 . 5</b>	<b>4</b>	<b>9</b>

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**TEF and TCDD-EQ From H4IIE Assay**

**Giesy & Kubiak, 1993**

# **CALCULATION OF BIOMAGNIFICATION FACTORS ( BMFs )**

$$\text{BMF} = \frac{\text{Concentration in Mink Liver}}{\text{Concentration in Fish}}$$

**BMFs were calculated for:**

**Total PCBs**

**TCDD-EQ ( Calculated )**

**TCDD-EQ ( H4IIE )**

**BMFs can be on wet weight or lipid basis**

# **BIOMAGNIFICATION FACTORS**

## **10% SAGINAW RIVER : FISH TO LIVER**

<b>Measure</b>	<b>BMF</b>
<b>TCDD-EQ ( Calc. )</b>	<b>11 . 3</b>
<b>TCDD-EQ ( H4IIE )</b>	<b>25 . 5</b>
<b>Total PCBs</b>	<b>3 . 0</b>

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# **BMF of TCDD-EQ**

## **Based on Different TEFs**

<b>Safe</b>	<b>Smith</b>	<b>H4IIE</b>	<b>Mean</b>
<b>6.5</b>	<b>12.0</b>	<b>11.8</b>	<b>10.1</b>

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# **LOWEST OBSERVABLE EFFECTS**

## **LOAEL-PCBs**

**Calculated from 10% Carp Diet**

<b>Route or Tissue</b>	<b>PCB</b>
<b>Conc. in Diet ( mg / kg )</b>	<b>0 . 72</b>
<b>Daily Dose ( mg / mink / day )</b>	<b>0 . 16</b>
<b>Daily Dose ( mg / kg, BW / day )</b>	<b>0 . 13</b>
<b>Dose-Liver ( mg / kg, WW )</b>	<b>2 . 2</b>
<b>Dose-Liver ( mg / kg, lipid )</b>	<b>44 . 6</b>

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# LOWEST OBSERVABLE EFFECTS LOAEL

Calculated from 10% Carp Diet

Route or Tissue	TEQ (Assay)	TEQ (Calc.)
Conc. in Diet ( ng / kg )	19 . 4	5 . 0
Daily Dose ( ng / mink / day )	4 . 2	1 . 1
Daily Dose ( ng / kg, BW / day )	3 . 6	0 . 93
Dose-Liver ( ng / kg, WW )	495	59
Dose-Liver ( ng / kg lipid )	10,419	1,242

C-364

TEFs or assay based on H4IIE

Giesy & Kubiak 1992

# **LOWEST OBSERVABLE EFFECTS**

**TCDD-EQ ( H4IIE Assay )**

**Calculated from 10% Carp Diet**

<b>Route or Tissue</b>	<b>LOAEL</b>
<b>Conc. in Diet ( ng / kg )</b>	<b>19 . 4</b>
<b>Daily Dose ( ng / mink / day )</b>	<b>4 . 2</b>
<b>Daily Dose ( ng / kg, BW / day )</b>	<b>3 . 6</b>
<b>Dose-Liver ( ng /kg, WW )</b>	<b>495</b>
<b>Dose-Liver ( ng / kg lipid )</b>	<b>10,419</b>

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# LOWEST OBSERVABLE EFFECTS LOAEL

**Calculated from 10% Carp Diet**

Route or Tissue	PCB ( mg / kg, WW )	TEQ-A ( ng / kg, WW )	TEQ-C ( ng / kg, WW )
<b>Conc. in Diet ( pg / g )</b>	<b>0.72</b>	<b>19.4</b>	<b>5.0</b>
<b>Daily Dose ( ng / mink / day )</b>	<b>0.16</b>	<b>4.2</b>	<b>1.1</b>
<b>Daily Dose ( ng / kg, BW / day )</b>	<b>0.13</b>	<b>3.6</b>	<b>0.93</b>
<b>Dose-Liver ( pg / g, WW )</b>	<b>2.19</b>	<b>495</b>	<b>59</b>
<b>Dose-Liver ( pg / g lipid )</b>	<b>44.6</b>	<b>10,419</b>	<b>1,242</b>

C-366

**TEQ-A Determined by H4IIE Assay**

**TEQ-B Calculated from H4IIE-Derived TEFs**

# CALCULATION OF HAZARD RATIOS

$$\text{HR} = \frac{\text{Concentration in Fish}}{\text{NOAEL}}$$

HRs were calculated for:

Total PCBs

TCDD-EQ ( Calculated )

TCDD-EQ ( H4IIE )

Calculation of HR assumes Mink eat only fish of Interest. This is a conservative or "worst-case" Estimate

$$\text{NOAEL} = \frac{\text{LOAEL}}{10}$$

Current Studies Support a NOAEL of < 1% Carp in Diet

# HAZARD RATIOS

## TOTAL PCBs SAGINAW BAY FISH

Fish	Total PCBs ( mg / kg )	Hazard Ratio
Walleye	1 . 0	13 . 4
"	0 . 9	12 . 0
"	1 . 8	25 . 0
"	2 . 8	38 . 8
"	1 . 2	16 . 7
"	1 . 4	19 . 4
Alewife	2 . 9	40 . 3
Shad	0 . 5	6 . 7
Carp	7 . 2	100
*MEAN-1	1 . 6	22 . 2
MEAN-2	2 . 2	30 . 4

**\* Mean-1 excludes carp, Mean-2 includes carp  
Based on Concentrations in Fishes & NOAEL**

# HAZARD RATIOS

## TCDD-EQ ( H4IIE ) Saginaw Bay Fish

Fish	TCDD-EQ ( ng / kg )	Hazard Ratio
Walleye	4 . 0	2 . 1
"	13	6 . 8
"	14	7 . 4
"	87	45 . 8
"	14	6 . 8
"	0 . 4	6 . 8
Alewife	13 . 0	6 . 8
Shad	11 . 0	5 . 7
Perch	4	2 . 1
Carp	194	100
Mean	38 . 3	19 . 7

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# SUMMARY OF HAZARD RATIOS TCDD-EQ ( H4IIE ) & Total PCBs Saginaw Bay Fishes

	PCBs	* TCDD-EQ-A
<b>Mean</b>	<b>30 . 4</b>	<b>19 . 7</b>
<b>Min</b>	<b>6 . 7</b> <b>( Shad )</b>	<b>2 . 1</b> <b>( Perch )</b>
<b>Max</b>	<b>100</b> <b>( Carp )</b>	<b>100</b> <b>( Carp )</b>

**\* TCDD-EQ Derived by H4IIE Assay**

# ALLOWABLE DAILY CONSUMPTION

## Saginaw Bay Fishes

$$\text{ADC} = \frac{\text{NOAEL}}{\text{Concentration in Fish}} \frac{(\text{pg / g or ug / g})}{(\text{same units})}$$

NOAEL = Reference Dose

NOAEL = LOAEL / 10

This is equivalent to approximately 1% carp in the diet which, based on more recent studies seems to be the approximate threshold for effects

Percent of Daily diet which ADC represents

Food consumption by mink = 218 g / d

**ALLOWABLE DAILY CONSUMPTION**  
**Saginaw Bay Fishes**  
**Based on Total PCBs**

<b>FISH</b>	<b>ADC ( g / d )</b>	<b>% ALLOWABLE IN DIET</b>
<b>Walleye-1</b>	<b>16</b>	<b>7 . 4</b>
<b>Walleye-2</b>	<b>18</b>	<b>8 . 3</b>
<b>Walleye-3</b>	<b>9</b>	<b>4 . 0</b>
<b>Walleye-4</b>	<b>6</b>	<b>2 . 6</b>
<b>Walleye-5</b>	<b>13</b>	<b>6 . 0</b>
<b>Walleye-6</b>	<b>5</b>	<b>2 . 5</b>
<b>Alewife</b>	<b>11</b>	<b>5 . 1</b>
<b>Shad</b>	<b>31</b>	<b>14 . 4</b>
<b>Carp</b>	<b>2</b>	<b>1 . 0</b>
<b>Perch</b>	<b>ND</b>	<b>ND</b>
<b>Average</b>	<b>13</b>	<b>5 . 8</b>

**Daily = Allowable % That Could be in Diet  
at The Threshold for Effects**

**Giesy & Kubiak, 1993**

**ALLOWABLE DAILY CONSUMPTION**  
**Saginaw Bay Fishes**  
**Based on TCDD-EQ ( Calc. )**

<b>FISH</b>	<b>ADC ( g / d )</b>	<b>% ALLOWABLE IN DIET</b>
<b>Walleye-1</b>	<b>6 . 0</b>	<b>2 . 7</b>
<b>Walleye-2</b>	<b>4 . 0</b>	<b>1 . 8</b>
<b>Walleye-3</b>	<b>3 . 0</b>	<b>1 . 4</b>
<b>Walleye-4</b>	<b>1 . 5</b>	<b>0 . 6</b>
<b>Walleye-5</b>	<b>3 . 8</b>	<b>1 . 7</b>
<b>Walleye-6</b>	<b>1 . 2</b>	<b>0 . 6</b>
<b>Alewife</b>	<b>5 . 5</b>	<b>2 . 5</b>
<b>Shad</b>	<b>4 . 5</b>	<b>2 . 1</b>
<b>Perch</b>	<b>4 . 5</b>	<b>2 . 1</b>
<b>Carp</b>	<b>1 . 0</b>	<b>0 . 6</b>
<b>Average</b>	<b>3 . 5</b>	<b>1 . 6</b>

**Daily = Allowable % That Could be in Diet  
at The Threshold for Effects**

**Giesy & Kubiak, 1993**

**ALLOWABLE DAILY CONSUMPTION**  
**Saginaw Bay Fishes**  
**Based on TCDD-EQ ( Assay )**

<b>FISH</b>	<b>ADC ( g / d )</b>	<b>% ALLOWABLE IN DIET</b>
<b>Walleye-1</b>	<b>105</b>	<b>48</b>
<b>Walleye-2</b>	<b>32</b>	<b>14 . 7</b>
<b>Walleye-3</b>	<b>30</b>	<b>13 . 8</b>
<b>Walleye-4</b>	<b>5</b>	<b>2 . 2</b>
<b>Walleye-5</b>	<b>30</b>	<b>13 . 8</b>
<b>Walleye-6</b>	<b>38</b>	<b>17</b>
<b>Alewife</b>	<b>13 . 5</b>	<b>6</b>
<b>Shad</b>	<b>38</b>	<b>17</b>
<b>Carp</b>	<b>2 . 2</b>	<b>1</b>
<b>Perch</b>	<b>105</b>	<b>48</b>
<b>Average</b>	<b>40</b>	<b>18</b>

**Daily = Allowable % That Could be in Diet  
at The Threshold for Effects**

**Giesy & Kubiak, 1993**

# DAILY ALLOWABLE CONSUMPTION

Saginaw River & Bay Fish  
Grams of Fish = NOAEL Dose

PARAMETER	TOTAL PCBs	TEQ-A	TEQ-C
Mean All Fish	13	40	3.5
SD	16	36	1.8
Min (Carp)	2.1	2.2	1
Max	31 (Shad)	105 (Walleye)	6 (Walleye)

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TEQ-A = H4IIE Assay

TEQ-C = Calculated from H4IIE TEFs

Giesy & Kubiak, 1993

# PERCENT ALLOWABLE IN DIET

## Saginaw River & Bay Fish

PARAMETER	TOTAL PCBs ( % )	TEQ-A ( % )	TEQ-C ( % )
Mean All Fish	5.8	18	1.6
SD	3.8	16.7	0.8
Min ( Carp )	1	1	0.5
Max	14 ( Shad )	48 ( Walleye-Fry )	2.7 ( Walleye-Fry )

TEQ-A = H4IIE Assay TEQ-C Calculated from H4IIE TEFs  
Assumes no Contribution From Other Items in Diet

Giesy & Kubiak, 1993

# OVERALL AVERAGES ADI & % OF ADC

## Saginaw River & Bay Fish

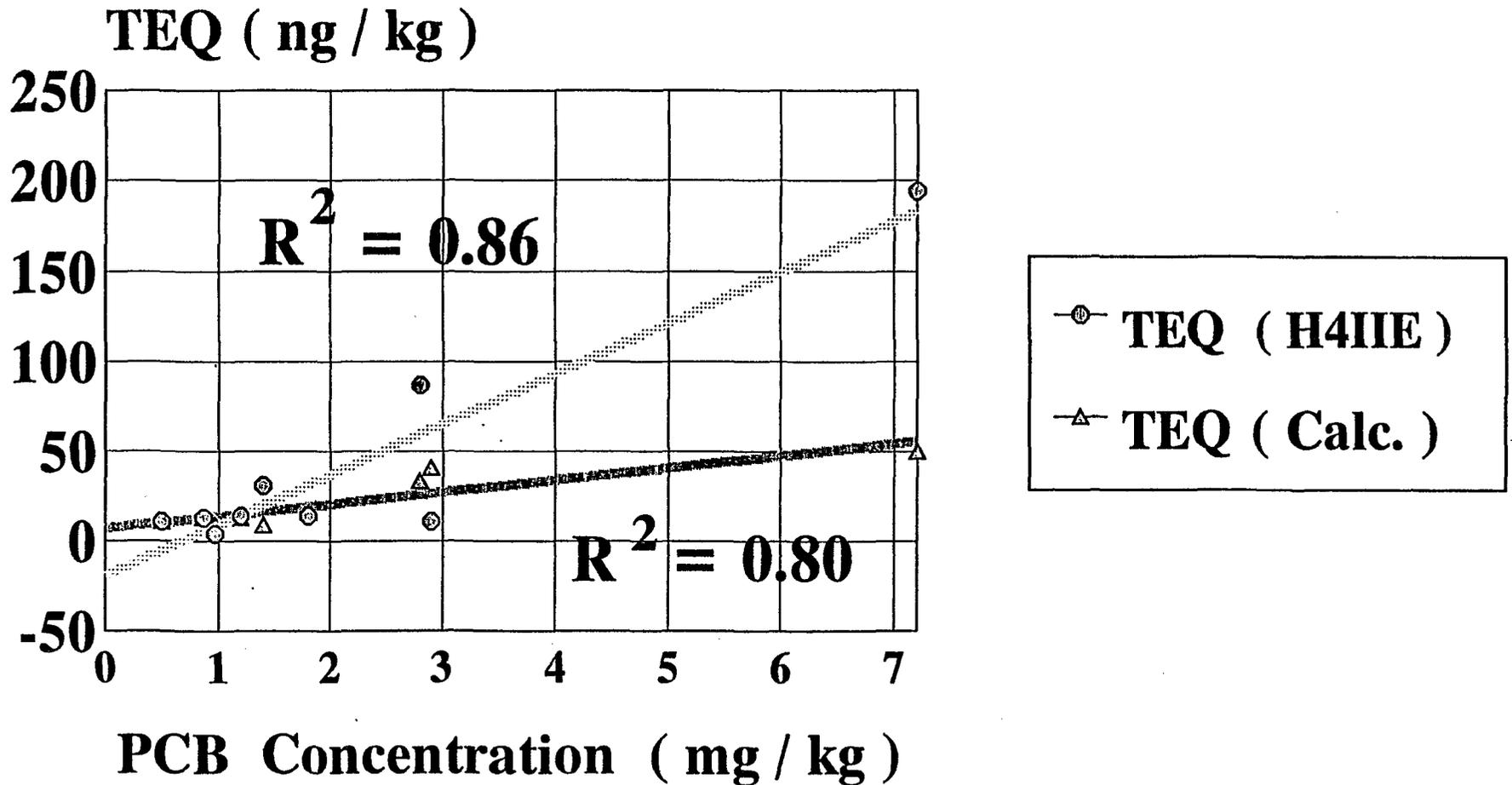
PARAMETER	ADC ( g )	% Allowable in Diet
Mean ( All Fish )	18	8 . 4
Min ( Carp )	1 . 0 ( TEQ - A )	0 . 5 ( TEQ - C )
Max	105 ( Walleye-Fry ) ( TEQ - A )	48 ( Perch ) ( TEQ - A )

\* Average of all Three Toxicants

Giesy & Kubiak, 1993

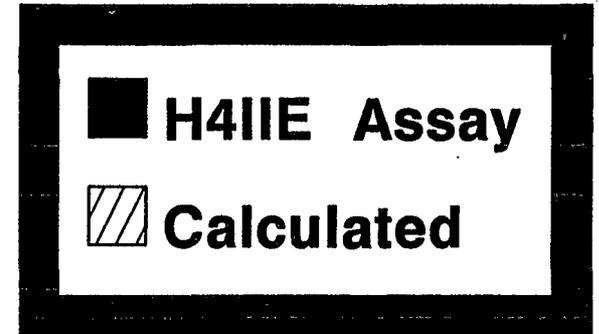
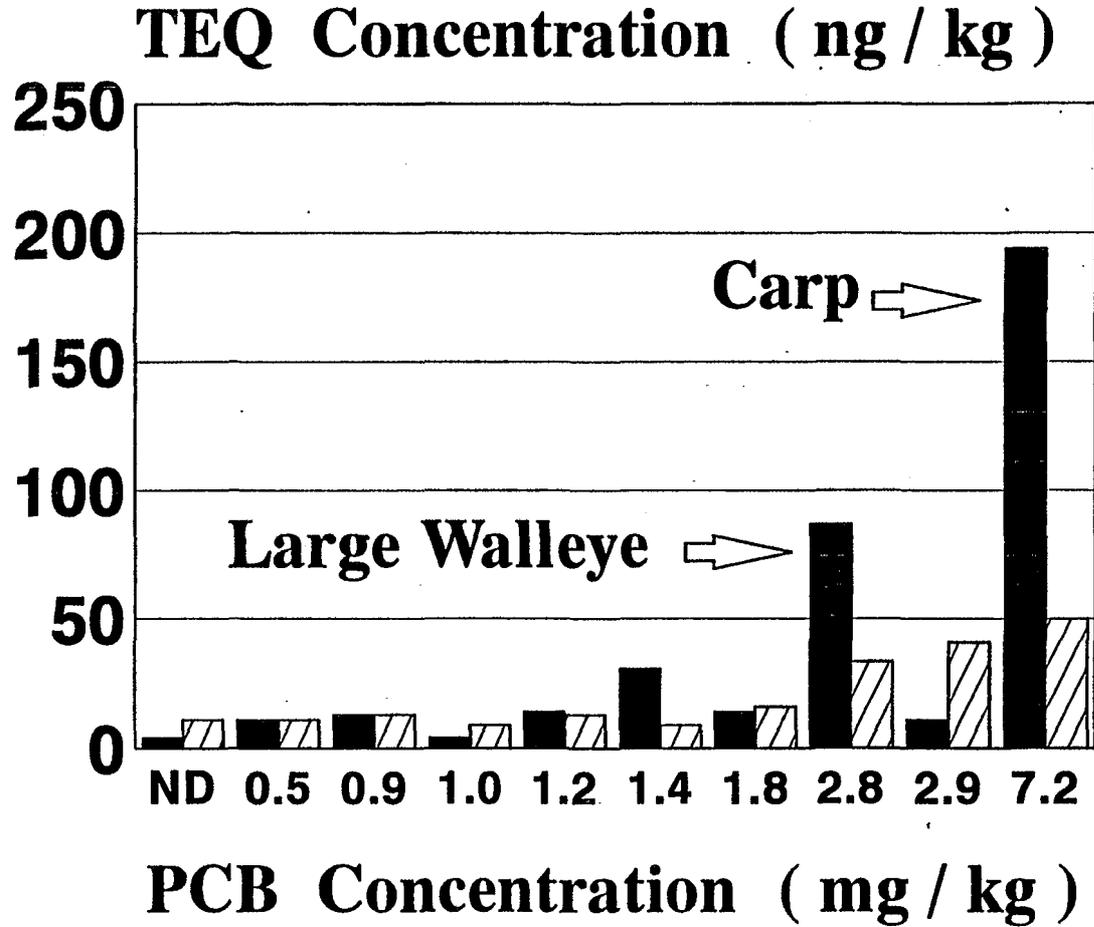
# CONCENTRATIONS OF PCBs & TEQs FISHES FROM SAGINAW BAY

C-378

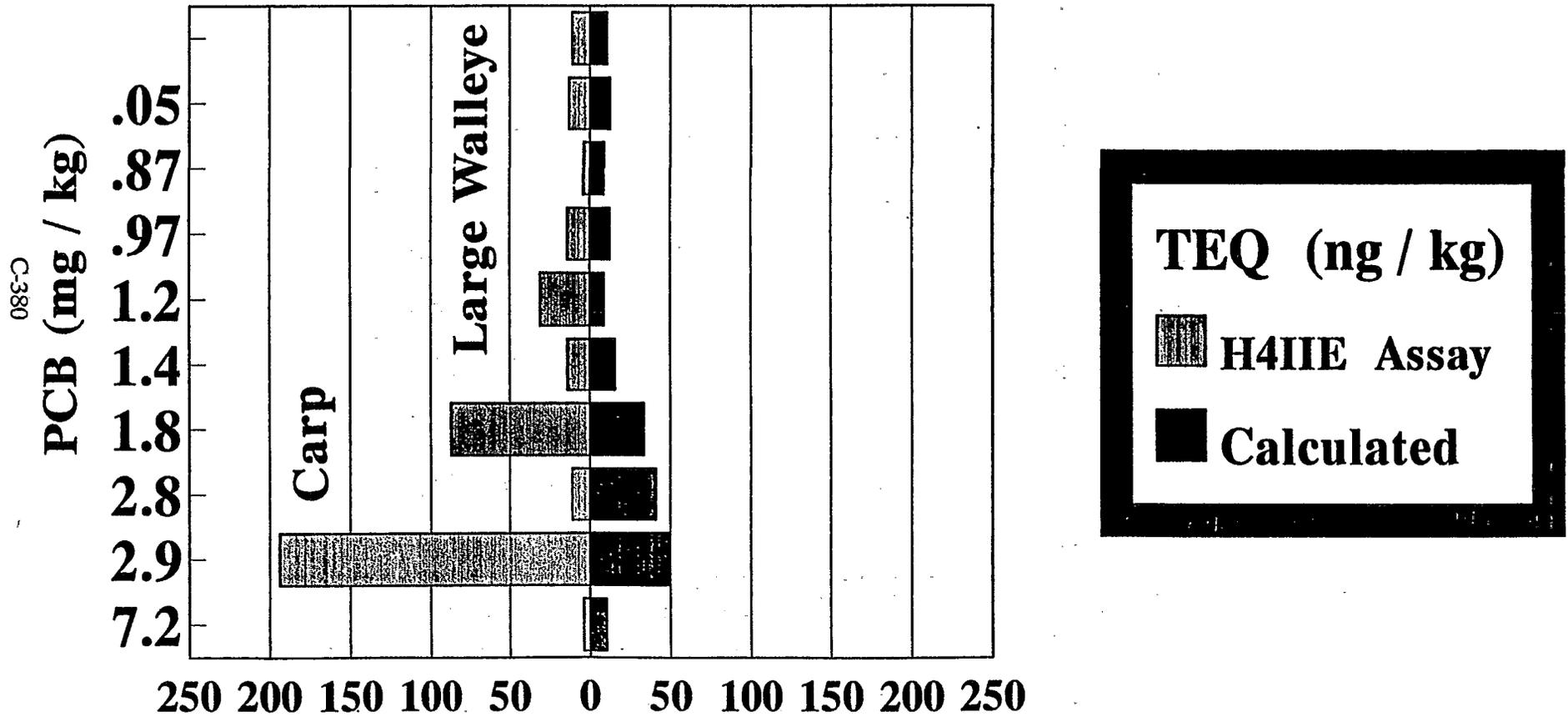


# CONCENTRATIONS OF PCBS & TEQS FISHES FROM SAGINAW BAY

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# PCBs & TEQs SAGINAW BAY FISHES



# CALCULATED TCDD-EQs IN SAGINAW BAY FISHES

## Percent Contribution

FISH	% PCDD	% PCDF	% PCB
Walleye 1	37	5	58
Walleye 2	49	6	45
Walleye 3	33	9	58
Walleye 4	48	7	45
Walleye 5	34	5	61
Walleye 6	48	10	42
Alewife	27	4	69
Shad	38	14	48
Perch	33	7	60
Carp	53	4	43
Mean	40	7	53

Calculated from H4IIE TEFs & Additive Model  
Reported as Percent of Total Calculated TEQs  
Giesy & Kubiak, 1993

# CALCULATED TCDD-EQs PCB IN SAGINAW BAY FISHES

## Percent Contribution

FISH	% PCB
Walleye 1	58
Walleye 2	45
Walleye 3	58
Walleye 4	45
Walleye 5	61
Walleye 6	42
Alewife	69
Shad	48
Perch	60
Carp	43
Mean	53

Calculated from H4IIE TEFs & Additive Model  
 Reported as Percent of Total Calculated TEQs  
 Giesy & Kubiak, 1993

# CALCULATED TCDD-EQs PCDD IN SAGINAW BAY FISHES

## Percent Contribution

FISH	% PCDD
Walleye 1	37
Walleye 2	49
Walleye 3	33
Walleye 4	48
Walleye 5	34
Walleye 6	48
Alewife	27
Shad	38
Perch	33
Carp	53
Mean	40

Calculated from H4IIE TEFs & Additive Model  
 Reported as Percent of Total Calculated TEQs  
 Kiesy & Kubiak, 1993

# CALCULATED TCDD-EQs PCDD IN SAGINAW BAY FISHES

## Percent Contribution

FISH	% PCDF
Walleye 1	5
Walleye 2	6
Walleye 3	9
Walleye 4	7
Walleye 5	5
Walleye 6	10
Alewife	4
Shad	14
Perch	7
Carp	4
Mean	7

Calculated from H4IIE TEFs & Additive Model  
 Reported as Percent of Total Calculated TEQs  
 Giesy & Kubiak, 1993

# RELATIVE CALCULATED TCDD-EQs IN SAGINAW BAY FISHES

## Percentage of Total Calculated TCDD-EQ

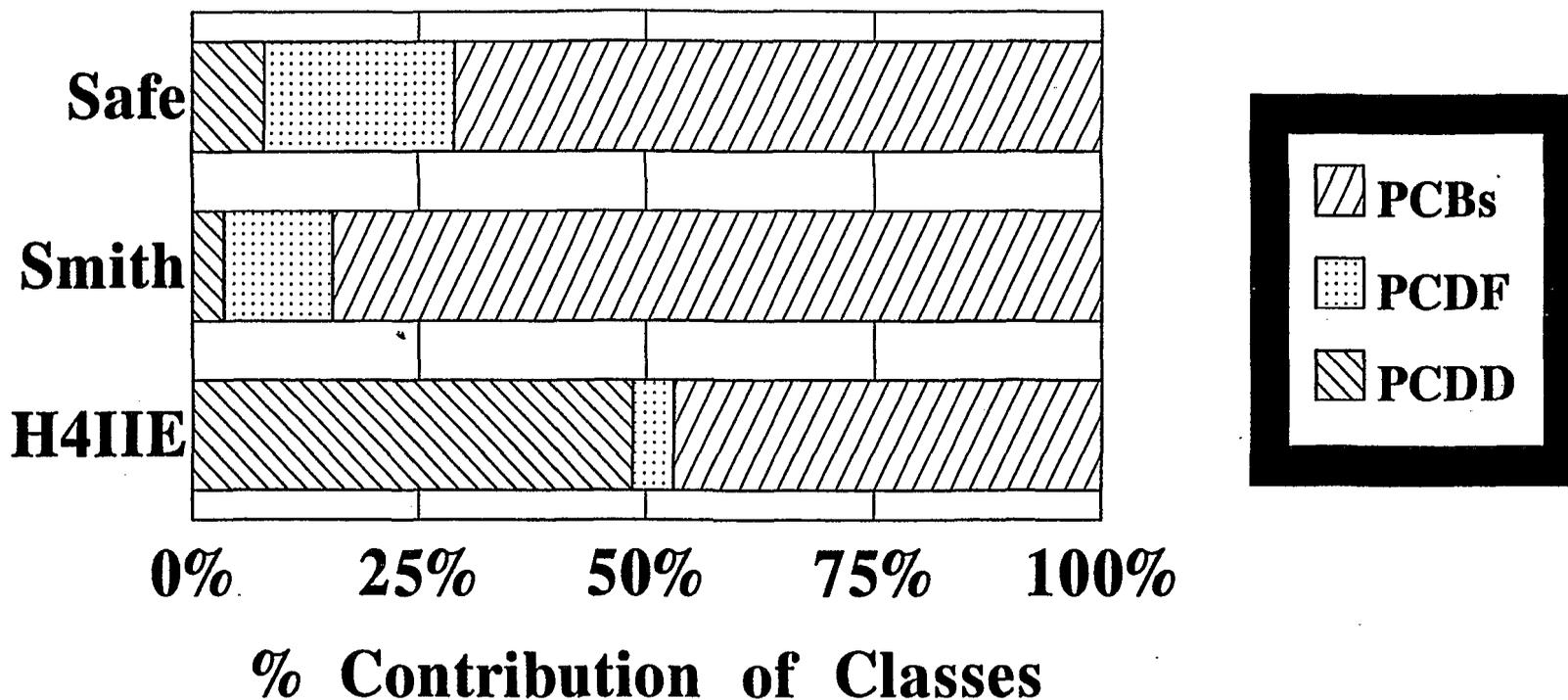
Parameter	PCDD (ng / kg)	PCDF (ng / kg)	PCB (mg / kg)
Mean	40	7	53
SD	8 . 7	3 . 1	9 . 4
Min	27 . 0	3 . 53	69 . 6
Max	49 . 2	17 . 5	42 . 4

Calculated from H4IE TEFs & Additive Model

Giesy & Kubiak, 1993

# RELATIVE CONTRIBUTIONS OF Classes to TCDD-EQ in Mink Liver

TEF Method



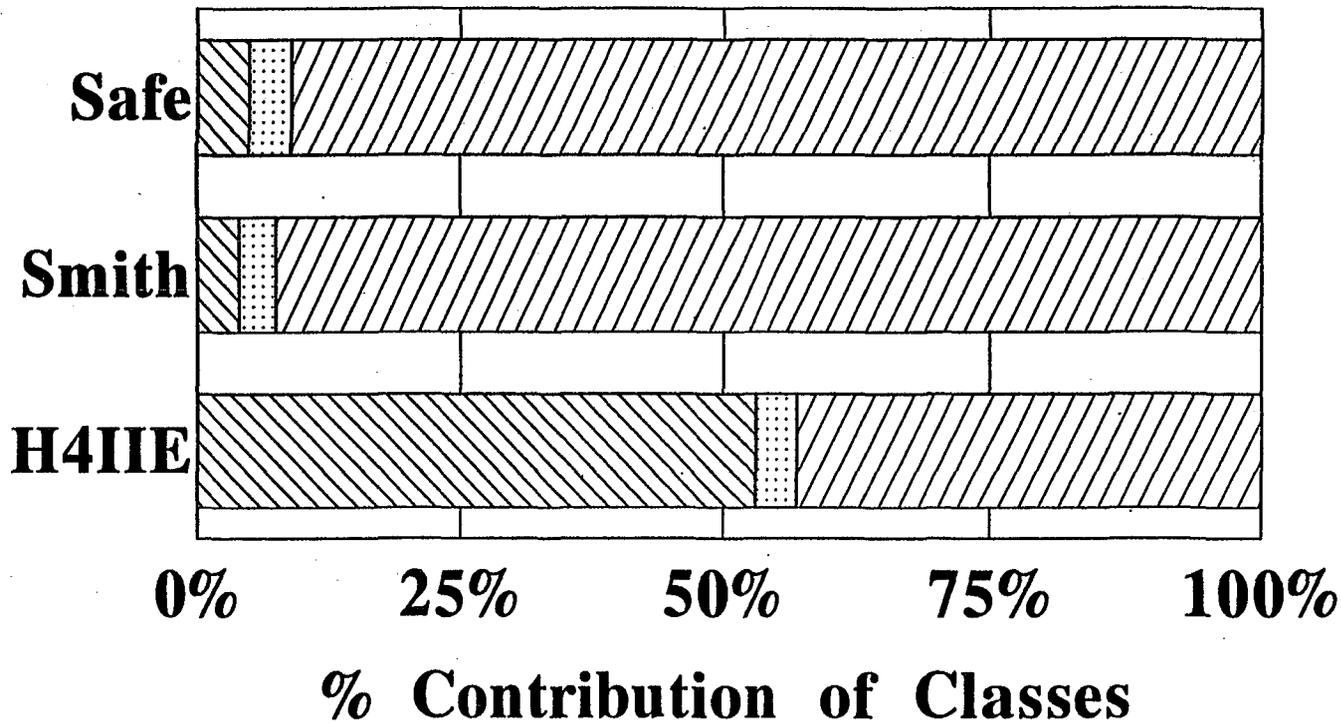
C-386

Based on Total TEQ By Additive Model

Giesy & Kubiak, 1993

# RELATIVE CONTRIBUTIONS OF Classes to TCDD-EQ in Carp

TEF



C-387

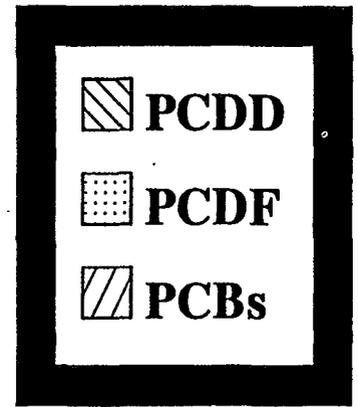
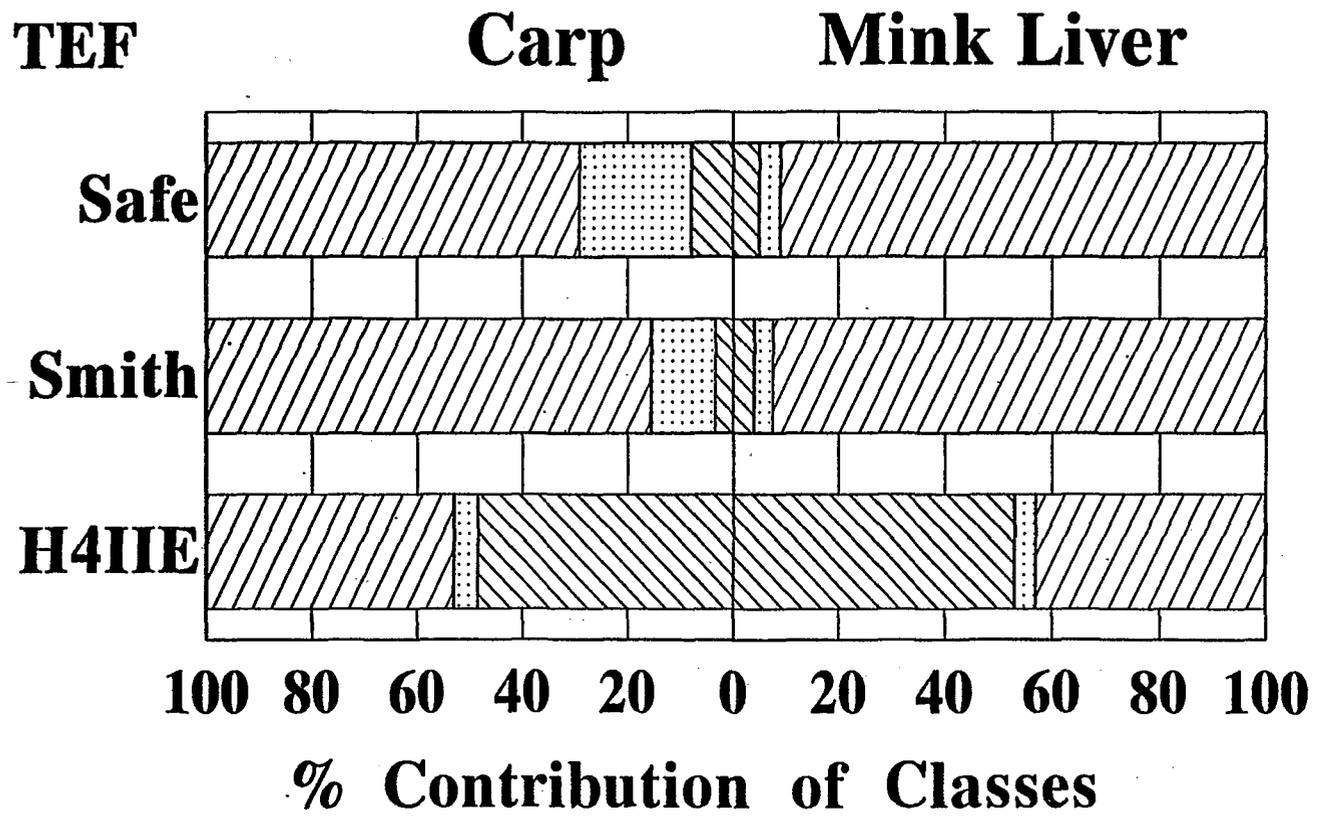
Based on Total TEQ calculated from Additive model

Giesy & Kubiak, 1993

# RELATIVE CONTRIBUTIONS

## Classes to TCDD-EQ

### Mink Liver & Carp



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Based on TEQ from Additive Model

Giesy & Kubiak, 1993

# **RELATIVE CONTRIBUTIONS** **of Classes to TCDD-EQ** **Saginaw Bay Carp**

**TCDD-EQ ( H4IIE ) = 50 . 37 pg / g, WW**

**\*TCDD-EQ ( Calc. ) = 19 . 4 pg / g, WW**

**Percent Total TCDD-EQ Accounted for by**  
**Quantified Congeners = 26%**

**PCDD: 53 %**

**PCDF: 4 %**

**PCBs: 43 %**

**\*TEFs for individual congeners determined in**  
**the H4IIE assay**

# RELATIVE POTENCY Calculation

$$\text{Relative Potency} = \frac{\text{Concentration of TCDD-EQ ( pg / g )}}{\text{Concentration of Total PCBs ( ug / g )}}$$

Used as a measure of contributions of non PCB components to TCDD-EQ or changes in TCDD-EQ Relative to the total PCBs in technical mixtures

Units of Relative Potency = mg / kg ( ppm )

# RELATIVE POTENCY & TOTAL PCBs SAGINAW BAY FISH

<b>Fish</b>	<b>Total PCBs ( mg / kg, WW )</b>	<b>Relative Potency ( ppm )</b>
<b>Walleye</b>	<b>1 . 0</b>	<b>4 . 1</b>
"	<b>0 . 9</b>	<b>14 . 9</b>
"	<b>1 . 8</b>	<b>7 . 8</b>
"	<b>2 . 8</b>	<b>31 . 0</b>
"	<b>1 . 2</b>	<b>11 . 6</b>
"	<b>1 . 4</b>	<b>3 . 8</b>
<b>Alewife</b>	<b>2 . 9</b>	<b>9 . 2</b>
<b>Shad</b>	<b>0 . 5</b>	<b>22</b>
<b>Carp</b>	<b>7 . 2</b>	<b>27</b>
<b>Mean</b>	<b>1 . 6</b>	<b>14 . 6</b>

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# **RELATIVE POTENCY**

## **In Carp and Mink Liver**

<b>TISSUE</b>	<b>POTENCY</b>
<b>Carp</b>	<b>27</b>
<b>Mink Liver</b>	<b>226</b>

C-392

**Based on TCDD-EQ from H4IIE assay & Total PCBs  
( mg TEQ / Kg PCB )**

# RELATIVE POTENCY

## In Tissues & Aroclors

SAMPLE	POTENCY
Mink Liver	226
Carp	27
Corm. Egg	28
Aroclor 1242	7.9
Aroclor 1248	12.4
Aroclor 1254	10.8
Aroclor 1260	3.2

Based on TCDD-EQ From H4IIE Assay & Total PCBs  
( mg TEQ / Kg PCB )

Giesy & Kubiak, 1993

# RELATIVE POTENCY

## SAGINAW BAY FISH

	<b>Fish</b>	<b>Relative Potency ( ppm )</b>
<b>Mean</b>	<b>All</b>	<b>14 . 6</b>
<b>SD</b>	<b>All</b>	<b>9 . 9</b>
<b>Max</b>	<b>Lg. Walleye</b>	<b>31</b>
<b>Min</b>	<b>Walleye Fry</b>	<b>4 . 1</b>

C-394

**WATER QUALITY CRITERION : Total PCBs  
Saginaw River & Bay  
Calculations**

<b>DIETARY NOAEL ( mg / kg )</b>	<b>BMF Water - Fish</b>	<b>WQC ( ng / l )</b>
<b>0 . 072</b>	<b>9 . 9 x 10 + 5</b>	<b>7 . 2 x 10 -2</b>

C-395

**WATER QUALITY CRITERION : Total PCBs  
Saginaw River & Bay  
Based on Mink**

<b>PARAMETER</b>	<b>VALUE</b>
<b>WQC ( ng / l )</b>	<b>7 . 2 x 10 - 2</b>
<b>Total PCBs ( ng / l )</b>	<b>22 . 3</b>
<b>Current Ratio</b>	<b>310 X NOAEL</b>

C-396

# **RESULTS & CONCLUSIONS**

## **BIOMAGNIFICATION FACTORS ( BMFs )-I**

- 1. Both Total PCBs and TEQs Were Biomagnified from Fish to Mink Liver**
- 2. The Least BMF was Observed For Total PCBs. This is Most Likely Due to the Fact That Some of the Congeners can be Metabolized and Excreted**
- 3. The BMFs Derived with Different TEF values for Calculated TCDD-EQ Were Similar**

C-397

# **RESULTS & CONCLUSIONS**

## **BIOMAGNIFICATION FACTORS ( BMFs )-II**

- 1. The BMF of TEQs Measured in the H4IIE Assay Were Greater Than for Those Calculated using TEFs. This was Most Likely Due to the Fact That the Assay was Measuring TEQs Contributed by Compounds Other than from PCBs, PCDDs and PCDFs**

# **RESULTS & CONCLUSIONS**

## **Relative Proportions of TEQs-I**

- 1. The Concentrations of TEQs Were Correlated With the Total Concentrations of PCBs**
- 2. The Concentrations of Bioassay-Derived TEQs Were Correlated with the Concentrations of TEQs Predicted From TEF**
- 3. Concentrations of TEQs Based on the Two Methods Were Similar for Small, Young Fish; For Larger Fish, Greater Concentrations of TEQs were Measured by the H4IIE Assay Than Predicted From an Additive Model and TEF Values.**

# **RESULTS & CONCLUSIONS**

## **Relative Proportions of TEQs-II**

- 1. While there was Good Agreement Between the Assay-Derived and Calculated TEQs for Smaller Fish only 25 % of the TEQ measured by the H4IIE in Carp Could Be Accounted for by TEQ Contributed by PCBs, PCDD & PCDF**
- 2. PCB Congeners Contributed an Average of 50 % of the TEQ Predicated by the Additive Model. PCDD Contributed Approximately 40 % While PCDF contributed Less Than 10 %**

# **RESULTS & CONCLUSIONS**

## **PCBs**

- 1. Concentrations of PCBs in Fishes Ranged From 0.5 mg / kg in Shad to 7.2 mg / kg in Carp**
- 2. Total Concentrations of PCBs Were Biomagnified from Fish to Mink Liver, Less so Than Were TCDD-EQs**
- 3. Concentrations of PCBs in all Species of Fishes Sampled from Saginaw Bay Exceeded the NOAEL for Mink**

C-401

# **RESULTS & CONCLUSIONS**

## **PCBs-II**

- 1. Hazard Ratios Ranged From 6.7 for Shad to 100 for Carp**
- 2. The Percent of PCB-Containing Fish Which Would be Allowed in the Diet of Mink to Keep Exposure to PCBs Less Than the NOAEL Ranged From 1 % for Carp to 14 % for Shad**

C-402

# **RESULTS & CONCLUSIONS**

## **Relative Potency**

- 1. The Relative Potency in Fishes from Saginaw Bay Were Equal to or Greater Than That of Aroclor Mixtures. Relative Potency Values Ranged from 3.8 in Small Walleye to 27 and 31 in Carp and Larger Walleye**
- 2. The Relative Potency in Mink Liver was 226, Which is Approximately 8 Times Greater Than in Carp, Which They Were Fed.**
- 3. These Results indicate Enrichment of the Ah-r-Congeners or the Presence of non-identified Congeners**

# **RESULTS & CONCLUSIONS**

## **Overall-I**

- 1. Current Concentrations of Both TEQs and PCBs in Fishes from Saginaw Bay Exceed the NOAEL for Mink by from 2 to 100 fold**
- 2. The Percentage of Fish From Saginaw Bay That Would be Allowable in the Diets of Mink Without Exceeding the NOAEL Ranged From 0.5 Based on TCDD-EQ ( Calculated ) in Carp to 48 % for Walleye Fry. The Overall Average was 8.4 % Based on all Fishes and all three Toxicants Studied**

C-404

# **RESULTS & CONCLUSIONS**

## **Overall-II**

- 1. The Hazard Posed by Consumption of Fishes from Saginaw Bay by Mink Were Similar for TEQs and Total PCBs. TCDD-EQ Calculated from Measured Congener Concentrations Would Result in the Most Stringent Regulation. PCBs Would Result in Regulations, Which Would be Approximately 3-Fold Less Stringent. The Least Stringent Regulation or Remediation Target Would Result From Assay-Derived TEQs. These Guidelines Would be Approximately 3-Fold Less Stringent Than Those Based on Total PCBs**

C-405

# **RESULTS & CONCLUSIONS**

## **Overall-III**

- 1. All of the TEQs Determined by the H4IIE Assay Were Accounted for by the Instrumental Analyses of PCBs, PCDDs and PCDFs in Small or Young Fish. However, in Larger Walleye and Carp, Only 25 % of the TEQs Measured by the H4IIE Assay Could be Accounted for**
- 2. PCB and PCDD Congeners Contributed Nearly Equal Proportions of the TEQs, Which Could be Accounted for by the Quantified Congeners. PCDFs Contributed less than 10 % of the TEQs**

C-406

# **RESULTS & CONCLUSIONS**

## **Overall-IV**

- 1. The Concentrations of Either PCDDs or PCBs found in Fish from Saginaw Bay Expressed Either as Total Concentrations of PCBs or TEQs Would Have Exceeded the NOAEL for Mink**
- 2. Both Total Concentrations of PCBs and TEQs Were Enriched in Fish and Mink Liver, Relative to Original Aroclor Mixtures**

C-407

# **RESULTS & CONCLUSIONS**

## **Overall-V**

- 1. Based on the BMFs Estimated for Accumulation of PCBs by Fish from the Water in Saginaw Bay the Water Quality Criterion to Protect Mink Should be  $7.2 \times 10^{-2}$  ng / l**
- 2. Current Total Concentrations of PCBs in Saginaw River Water Exceed the NOAEL for Mink That Eat 100 & Saginaw Bay Fish in Their Diet, by a Factor of 300-Fold**

C-408

# **RESULTS & CONCLUSIONS**

## **TCDD-EQ-I**

- 1. Concentrations of TEQs Determined by the H4IIE Assay or Calculated with an Additive Model based on TEFs derived with the H4IIE Assay, Were Similar for Small or Young Fish**
- 2. The TCDD-EQ Measured with the Assay were generally Greater than those Predicted from TEFs in the Larger Fish**
- 3. Concentrations of TEQs Exceeded the NOAEL for Mink in all of the Fishes, Which Were Sampled**

**Giesy & Kubiak, 1993**

# **RESULTS & CONCLUSIONS**

## **TCDD-EQ-II**

- 1. The Calculated TEQs Accounted for Only 25 % of the TEQs Measured by the H4IIE Assay in Larger Fish. This is Most Likely Due to the Presence of Ah-r-active Compounds Other than PCBs, PCDDs or PCDFs**
- 2. Hazard Ratios Ranged from 2.1 for Gizzard Shad 100 for Carp**

C-410

# **SUGGESTED STUDIES**

- 1. Assess Populations of Mink Which Could Be Exposed to Fish From The Saginaw River and Saginaw Bay in Their Diet**
- 2. Determine Concentrations of Total PCBs and TEQs in Liver: Compare these Values to the Dose-Response Relationship to Determine How Far They are From the Threshold for Adverse Effects**
- 3. Determine the Relationship Between PCBs & TEQs in Mink Feces & Their Food or Liver**

C411

# **HAZARD ASSESSMENT**

## **Refinement**

**A MORE ACCURATE DETERMINATION OF HAZARD WOULD REQUIRE DETAILED INFORMATION ON THE RELATIVE PROPORTION OF FOODS IN THE DIET AND THE DEGREE OF CONTAMINATION OF EACH FOOD ITEM**

**WE HAVE MADE A HAZARD ASSESSMENT BASED ON FISH (CARP) WHICH CONTAINED A COMPLEX MIXTURE OF TOXICANTS. ONE SOURCE OF UNCERTAINTY IN THE ANALYSIS WOULD BE CHANGES IN THE MIXTURE SPATIALLY OR TEMPORALLY**

C-412

**APPENDICES D-F**  
**WORKSHOP MATERIALS**



**APPENDIX D**  
**WORKSHOP AGENDA**





U.S. Environmental Protection Agency  
Risk Assessment Forum

**Workshop on Ecological Risk Assessment Issues for  
2,3,7,8-Tetrachlorodibenzo-p-Dioxin**

Radisson Hotel Metrodome  
Minneapolis, MN  
September 14-15, 1993

## Agenda

**Workshop Chair:**  
*Robert Huggett*  
*Virginia Institute of Marine Science*

### Tuesday, September 14

7:30AM           Registration and Onsite Check-In  
*Eastern Research Group, Inc. (ERG)*

#### PLENARY SESSION

8:30AM           **Welcome and Introduction**  
*Jack Gentile, U.S. Environmental Protection Agency (U.S. EPA),  
Risk Assessment Forum (RAF)*

8:45AM           **Workshop Objectives and Format**  
*Robert Huggett, Virginia Institute of Marine Science*

9:00AM           **Use of EPA's Framework for Ecological Risk Assessment**  
*William van der Schalie, U.S. EPA, RAF*

9:15AM           **Ecological Effects and Endpoint Selection Issues**  
*Randall Wentzel, U.S. Army*

9:30AM           **Stressor Characterization Issues**  
*William Adams, ABC Laboratories*

9:45AM           B R E A K

## **BREAKOUT GROUPS**

10:00AM

### **Discussion**

**Exercise 1:** Ecological Effects and Endpoint Selection  
*Randall Wentzel, Workgroup Leader*

**Exercise 2:** Stressor Characterization  
*William Adams, Workgroup Leader*

12:15PM

### **LUNCH**

1:30PM

### **Discussion (continued)**

**Exercise 1:** Ecological Effects and Endpoint Selection  
*Randall Wentzel, Workgroup Leader*

**Exercise 2:** Stressor Characterization  
*William Adams, Workgroup Leader*

3:00PM

### **BREAK**

## **PLENARY SESSION**

3:15PM

### **Summary Presentation and Discussion**

**Exercise 1:** Ecological Effects and Endpoint Selection  
*Randall Wentzel, Robert Huggett*

4:00PM

### **Summary Presentation and Discussion**

**Exercise 2:** Stressor Characterization  
*William Adams, Robert Huggett*

4:45PM

### **Observer Comments**

5:15PM

### **ADJOURN**

**Wednesday, September 15**

**PLENARY SESSION**

**8:30AM**      **Conceptual Model Development Issues**  
*Charles Menzie, Menzie-Cura & Associates*

**BREAKOUT GROUPS**

**8:45AM**      **Discussion**

**Exercise 3:**    **Conceptual Model Development**  
*Charles Menzie, Workgroup Leader*

**Exercise 3:**    **Conceptual Model Development**  
*Robert Huggett, Workgroup Leader*

**10:00AM**      **B R E A K**

**10:15AM**      **Discussion (continued)**

**Exercise 3:**    **Conceptual Model Development**  
*Charles Menzie, Workgroup Leader*

**Exercise 3:**    **Conceptual Model Development**  
*Robert Huggett, Workgroup Leader*

**12:00NOON**    **L U N C H**

**PLENARY SESSION**

**1:15PM**      **Observer Comments**

**1:45PM**      **Summary Presentation and Discussion**

**Exercise 3:**    **Conceptual Model Development Issues**  
*Charles Menzie, Robert Huggett*

**2:30PM**      **Identification of Major Uncertainties and Research Needs**  
*Robert Huggett, Workgroup Leaders*

**3:30PM**      **A D J O U R N**



**APPENDIX E**

**WORKSHOP PARTICIPANTS AND FINAL OBSERVER LIST**

1. The first part of the document is a list of names and addresses.

2. The second part of the document is a list of names and addresses.

3. The third part of the document is a list of names and addresses.

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10. The tenth part of the document is a list of names and addresses.



U.S. Environmental Protection Agency  
Risk Assessment Forum

**Workshop on Ecological Risk Assessment Issues for  
2,3,7,8-Tetrachlorodibenzo-p-Dioxin**

Radisson Hotel Metrodome  
Minneapolis, MN  
September 14-15, 1993

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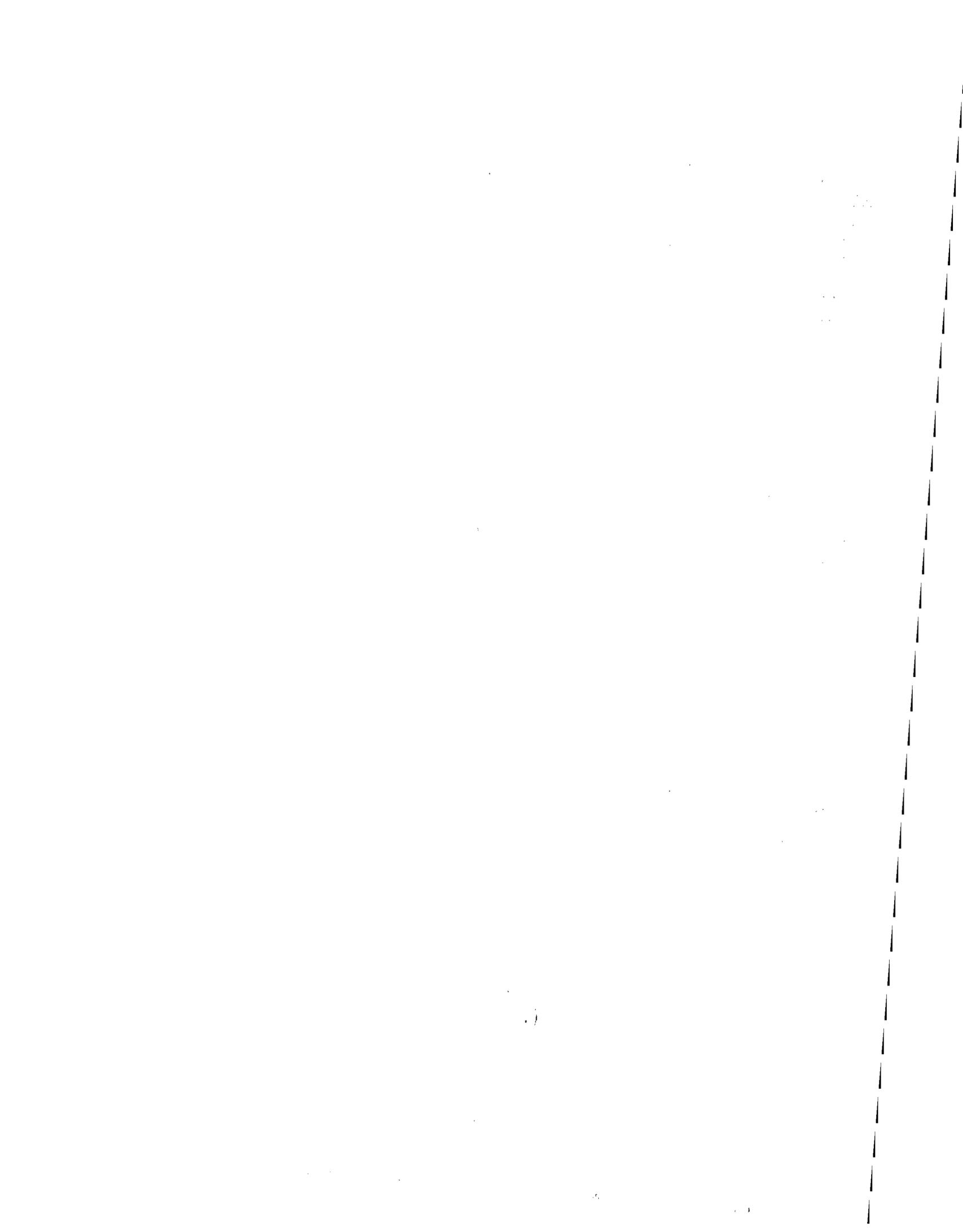
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U.S. Environmental Protection Agency  
Risk Assessment Forum

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**APPENDIX F**  
**WORK GROUP ASSIGNMENTS**



**U.S. Environmental Protection Agency  
Workshop on Ecological Risk Assessment Issues for  
2,3,7,8-Tetrachlorodibenzo-p-Dioxin  
September 14-15, 1993**

**WORKGROUP ASSIGNMENTS**

**Tuesday, September 14, 1993**

***Exercise 1: Ecological Effects  
and Endpoint Selection***

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***Exercise 2: Stressor Characterization***

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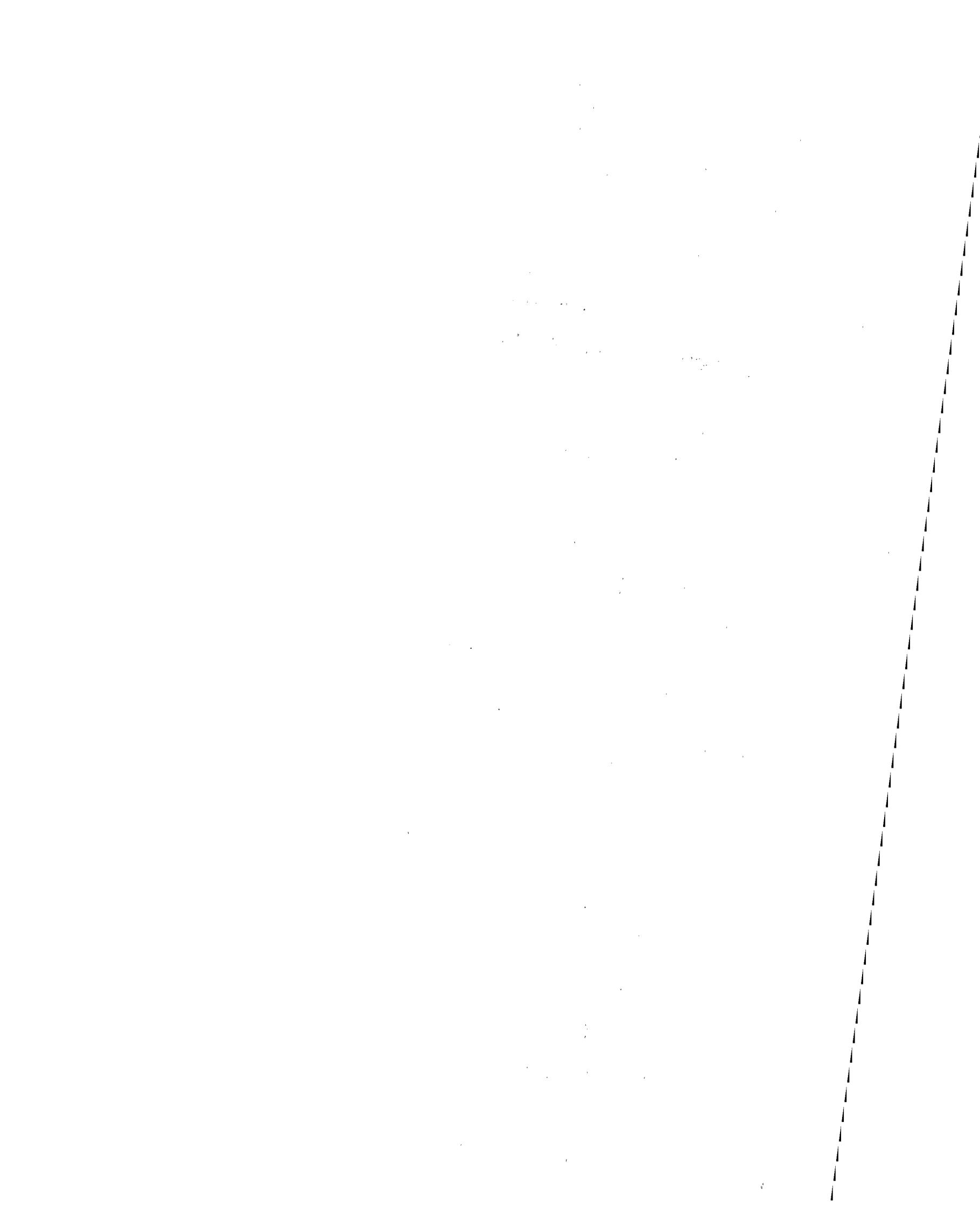
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PTI Environmental Services

Paul Rodgers  
LTI, LimnoTech, Inc.

**Wednesday, September 15, 1993**

***Exercise 3: Conceptual Model Development***

The Conceptual Model Development group will be divided into two subgroups as above, with Robert Huggett and Charles Menzie as workgroup leaders.



**APPENDIX G**  
**REVISED RISK ASSESSMENT CONCEPTUAL MODEL**



## Revised Conceptual Model for the Southern Reservoir

**Note:** See appendix A for the original conceptual model. Comments on the conceptual model figures in appendix A are provided in the text boxes. A revised diagram of the overall conceptual model is shown in figure 4 of the main report.

The foundation for the conceptual model is the tissue residue approach contained in the TCDD *Interim Report*. Concentrations of TCDD and related chemicals (PCDDs, PCDFs, coplanar PCBs, and monoorthochlorine-substituted analogues of the coplanar PCBs) that act by an Ah receptor mechanism in eggs of fish and in fish consumed by piscivorous birds and mammals are presently the exposure metrics upon which the estimation of the potential for adverse effects to the organism must be based. In this case, figure 2 (appendix A; see text box) shows the logical flow of assessment information when thresholds for adverse ecological effects on fish and wildlife population protection goals are to be related to safe chemical loadings to the ecosystem. A novel feature of this model is the transformation of reproductive effects to populations and community level impacts. The boxes in this conceptual model include endpoints that are generally quantitative. The arrows between boxes are specific types of models that are used to interrelate the assessment endpoints. All the models are reversible; hence the two-way arrows. The conceptual model applies equally to assessments that seek to determine risks associated with a known or predicted chemical loading (right to left flow of steps). This model may be used, following further review and validation, in the establishment of acceptable residue levels that form the basis for translation to permit conditions and associated effluent treatment standards. Within the conceptual model, exposure characterization is performed with the assistance of appropriate exposure models. Uncertainty associated with these models is related to the uncertainty associated with measurements of physicochemical properties, lipid and organic carbon relationships, and spatial-temporal heterogeneity of chemical concentrations in the ecosystem.

### Conceptual Model Comments: Figure 2 (Appendix A)

This figure represents an ideal that may not be attainable, in that our ability to do population- and ecosystem-level evaluations is quite limited. The challenge of this case study is to relate chemical loadings in the effluent to levels in food, water, and sediment to residues and effects in aquatic organisms and associated wildlife. The level of sophistication required in the analysis will depend on the resources available and specific questions being asked. A *tiered approach* is advisable—starting with simple methods and assumptions and proceeding to more complex analyses (e.g., those requiring reservoir segmentation) only if necessary.

Figure 2 shows effects on ecological systems linked to exposure levels through chemical residues. While the food chain is recognized as the primary route of exposure for consumers, uptake of TCDD-like chemicals directly from the water by primary producers (e.g., algae) is important. Since the known adverse effects of TCDD and related chemicals for fish are directly attributable to exposure of the embryo, the chemical residue levels in eggs are at present the exposure metric of primary interest. The exposure metric of interest for piscivorous avian and

mammalian wildlife is the concentration of TCDD and related chemicals in fish and other prey species consumed by these animals that causes reproductive toxicity. If more sensitive endpoints are found, they may be included. Care must be taken to ensure that appropriate exposure models are chosen for each aquatic and wildlife species of concern.

Atmospheric deposition of PCBs into Omigoshie Reservoir resulted in fish having background total PCB concentrations of 500 ng/g. The background levels of TCDD-like PCBs could result in fish inhabiting this reservoir being at greater risk of approaching an adverse body burden. The background body burden of TCDD-like PCBs would decrease the amount of TCDD-like PCDDs and PCDFs that could be released into the reservoir from the paper mill. Based on the occurrence of background levels of PCBs, site-specific BSAF factors could be determined. This would allow for pre-paper mill site information for modeling the reservoir.

Figure 3 (appendix A; see text box) illustrates the pathways for TCDD exposures and bioaccumulation in Omigoshie Reservoir biota. Selection of which boxes and arrows require inclusion in the assessment is a reflection of the management question being addressed and what supporting data are likely to be available. Thus TCDD exposure to fish and wildlife in natural systems is expected to be primarily via contaminated food, and effects are often best referenced to concentration in food or in the receptor organism itself. Thus TCDD residues in aquatic organisms, and the distribution and bioavailability of TCDD in water and sediments, will be of central concern in this assessment. Concentrations and their spatial-temporal heterogeneity in sediments, suspended solids, and water should be estimated using suitable fate-and-transport models. Tissue residue levels can be estimated via transport models and either BSAF, BSSAF, or food chain models, such as shown in figure 4 (appendix A; see text box). The

concentration of chemical predicted for the whole organism can be related to specific tissue concentrations through lipid normalization or a more specific toxicokinetic model. The variability of tissue residues among different organisms and their relationship to organic carbon in suspended solids and lipid in organisms are major uncertainties that must be considered. A third bioaccumulation approach is to estimate chemical concentrations of concern in the surface

**Conceptual Model Comments:  
Figures 3, 4 (Appendix A)**

Panelists felt figure 3 was adequate as a general descriptive tool but not for indicating the relative importance of different pathways. It was suggested that the benthic invertebrates be divided into two groups to better show links to birds, fish, and mammals. Spatial heterogeneity in the distribution of TCDD in the reservoir (main channel vs. side arms) will be especially important if there is parallel heterogeneity in the distribution of aquatic species of concern.

Panelists suggested that figure 4 should include crayfish and mussels. It was suggested that it would be important to know the dietary proportion of different organisms for species of concern.

Panelists developed additional figures for the conceptual model, as discussed in section 5 of this report.

sediments of the organism's habitat by application of measured or estimated biota-sediment accumulation factors (BSAF; see section 3.5 of the *Interim Report*) to the tissue residue-toxic response relationship for each species of concern. The BSAF approach has an advantage of using an accumulation factor that can be directly measured in contaminated ecosystems.