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

PB93-202828

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A related publication has been released and is now available. It is the "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. Held in Minneapolis, Minnesota on September 14-15, 1993."

NTIS order number: PB94-214822LEU, May 94, 444p. Source: Environmental Protection Agency, Washington, DC. Risk Assessment Forum. See also PB93-202828.
Summary: 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 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.

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16. ABSTRACT

In April, 1991 the administrator of the U.S. Environmental Protection Agency (EPA) announced that the Agency would conduct a scientific reassessment of the risk of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and similar chemicals, to human health and the environment. The reassessment plan includes a component on the risks of TCDD to aquatic life and associated wildlife. Research to provide needed exposure and effects information to better characterize these risks was initiated in September 1991. Because the results of this research effort will not be available until June 1995, this interim report was prepared to critically review and evaluate data and models currently available for analyzing TCDD exposure to, and effects on, aquatic life and wildlife and to identify major uncertainties that limit how well risks can be characterized. The report addresses TCDD exposure to, and bioaccumulation in, aquatic organisms, TCDD toxic effects on aquatic life and wildlife, and aspects of risk characterization to exemplify approaches and applicability of current information. The report will serve as a working document for a public meeting of scientists and risk assessment experts who will further evaluate present data limitations and uncertainties and who will also evaluate the applicability of the data and methods to actual TCDD risk assessments following the EPA's framework for ecological risk assessment.

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

Environmental Research Laboratory
Office of Research and Development
United States Environmental Protection Agency
Duluth, MN

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LIST OF SELECTED ABBREVIATIONS

Ah	Aryl hydrocarbon
AHH	Aryl hydrocarbon hydroxylase
BAF	Bioaccumulation factor
BAF_l	Lipid-normalized bioaccumulation factor
BCF	Bioconcentration factor
BCF_l	Lipid-normalized bioconcentration factor
BEF	Bioaccumulation equivalency factor
BKME	Bleached kraft pulp mill effluent
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factor
BSSAF	Biota-suspended solids accumulation factor
DOC	Dissolved organic carbon concentration in water
EC50	Concentration causing a 50% effect
ELIZA	Enzyme-linked immunosorbent assay
ER50	Residue concentration causing a 50% effect
EROD	7-Ethoxyresorufin-O-deethylase
FAF	Food accumulation factor
f_d	Fraction of chemical freely dissolved in water
f_l	Fraction of lipid in organism
f_{oc}	Fraction of organic carbon in sediment or suspended solids
HPLC	High pressure liquid chromatography
HRGC/HRMS	High resolution gas chromatography/high resolution mass spectrometry
IHNV	Infectious hematopoietic necrosis virus
K_{aw}	Aquatic organism/water equilibrium partition coefficient
k_1	Chemical uptake rate constant
k_2	Chemical elimination rate constant
K_{oc}	Organic carbon/water partition coefficient
K_{ow}	Octanol/water partition coefficient
LD50	Lethal dose to 50% of exposed organisms
LOAEL	Lowest observed adverse effect level
LR50	Residue concentration causing 50% lethality
MFO	Mixed-function oxygenase
MLD	Minimum level of detection
MOA	Mode of action
NOAEL	No observed adverse effect level
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PCH	Polychlorinated hydrocarbons
PeCDD	Pentachlorodibenzodioxins

PeCDF	Pentachlorodibenzofurans
POC	Particulate organic carbon concentration in water
R_{as}	Aquatic organism/sediment disequilibrium ratio
R_{aw}	Aquatic organism/water disequilibrium ratio
R_{ws}	Water/sediment disequilibrium ratio
S_m	Molar solubility in water
ssBAF	Steady-state bioaccumulation factor
ssBCF	Steady-state bioconcentration factor
S_w	Water solubility
TBF_{oc}	Total organic carbon binding factor
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCDF	2,3,7,8-Tetrachlorodibenzofuran
TEC	Toxicity equivalency concentration
TEF	Toxicity equivalency factor

EXECUTIVE SUMMARY

INTRODUCTION

Background

In April, 1991 the Administrator of the U.S. Environmental Protection Agency (EPA) announced that the Agency would conduct a scientific reassessment of the risk of 2,3,7,8-tetrachlorodibenzo-p-dioxin (hereafter referred to as TCDD), and similar chemicals, to human health and the environment. Since 1985, EPA has classified TCDD, which it considers the most potent known animal carcinogen, as a probable human carcinogen. Sources of TCDD in the environment were subsequently regulated on the basis of animal cancer rates extrapolated to doses associated with human exposures. Recently, consensus has developed that the toxic effects of TCDD appear to be mediated by its binding to a receptor protein, the Ah cytosolic receptor. This conclusion has led to the supposition that a receptor-based model is appropriate for characterizing TCDD risk. As a consequence, EPA designed and implemented a reassessment research plan, founded on biologically-based dose-response models for TCDD and related chemicals, to establish a more scientific basis for credible risk assessments.

In addition to addressing human health risks of TCDD, the reassessment plan includes a component on the risks of this compound to aquatic life and associated wildlife. This component is included because EPA not only recognizes that TCDD in aquatic environments can be a major contribution to overall human exposure through fish and shellfish consumption, but also that piscivorous fish and wildlife may be particularly at risk due to their large exposure through aquatic food chains. The limited available toxicological data indicate that fish, especially salmonid sac fry, and mink (*Mustela vison*) are among the most sensitive animals to TCDD and related compounds. Therefore, independent of the human health risks, there is an increased concern for assessing ecological risks.

Research to provide the needed exposure and effects information to characterize the risk of TCDD to aquatic life began in September, 1991, and is continuing. The experimental design for this research was formulated on existing data gaps and employs an approach founded on the Ah receptor-based mode of action. The design also addresses the definition of sensitive and ecologically-relevant toxic effect endpoints, a variety of potentially threatened species and complex exposure relationships. Overall, the research is based on major uncertainties regarding TCDD exposures and toxic effects, but is also designed to provide scientific data of the quality and quantity required for the development of an aquatic life-based EPA water quality criterion for TCDD. The goal of the EPA Office of Research and Development's aquatic effects research plan is to develop data and models needed to

complete a comprehensive final report on data and methods for TCDD aquatic risk assessment for review in June, 1995.

Goal and Scope of the Interim Report

The goal for this report was to critically review and evaluate relevant published, and in some instances unpublished, data and models currently available for analyzing TCDD exposure to, and effects on, aquatic life and wildlife and to identify major areas of uncertainty that are expected to limit how well related risks can be characterized. It offers a critical analysis of currently available exposure and effects information and outlines important principles and concepts that must be considered when these data are later integrated to characterize risk. Specifically, this report addresses TCDD exposure to and bioaccumulation in aquatic organisms, TCDD toxic effects on aquatic life and wildlife, and aspects of risk characterization to exemplify approaches and applicability of current information.

The resulting analyses have two uses. First, as discussed previously, analysis of available data assists research planning by identifying data gaps. Second, by collecting and organizing existing information in line with EPA's recent report "Framework for Ecological Risk Assessment", such analyses serve as first steps in planning a risk assessment. That is, for future risk assessments associated with TCDD and related chemicals in aquatic food webs, the available data can be examined specifically in terms of its suitability to form a "conceptual model", consistent with the EPA framework for ecological risk assessment.

This interim report has a limited scope. The analyses presented specifically address the direct toxic effects of TCDD to aquatic life and wildlife based on uptake from aquatic prey, sediment and surface water. This report does not provide generic or site-specific TCDD risk assessments nor TCDD risk assessments for specific aquatic life or wildlife species. Information on polychlorinated and polybrominated dibenzo-p-dioxins, dibenzofurans, and planar polychlorinated biphenyls, that have the same mode of toxic action as TCDD, is not treated in detail; however, mixtures of these compounds certainly must be considered to ultimately characterize the ecological risk of TCDD. The final report on aquatic ecological risk assessment elements will attempt to incorporate the contribution of TCDD-related chemicals in assessing the risk of complex mixtures to aquatic life and wildlife.

Techniques to assess TCDD risk in aquatic ecosystems are hampered by limited exposure and effects data as well as generic shortcomings that confront most, if not all, ecological risk assessments for chemical stressors. Using currently available dose-response relationships for reproductive and/or developmental impairment, the interim risk characterization approach provides techniques to relate TCDD concentrations in water, sediment and fish tissues to a low or high likelihood of population failure in aquatic life and wildlife. More precise probability estimates of aquatic life and wildlife population responses are not currently possible because of the

limited dose-response relationships available and because, in general, validated species-specific population dynamic models do not exist. With more refined TCDD dose-response relationships, well-developed aquatic life and wildlife population models, and a better understanding of the interaction of additional chemical and non-chemical stressors, the contribution of TCDD exposure to the probability of changes in population dynamics could be quantified with increased certainty in the future. Finally, this report does not discuss the impact of TCDD on community or ecosystem structure and function and the resultant interrelated effects on aquatic life or wildlife populations. To assess the risk of TCDD to communities and ecosystems will require models, with parameters specified appropriately for intended applications, to evaluate and forecast responses as a function of current or future TCDD concentrations in water, sediment and biota.

Peer Review

As outlined in the Acknowledgements Section, this report has undergone extensive review. The report was first reviewed by U.S. EPA scientists within the Office of Research and Development and two non-EPA scientists who are undertaking particularly relevant research on TCDD and related chemicals. A subsequent draft of the document was then reviewed by twelve scientists from academia, industry, non-EPA governmental agencies and private organizations. The current document reflects the comments and suggestions provided through this review process.

This report provides an initial base of information and analyses that EPA is planning to use for assessing risks of TCDD to aquatic life and wildlife. This report will provide a working document for a meeting of EPA scientists and ecological risk assessment experts who will further evaluate the present data limitations and uncertainties that should be incorporated into plans for completing a comprehensive final report on TCDD risk assessment in 1995. The peer panel will also evaluate the applicability of the data and methods for actual TCDD risk assessments following the U.S. EPA's framework for ecological risk assessment.

EXPOSURE

In natural environments, TCDD is typically associated with sediments, biota and the organic carbon fraction of ambient waters. This distribution of TCDD in aquatic systems is a function of its low water solubility and extreme hydrophobicity. Water solubility measurements for TCDD indicate that a range of 12 to 20 ng/L is likely for colder water (4 to 12°C), but the extent to which the solubility increases at warmer temperatures is more uncertain due to the limited data and variable results reported. The true activity of TCDD in water (freely dissolved or bioavailable concentrations) is uncertain because current analytical chemistry techniques and associated minimum levels of detection are not sufficiently sensitive. Concentrations of total TCDD (freely dissolved and organic carbon-bound) in ambient waters have also not been measured

to date; however, lower levels of detection through improved high resolution gas chromatography/high resolution mass spectrometry techniques may permit such measurements in the future. Estimates for log octanol/water partition coefficients (log K_{ow}s) and log carbon/water partition coefficients (log K_{oc}s) range from approximately 6.5 to 8.0 and from 6.5 to 7.5, respectively. Based on an analysis of a variety of measurements and models, the log K_{ow} and log K_{oc} for TCDD were both estimated to be 7.0 for this report; however, a wide range of uncertainty exists and the "true" log K_{ow} and log K_{oc} are likely to be greater than 7.0.

Because of its lipophilicity, and low rates of chemical and biological degradation in aquatic environments, TCDD does accumulate in biota to detectable levels. When interpreting and comparing TCDD residue accumulation in aquatic organisms, it is important to realize that exposure occurs through combinations of water, sediment, and dietary routes that are influenced by species-specific differences in physiology, bioenergetic condition and habitat, as well as site-specific TCDD bioavailability. Two EPA national surveys undertaken during the mid 1980s indicate that most areas across the United States had TCDD fish concentrations ranging from non-detectable (less than 0.5 to 1.0 pg/g) to 1.0 pg/g on a whole body, wet weight basis. Fish from open Great Lakes sites and some rivers and estuaries downstream from kraft paper mills had concentrations in the range of 1 to 20 pg/g, while fish taken from specific sites in Lake Ontario and other inland freshwater sites had concentrations in the range of 20 to 100 pg/g. There have also been reports of occasional fish collected with concentrations in excess of 100 pg/g. On the basis of limited samples and sites, TCDD residues in fish appear to have decreased over the past decade.

TCDD also accumulates in sediments to measurable levels; however, there have been no national surveys undertaken that are comparable to those for fish. Sediment monitoring studies have tended to focus on sediments from areas known to be contaminated. Based on dated sediment core samples from Lake Ontario sites contaminated by loading from the Niagara River, TCDD levels were nondetectable prior to 1940 and reached a maximum level of approximately 500 pg/g dry sediment in 1962. By 1987, the average surface sediment TCDD concentrations declined to 68 pg/g. Sediment samples taken from the Newark Bay, New Jersey estuary have had TCDD concentrations ranging from 730 to 7,600 pg/g dry sediment in association with herbicide production from 1948 to 1969.

BIOACCUMULATION

Lipid-normalized bioaccumulation factors (BAF_ls) for TCDD derived from lake trout (*Salvelinus namaycush*) and sediment data from Lake Ontario combined with chemical mass balance model predictions of TCDD concentrations in water, are approximately $1 \cdot 10^6$ for total TCDD concentration in water and $3 \cdot 10^6$ for freely dissolved TCDD, assuming a log K_{ow} of 7.0. BAFs for whole fish or fish tissue of a particular lipid content are determined by multiplying the BAF_l by the fraction of lipid in

the fish. The BAF_ts can be applied to waters other than Lake Ontario by adjusting for the effect of dissolved organic carbon on the fraction of TCDD that will be freely dissolved (f_d). The BAF_t for total TCDD is approximately $3 \cdot 10^6 \cdot f_d$ or $0.2 \cdot 10^6$/POC (where POC is the concentration of particulate organic carbon in the water). The BAF_t for freely dissolved TCDD is $3 \cdot 10^6$ for all waters, but is uncertain (as is f_d) due to the uncertainty in the log K_{ow} assumption. Steady-state lipid-normalized bioconcentration factors ($ssBCF_t$s) reported for fish exposed under constant flow conditions in laboratory experiments are approximately $1 \cdot 10^6$ (or $1 \cdot 10^4$ for each 1% lipid in the fish). TCDD does not appear to undergo biomagnification in fish, most likely due to the counteracting effect of biotransformation.

The biota-sediment accumulation factor (BSAF) can be measured for TCDD. The BSAF is not influenced by the uncertainty for log K_{ow} since it relates TCDD in lipids of organisms to TCDD in organic carbon of sediment. Data, which are limited by the paucity of appropriate measurements of TCDD concentrations in surface sediments, provide BSAFs for TCDD in fish that range from 0.03 to 0.30. Predicted equilibrium BSAFs for TCDD at equilibrium between sediment and organisms are on the order of one or slightly greater. These observed BSAFs indicate that disequilibrium exists between fish and sediment, which is the product of disequilibria between fish and water and between water and sediment. The limited data available suggest these disequilibria are common for fish at different sites, although some variation in magnitude among species and sites is apparent.

EFFECTS

The Ah receptor has been detected in teleost and elasmobranch fishes, but high quality data for amphibians and reptiles are unavailable. The Ah receptor has not been detected in aquatic invertebrates. Although there appear to be no studies available in the literature that address Ah receptor presence in mammalian or avian wildlife species, it is reasonable to assume its presence based upon data from closely related species such as the common rat and chicken (*Gallus domesticus*). Available toxicity data, as well as information concerning the phylogenetic distribution of the Ah receptor, suggest that a toxicity model based upon the Ah receptor is appropriate for most fish and wildlife species.

Aquatic Life

In general, toxicity test results with aquatic organisms indicate that TCDD is not toxic during standard acute test periods of 24 to 96 hours but can cause delayed adverse effects, days, weeks or even months after exposure. Water concentrations up to 1,330 ng/L (approximately 70 times higher than water solubility) did not affect plants (both algae and vascular plants) and invertebrates (snails, worms and cladocerans) after several days of exposure, whereas concentrations as low as 0.05 to 1 ng/L caused 50% mortality to rainbow trout (*Oncorhynchus mykiss*) and northern pike (*Esox lucius*) fry, respectively.

Because of uncertainties in establishing the bioavailability of TCDD in aqueous solutions, measured TCDD concentrations in test organisms, as opposed to aqueous TCDD concentrations, are a more useful metric of expressing aquatic toxicity dose-response relationships. Largest TCDD concentrations reported not to cause effects in algae, snails, cladocerans and bullfrogs (*Rana catesbeiana*) are 2,295,000, 502,000, 1,570,000 and 1,000,000 pg/g, respectively, in contrast to a concentration range of 47-65 pg/g in lake trout eggs which can cause significant mortality in the hatched fry. Measured concentrations in the eggs and tissues of fish indicate that young fish are more sensitive to TCDD than older fish, and that delivered doses to eggs that elicit adverse effects in salmonid sac fry are similar regardless of the route of egg exposure (i.e., waterborne, egg injection or maternal transfer). There is no evidence that adverse effects occur in any fish species if egg concentrations are less than 34 pg/g. This likely corresponds to an accumulation in parent fish, with lipid content similar to the eggs, of less than 50 pg/g. However, depending on fish species, effects on fish fry survival are expected to be substantial when accumulations in the eggs are 50 to 500 pg/g, which corresponds to a range of 75 to 750 pg/g in parent fish. Substantial mortality to older fish is expected to occur when total body accumulations are in the range of 1,000 to 15,000 pg/g.

To date, lake trout sac fry mortality due to low concentrations of TCDD in eggs has been one of the most sensitive and ecologically-relevant endpoints identified. This mortality in salmonids has been associated with a stress-syndrome commonly referred to as blue-sac disease. The dose-response curve for survival of hatched fry is very steep. For example, a lake trout egg concentration of 34 pg/g has not been shown to cause adverse effect but 104 pg/g causes complete mortality. Preliminary analyses based on assumptions of TCDD bioavailability during waterborne egg exposures suggest that other fish, such as the northern pike, are nearly as sensitive, if not more so, than lake trout. Sensitivity also appears to vary between salmonid species and within different strains of the same species.

In juvenile and adult fish, sublethal effects such as fin necrosis, lesion development, histopathological changes, enzyme induction (cytochrome P4501A1) and immune suppression appear to occur in fish at TCDD tissue concentrations that ultimately cause lethal effects. These results indicate that such responses may have applicability as screening parameters for assessing contamination due to TCDD and related compounds.

The laboratory toxicity information shows that fish are generally more sensitive than aquatic plants, aquatic invertebrates and other aquatic vertebrates such as amphibians. However, the database regarding this sensitivity is limited and the exposures are not always readily comparable to the data on fish. It is possible that aquatic ecosystem components other than fish are sensitive to TCDD, but simply have not been adequately tested. Additional long-term tests that utilize a greater portion of

the species' life cycle are needed to more definitively establish dose-response curves for these groups of seemingly insensitive organisms.

Few TCDD epidemiological studies of aquatic life populations have been reported. Such studies are complicated because TCDD exposures to aquatic organisms in natural systems are confounded by the presence of other chemical and non-chemical stressors that may add to, or otherwise modify, the effects of TCDD. Despite these difficulties, investigations of lake trout populations in Lake Ontario provide some insights into the relationship of TCDD exposure to reproductive success that are consistent with laboratory findings that lake trout sac fry are very sensitive to TCDD.

Prior to the onset of severe TCDD contamination in Lake Ontario in about 1950, lake trout populations had declined precipitously due to overfishing, sea lamprey predation and habitat degradation. Despite a lake trout stocking and management program (i.e., controls on the lamprey and habitat improvement) which began in 1971, populations of naturally reproducing lake trout have not been achieved. In 1978, lake trout eggs contained approximately 30 pg TCDD/g and a 48% incidence of blue-sac disease, and associated mortality, was reported for hatchery-raised sac fry collected as eggs in 1979. If all of this reported incidence of blue-sac disease were due to TCDD and toxicologically-related chemicals, TCDD is estimated to have accounted for half of the toxicity equivalents. Recent reports, which indicate that some lake trout sac fry are reappearing in Lake Ontario and that there is an absence of blue-sac disease, suggest an improvement in reproduction may be occurring. In 1988, TCDD lake trout egg concentrations were reduced to 10 pg/g, which is consistent with declines in TCDD concentrations in the sediments (since the 1960s) and adult lake trout, since their reintroduction in the 1970s. The most recent measurements of TCDD concentrations in lake trout eggs are approximately one third of the no-effect level derived from laboratory toxicity tests. Although there is consistency between single chemical dose-response curves for TCDD and related compounds and the recent history of Lake Ontario lake trout populations, further study is needed to define dose-response curves for the specific chemical mixtures accumulating in lake trout eggs.

In a potentially complementary approach to assessing risks of chemical mixtures, cytochrome P4501A1 induction in some populations of fish has been explored as an indicator of chronic exposure to TCDD and related chemicals. To date, the biochemical indicator studies have not established a clear linkage between toxic effects and TCDD exposure.

Wildlife

There are few TCDD toxicity studies reported for mammalian and avian wildlife species and apparently no information exists for TCDD toxicity to reptiles.

Based on a single dose LD50 value of 4,200 pg/g, the mink is one of the most sensitive mammals evaluated thus far and is comparable in sensitivity to the guinea pig. TCDD administered by intraperitoneal (i.p.) injection to newborn mink at 100 and 1,000 pg/g/day for 12 consecutive days resulted in 100% mortality within 14 days at the high dose and 62% mortality by 19 weeks at the lower dose. No reproduction and developmental bioassays for mammalian wildlife were found in the literature; however, rat and Rhesus monkey (*Macaca mulatta*) bioassays indicated threshold effect levels for offspring production and survival of 1 and 0.13 pg TCDD/g/day, respectively. Based on these values, and the greater sensitivity of the mink compared to the rat based on acute and subchronic exposures, a threshold level of 0.1 pg TCDD/g/day was estimated for primarily piscivorous mammalian wildlife species, such as the mink and river otter (*Lutra canadensis*). This oral intake value is equivalent to a dietary fish concentration of 0.5 to 1.0 pg TCDD/g, assuming dietary consumption rates as a percentage of body weight equal to 10 to 20%.

Based on currently available data for avian species, it appears that gallinaceous birds are the most sensitive to TCDD. Of these birds, the ring-necked pheasant (*Phasianus colchicus*) and the chicken are among the most sensitive with mortality rates of 80 to 100%, respectively, reported for a single dose of 25,000 pg/g. The available evidence suggests that effects on reproduction are of particular concern; however, only one reproduction bioassay has been reported. Results from this study, which consisted of a 10 week i.p. injection dosing regime to female ring-necked pheasant, indicated that a female adult dose equivalent to 140 pg/g/day resulted in 100% embryo mortality, while a dose of 14 pg/g/day appeared to be a threshold level for no effect. Several egg injection studies completed with the chicken and ring-necked pheasant indicate that egg concentrations of 100 to 500 pg/g are threshold levels for embryo mortality, the chicken being the more sensitive species. Considering uncertainties associated with the ring-necked pheasant reproduction study (i.e., study duration and interspecies sensitivity), the adult pheasant threshold value of 14 pg TCDD/g/day for embryo mortality was extrapolated to a value of 1.4 pg TCDD/g/day for representative piscivorous avian wildlife species, such as raptors, wading birds and diving ducks. This oral intake value is equivalent to a dietary fish concentration of 3 to 14 pg TCDD/g, assuming dietary consumption rates as a percentage of bird body weight equal to 10 to 50%.

Results from limited epidemiology studies are consistent with laboratory-derived threshold levels for TCDD impairment of reproduction in avian wildlife. Population declines in herring gulls (*Larus argentatus*) on Lake Ontario during the early 1970s coincided with egg concentrations of TCDD and related chemicals expected to cause reproductive failure based on laboratory experiments (TCDD concentrations in excess of 1,000 pg/g). Improvements in herring gull reproduction through the mid-1980s were correlated with declining TCDD concentrations in eggs and lake sediments. Studies at sites in British Columbia, Canada also showed an inverse relationship between Great Blue heron (*Ardea herodias*) chick growth and incidence of edema and TCDD egg

concentrations. The herring gull and heron data indicate that successful reproduction exists in wild bird colonies even though TCDD is present in the eggs at concentrations between 200 to 500 pg/g. In turn, toxic effects at the individual level are associated with concentrations above 100 pg/g. These concentrations are consistent with findings from laboratory-based egg injections studies, summarized previously. Based on residue monitoring studies in Lake Ontario that relate TCDD herring gull egg concentrations to TCDD fish concentrations, the threshold value for TCDD concentrations in fish associated with successful herring gull reproduction in Lake Ontario was calculated to be 24 pg/g. This value is consistent with the independently derived fish concentration threshold value of 3 to 14 pg/g extrapolated from the ring-necked pheasant reproduction study.

The limited data available for TCDD contribute to uncertainty in establishing the mammalian and bird effect profiles. The estimates of fish TCDD contamination that pose a risk to wildlife are for organisms which are completely piscivorous; other dietary sources can alter exposures. For example, mink that do not primarily forage on fish and aquatic invertebrates could have approximately 50% lower TCDD exposures, while raptors that may consume fish-eating birds could have approximately 50% higher TCDD exposures. The limited toxicological databases also contribute to uncertainties, as reflected in the use of interspecies and subchronic to chronic extrapolation factors in the mammalian and avian profiles, respectively. Because there are essentially no toxicokinetic and toxicodynamic data, it is very difficult to address these extrapolation uncertainties quantitatively. Finally, the dose-response curves currently available are based on large differences in treatment levels, which also contributes to uncertainty for establishing the probability of effects for a given exposure.

RISK CHARACTERIZATION METHODOLOGY

In an ecological risk assessment for a chemical stressor, risk characterization is the phase in which the results of exposure and effects analyses are integrated to evaluate the likelihood of adverse effects in exposed organisms, populations, communities, or ecosystems based on actual or projected exposures of organisms to the chemical, or suite of chemicals, in the environment. The degree to which risk is characterized can vary markedly, but ideally involves a quantitative scale of effects and estimation of probabilities and uncertainties. Current information is insufficient to provide such a thorough description for TCDD risk to aquatic life and associated wildlife, with quantification of uncertainty being particularly difficult given the limited knowledge base. Furthermore, a thorough assessment of TCDD risk should consider its joint action with other contaminants and non-chemical stressors and the expression of effects on individual organisms at a population and community level; such techniques are even less well established and must await further development. However, the adequacy of a risk characterization depends on the nature of the

specific problem of interest, so current information can be adequate to characterize TCDD risk to aquatic life and associated wildlife in some cases.

The principal goal of this report was to evaluate and summarize data and methods that are available for the assessment of TCDD risk to aquatic life and associated wildlife, and to identify the major uncertainties that currently limit how well risks can be characterized. A definitive risk assessment for a specific problem was not a goal of this report. However, it is necessary to discuss how exposure and effects information should be integrated into a description of risk and it is most effective to do this in the context of actual problems regarding the risk of TCDD to aquatic life and associated wildlife. The following examples are presented to illustrate methods that can be used in risk characterizations and to identify major uncertainties that should be of concern. The degree to which these problems were evaluated was limited to that needed to accomplish this limited purpose, so these examples should not be treated as complete risk characterizations.

Fish Contamination in the United States: Risk to Aquatic Life and Associated Wildlife

In two national EPA surveys for a large number of diverse sites, fish were analyzed for TCDD and other chemical contaminants. These surveys provide the best data set for consideration of TCDD risk on a national basis and at different classes of sites, although effects can only be considered on the basis of accumulation in fish and cannot reliably be related back to environmental concentrations and source loadings. Although there are limitations in the geographical sampling designs associated with these fish residue data, they can be used to judge how extensive and severe risk of TCDD to aquatic life and associated-wildlife might be on a national and regional scale. When contrasted with TCDD concentrations in fish established in this report to be of low to high risk to sensitive fish, birds, and mammals, the survey data suggest that TCDD contamination is below levels of concern for aquatic life at all but a small percentage of the sites nationwide. However, there are sites where TCDD concentrations are high enough to pose significant risk to fish. This is especially true if joint action with other chemicals is considered. For wildlife, significant risk is potentially more widespread than for aquatic life, which is expected since the effects profile developed in this report suggests completely piscivorous wildlife are much more susceptible to TCDD than fish. In particular, TCDD residues in fish associated with a low risk for mammals are exceeded for about half of the samples in these national surveys. This characterization must be qualified by the recognition of uncertainties cited earlier for wildlife effects data and uncertainties associated with actual wildlife exposures. Nevertheless, despite these uncertainties, this comparison of fish survey results and wildlife effects data does raise significant concerns about the present risk of TCDD to piscivorous wildlife.

Lake Trout Populations in Lake Ontario

Lake trout are a Great Lakes ecosystem quality indicator species and are the subject of extensive efforts for restoration of naturally reproducing populations. Prior to the onset of severe TCDD contamination in Lake Ontario in about 1950, lake trout populations had declined precipitously due to overfishing, sea lamprey predation and habitat degradation. Despite a lake trout stocking and management program, which began in 1971, populations of naturally reproducing lake trout have not been achieved. Laboratory-based information on TCDD residues associated with toxic effects can be compared to TCDD concentrations observed in Lake Ontario lake trout and their eggs over the last 15 years. In 1987, eggs collected from Lake Ontario contained about 10 pg TCDD/g wet weight. This concentration is about three-fold lower than the threshold for sac fry mortality derived from toxicity studies using a variety of exposure routes. Therefore, even given the uncertainties in these bioassays, it is unlikely that lake trout reproduction is currently at risk in Lake Ontario due solely to TCDD effects on sac fry survival. However, this conclusion is not necessarily appropriate for past conditions in Lake Ontario. TCDD in surficial sediments and lake trout both declined two- to three-fold from 1978 to 1988. In 1978, lake trout had average concentrations of 78 pg TCDD/g wet weight, high enough that TCDD alone would be expected to have effects on reproduction as a result of sac fry mortality. Based on the sediment record, this concentration would have been several-fold higher in the early 1960s, and well above that which would have precluded successful reproduction.

Based on the data from 1987-1988, TCDD, in concert with other polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and planar polychlorinated biphenyls, may still be contributing to the continuing Lake Ontario lake trout reproduction problem. TCDD concentrations in eggs are at about one-fifth the lethal residue expected to cause 50% mortality, which could be a significant component in a complex mixture. The joint toxicity of TCDD and related chemicals is a major uncertainty that needs to be addressed. There also is the issue of whether the measurement endpoint used here, sac fry survival in a laboratory environment, is an adequate surrogate for the assessment endpoint of interest, namely lake trout reproduction in a natural system. Other toxicological endpoints associated with reproductive physiology may be more relevant and sublethal effects on fry might also affect their survival in a natural environment. These uncertainties need to be addressed to adequately assess risk of TCDD to Lake Ontario lake trout, and represent major uncertainties confronting aquatic life risk assessments in general.

Environmental Concentrations Associated with TCDD Effects

Some EPA regulatory activities, such as the establishment of water and sediment quality criteria, require the specification of environmental concentrations that are considered to represent acceptably low risk. These concentrations are set generically and are applied to specific sites to calculate allowable discharges or set goals for remedial actions. Such procedures do not fit the risk paradigm in which effects and exposure information are combined into a statement of risk, after which

decisions about managing risk are made. Rather, water and sediment quality criteria incorporate risk management decisions before specific exposure information is introduced into the process. Nevertheless, the setting of such criteria still contain major elements of risk assessment, albeit an incomplete one, and the entire regulatory process includes all the elements of risk assessment, although somewhat intertwined with risk management.

Information presented earlier on effects and their relationship to exposure conditions can be used to specify concentrations in water and sediment that will be associated with different levels of risk to aquatic life and aquatic-associated wildlife (see Table E-1). This exercise serves as a simple demonstration of how the information reviewed in this document can be used and is not intended to be a complete and definitive characterization of TCDD risk and associated uncertainties. Consistent with the presentation of aquatic life and wildlife effect profiles, the results of these analyses indicate that completely piscivorous wildlife, and especially piscivorous mammals, are at greatest potential risk to TCDD exposure in aquatic ecosystems.

Table E-1. Environmental concentrations associated with TCDD risk to aquatic life and associated wildlife.

Organism	Fish Concentration (pg/g)	Sediment Concentration (pg/g dry wt.)	Water Concentration (pg/L)	
			POC=0.2	POC=1.0
Low Risk				
Fish	50	60	0.6	3.1
Mammalian Wildlife	0.7	2.5	0.008	0.04
Avian Wildlife	6	21	0.07	0.35
High Risk to Sensitive Species				
Fish	80	100	1.0	5
Mammalian Wildlife	7	25	0.08	0.4
Avian Wildlife	60	210	0.7	3.5

Fish lipid of 8% and sediment organic carbon of 3% assumed where needed.
For risk to fish, BSAF of 0.3 used; for risk to wildlife, BSAF of 0.1 used.
Low risk concentrations are derived from no-effects thresholds for reproductive effects (mortality in embryos and young) in sensitive species.
High risk concentrations are derived from TCDD doses expected to cause 50 to 100% mortality in embryos and young of sensitive species.

RESEARCH NEEDS FOR REDUCING UNCERTAINTIES

In the preparation of this interim report important and immediate uncertainties associated with characterizing the risks of TCDD to aquatic life and wildlife have been identified. The major knowledge gaps that are highlighted below are intended to be specific to TCDD only and do not address uncertainties associated with mixtures of polyhalogenated dibenzodioxins, dibenzofurans and biphenyls. The following discussion also does not address uncertainties associated with the lack of appropriate population, community and ecosystem level models and their linkage to toxicological inputs. Clearly, the development of such models are needed for improving ecological assessments of chemical stressors in general.

Exposure

Research is needed to better establish the K_{ow} for TCDD because current uncertainty in this parameter leads to increased uncertainty in extrapolating TCDD BAFs across aquatic habitats and in estimating the bioavailable fraction of TCDD in water. Current understanding of the role of organic carbon on the partitioning and bioavailability of TCDD from water and sediment is primitive. Analytical procedures are currently inadequate to reliably measure TCDD in water at the concentrations expected to elicit adverse effects in aquatic life and wildlife; this situation is especially critical for monitoring dissolved TCDD. As a consequence of this analytical deficiency, there are very few reports of TCDD concentrations in natural waters and the values that are reported are uncertain. With the development of new techniques and instrumentation that lower detection limits, measurements of total and dissolved TCDD in a variety of water systems need to be made to better establish current and future exposures.

Bioaccumulation

There are currently very few reliable TCDD BAFs available for natural systems, which makes predictions of TCDD concentrations in fish tissues based on TCDD concentrations in water or surficial sediments difficult to assess. Measurements of TCDD in biota, surficial sediments and water are needed for a variety of natural systems to develop a database of sufficient scope to calibrate and validate predictive bioaccumulation models. In gathering such data particular attention must be given to organism attributes (e.g., lipid content) and water column and sediment properties (e.g., particulate and dissolved organic matter) that might influence bioaccumulation.

Effects

Current data suggests fish are more sensitive than aquatic invertebrates and amphibians to TCDD, perhaps due to the absence of the Ah receptor, or a comparably sensitive receptor. A more rigorous assessment is needed to determine if the Ah receptor is present in aquatic invertebrates, amphibians and reptiles. Based on results from such a study specific toxicity studies could be undertaken to insure that current conclusions on interspecies differences in TCDD sensitivity are valid. Associated with

this testing, it is critical that reproductive studies be incorporated and that TCDD accumulation be monitored to establish the basis to characterize risk with increased certainty.

Current data suggest fry survival as the most critical endpoint for fish; however, the current threshold values are based on exposures of limited durations in two salmonid species. As a consequence there is uncertainty regarding the species sensitivity distribution, as well as the impact of chronic exposures on reproduction. Effects on reproductive physiology, early life stage development, and immune response should be studied in a diversity of species to reduce these uncertainties. Associated with such studies it is critical that TCDD accumulation be monitored and that supportive toxicokinetic studies be undertaken to establish biologically-based dose-response models.

With both the mammalian and avian wildlife risk characterizations, there is a lack of quality reproduction bioassays and toxicokinetic information to establish well-defined dose response relationships. For the mammalian assessment there were no reproduction bioassays available for a representative piscivorous wildlife species (e.g., the mink) and for the avian assessment, a ring-necked pheasant reproduction bioassay of limited duration and incorporating an i.p. exposure regime was the only study reported in the literature. Finally, there are apparently no data available to assess the toxicity of TCDD to reptiles. To reduce current uncertainties in the wildlife risk characterization, long-term (i.e., one generation) feeding studies in the mink and an avian species are needed to more adequately assess reproductive and developmental effects. These bioassays should be supported by toxicokinetic studies and Ah receptor investigations to develop biologically-based dose response models to better establish critical parameters in species extrapolations and in linking TCDD accumulation to toxic effects. The avian study should also be designed in such a way to better quantify the uncertainties of using egg injection studies as a source of toxicological data in avian hazard assessments.

Finally, there is a need to assess TCDD concentrations in aquatic life and wildlife in association with population assessments in appropriately selected natural systems. Such studies would further refine and validate the reliability of relationships between TCDD accumulation and toxic effects that have been developed based on currently available laboratory and ecoepidemiological investigations.

1. INTRODUCTION

1.1 BACKGROUND

In April, 1991 the Administrator of the U.S. Environmental Protection Agency (EPA) announced that the Agency would conduct a scientific reassessment of the risk of 2,3,7,8-tetrachlorodibenzo-p-dioxin (hereafter referred to as TCDD), and similar chemicals, to human health and the environment. Since 1985, EPA has classified TCDD, which it considers the most potent known animal carcinogen, as a probable human carcinogen. Sources of TCDD in the environment were subsequently regulated on the basis of animal cancer rates extrapolated to doses associated with human exposures. Two major activities, however, prompted the decision to reassess this approach in evaluating TCDD toxicity and its associated risks. First, an epidemiological study of cancer mortality in U.S. chemical workers (Fingerhut et al., 1991) by the National Institute of Occupational Safety and Health provided evidence of TCDD-mediated human carcinogenicity. Second, a conference at the Banbury Center of the Cold Spring Harbor Laboratory in New York in October, 1990 resulted in general agreement that TCDD's mode of action involves the activation of a TCDD-receptor complex and its subsequent translocation into the cell nucleus as a necessary, but not sufficient, prerequisite for any TCDD related effects (Scheuplein et al., 1991). It was also generally agreed that: 1) animals which possess an aryl hydrocarbon (Ah) receptor respond similarly to TCDD; 2) multiple effects, including enzyme induction, immunotoxicity, reproductive toxicity, developmental toxicity and carcinogenicity, occur in all susceptible species; 3) chemicals which are isostereomers of TCDD (i.e., polychlorinated and polybrominated dibenzo-p-dioxins, dibenzofurans) and planar biphenyls may act through the same mode of toxic action; and 4) since all toxic effects of TCDD appear to be mediated by the chemical's binding to a receptor protein within the cytoplasm of a cell, a receptor-based risk assessment model for this chemical is appropriate. Based on these findings, EPA identified a need for biologically-based dose-response models for TCDD, and related chemicals, to establish a scientific base for more credible risk assessments. As a consequence, the EPA designed and implemented a TCDD reassessment research plan.

In addition to addressing human health risks of TCDD and related chemicals, the reassessment plan includes a component on the risks of these compounds to aquatic life and associated wildlife. This component is included because EPA not only recognizes that these chemicals in aquatic environments can be a major contribution to overall human exposure, through fish and shellfish consumption, but piscivorous fish and wildlife may be at risk due to their exposures through aquatic food chains. The limited available toxicological data also indicate that fish, especially salmonid sac fry (Walker et al., 1991; Cook et al., 1991), and mink (*Mustela vison*) (Aulerich et al., 1988; Hochstein et al., 1988; Aulerich et al., 1985; Bleavins et al., 1980; Aulerich and Ringer, 1977) are among the most sensitive animals to TCDD and related compounds.

Research to generate data and models for characterizing the risk of TCDD and related compounds to aquatic life (Cook et al., 1992) began in September, 1991 and is continuing. The experimental design is based on a toxicological perspective founded on the Ah receptor-based mode of action and addresses existing ecotoxicological data gaps. The design is formulated on the definition of sensitive and ecologically-relevant toxic effect endpoints, a variety of species and complex exposure relationships. Overall, the plan not only treats major uncertainties regarding exposures and toxic effects, but is also designed to provide scientific data of the quality and quantity required for the development of an aquatic life-based EPA water quality criterion for TCDD. The goal of the EPA Office of Research and Development's aquatic effects research plan is to develop data and models needed to complete a comprehensive final report on data and methods for TCDD aquatic risk assessment for review in June, 1995. This report will contain the data and scientific interpretation of TCDD-related risk elements for aquatic life and associated wildlife that are needed by a number of EPA program and regional offices for their regulatory activities.

1.2 GOAL AND SCOPE OF THE INTERIM REPORT

The goal for this report was to critically review and evaluate relevant published, and in some instances unpublished, data and models currently available for analyzing TCDD exposure to, and effects on, aquatic life and wildlife and to identify major areas of uncertainty that limit how well this risk can be characterized. It offers a critical analysis of currently available exposure and effects information and outlines important principles and concepts that must be considered when integrating these data to characterize risk. This interim report addresses TCDD exposure to and bioaccumulation in aquatic organisms, TCDD toxic effects on aquatic life and wildlife, and aspects of risk characterization to exemplify approaches and applicability of current information. The report is intended for those individuals familiar with the scientific literature concerning TCDD; however, key reviews, in addition to primary references, are cited throughout the document for those readers who wish to consult additional background material.

The resulting analyses have two uses. First, as discussed previously, analysis of available data assists research planning by identifying data gaps. Second, by collecting and organizing existing information in line with EPA's recent report on a framework for ecological risk assessment (U.S.EPA, 1992c), such analyses serve as first steps in planning a risk assessment. That is, for future risk assessments associated with TCDD and related chemicals in aquatic food webs, the available data can be examined specifically in terms of its suitability to form a "conceptual model", consistent with the EPA framework for ecological risk assessment.

This interim report has a limited scope. The analyses presented specifically address the direct toxic effects of TCDD to aquatic life and wildlife based on uptake from aquatic prey, sediment and surface water. This report is not intended to provide

detailed generic or site-specific TCDD risk assessments nor TCDD risk assessments for specific aquatic life or wildlife species. Information on polychlorinated and polybrominated dibenzo-p-dioxins, dibenzofurans, and planar polychlorinated biphenyls, that have the same mode of toxic action as TCDD, is not directly treated in this interim report; however, mixtures of these compounds certainly must be considered to ultimately characterize ecological risk. The final report on aquatic ecological risk assessment elements will attempt to incorporate the contribution of TCDD-related chemicals in assessing the risk of complex mixtures to aquatic life and wildlife.

Techniques to assess TCDD risk in aquatic ecosystems are hampered by limited exposure and effects data as well as generic shortcomings that confront most, if not all, ecological risk assessments for chemical stressors. Using currently available dose-response relationships for reproductive and/or developmental impairment, the interim risk characterization approach provides techniques to relate TCDD concentrations in water, sediment and fish tissues to a low or high likelihood of population failure in aquatic life and wildlife. More precise probability estimates of aquatic life and wildlife population responses are not currently possible because of the limited dose-response relationships available and because, in general, validated species-specific population dynamic models do not exist. With more refined TCDD dose-response relationships, well-developed aquatic life and wildlife population models, and a better understanding of the interaction of additional chemical and non-chemical stressors, the contribution of TCDD exposure to the probability of changes in population dynamics could be quantified with increased certainty in the future. Finally, this report does not discuss the impact of TCDD on community or ecosystem structure and function and the resultant inter-related effects on aquatic life or wildlife populations. To assess the risk of TCDD to communities and ecosystems will require physical and mathematical models, with parameters specified appropriately for intended applications, to evaluate and forecast responses as a function of current or future TCDD concentrations in water, sediment and biota.

Peer Review

As outlined in the Acknowledgements Section, this report has undergone extensive review. The report was first reviewed by U.S. EPA scientists within the Office of Research and Development and two non-EPA scientists who are undertaking particularly relevant research on TCDD and related chemicals. A subsequent draft of the document was then reviewed by twelve scientists from academia, industry, non-EPA governmental agencies and private organizations. The current document reflects the comments and suggestions provided through this review process.

This report provides an initial base of information and analyses that EPA is planning to use for assessing risks of TCDD to aquatic life and wildlife. This report will provide a working document for a meeting of EPA scientists and ecological risk

assessment experts who will further evaluate the present data limitations and uncertainties that should be incorporated into plans for completing a comprehensive final report on TCDD risk assessment in 1995. The peer panel will also evaluate the applicability of the data and methods for actual TCDD risk assessments following the U.S. EPA's framework for ecological risk assessment.

2. EXPOSURE

The distribution of TCDD and other planar halogenated aromatic chemicals in aquatic ecosystems and their accumulation in specific animal tissues susceptible to TCDD intoxication must be understood to assess risks to aquatic life and wildlife. A residue-based approach is an essential component of ecological risk assessment and water quality criteria development for TCDD and related chemicals because long term chemical accumulation in tissues of fish and other aquatic organisms is the best correlate of dose for evaluating toxicity. Thus, exposure information that impacts bioaccumulation, tissue-residue, and wildlife dietary exposure relationships used for this interim report on TCDD aquatic risk assessment are emphasized in this section. Information on physical-chemical properties; chemical sources; environmental concentrations in water, sediment and organisms; and aspects of chemical fate and transport will be included only to the extent necessary to complete a generic TCDD aquatic risk characterization. More detailed information concerning all aspects of exposure assessment for chemicals with a toxic mode of action like TCDD can be obtained from the EPA draft report "Estimating Exposure to Dioxin-like Compounds" (U.S. EPA, 1992a) which presents procedures for conducting site-specific exposure assessments. The occurrences and magnitudes of TCDD residues in aquatic organisms are reviewed in this section, but TCDD residues in avian and mammalian wildlife are not included because TCDD risks to wildlife in this report are estimated from exposure through ingestion of aquatic organisms, rather than from accumulation of TCDD in the tissues of wildlife.

2.1 PHYSICAL/CHEMICAL PROPERTIES OF TCDD AND RELATED CHEMICALS

Since fate and transport modeling of TCDD and related chemicals is not within the scope of this report, only those properties that influence bioavailability during exposure of aquatic biota are described here. TCDD has a molecular weight of 321.977 daltons and is a crystalline solid at ambient temperatures with a melting point of 305°C (Boer et al., 1972). The water solubility (S_w) of TCDD is approximately 12-20 ng/L for cold water (ca. 4-12°C). Solubility measurements based on water equilibration with a thin film of TCDD on glass (Marple et al., 1986a) indicate a range in S_w of 12.5 to 19.3 ng/L at 22°C. A generator column experiment indicated 12.9 ng/L at 4.3°C but 480 ng/L at 17.3°C (Lodge, 1989). Increase in S_w with water temperature is expected but the 17.3°C result of Lodge appears to be inconsistent with the measurements of Marple et al. (1986a) at 22°C. Water with excess TCDD allowed to equilibrate for 7 months with soil particles and flask surfaces contained only 8 ng/L at 20 to 22°C (Adams and Blaine, 1986). However, a S_w of 420 ng/L at 22.7 °C has been reported for 2,3,7,8-tetrachlorodibenzofuran (TCDF) based on a generator column experiment (Friesen et al., 1990). Friesen et al. (1990) reported equivalent correlations of log S_w with chlorine number for both polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) using generator

column data obtained at warmer water temperatures and which included the measurement for TCDD at 17.3 °C from Lodge (1989).

The most important physical-chemical parameter used in TCDD exposure assessments is the octanol/water partition coefficient (K_{ow}). The log K_{ow} is also referred to as log P and is used as a measure of the hydrophobicity and lipophilicity of organic chemicals. Measurement of K_{ow} introduces a complication into its definition, which is the need to recognize the distribution of water into octanol and octanol into water when equilibrium between the phases is achieved (Miller et al., 1985). Thus:

$$K_{OW} = \frac{C_{OW}}{C_{WO}} \propto \frac{\gamma_{WO}}{\gamma_{OW}} \qquad (2\text{-}1)$$

where C_{ow} and C_{wo} are the concentrations of the solute in octanol saturated with water and water saturated with octanol, respectively, and γ is the activity coefficient of the solute in each solvent. If octanol in the water does not significantly decrease the solute activity, as suggested by Miller et al. (1985), the activity coefficient for pure water (i.e., $\gamma_w=\gamma_{wo}$) and the measured K_{ow} (assuming equilibrium is reached; the solute is not present as micelles, etc.) can be used as a first approximation of the activity of the solute in water in comparison to organic carbon phases in water, sediments and biota. For DDT, γ_{wo}/γ_w was measured to be 2.8 (Chiou et al., 1982). Since TCDD is very insoluble in water and more hydrophobic than DDT, the possibility for overestimation of γ_w should be considered. The value of octanol as a surrogate for naturally occurring organic phase interactions with chemicals such as TCDD may also have limitations (Miller et al., 1985). The importance of interactions between organic solutes and organic solvents is indicated by the observation of increase in free energy of solution for chlorinated dibenzo-p-dioxins with increasing degree of chlorination due to an increase of the free energy of vaporization with increasing solute size (Gobas and Zhang, 1992).

Only two directly measured K_{ow} values for TCDD have been reported. A slow water phase stirring technique with mutually presaturated octanol and water phases reached equilibrium within one week and a log K_{ow} range of 6.54 to 6.95 was measured (Marple et al., 1986b). Slow stirring with an extract of municipal incinerator fly ash for 9 days gave a TCDD log K_{ow} estimate of 6.4 (Sijm et al., 1989). After 21 days of slow stirring, Sijm et al. (1989) observed decreases in all K_{ow} measurements possibly, due to slow micelle formation in the water phase. The degree to which the K_{ow} measured at 9 days was so influenced is unknown. A generator column method was used to measure a log K_{ow} of 6.2 for another tetrachlorinated congener, 1,2,3,4-tetrachlorodibenzo-p-dioxin (Shiu et al., 1988).

Reverse-phase high pressure liquid chromatography (HPLC) with liquid chromatography/mass spectrometry detection was employed to estimate a log K_{ow} of 7.02 for TCDD from an HPLC retention time - log K_{ow} linear regression equation

derived from seven chemicals with measured K_{ow} values (Burkhard and Kuehl, 1986). Burkhard and Kuehl (1986) also estimated a log K_{ow} of 7.2 for both 1,2,3,4- and 1,3,6,8- tetrachlorodibenzo-p-dioxin. However, HPLC retention was used with three different calibration chemicals with measured K_{ow} values to estimate log K_{ow} values of 8.6 and 8.7 for 1,2,3,4- and 1,3,6,8-tetrachlorodibenzo-p-dioxin, respectively (Webster et al., 1985).

CLOGP version 3.54 (Leo and Weininger, 1989) predicts a log K_{ow} of 7.31 from the molecular structure of TCDD. Since water solubility of TCDD is probably more reliably measured than K_{ow}, the relationship between molar solubility in water (S_m), melting point (MP) and K_{ow} (Yalkowsky et al., 1983) has been used to estimate K_{ow}:

$$\log K_{ow} = \frac{\log S_m + .01MP - 0.323}{-0.944} \qquad (2\text{-}2)$$

Using TCDD's melting point of 305°C and $S_m = 6 \cdot 10^{-11}$ (20 ng TCDD/L), log K_{ow} is calculated to be 7.94.

Related to K_{ow} is the organic carbon/water partition coefficient, K_{oc}:

$$K_{oc} = \frac{C_{oc}}{C_w^d} = \frac{C_s}{f_{oc} \cdot C_w^d} \qquad (2\text{-}3)$$

where C_{oc} is the concentration of chemical in organic carbon associated with suspended solids or sediment, C_w^d is the concentration of freely dissolved chemical in water (theoretically, the chemical activity), C_s is the concentration of chemical in suspended solids or sediment (dry weight) and f_{oc} is the fraction of organic carbon in suspended solids or sediment (dry weight).

Desorption of TCDD from contaminated soils, followed by 0.45 µm membrane filtration to remove particles and associated TCDD from the soil leachates, resulted in measured log sediment organic carbon - water partition coefficients (log K_{oc}) of 7.4 to 7.6 (Jackson et al., 1985). Although data were not presented to demonstrate that an equilibrium was reached between soil and water, the consistency of results for a single extraction compared to three successive extractions of fresh soil samples with the same water suggests that equilibrium was probably achieved. Sorption experiments using [^{14}C]TCDD with two uncontaminated soils by batch shake testing and isolation of the water phase by centrifugation (3500 rpm for 30 min) resulted in an estimated log K_{oc} of 6.66 (Walters et al., 1989). One to five successive prewashings to remove nonsettleable matter resulted in an observation of K_{oc} dependence on soil water contact period and/or prewashing. No measurements of the amount of TCDD associated with organic carbon remaining in the centrifugates were provided. In a related study, sorption of TCDD to soil from water/methanol mixtures gave an

estimated log K_{oc} of 6.6 based on co-solvation theory (Walters and Guiseppi-Elie, 1988).

Only one attempt to measure K_{oc} on the basis of freely dissolved TCDD has been reported (Lodge and Cook, 1989). In this experiment, the equilibrium distribution of TCDD between water containing decreasing amounts of non-settled organic matter and settled sediment was measured with the intent to approach the lowest solids concentration in water that would still allow detection of TCDD. These data established a minimum value of K_{oc} and, through extrapolation to a pure water condition, allowed an estimate of K_{oc} based on TCDD activity in water. At 10°C extrapolation to zero solids concentration in water gave an estimated log K_{oc} of 7.6. Subsequent measurements have confirmed the attainment of equilibrium between solids and water and the probability that log K_{oc} for TCDD is equal to or exceeds 7.3 (Lodge, 1992).

"Apparent" log K_{oc} values for seventeen PCDD and PCDF congeners were calculated on the basis of filtered particulate and filtrate ("apparently dissolved") samples obtained from 1.5 to 2.2 m^3 samples of Baltic Sea water (Broman et al., 1991). The log K_{oc} for TCDD was 6.8 with values of 6.8 to 7.9 for congeners with greater chlorination. The effect of residual colloidal organic carbon in the filtrates is to increase the fraction of chemical measured as apparently dissolved and this effect is greater for the more hydrophobic congeners. Thus both individual congener log K_{oc} values and the range of log K_{oc} values for PCDDs and PCDFs would be greater if the freely dissolved concentrations of each chemical determined on the basis of residual organic carbon in the filtrate is used to calculate the log K_{oc}. Many of the congeners, including TCDD, were not detectable in the filtrates and the log K_{oc}s were calculated on the basis of half the analytical detection limit. The detection limits for TCDD in filtrates were much greater than for TCDD in filtered particulate samples so the half detection limit approximation may overestimate actual TCDD concentrations and contribute to underestimation of the K_{oc}.

Correlations between K_{ow} and K_{oc} such as that of Karickhoff (1981) are frequently used to estimate K_{oc}. Increasingly, chemical partitioning models set K_{oc} equal to K_{ow} (DiToro, 1985). Since chemical fate and bioaccumulation models used for this report depend on estimates of the activity or freely dissolved concentration of the chemical in water, this risk assessment will consider K_{ow} equal to K_{oc} with a possible range of 10^7 to 10^8 for TCDD. This range exceeds the average values frequently selected for modeling TCDD fate and transport because inaccuracies associated with measurements of K_{ow} and K_{oc} for very hydrophobic chemicals such as TCDD tend to result in underestimates. If headspace analysis of hydrophobic organic chemicals in air equilibrated with water solutions containing co-solvents or sorbents (Resendes et al., 1992) can be applied to TCDD, uncertainties for TCDD activity or freely dissolved concentrations may be reduced in the future. Regardless of the elusive true activities of TCDD and related chemicals in water and organic carbon

phases, exposure modeling errors can be minimized by consistent use of a single best estimate value of K_{ow} for each chemical in all components of the models and their application.

2.2 ANALYTICAL LEVELS OF DETECTION

Large uncertainties associated with estimating aquatic exposures to TCDD and related chemicals could be greatly reduced if minimum levels of detection (MLD) could be lowered to allow measurements of concentrations in water. High resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) is used to provide the most sensitive and reliable measurements of TCDD concentrations in sample extracts. MLD generalized in this discussion are based on the minimum amount of chemical present in a sample that allows attainment of a 3:1 signal/noise ratio. Table 2-1 summarizes the present routinely achievable MLD for TCDD in the various sample matrices analyzed for exposure assessments. These estimates are based on typical sample sizes, established sample preparation methods and routine HRGC/HRMS detection of 20 pg TCDD (Marquis et al., 1992). Large reductions in these MLD can now be achieved with use of the best research HRGC/HRMS instrumentation available. Through large volume sampling, optimum sample clean-up and maximum instrument sensitivity, MLD for water samples may be lowered by as much as a factor of 10^4. Lesser but significant reductions in MLD could occur for sediment and tissue samples.

Table 2-1. Minimum levels of detection (MLD) for routine high resolution gas chromatography/high resolution mass spectrometry analyses of TCDD in samples from aquatic ecosystems.

Sample Matrix	Environmental Range	MLD
Tissue (pg/g)	0-1000	0.5
Sediment (pg/g)	0-10,000	1.0
Water-solids (ng/L)	0-0.002	0.005
Water-dissolved (ng/L)	0-0.0002	0.0005

2.3 DISTRIBUTION IN WATER, SEDIMENTS AND FOOD CHAINS

TCDD and other persistent, hydrophobic non-polar organic chemicals partition appreciably into organic matter in water and sediments and into biota. Despite the very low solubility and great hydrophobicity of these chemicals, water acts as the medium for their transport and partitioning within aquatic systems. Equilibrium distributions are approached to the extent that rates of transfer to, from and within an ecosystem allow. Thus when chemical loading is constant for an extended period of time, steady-state distributions can occur. Concentrations of TCDD in the water column respond much faster to changes in chemical loading than do concentrations in sediments. Ecosystems like the Great Lakes may have elevated concentrations in water for decades after loading reductions due to the redistribution of chemicals from contaminated sediments. Chemical mass balance models are required in order to determine the fraction of chemical in water and biota from active external loadings in comparison to sediment sources.

Only one report of detectable ambient TCDD concentrations in water can be found. Very large volume (1.5 - 2.2 m^3) water samples from the Baltic Sea were separated into filtered solids and filtrate fractions for determination of concentrations of PCDDs and PCDFs (Broman et al., 1991). Since some colloidal organic carbon could pass through the 0.45 µm filter used, the filtrate TCDD concentration was regarded as "apparently dissolved" and not 100% freely dissolved. Only two of nine water filtrate samples were reported to have detectable TCDD. The concentrations were 0.0002 and 0.0003 pg/L (0.2 and 0.3 parts per quintillion). Four of nine filtered solids samples were reported to have detectable TCDD at 0.0002 pg/L. These levels of detection, if applied to Lake Ontario water, would result in extraordinary new insight into TCDD partitioning model accuracy since concentrations 1000x greater than these Baltic Sea results are expected (Endicott et al., 1990).

Sediments can contain measurable concentrations of PCDDs and PCDFs because of the transport and deposition of hydrophobic organic chemicals on waterborne biotic and abiotic solids. The distribution of TCDD throughout the surface sediments of Lake Ontario after several decades of loading through the Niagara River is remarkably uniform, especially if the differences in organic carbon distribution between depositional basins and other regions are considered (Short et al., 1990). The Lake Ontario mean surface sediment concentration of TCDD was determined to be 68 pg/g dry sediment for samples collected in 1987. One centimeter increments of sediment cores collected with the Lake Ontario surface sediments in 1987 were dated by radionuclide methods and analyzed by HRGC/HRMS for PCDDs, PCDFs, polychlorinated biphenyls (PCBs) and other organochlorine chemicals (Cook et al., 1993a). TCDD levels rose from non-detectable before 1940 to maximum sediment concentrations of approximately 500 pg/g dry sediment in about 1962, and then steadily declined to the 1987 surface sediment conditions measured in conjunction with the Lake Ontario TCDD Bioaccumulation Study (Short et al., 1990). The

sediment core profiles for other PCDDs and PCDFs were similar. The PCBs and other organochlorine chemicals appeared to follow the same general historical pattern but maximum sediment concentrations occurred in different years with different rates of change.

The sediments of Lake Ontario contain relatively large concentrations of TCDD in comparison to other PCDDs and PCDFs because of past chlorophenol production as a source of TCDD to the lake. A similar pattern was found in the Newark Bay, New Jersey estuary where surface sediments were found to contain up to 730 pg TCDD/g overlying mid-1960s sediments with 7,600 pg TCDD/g (Tong et al., 1990). The highly contaminated Newark Bay sediments are associated with 2,4,5-trichlorophenoxyacetic acid production between 1948 and 1969. Contaminated sediments from many other locations have less TCDD and relatively more TCDF and other PCDFs, frequently in association with large concentrations of PCBs. New Bedford Harbor, Massachusetts sediments were found to contain TCDF concentrations up to 1,100 pg/g dry sediment in association with large concentrations of PCBs but with TCDD concentrations ≤4 pg/g dry sediment (Pruell et al., 1990). Another example is the lower Fox River in Wisconsin in which the sediments (dry weight) contained 1 to 7 pg TCDD/g in comparison to 3 to 61 pg TCDF/g and 0.31 to 6.57 µg total PCBs/g (Ankley et al., 1992a). Fox River sediment concentrations of each chemical varied more in relation to sediment organic carbon concentration distribution than to a downstream distribution gradient associated with transport from contaminated sediments. The upper Great Lakes sediments have PCDD and PCDF congener distributions that are indicative of atmospheric deposition from regional combustion sources since the 1940s (Czuczwa and Hites, 1984; Czuczwa et al., 1985).

Fish are good detectors for monitoring the concentrations of TCDD and similar bioaccumulative chemicals in aquatic systems. The National Dioxin Survey initiated by EPA in 1983 included a nationwide survey of TCDD residues in fish from 395 sites (U.S. EPA, 1987; Kuehl et al., 1989). TCDD concentrations in fish (reported throughout this report as pg TCDD/g wet weight of whole organism unless specified otherwise) ranged from below detection at 1 pg/g (72% of all samples) to a maximum concentration of 85 pg/g. TCDD was detectable (≥1 pg/g) in whole fish samples from 17 of 90 randomly selected sites but 23 of 29 Great Lakes sites (U.S. EPA, 1987). Highest concentrations among all fish sampled were associated with the open Great Lakes and river sites downstream from kraft paper mills. In another study (U.S. EPA, 1992b) TCDD was detected in fish from 70% of sites sampled across the United States in 1986-1988 for a wide range of organic chemical residues. The maximum TCDD concentration was 204 pg/g with an average of 6.8 pg/g. Although the selection of sites in this study was biased toward sites where TCDD and other PCDDs and PCDFs were likely to be found, the survey probably provides the most extensive fish residue data set for PCDDs and PCDFs based on a single analytical effort.

Although sediment samples were not collected, it is likely that sediments associated with sites having contaminants in fish are similarly contaminated.

TCDD (O'Keefe et al., 1983) and other PCDDs and PCDFs (Zacharewski et al., 1989) are ubiquitous contaminants of Great Lakes fish. Whereas concentrations of congeners such as TCDF vary little in fish among the lakes (16 pg/g for Lake Superior versus 35 pg/g for Lake Michigan), Lake Ontario's fish have much greater concentrations of TCDD; 40 pg/g for Lake Ontario versus 1 pg/g for Lake Superior (Zacharewski et al., 1989). TCDD residues in Lake Ontario lake trout (*Salvelinus namaycush*) have decreased to approximately 25% of the levels present in 1977 shortly after lake trout populations were reestablished through stocking. These reductions in TCDD and other PCDD and PCDF residues parallel the changes recorded in sediment profiles from depositional basins (Cook et al., 1993a). In contrast to the findings of Cook et al. (1993a), Whittle et al. (1992) has reported a significant increase in mean TCDD concentrations over the period of 1981 to 1990, despite evidence for decreased contaminant loading to Lake Ontario.

Striped bass (*Morone saxatilus*) from the lower Hudson River and its estuary were found to contain TCDD concentrations up to 120 pg/g in comparison to 1-4 pg/g in striped bass from Chesapeake Bay (O'Keefe et al., 1984). Two striped bass collected from Newark Bay contained 84 and 734 pg TCDD/g fillet (Rappe et al., 1991). The large difference in TCDD concentrations in these samples was not paralleled for other PCDD and PCDF congeners which differed by a factor of only two. Rappe et al. (1991) commented that the striped bass with the greater TCDD concentration probably resided in the contaminated area for a longer period. TCDD concentrations in crab and lobster meat averaged 111 and 5.5 pg/g, respectively, while hepatopancreas concentrations were 4,900 and 435 pg/g, respectively.

Adult bullfrogs, collected in 1984-1985 near a former 2,4,5-trichlorophenoxyacetic acid production site along Rockey Branch Creek in Arkansas, contained 640 - 48,000 pg TCDD/g of liver (Korfmacher et al., 1986a). Bullfrog muscle tissue samples contained less than 10% of the liver TCDD concentrations (Korfmacher et al., 1986b). Fish collected in 1979-1981 from Bayou Meto, which receives Rockey Branch Creek drainage, contained 37 to 480 pg TCDD/g (Mitchum et al., 1980). The largest TCDD concentrations found in bullfrogs indicate the potential for TCDD residues in fish, if present in a highly contaminated stream like Rockey Branch Creek, to exceed 2,000 pg/g.

Mussels (*Elliptio complanata*) were placed in cages to detect TCDD in water associated with pulp and paper mills and other sources in the Rainy River, Ontario watershed. The mussels accumulated up to 10 pg/g after 21 days (Hayton et al., 1990). Invertebrate species, which do not appear to biotransform PCDDs, PCDFs and PCBs, can be used to monitor distribution patterns for complex mixtures of these chemicals that are not detectable in water through direct chemical analyses.

2.4 EXPOSURE ROUTES FOR AQUATIC ORGANISMS

TCDD and related chemicals are accumulated by aquatic organisms through exposure routes that are determined by the habitat and physiology of each animal. Site-specific differences in exposure can occur as a result of temporal and spacial variation of the distribution of the chemical between the water, sediment and food that organisms contact. With log K_{ow}>5, exposure through ingestion of contaminated food becomes an important route for uptake in comparison to respiration of water (Thomann, 1989). Thus all biosignificant PCDDs, PCDFs and PCBs are significantly accumulated through food ingestion, although the net result for TCDD and other PCDDs and PCDFs, because of metabolism, is to approach, rather than exceed, tissue levels that are at equilibrium with their activities in water (Opperhuizen and Sijm, 1990).

The relative contributions of water, sediment and food to uptake of TCDD by lake trout in Lake Ontario was examined in a laboratory study by exposing yearling lake trout to a diet prepared from Lake Ontario rainbow smelt (*Osmerus mordax*) and to sediment from Lake Ontario along with water at a TCDD concentration simulated to be at equilibrium with the sediments (Batterman et al., 1989). Each exposure route was studied individually and in combination with the other routes for two 10-fold different TCDD exposure levels. Food ingestion was found to contribute approximately 75% of total TCDD uptake (Cook et al., 1990). The amount of TCDD uptake from water in the absence of contaminated sediment was negligible in relation to uptake from food. TCDD uptake associated with sediment exposure was thought to be exaggerated due to suspended sediment concentrations much greater than trout normally experience, and the presentation of food to the trout at the sediment-water interface. In the water exposures, TCDD was introduced continuously to the water flowing into the aquaria at twice the estimated sediment equilibrium concentration in an attempt to accommodate sorption to organic material not at steady-state. There may still have been significant loss of bioavailable TCDD to organic carbon that accumulated in the water exposure system. However, when TCDD was added to water in exposures with contaminated sediment, no increase in TCDD uptake (over TCDD uptake associated with sediment alone) occurred. Three interpretations of this result are possible: (1) desorption of TCDD from sediment was sufficiently rapid to allow maintenance of equilibrium between TCDD in sediment and the water flowing through the aquaria and thus uptake in the sediment exposures could be from both sediment and water ; (2) the TCDD added to water in the presence of sediment was rapidly sorbed to the suspended sediment because the assumed TCDD log K_{oc} of 7.0 was an underestimation, and thus uptake was due to direct exposure to sediment and the reduced TCDD concentration in water at equilibrium with the sediment; or (3) the TCDD added continuously to the water increased the availability of TCDD from water but the water was a minor source for uptake in comparison to TCDD on sediment.

Included with the Lake Ontario sediment study (Cook et al., 1990) were exposures of lake trout to Lake Ontario sediments with a TCDD concentration ten-fold greater (10X sediments) than the 1987 lake wide average. Measurements of large volume samples of filtered water from the 10X exposures indicated that freely dissolved TCDD concentrations in the water were less than 0.3 pg/L. Cook et al. (1990) concluded that direct ingestion of sediment, and possibly gill contact with suspended sediment were probably more important routes of uptake than ventilation of water containing freely dissolved TCDD resulting from desorption from suspended sediment. An independent analysis (Barber et al., 1991) of data selected from a preliminary report (Batterman et al., 1989) of the 10X sediment study, and an assumed freely dissolved TCDD concentration of 0.75 pg/L in equilibrium with sediment, resulted in the conclusion that gill uptake of freely dissolved TCDD in water associated with the Lake Ontario sediments was primarily responsible for the TCDD accumulation observed in lake trout by Cook et al. (1990). The analysis of Barber et al. (1991) did not consider the alternative interpretations (2) and (3) above or the water analysis data presented in Cook et al. (1990).

The availability of TCDD and related chemicals to aquatic organisms through uptake by ingestion or contact with sediments is an important consideration for this risk characterization. Sediment dwelling invertebrates and bottom feeding fish may have exceptional exposures that can increase human or wildlife exposures, increase concentrations in aquatic food chains and cause sensitive fish species to be at increased risk. Many aquatic organisms, including fish, ingest sediment and/or suspended particles either as a source of food or inadvertently while feeding. The diets of minnows and suckers include large quantities of detritus as a major food source. Gizzard shad (*Dorosoma cepedianum*) can digest 50 to 66% of the organic matter in ingested sediments which are consumed at a rate of up to 20% of their wet weight in dry sediments each day (Mundahl, 1991). No studies of fish gut assimilation efficiency from ingested sediment have been reported for TCDD or similar nonpolar organic chemicals. Carp (*Cyprinus carpio*) (250 g) were exposed by oral gavage to 500 mg of sediment contaminated with PCDFs but this was an insufficient amount of sediment to allow detection of chemical assimilation (van der Weiden et al., 1989).

Since trout gut assimilation efficiency for TCDD in natural food is about 40% (Cook et al., 1990) to 50% (calculated from the data of Kleeman et al., 1986a) and some fish appear to digest organic matter associated with ingested sediment, TCDD uptake from ingested sediments may be an important route of exposure for some species of fish. A recent nationwide survey of organic chemical residues in fish indicated that TCDD and other bioaccumulative chemicals were more frequently detected in bottom feeding fish, such as carp, and that the average concentrations in the bottom feeders were greater than for other species (U.S. EPA, 1992b). However, comparison of lipid-normalized residues is needed in order to confirm the tendency for greater bioacccumulation by bottom feeding fish. Carp exposed to TCDD only through water do not appear, on a lipid-normalized basis, to accumulate more than rainbow

trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*) (Cook et al., 1991) but do appear to have five-fold greater TCDD bioaccumulation in environmental exposures where sediment ingestion can occur (Kuehl et al., 1987).

Accumulation of PCBs by sandworms (*Nereis virens*) from ingestion of and contact with contaminated sediments was estimated to exceed accumulation possible from water alone, even with maximum water concentrations of PCBs from sediment elutriates (Rubinstein et al., 1983). TCDD, TCDF and PCBs in sediment from the Passaic River, New Jersey were accumulated by sandworms, clams (*Macoma nasuta*) and shrimp (*Palaemonetes pugio*) in laboratory exposures involving flowing filtered water over the sediment (Rubinstein et al., 1990). The Passaic River sediment exposure data indicate that each invertebrate species at steady-state would accumulate each chemical to a level expected for equilibrium partitioning between sediment organic carbon and organism lipid. Only a few measurements of gut uptake efficiency for any hydrophobic organic chemicals on ingested sediments have been made for aquatic invertebrates. Assimilation efficiencies for 2,4,5,2',4',5'-hexachlorobiphenyl on Lake Michigan sediments ingested by oligochaetes ranged from 15 to 36%, depending on gut clearance times (Klump et al., 1987). Efficiency of gut uptake of hexachlorobenzene from ingested sediment by the deposit-feeding clam (*Macoma nasuta*) was measured to be 38 to 56% (Lee et al., 1990).

3. BIOACCUMULATION

Relating information on effects of TCDD to environmental concentrations is made difficult by several factors. A variety of routes and durations of exposure are employed in laboratory tests, which are usually quite dissimilar from what occurs in natural systems. In natural systems, multiple and variable routes of exposure also must be considered. Association of TCDD with organic matter can affect its availability to organisms both in laboratory and natural systems. Effects of TCDD are often expressed in terms of concentrations in the diet or in the test animal itself, rather than environmental concentrations. Because of these considerations, the amount of TCDD accumulated by an organism generally will be an important component in integrating exposure and effects information. This chapter will review concepts and information on the bioaccumulation of TCDD and consider how it should be used in a risk assessment.

3.1 CONCEPTUAL FRAMEWORK AND DEFINITIONS

Discussions of TCDD bioaccumulation frequently are complicated by use of poorly defined terms and parameters. As a prelude to a review of specific information on TCDD bioaccumulation, key concepts and terms will be defined.

3.1.1 Bioconcentration

For aquatic organisms, bioconcentration refers to the accumulation of a chemical from exposure via water only. Bioconcentration is a dynamic and often complicated process, involving uptake via gills and skin; elimination of the chemical via gills, skin, urine, and feces; and metabolic transformation of the compound. For chemicals that only slowly come to steady state, growth of the organism can also dilute the accumulated chemical and affect the apparent bioconcentration. Additionally, organisms consist of multiple tissues and organs with different toxicokinetic characteristics, which can complicate the time course of accumulation. A useful approximation often employed for bioconcentration is to consider the organism as a single compartment at internal equilibrium and to describe the change in chemical concentration with time by a first-order kinetic expression such as the following:

$$\frac{dC_a}{dt} = (k_{ug} + k_{us}) \cdot C_w - (k_{eg} + k_{es} + k_{ef} + k_m + g) \cdot C_a \qquad (3\text{-}1)$$

$$= \quad k_1 \cdot C_w - \quad k_2 \cdot C_a$$

where: C_w and C_a are chemical concentrations in the water and aquatic organism; k_{ug} and k_{us} are uptake rate constants via gill and skin; k_{eg}, k_{es} and k_{ef} are elimination rate

constants via gill, skin, and feces; k_m is a metabolic transformation rate constant; and g is the proportional growth rate of the organism. The rate constants k_1 and k_2 are the uptake and loss constants that would be derived from net uptake versus time in classical bioconcentration experiments. As indicated here, k_1 and k_2 are the summation of a variety of constants. In some bioconcentration measurements, k_2 is adjusted for growth and therefore does not include the parameter g.

A bioconcentration factor (BCF) is defined as the ratio of the chemical concentration in the aquatic organism (C_a) to that in the water (C_w):

$$BCF = \frac{C_a}{C_w} \qquad (3\text{-}2)$$

A BCF can apply at any time during the process of bioconcentration and is premised on a reasonably constant chemical concentration in water. After sufficiently long time, the concentration of chemical in the organism will tend to approach a constant value, termed the steady-state bioconcentration factor (ssBCF). For highly hydrophobic chemicals, steady-state takes a long time to reach and often cannot be closely approached in practical experiments, especially for larger organisms which tend to have slower uptake rates and longer half-lives for elimination. ssBCFs must then be estimated from the kinetic rate constants for uptake and loss, which for the single-compartment, first-order case is:

$$ssBCF = \frac{k_1}{k_2} = \frac{k_{ug} + k_{us}}{k_{eg} + k_{es} + k_{ef} + k_m + g} \qquad (3\text{-}3)$$

The driving force for bioconcentration is the difference in activities of the chemical between the water and organism. At equilibrium, these activities are equal and C_a/C_w would equal K_{aw}, an equilibrium constant for partitioning of the chemical between the aquatic organism and water. Because exchange of chemical across gills and skin is a two-way, diffusive process driven by the activity differences, $k_{eg}=k_{ug}/K_{aw}$ and $k_{es}=k_{us}/K_{aw}$ under the model used here of a single compartment organism at internal equilibrium. The relationship between ssBCF and K_{aw} therefore is:

$$ssBCF = K_{aw} \cdot \frac{k_{eg} + k_{es}}{k_{eg} + k_{es} + k_{ef} + k_m + g} = K_{aw} \cdot \left(1 - \frac{k_{ef} + k_m + g}{k_2}\right) \qquad (3\text{-}4)$$

This equation illustrates how steady-state bioconcentration can fall below the expected equilibrium. Routes of elimination other than the gills and skin can contribute to loss of chemical without providing a corresponding uptake. In particular, for highly hydrophobic chemicals, elimination via feces may be important if the organisms are fed uncontaminated food. Metabolic transformation of a chemical will have a similar effect, and be especially important if rates of elimination of the parent compound are low as they typically are for highly hydrophobic chemicals. Dilution of chemical

concentration due to growth can also cause steady-state estimates to fall below the expected equilibria if the growth rate is appreciable compared to rates of elimination.

Bioconcentration can depend on the composition of the water and organism since this composition can affect the relationship between chemical activity and the total concentration of chemical. For highly hydrophobic organic chemicals, a significant fraction of the chemical concentration in the water can be associated with suspended particles and dissolved organic matter and be less available for uptake by an organism. The total chemical concentration in water (C_w^t) can be expressed as:

$$C_w^t = C_w^d + POC \cdot C_{POC} + DOC \cdot C_{DOC} \quad (3\text{-}5)$$

where C_w^d refers to the concentration of chemical which is freely dissolved and is considered here as equivalent to the chemical activity; POC and DOC are the concentrations of particulate and dissolved organic carbon in the water; and C_{POC} and C_{DOC} are the concentrations of chemical associated with the particulate and dissolved organic carbon (on an organic carbon weight basis). If these different constituents in the water are at equilibrium with each other, this expression can be rewritten as:

$$C_w^t = C_w^d \cdot (1 + POC \cdot K_{POC} + DOC \cdot K_{DOC}) = \frac{C_w^d}{f_d} \quad (3\text{-}6)$$

where K_{POC} and K_{DOC} are the equilibrium constants for partitioning of the chemical between water and POC and DOC, respectively, and f_d is the fraction of chemical which is freely dissolved (i.e., $[1+POC \bullet K_{POC}+DOC \bullet K_{DOC}]^{-1}$). To relate the freely dissolved concentration just to POC, this expression will be further modified for use here as:

$$f_d = \frac{1}{1 + TBF_{oc} \cdot POC \cdot K_{OW}} \quad (3\text{-}7)$$

where TBF_{oc} ("total binding factor for organic carbon") is an empirical adjustment to express binding by all organic carbon in water in terms of POC equivalents (e.g., a TBF_{oc}=2 indicates that the chemical is as much associated with DOC is POC) and K_{ow} is used as a surrogate for K_{POC}.

Bioconcentration factors therefore should specify what water concentration basis is used, total (BCF^t, C_w^t) or freely dissolved (BCF^d,C_w^d):

$$BCF^t = \frac{C_a}{C_w^t} \qquad BCF^d = \frac{C_a}{C_w^d} \tag{3-8}$$

Similarly, the uptake rate constants (k_{ug}, k_{us}) and equilibrium partition coefficient (K_{aw}) must clearly be based on either dissolved or total water concentration.

BCF^d is expected to be largely independent of site water characteristics and thus to be similar among different sites. BCF^t between two sites (x and y) therefore should follow the relationship:

$$\frac{BCF^{t,x}}{BCF^{t,y}} = \frac{f_d^x}{f_d^y} \tag{3-9}$$

For sites in which $f_d << 1$ and in which TBF_{oc} is similar, this can be further simplified to:

$$\frac{BCF^{t,x}}{BCF^{t,y}} = \frac{POC^y}{POC^x} \tag{3-10}$$

Analogous to the expression above (equation 3-5) for water, the total concentration of chemical in an aquatic organism (C_a^t) can be expressed as a sum across different constituents of an organism's body. For example, Barber et al. (1991) considered three constituents - water, lipid, and nonlipid organic matter:

$$C_a^t = f_w \cdot C_a^d + f_l \cdot C_l + f_{nl} \cdot C_{nl} \tag{3-11}$$

where C_a^d is the concentration of chemical freely dissolved in water in the organism and, as for C_w^d, is assumed to be equivalent to chemical activity; C_l and C_{nl} are, respectively, the concentration of chemical in the lipid, and nonlipid phases; and f_w, f_l, and f_{nl} are the fraction of these phases on the basis of an organism's wet weight. The proportion of chemical bound to a receptor such as the case for TCDD and the Ah receptor is incorporated into f_{nl}. If the organism is at internal equilibrium, this can be expressed as:

$$C_a^t = C_a^d \cdot (f_W + f_l \cdot K_l + f_{nl} \cdot K_{nl}) \tag{3-12}$$

where K_l and K_{nl} are the lipid/water and nonlipid/water partition coefficients for the chemical. For hydrophobic chemicals, lipids are thought to dominate partitioning in many cases and this expression is often simplified to the following:

$$C_a^t \approx C_a^d \cdot f_l \cdot K_{ow} \quad (3\text{-}13)$$

where the octanol/water partition coefficient (K_{ow}) is used as an estimate for the lipid/water partition coefficient. The equilibrium partitioning between an organism and chemical freely dissolved in the water is therefore expected to be:

$$K_{aw}^d \approx f_l \cdot K_{ow} \quad (3\text{-}14)$$

Because of the importance of the lipid phase, it is sometimes useful to express BCFs on the basis of organism lipid content in order to reduce variability among organisms and to better contrast activities. In this case, the chemical concentration in the organism is denoted C_l and is calculated as the mass of chemical in an organism divided by the lipid content rather than the wet weight of the organism:

$$BCF_l = \frac{C_l}{C_w} = \frac{C_a^t / f_l}{C_w} = \frac{BCF}{f_l} \quad (3\text{-}15)$$

This expression can be based on either total or freely dissolved water concentrations. The equilibrium partitioning between the lipid-normalized concentration in an organism and chemical freely dissolved in the water is expected to be approximately equal to K_{ow} (i.e., a $ssBCF_l^d$ will equal K_{ow} in the absence of growth dilution, metabolism, and elimination other than across the gills and skin).

3.1.2 Bioaccumulation

For aquatic organisms, bioaccumulation refers to the net accumulation of a chemical from exposure via food and sediments as well as water. The kinetic expression for bioaccumulation is simply that for bioconcentration with an additional term for chemical absorbed in the gastrointestinal tract:

$$\frac{dC_a}{dt} = (k_{ug} + k_{us}) \cdot C_w + k_{uf} \cdot C_f - (k_{eg} + k_{es} + k_{ef} + k_m + g) \cdot C_a \quad (3\text{-}16)$$

where C_f is concentration in the food and k_{uf} is a rate constant for absorption of chemical from food; this rate constant depends on the amount and nature of the food consumed. Under the assumption of a single-compartment organism at internal equilibrium, the other rate constants should be the same for bioconcentration and bioaccumulation, although in practice different internal distribution of chemical

absorbed via the gastrointestinal tract might produce somewhat different elimination rate constants.

A bioaccumulation factor (BAF) is the ratio of the chemical concentration in the organism to that in the water. As such, the basic definitions for BCFs above apply also to BAFs. The previous discussion regarding the importance of specifying the form of the chemical in the water and the value of lipid normalization also applies. Thus, there are four important BAF variations, and care must be taken to clearly specify which variation is used:

$$BAF^t = \frac{C_a}{C_w^t} \qquad BAF^d = \frac{C_a}{C_w^d} \qquad BAF_l^t = \frac{C_l}{C_w^t} \qquad BAF_l^d = \frac{C_l}{C_w^d} \qquad \text{(3-17)}$$

The difference between BAFs and BCFs is primarily in the routes of exposure involved and the levels of accumulation therefore attained. BCFs must be measured for water exposure alone in the laboratory or from environmental data with the assumption that chemical uptake by the organism from water is the predominant route of accumulation. BAFs are usually determined from measurements of chemical concentration in water and organism tissue samples.

The steady state BAF has the following form under the model used here:

$$ssBAF = \frac{k_{ug}+k_{us}+k_{uf}\cdot FAF}{k_{eg}+k_{es}+k_{ef}+k_m+g} \qquad \text{(3-18)}$$

where FAF is the ratio of the chemical concentration in food to that in water. BAFs will therefore exceed BCFs to the extent that uptake via the gastrointestinal tract is significant compared to uptake from water via the gills and skin. In fact, whereas ssBCFs must be at or below equilibrium with the water, ssBAFs can exceed the equilibrium expected based on water and food concentrations because the removal of lipids and other organic material from food during digestion may increase the activity of chemical in the gut contents and promote uptake (Connolly and Pedersen, 1988). Thomann (1989) defined the condition of $BAF_l/BCF_l > 1.0$ as "food chain accumulation". The increase in the concentration of chemical in an organism relative to its food has also been termed biomagnification, with the ratio of these concentrations being the biomagnification factor (BMF). Ideally, BMFs should be calculated on the basis of lipid-normalized food and organism concentrations of the chemical so that they actually represent differences in chemical activity, rather than just a response to different lipid contents and thus different affinities for the chemical.

For chemicals that are not highly hydrophobic, the distinction between bioconcentration and bioaccumulation is of little consequence because accumulation rates via water are much greater than those via food ($k_{ug}+k_{us} >> k_{uf}\bullet FAF$). This is due

to the rate constants being roughly proportional to the volume of material processed. For food intake, this is generally a few percent of body weight a day, whereas water passing over gills this can vary from about two hundred to several thousand times the organism weight per day, depending on the size and type of organism, its activity, and the temperature. For food uptake to be as important as uptake via water, the FAF therefore must be a few thousand to as much as one hundred thousand (corresponding to a K_{ow} of 10^4 to more than 10^5), depending on the organism and its environment. For chemicals with lower hydrophobicities, measured accumulations would therefore be similar in water only and water+food exposures.

Although the complex kinetics of exchange make it unlikely that a highly hydrophobic chemical in an organism will be at equilibrium with the chemical in the surrounding water, this equilibrium is still a useful reference point in interpreting and applying BAFs. A measure of the disequilibrium between an aquatic organism and water is:

$$R_{aw} = \frac{C_a^d}{C_w^d} = \frac{BAF^d}{K_{aw}^d} = \frac{BAF_l^d}{K_{ow}} \qquad (3\text{-}19)$$

3.1.3 Biota-Sediment Relationships

For many extremely hydrophobic chemicals such as TCDD, reliable measurements of ambient water concentrations, especially dissolved concentrations, are not available. Therefore, accumulation of chemical by an organism cannot be referenced to a water concentration as required for a BCF or BAF. However, concentrations are generally measurable in sediments as well as in organisms because these chemicals distribute predominantly in association with organic carbon. Analogous to equations 3-5 and 3-9, presented previously for water and aquatic organisms, the relationship of total chemical concentration in the sediment (C_s^t) to freely dissolved chemical in pore water (C_s^d) can be described as follows:

$$C_s^t = f_{ws} \cdot C_s^d + f_{oc} \cdot C_{oc}' = C_s^d \cdot (f_{ws} + f_{oc} \cdot K_{oc}) \approx C_s^d \cdot f_{oc} \cdot K_{oc} \qquad (3\text{-}20)$$

where f_{ws} is the fraction of water in the sediment, f_{oc} is the fraction organic carbon in the sediment, C_{oc}' is the concentration of chemical bound to sediment organic carbon (this is different from the organic carbon normalized sediment concentration, C_{oc}, which equals C_s^t/f_{oc} and thus includes both bound and free chemical), and K_{oc} is the equilibrium constant for partitioning of chemical between organic carbon and water.

The relationship between chemical concentrations in organisms and sediment is defined by Ankley et al. (1992b) as the biota-sediment accumulation factor (BSAF):

$$BSAF = \frac{C_l}{C_{oc}} = \frac{C_a^t / f_l}{C_s^t / f_{oc}} = \frac{C_a^d \cdot K_{ow}}{C_s^d \cdot K_{oc}} \qquad (3\text{-}21)$$

Concentration of chemical in the organism is normalized to a lipid basis and in the sediment it is normalized to an organic carbon basis to make the BSAF more independent of the effect of these factors on chemical partitioning and more indicative of activity differences. The BSAF has previously been named the bioavailability index (BI) (Kuehl et al., 1987), the accumulation factor (AF) (Lake et al., 1990) and the biota-sediment factor (BSF) (Thomann et al., 1992). It can be used to quantify bioaccumulation relationships in the field and to help evaluate risk. Selection of surface sediment samples that quantitatively represent the average sediment/water/food chain exposure environment of an organism is difficult. Underestimation of the concentration of chemical in thin sediment surface layers acting as sources of the chemical to water and food chains may cause large overestimates of BSAFs.

BSAF measurements reflect disequilibrium between the organism, water and sediment. The form of the aquatic organism/water disequilibrium factor (R_{aw}) in equation 3-17 can be applied to disequilibrium between water and sediment (R_{ws}) and organism and sediment (R_{as}). Thus:

$$BSAF = \frac{C_a^d \cdot K_{ow}}{C_s^d \cdot K_{oc}} = R_{as} \cdot \frac{K_{ow}}{K_{oc}} = R_{aw} \cdot R_{ws} \cdot \frac{K_{ow}}{K_{oc}} \qquad (3\text{-}22)$$

If fish and sediment are at equilibrium ($R_{as} = R_{aw} \bullet R_{ws} = 1$), the BSAF is expected to equal the ratio of the lipid-normalized partition coefficient for aquatic organisms to the organic-carbon normalized partition coefficient for sediment. Because K_{oc} is of similar magnitude to and varies proportionally with K_{ow}, the expected equilibrium BSAF is unity or slightly greater and is insensitive to uncertainty in K_{ow}. The degree to which the BSAF deviates from the expected value is indicative of the degree of the disequilibrium between fish and sediment. Such disequilibrium can occur due to factors such as the following: (1) kinetic limitations for chemical transfer from sediments to water ($R_{ws}<1$ for systems which have a net flux of chemical from sediment); (2) surface sediment concentrations of the chemical that have not reached steady-state with water ($R_{ws}>1$); (3) organic carbon diagenesis in sediments which commonly results in $C_s^d > C_w^d$ ($R_{ws}<1$) ; and (4) biological processes which cause accumulation to be lower ($R_{aw}<1$) such as for biotransformation by the organism or greater ($R_{aw}>1$) such as for biomagnification.

Analogous to the BSAF, and perhaps more directly applicable to bioaccumulation by fish if the chemical can be detected on suspended solids, is the biota-suspended solids accumulation factor (BSSAF):

$$BSSAF = \frac{C_l}{C_{poc}} = \frac{C_a / f_l}{C_{ss} / f_{oc}} \qquad (3\text{-}23)$$

where C_{ss} is the concentration of chemical in suspended solids and f_{oc} is the fraction of organic carbon in suspended solids. Like the BSAF, this term should be of the order of one at equilibrium and disequilibria among system components will result in deviations from this expected value. For the BSSAF, the important disequilibrium is between the organism and water, because the disequilibrium between sediment and overlying water does not affect this parameter and because the extent of disequilibrium in water between the free chemical and that bound to POC and DOC should be much less than between the organism and water. DOC need not be explicitly considered in this relationship since it should have chemical activity similar to POC; also, DOC tends to be correlated with suspended solids concentrations and partitioning of the chemical to DOC is probably less than to POC. The BSSAF should not vary with differences in average suspended solids concentration or f_{oc} between ecosystems, but field measured BSSAFs should fluctuate some due to the slow response of C_l to changes in suspended solids and f_{oc}.

Since the BSSAF and the BSAF do not vary significantly with K_{ow}, the great uncertainty existing for the K_{ow} of TCDD is not incorporated into these bioaccumulation factors. Unfortunately, the K_{ow} uncertainty still is important when the application of either BSSAF or BSAF involves the prediction of C_{poc} or C_{oc} on the basis of chemical loading to the water. If the K_{oc}s for both sediment organic carbon and suspended organic carbon in water are similar, the difference between the BSAF and BSSAF for TCDD should be attributable to the disequilibrium present between surface sediment and the overlying water body. The difference between a measured BSSAF and the theoretical equilibrium partitioning value (perhaps 1 to 2) could be attributable to the combined effects of metabolism, biomagnification and choice of a nonequilibrium C_{poc}. Unfortunately, there are almost no C_{poc} data available for TCDD at this time. When available, these data could provide a better water-based bioaccumulation relationship.

3.2 TCDD BIOCONCENTRATION FACTORS

The expected equilibrium value for $ssBCF_l^d$ is K_{ow}, which is approximately 10^7 for TCDD (Burkhard and Kuehl, 1986). In practice, the extreme hydrophobicity of this chemical and the inability to determine C_w^d requires bioconcentration factors to be calculated only on the basis of total water concentrations and to be significantly less than this expected value. The absence of food uptake can also reduce accumulation and keep it below equilibrium values. However, if bioconcentration factors are

measured and used with these limitations in mind, they can still provide useful estimates of bioaccumulation potential.

A summary of TCDD ssBCF$_t^t$ determinations for fish (Table 3-1) indicates a range of 81,300 to 4,300,000. The variability of these results seems to be mostly due to differences in the uptake rate constants (k_1), since the elimination rate constants (k_2) differ only by a factor of 7. The large variability in k_1 is likely due to reduced bioavailability of TCDD associated with organic carbon buildup and the limited mass of TCDD added in static exposure systems. In these types of experiments, available TCDD is removed through bioconcentration by the fish, leaving only TCDD that is primarily absorbed to organic matter in the water. The results suggest that C_w^d may be further reduced from equilibrium with fish and absorbents due to a desorption rate slower than the rate of gill uptake. Renewal exposures are probably characterized by rapidly declining TCDD uptake between periodic additions of TCDD. The exposure reported for rainbow trout (Branson et al., 1985) is 27 times greater than the solubility of TCDD in water at 10°C (Lodge, 1989). Although the water concentration used for this BCF determination was reduced to 107 ng/L to adjust for bioavailability, it is likely that the actual fraction of TCDD available for bioconcentration during the 6-hour static exposure was only a small fraction of this value.

Fish size, age, and lipid content influence bioconcentration kinetics, but the impact of exposure water conditions on TCDD bioavailability is most difficult to evaluate because only total TCDD in the water can be measured. Steady-state BCF$_t^t$s for the flow-through exposures of rainbow trout (Mehrle et al. 1988), carp (Cook et al. 1991) and fathead minnows (ibid) ranged from 510,000 to 837,000 (mean of 728,000). These exposures involved TCDD added to the water with a solvent carrier and are expected to have a significant reduction in bioavailability of TCDD due to both organic carbon binding and a co-solvation effect due to the solvent carrier. The recently reported ssBCF$_t^t$ of 4,300,000 for medaka (*Oryzias latipes*) (Schmieder et al., 1992) is the only determination using a generator column for adding dissolved TCDD to the water. If log K_{ow} for TCDD is 7 and log BCF$_t^d$ is equal to log K_{ow} in the absence of a metabolism or enhanced elimination rate, this result indicates that most of the TCDD in the water was dissolved (or at least bioavailable) and metabolism of TCDD by the fish was too slow to greatly reduce bioconcentration. However if the log K_{ow} for TCDD is 8, the combined impact of low bioavailability and metabolism results in a BCF$_t^t$ that is approximately 20 times less than the theoretical equilibrium BCF$_t^d$ for TCDD.

The differences in k_2s for the ssBCF determinations (Table 3-1) are primarily attributable to differences in size, life-stage and lipid content of the test species. Unfortunately, all of the BCF determinations involved measurements of k_2 for fish with toxic symptoms. Also, the fathead minnow k_2 reported by Adams et al. (1986) was calculated without growth correction of TCDD concentrations in the fish and thus may overestimate the true k_2. The rainbow trout k_2 values appear large for a cold water

Table 3-1. Summary of TCDD steady-state bioconcentration factor determinations for fish.

Study	Species	Initial Size (g)	%Lipid	Temp (C)	TCDD conc. (pg/liter)	Exposure period (days)	Depuration period (days)	Uptake k_1 (ml/g/day)	Elimination k_2 (1/day)	ssBCF	ssBCF$_l$*
Branson et al. (1985)	rainbow trout	35	11	10	320,000	0.25 static	139	108	0.012	9,270	81,300
Mehrle et al. (1988)	rainbow trout	0.38 fry	n.r (est. 5)	11	38	28 flow-through	none	1852	0.047	39,000	780,000
Adams et al. (1986)	fathead minnow	0.5-1.0 juvenile	n.r. (est. 7)	25	1,000	28 static renewal	20	381	0.048	7,900	113,000
Cook et al. (1991)	fathead minnow	1.0 young adult	19	35	49-67	71 flow-through	61	1280-1890	0.012-0.013	97,000-159,000	510,000-837,000
Cook et al. (1991)	carp	15	9	25	62	71 flow-through	61	700	0.010	66,000	733,000
Schmieder et al. (1992)	medaka	0.175	8	25	101	12 flow-through no solvent	175	2306	0.0067	344,000	4,300,000

* Where not reported (n.r.), % lipid is estimated on basis of fish species, size, and age.

species (Table 3-1). The k_2 of 0.047 reported by Mehrle et al. (1988) was calculated on the basis of gill uptake kinetic data only. Lake trout (approximately 40 g), exposed to environmental, non-toxic levels of TCDD for 120 days via combinations of food, sediment, and water (TCDD from a generator column), eliminated TCDD at a slower rate with k_2 values in the range of 0.002-0.011 without growth correction (Cook et al., 1991). The nominal water exposure concentration for lake trout was only 0.7 pg/L and limited water analyses indicated that almost all of the TCDD was bound to suspended solids. On the basis of the nominal TCDD water concentration and a k_1 of 10, the ssBCF$_t^1$ for these lake trout is only 104,000 despite the slow elimination rate observed.

TCDD elimination was measured in adult carp with long-term exposure through water, food and sediment (Kuehl et al., 1987). Carp (1.5 kg) with 16% lipid were netted from the Wisconsin River and maintained in Lake Superior water for up to 336 days. The $t_{1/2}$ for TCDD elimination was approximately 320 days, with a k_2 of 0.0022 days^{-1}, in comparison to a $t_{1/2}$ of 63 days and a k_2 of 0.011 days^{-1} for smaller carp (Cook et al., 1991). The fivefold slower elimination rate for Wisconsin River adult carp may be attributable to size and lipid differences between the two groups of carp, without invoking consideration of long-term environmental exposure versus short-term laboratory water exposure.

The k_2 of 0.0067 for medaka (Schmieder et al., 1992) is the smallest reported for a small, warm water species. The medaka TCDD concentrations were corrected for growth and no solvent was present that might increase TCDD elimination across the gills. Insufficient kinetic data exist for induction of TCDD metabolism over time of exposure and depuration to allow a determination of the possible influence of the short 12 day exposure period on k_2.

3.3 TCDD BIOACCUMULATION FACTORS

Measurements of BAFs are usually based on presumed steady-state or pseudo-steady-state field exposure conditions. The ideal BAF$_t$s, for risk assessments in general and water quality criteria in particular, would be calculated on the basis of, and applied to, concentrations of freely dissolved or bioavailable organic chemical in water averaged over a time period greater than that required to reach 90% of steady-state (approximately one year for TCDD in fish). This would reduce variability due to fluctuations in water concentrations experienced by an organism and its food chain as a result of seasonal, meteorological, chemical loading or biota migrational patterns. Weather-induced fluctuations in water flow probably have considerable site-specific variation in the magnitude of influence on chemical concentration. This variability may be reduced through use of an annual mean concentration estimated on the basis of all factors influencing the concentration of the chemical which is bioavailable to the organism.

Great uncertainty exists for the estimation of such water concentrations, especially for very hydrophobic chemicals. In some cases, such as for TCDD and toxicologically related chemicals, ambient water concentrations can not be measured even on the basis of the total chemical present (see section 2.2). This makes direct validation of predicted water concentrations for these chemicals impossible at this time. Surface sediment contamination levels in systems approaching steady-state can be measured and thus provide an indirect and partial check on fate and transport model predictions of chemical concentrations in water.

Although $ssBAF_t^d$ should more readily extrapolate across sites than $ssBAF_t^t$, both factors will be estimated here because present EPA regulatory and risk assessment actions routinely estimate bioaccumulation on the basis of total chemical concentration in water. If BAF_t^t is estimated on the basis of water having small concentrations of DOC and suspended solids, the BAF_t^t will overestimate bioaccumulation of TCDD in systems with greater DOC and suspended solids. However, site-specific BAF_t^ts for fish can be readily calculated on the basis of the fraction of chemical freely dissolved (f_d) in the water:

$$BAF_t^t = BAF_t^d \cdot f_d \qquad (3\text{-}24)$$

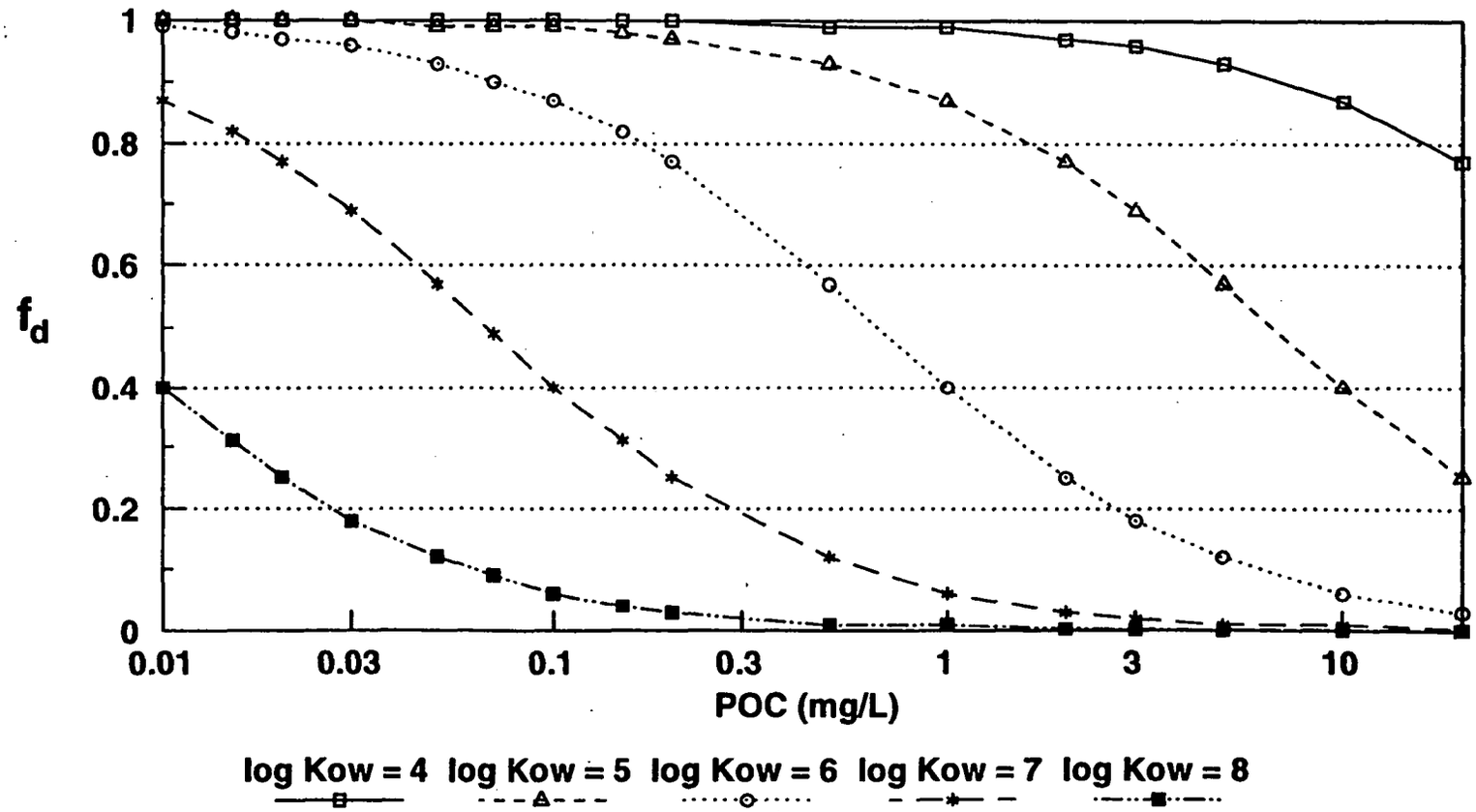
Equation 3-7 and its derivation describe f_d. Figure 3-1 demonstrates how f_ds and thus BAF_t^ts vary with POC and log K_{ow}.

Variability in TCDD BAF_ts, that may be attributable to interspecies differences in biomagnification, bioenergetics and chemical biotransformation kinetics, should be considered in addition to site-specific bioavailability conditions. These factors can only be included in bioaccumulation calculations through the use of site-specific bioaccumulation models requiring extensive calibration or through development of generic bioaccumulation models that allow a choice of BAFs based on site-specific food web and ecosystem characteristics (Cook et al., 1991). All BAF measurements or estimates also have variability and inaccuracy associated with movement of organisms over time through regions with different concentrations of chemical in water, sediment and food chain.

The best data for calculating a bioaccumulation factor for TCDD are provided by EPA studies of TCDD bioaccumulation for fish in Lake Ontario (Carey et al., 1990; Cook et al., 1990) coupled with estimates of sediment and water concentrations of TCDD (Endicott et al., 1990). Five species of fish were sampled at ten different locations throughout the lake in 1987. Rainbow smelt and slimy sculpin (*Cottus cognatus*) were also sampled in 1986 to determine lake trout food exposure (Cook et al., 1990). Since water concentrations of TCDD were expected to be nondetectable, the bioavailability index (BI = BSAF) approach (Kuehl et al., 1987; Ankley et al., 1992b) was attempted for a direct measure of bioaccumulation. Surface sediment (0-3 cm) samples were collected from 60 locations throughout the lake. In general, the

Figure 3-1. Fraction of organic chemical freely dissolved in water (f_d) if the total organic carbon binding factor TBF_{OC}=1.5 and log K_{OW}=4, 5, 6, 7, or 8.

$$f_d = \frac{1}{1 + TBF_{OC} \cdot POC \cdot K_{OW}} \qquad TBF_{OC} = 1 + \frac{DOC \cdot K_{DOC}}{POC \cdot K_{POC}}$$



TCDD distribution in surface sediments was found to follow organic carbon distribution. TCDD concentrations in fish did not reveal any distinct association with location (Carey et al., 1990). Lipid-normalized TCDD concentrations in different fish species ranged only ± 50% from the mean, except for older fish which had greater concentrations. There was a tendency for deeper water fish (lake trout, sculpin, smelt) to have greater lipid-normalized TCDD concentrations than near-shore, shallower water species, including brown trout (*Salmo trutta*), yellow perch (*Perca flavescens*) and smallmouth bass (*Micropterus salmoides*). White perch (*Morone americana*), an introduced species with a less known habitat and feeding preference in Lake Ontario, were found to have the greatest concentration of TCDD, at least in part due to their age. The lake wide average BSAFs found for each species were; lake trout - 0.07, brown trout - 0.03, yellow perch- 0.03, white perch - 0.20, smallmouth bass - 0.05, smelt - 0.06 and slimy sculpin - 0.12.

Lake Ontario lake trout TCDD residues declined approximately 65% between 1978 and 1988 (Cook et al., 1993a). The problem of estimating the Lake Ontario water concentration of TCDD associated with the measured TCDD residues in fish and wildlife can only be approached from the sediment contamination record. Surface sediment contamination in depositional basins has steadily decreased to the present 10% of the peak level that occurred around 1960 (Cook et al., 1993a). A mass balance model for TCDD loading of Lake Ontario (Endicott et al., 1990) estimated that 1 kg TCDD/year would result in a steady-state average surface sediment concentration of 55 pg TCDD/g dry weight sediment for a log K_{oc} of 6.5 and a f_{oc} of 0.03. The 1987 lake wide average sediment concentration of TCDD measured for Lake Ontario was 68 pg/g (Short et al., 1990) with 2.34% organic carbon (2,906 pg TCDD/g sediment organic carbon). Since the sediment record indicates that Lake Ontario is not at steady-state due to large reduction in TCDD loading since 1962, TCDD concentrations in the water and the pelagic food web are likely to be controlled by contaminated sediment interaction with the lake water, principally through resuspension of sediment.

The dynamic mass balance model for Lake Ontario (Endicott et al., 1990) predicted that a longterm 100% reduction in TCDD loading causes the water concentration of TCDD to decline for decades in proportion to the decrease in surface sediment concentrations. The ratio between water and sediment TCDD concentrations (C_w/C_s) associated with reduced TCDD loading was predicted to be 40% of the ratio associated with a steady-state condition. This model, with slight changes in organic carbon partitioning parameters (Endicott et al., 1993), was used for this report to estimate freely dissolved TCDD (C_w^d) and total TCDD (C_w^t) concentrations under either steady-state or major load reduction conditions.

Three TCDD loading scenarios were simulated for the purpose of estimating the 1987 Lake Ontario average concentration of TCDD in water and BAF_fs for lake trout: (1) steady-state; (2) a 90% reduction in TCDD loading for 20 years; and (3) a 100%

reduction in TCDD loading for 20 years. The lake wide average TCDD concentration in the model's 1.8 cm active surface sediment layer was set at 110 pg/g dry sediment or 3,667 pg/g organic carbon (f_{oc}=0.03). This sediment concentration was calculated from 29 surface sediment samples (0 to 3 cm) collected in 1987 (Short et al., 1990) from depositional basins in Lake Ontario. The model conservatively assumes an average suspended solids retention time of 43 days in a completely mixed water column and instantaneous equilibrium of TCDD in water with suspended solids. The suspended solids concentration is set at 1.2 mg/L with a f_{oc} of 0.15 and the amount of dissolved TCDD associated with colloidal or dissolved organic carbon is assumed to equal half the amount of dissolved TCDD associated with suspended particle organic carbon (TBF_{oc} = 1.5). Although the K_{oc} can be approximated to equal the measured K_{ow} of 10^7 (Burkhard and Kuehl, 1986), K_{ow} and $K_{oc} > 10^7$ may be more accurate (Lodge and Cook, 1989). Thus a K_{oc} of 10^8 is a reasonable upper bound for TCDD partitioning and is included in this estimation of C_w^d and C_w^t associated with the 1987 Lake Ontario lake trout average TCDD concentration (C_f = 194 pg/g lipid) and sediment active layer average TCDD concentration (C_{oc} = 3667 pg/g organic carbon).

Table 3-2 contains estimated concentrations of dissolved and total TCDD in Lake Ontario water for 1987 TCDD contamination levels under the three TCDD loading scenarios. Figure 3-2 shows the expected time course of the ratio of water to sediment concentrations for the two load reductions. The water concentrations only vary as a function of the loading scenario and K_{oc}. The BAF_f^d and BAF_f^t are calculated for lake trout. BAF_fs for other fish species can be calculated by dividing their average lipid-normalized TCDD residues by C_w. The same result is obtained by multiplying the lake trout BAF_fs by the ratio of the species' BSAF to the lake trout BSAF. BAF_f^t changes slightly when K_{oc} is increased from 10^7 to 10^8 but BAF_f^d is strongly influenced

Table 3-2. Steady-state TCDD bioaccumulation factors for lake trout, calculated from estimated Lake Ontario water concentrations in 1987.

Loading Scenario	K_{oc}	C_w^d pg/L	C_w^t pg/L	f_d	BAF_f^d	BAF_f^t
Steady-state	10^7	.102	.376	.27	1.90×10^8	5.16×10^5
Steady-state	10^8	.0102	.286	.036	1.90×10^7	6.78×10^5
20 y-90% load red.	10^7	.064	.235	.27	3.03×10^8	8.26×10^5
20 y-90% load red.	10^8	.0068	.190	.036	2.86×10^7	1.02×10^6
20 y-100% load red.	10^7	.050	.185	.27	3.86×10^8	1.05×10^6
20 y-100% load red.	10^8	.0057	.160	.036	3.40×10^7	1.21×10^6

by K_{oc}. This coincides with a ten-fold difference in the fraction of TCDD dissolved in water (f_d). The choice of loading scenario influences the estimated water/sediment TCDD concentration ratio (C_w^d/C_s^t) but only by a factor of two. Uncertainty associated with the choice of 20 years as the period of Lake Ontario response to TCDD loading reductions which probably began in the 1960s does not influence C_w^d/C_s^t for the zero loading scenario (Figure 3-2). The 90% TCDD loading reduction scenario, however, results in initial reduction in C_w^d/C_s^t, followed by many years of slow increase toward a new steady-state condition for the reduced TCDD loading.

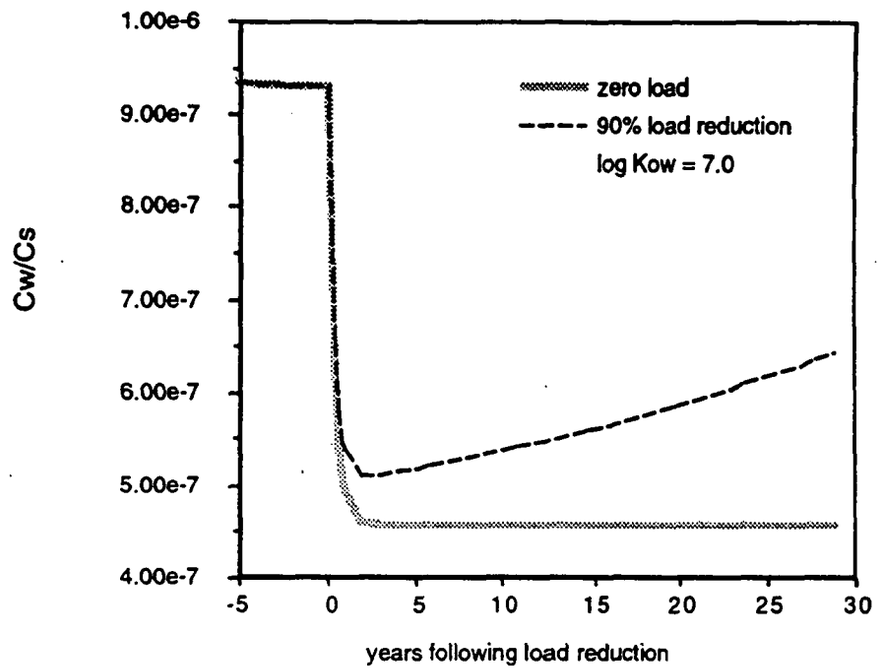
On the basis of the sediment core record (Cook et al., 1993a), the 90% TCDD load reduction scenario for Lake Ontario is most probable and thus is used here to select the best estimate of lake trout BAF_t. K_{oc} and K_{ow} are both estimated to be 10^7; however, the uncertainties for the true values of K_{ow} and K_{oc} remain. On this basis, the BAF_t^d for lake trout is estimated to be $3.0 \cdot 10^6$ and the BAF_t^t is estimated to be $8.3 \cdot 10^5$. The latter value is close to an earlier BAF_t^t estimate of $1 \cdot 10^4$/1% lipid used for human health and wildlife exposure assessments in the Great Lakes Water Quality Initiative (U.S. EPA, 1991a). The BAF_t^d estimate for TCDD may be less than K_{ow} as the net result of biomagnification potential in the aquatic food web countered by a slow rate of TCDD metabolism in fish which can be shown to significantly decrease organic chemical bioaccumulation (de Wolf et al., 1992). If the Lake Ontario model overestimates the magnitude of sediment resuspension on lake water TCDD concentrations, the BAFs reported here are too small.

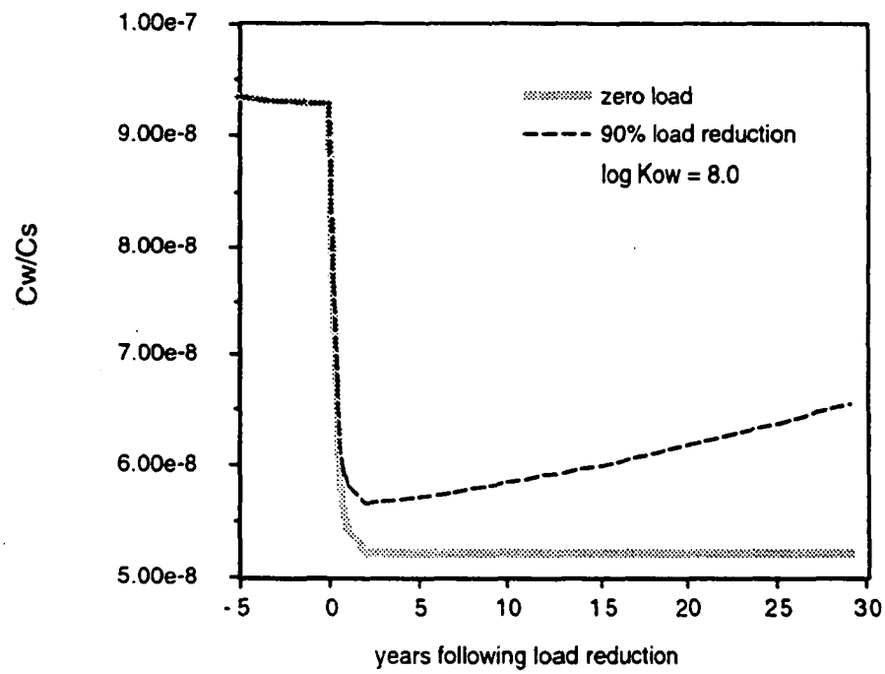
The applicability of the Lake Ontario TCDD BAF_ts to other aquatic systems is primarily a question of partitioning or bioavailability differences. Because the range of BAF_ts across fish species seems small, especially if age and sediment feeding habits are considered, the Lake Ontario BAF_t^d estimate may be the best predictor of TCDD residues in other systems if C_w^d can be estimated accurately. The Lake Ontario BAF_t^t estimate is based on a C_w^t estimate that is strongly influenced by suspended solids and DOC concentrations and thus could not be used directly to estimate bioaccumulation in aquatic systems having greater suspended solids and DOC concentrations. A site-specific fish BAF_t^t for TCDD, assuming a log K_{ow} of 7.0, is $3.0 \cdot 10^6 \cdot f_d$, where f_d is the fraction freely dissolved. Alternatively, since f_d is low even in Lake Ontario water, this can be further simplified to approximately $0.2 \cdot 10^6$/POC using equation 3-10. The extent to which fish accumulate super-hydrophobic organic chemicals like TCDD when bound to suspended solids in turbid water systems which have less than 1% of waterborne chemical in a freely dissolved state is uncertain.

3.4 TCDD BIOMAGNIFICATION FACTORS

Lake Ontario provides a good TCDD aquatic exposure site for studying BMFs because of the relatively uniform spatial distribution of TCDD and similar chemicals and the availability of monitoring data. Biomagnification of TCDD appears to be significant between fish and fish-eating birds but not between fish and their food

Figure 3-2. Predicted TCDD concentration response to loading reduction in Lake Ontario.





(Carey et al., 1990). When calculated for older predaceous fish such as lake trout eating young smelt, the BMF can equal 3. The lack of apparent biomagnification among fish is probably due to the influence of biotransformation of TCDD by the fish. Limited data for the base of the Lake Ontario lake trout food chain indicates little or no biomagnification between zooplankton and forage fish (Whittle et al., 1992). BMFs based on fish consuming invertebrate species probably are close to 1.0 because of the TCDD biotransformation by forage fish. BMFs greater than 1.0 may exist between some zooplankton species and their food due to the lack of TCDD biotransformation in invertebrates. The TCDD BMFs for herring gulls (*Larus argentatus*) and herring gull eggs in comparison to alewife (*Alosa pseudoharengus*) are 32 and 21, respectively (Braune and Norstrom, 1989). This compares to BMFs of 93 and 32 for total PCBs.

A pelagic [phytoplankton-zooplankton-herring (*Clupea harengus*)-cod (*Gadus morrhua*)] food chain and a littoral [phytoplankton/detritus-blue mussel (*Mytilus edulis*)-juvenile eider duck (*Somateria mollissima*)] food chain from the northern Baltic Sea were examined for biomagnification of PCDDs and PCDFs (Broman et al., 1992). The authors claimed that the combined concentrations of TCDD, 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,7,8-pentachlorodibenzo-p-dioxin increased with trophic level; however, the concentrations when lipid normalized do not appear to demonstrate biomagnification. TCDD was not detectable in zooplankton and gave a BMF a 1.3 for the blue mussel-eider duck relationship and a BMF of 0.84 for the herring-cod relationship.

3.5 BIOTA-SEDIMENT ACCUMULATION FACTORS

If the range in TCDD BSAFs for different fish species from different ecosystems with different food chain structures, contaminant distribution patterns and bioavailability conditions is relatively small, the TCDD BAF_i^d estimates obtained from Lake Ontario data should have general application to fish in other systems. BSAFs carefully determined for a wide range of bioaccumulative organic chemicals found in lake trout from Lake Ontario correlate well with BSAFs measured for brown bullheads (*Ictalurus nebulosus*) in the Fox River near Green Bay, WI (P. Cook, ERL-Duluth, unpublished data). BSAFs for PCBs in both oligochaetes and bullheads in the Fox River (Ankley et al., 1992b) demonstrate that these organic chemicals in the sediments are bioaccumulated to levels expected for equilibrium partitioning. In both Lake Ontario and the Fox River, BSAFs for TCDD in fish are approximately 20-fold less than for PCBs. Thus, despite the large differences in ecosystem characteristics and fish species, the BSAFs consistently predict each chemicals bioaccumulation potential. Agreement in BSAFs measured for TCDD and other bioaccumulative organic chemicals at different sites, however, does not eliminate the large uncertainty in BAF_i^ds associated with accuracy of K_{ow} measurements or estimates that translate directly into uncertainty for the estimated C_w^d.

Beside the TCDD BSAFs reported for fish in Lake Ontario, there are only a few studies in which sediment sampling and chemical analyses were performed so that BSAFs may be calculated and used as reliable quantitative estimates of bioaccumulation potential. BSAFs obtained from data sets having sediment TCDD concentrations likely to represent average surface sediment conditions associated with the region inhabited by the organisms are summarized in Table 3-3.

Table 3-3. Steady-state biota/sediment accumulation factors (BSAF) for TCDD.

Species	Location	Sed. Char.	BSAF	Reference
Brown trout	Lake Ontario	Lakewide mean	0.03	1
Lake trout	"	"	0.07	1
Small. bass	"	"	0.05	1
White perch	"	"	0.20	1
Yellow perch	"	"	0.03	1
Smelt	"	"	0.04	2
Sculpin	"	"	0.12	2
Herring gull	"	"	0.43	1,3
Carp	Wisconsin River	Reservoir mean	0.27	4
Bullhead	Fox River	Segment mean	0.05	5
Sandworm	Passaic River	Laboratory mean	0.48	6
Clam	"	"	0.93	6
Shrimp	"	"	0.73	6

References:
1. Carey et al. (1990).
2. Batterman et al. (1989).
3. Braune and Norstrom (1989).
4. Kuehl et al. (1986).
5. Cook (unpublished).
6. Rubinstein et al. (1983).

The larger fish BSAFs in Table 3-3 appear to result from factors such as age (white perch), association with sediments having TCDD concentrations that exceed the average used to calculate the BSAF (sculpins), or sediment ingestion exposure (carp). The Lake Ontario herring gull BSAF reflects biomagnification of TCDD. The saltwater benthic invertebrates have larger BSAFs due to direct exposure to sediment and the

lack of metabolism of TCDD. Additional BSAFs for TCDD were reported for suckers (*Catostomus spp.*), mountain whitefish (*Prosopium williamsoni*) and Lake whitefish (*Coregonus clupeaformis*) in Canadian rivers associated with bleached kraft paper mills (Muir et al., 1992a); however, the sediment samples were not collected for the specific purpose of obtaining the mean BSAF values, which ranged from 0.14 to 1.88. It is possible that the feeding habits of these fish and/or slight disequilibrium between sediment and water (R_{ws}) caused the approximately ten-fold greater BSAFs than reported above in Table 3-3 for fish but it is also possible that the sediment sample analyses underestimated the amount of TCDD available for bioaccumulation in the river water and food to which the fish were exposed.

In summary, few BSAF values that quantitatively measure the degree to which fish approach equilibrium with surface sediment have been reported. For the systems and fish species studied, the BSAF appears to vary within the 0.03 to 0.3 range. Larger BSAFs, which have been reported on the basis of less certain average surface sediment characterizations, may be biased by measurement of less contaminated layers than the average sediment/water interface. BSAFs much less than one indicate that fish generally are well below equilibrium with respect to the sediment (at least ten-fold for BSAF=0.1, or even more if $K_{ow}/K_{oc}>1$). Low BSAFs might reflect disequilibrium between water and sediment ($R_{ws}<1$) due to the effects of decreasing anthropogenic inputs into the water coupled with slow sediment resuspension; sediment diagenesis which increases chemical activity in the sediment; and loss processes from the water which keep TCDD concentrations depressed relative to sediment. The low BSAFs might also reflect effects of growth dilution and metabolism (in the entire food chain) which keep tissue concentrations below equilibrium values with the water ($R_{aw}<1$). Comparison of BSAFs for TCDD to BSAFs for PCBs which are approximately 20-fold greater suggests that metabolism of TCDD by fish is a major contributor to the overall TCDD disequilibrium ($R_{as}<<1$). The importance of metabolism is slightly uncertain due to uncertainty for the relative differences in biomagnification potential and R_{ws} between TCDD and PCBs.

BSAFs may be used to calculate BAFs for other PCDD, PCDF and PCB congeners that could contribute to Ah receptor mediated toxic effects. This is particularly important when toxicity equivalency factors (TEFs) are used to estimate the combined toxic potential of PCDDs, PCDFs and PCBs, as a toxicity equivalence concentration (TEC), in the exposure of fish, wildlife or humans through aquatic food chains:

$$TEC = \sum_i (C_{w,i}^t \cdot BAF_i^t \cdot TEF_i) \qquad (3\text{-}25)$$

The selection of BAF_i^t for each congener is an important step in the TEC calculation. Assuming that the relative amount of each organic chemical bound to sediment approximates the relative amount bound to suspended solids, bioaccumulation

equivalency factors (BEF) can be calculated from BSAFs for each congener (i) in comparison to the BSAF for TCDD (D):

$$BEF_i = \frac{BSAF_i}{BSAF_D} = \frac{BAF^b_{l,i}}{BAF^b_{l,D}} = \frac{BAF^t_{l,i} \cdot f_{b,D}}{BAF^t_{l,D} \cdot f_{b,i}} \qquad (3\text{-}26)$$

where BAF^b_l is the lipid normalized BAF based on the concentration of organic carbon-bound chemical in water and f_b is the fraction of chemical bound to organic carbon in water ($f_b = 1\text{-}f_d$). Many of the BEFs in Table 3-4 are estimates of maximum values based on nondetection of the congeners in fish collected from Lake Ontario in 1987-1988. Also, the Lake Ontario BEF for TCDF is large in comparison to values from other systems such as the Wisconsin River (Kuehl et al., 1987), possibly as a result of greater water concentrations of TCDF with respect to sediment concentrations of TCDF in Lake Ontario. The BAF for the ith congener (BAF^t_i) is :

$$BAF^t_i = (BEF_i)(BAF^t_{l,D})\left(\frac{f_{b,i}}{f_{b,D}}\right)(f_l) \qquad (3\text{-}27)$$

Table 3-4. Bioaccumulation equivalency factors (BEF) derived for PCDDs and PCDFs from Lake Ontario lake trout and sediment data.

Congener	Estimated[a] log K_{ow}	BSAF	BEF
2,3,7,8-TCDD	7.0	0.06	1.0
1,2,3,7,8-PeCDD	7.5	≤0.05	≤0.8
1,2,3,4,7,8-HxCDD	7.8	≤0.02	≤0.3
1,2,3,6,7,8-HxCDD	7.8	≤0.01	≤0.2
1,2,3,7,8,9-HxCDD	7.8	≤0.01	≤0.2
1,2,3,4,6,7,8-HpCDD	8.2	≤0.002	≤0.03
OCDD	8.6	≤0.001	≤0.02
2,3,7,8-TCDF	5.8	0.07	1.2
1,2,3,7,8-PeCDF	NR[b]	0.02	0.3
2,3,4,7,8-PeCDF	NR	0.11	1.8
1,2,3,6,7,8-HxCDF	NR	≤0.02	≤0.3
2,3,4,6,7,8-HxCDF	NR	≤0.03	≤0.5
1,2,3,7,8,9-HxCDF	NR	≤0.03	≤0.5
1,2,3,4,6,7,8-HpCDF	NR	≤0.0002	≤0.003
1,2,3,4,7,8,9-HpCDF	NR	≤0.01	≤0.1
OCDF	8.8	≤0.0003	≤0.005

[a] Burkhard and Kuehl (1987).
[b] NR = not reported.

4. EFFECTS

4.1 COMPARATIVE TOXICOLOGY

Hundreds of publications have focused upon the toxicology of TCDD in laboratory mammals and birds. In addition to being one of the most toxic synthetic molecules known, TCDD represents the prototypical compound for a variety of structurally-similar contaminants of environmental concern that appear to act via the same mode of action (MOA), which include other 2,3,7,8-substituted PCDDs and PCDFs, and several non-and mono-ortho-substituted (planar) PCBs (for reviews, see Goldstein, 1980; Poland and Knutson, 1982; Greenlee and Neal, 1985; Whitlock, 1987, 1990; Safe, 1990). The initial step by which TCDD is thought to exert its toxicity is through binding to the cytosolic Ah receptor (Poland and Glover, 1980). Endogenous ligands for the Ah receptor have not been identified, and some researchers have hypothesized that the functions of the Ah receptor may be regulated by exogenous materials (for reviews, see Nebert et al., 1981; Okey, 1983; Nebert and Gonzalez, 1987). After initial binding, the ligand-receptor complex is translocated to the nucleus of the cell where it becomes associated with DNA thereby causing initiation of transcription of one or more target genes (Okey et al., 1979; Gonzalez et al., 1984; Denison et al., 1989; Nebert, 1990; Whitlock, 1990). The subsequent suite of physiological effects observed are somewhat species-specific, but surprisingly consistent across vertebrate phylogenetic lines. In addition to lethality, common effects include weight loss ("wasting syndrome"), decreased immunocompetence, subcutaneous edema, reproductive effects (fetotoxicity, teratogenesis), alterations in lipid metabolism and gluconeogenesis, thymic atrophy, and induction of certain enzyme systems, most notably cytochrome P4501A1 (Goldstein, 1980; Poland and Knutson, 1982; Greenlee and Neal, 1985; Safe, 1990). Characteristic of TCDD-induced toxicity in mammals and birds is a delayed onset of mortality, even at relatively large doses (see following sections on the effects of TCDD on aquatic life and wildlife). For this reason, it is inappropriate to refer to the acute versus chronic effects of TCDD; these terms are often used to refer to both exposure duration and time-to-effects. In the case of TCDD, more accurate terminology would be short- versus long-term exposure and lethal versus sublethal effects.

Species-specific factors such as uptake, disposition and metabolism of TCDD, as well as interspecies differences in concentration, tissue distribution and ligand affinity of the Ah receptor, all likely play a role in determining the relative sensitivity of organisms to TCDD. However, the presence of the Ah receptor clearly appears to be a necessary prerequisite for TCDD (and related compounds) to exhibit toxicity. Moreover, the relative affinity of planar PCBs, and 2,3,7,8-substituted PCDFs and PCDDs for the Ah receptor appears to dictate the relative toxicity of these compounds to different test species (Poland and Glover, 1977; Bandiera et al., 1984; Mason et al., 1986). When sensitive techniques have been used, all mammals (including man) and birds investigated thus far have exhibited detectable concentrations of Ah receptor in a

number of different tissues, though often at quite different levels. This suggests that it is valid to extrapolate TCDD toxicity/receptor models developed for standard laboratory species, such as the rat or chicken (*Gallus domesticus*), to avian and mammalian wildlife. Results of initial studies using sucrose density gradient centrifugation to detect the Ah receptor in fishes and amphibians were somewhat ambiguous (Denison et al., 1985; 1986). However, subsequent experiments using more sensitive techniques to detect and quantify the Ah receptor conclusively demonstrated its presence in teleost and elasmobranch fishes and teleost cell lines (Lorenzen and Okey, 1990; Hahn et al., 1992). The Ah receptor has not been detected in some primitive fishes (hagfish, lamprey; class Agnatha), and has not been found in nine species of invertebrates representing eight classes of four phyla (Hahn et al., 1992). Some studies suggest the presence of a protein similar to the Ah receptor in certain terrestrial invertebrates (Bigelow et al., 1985; Muehleisen et al., 1989); however, it appears that the structure (and function) of that protein differs from the Ah receptor present in vertebrates (Hahn and Stegeman, 1992). The possible presence of the Ah receptor in amphibians or reptiles remains uncertain, as relatively sensitive detection techniques have not been applied to these animal classes.

The presence of the Ah receptor in fishes, and lack of the receptor in aquatic invertebrates, is consistent with the relative sensitivity of the two groups of species to TCDD and structurally-similar compounds. For example, TCDD has been shown to be lethal to a number of fish species when administered either through the diet or the water (Miller et al., 1973; 1979; Norris and Miller, 1974; Hawkes and Norris, 1977; Helder, 1980; 1981; Adams et al., 1986; Kleeman et al., 1988; Mehrle et al., 1988; Spitsbergen et al., 1988a; 1988b; Walker, 1991; Cook et al., 1991; Walker et al., 1993). Moreover, exposure of fishes to TCDD results in effects similar to those seen in mammals, such as delayed mortality (see numerous references in the following section on the effects of TCDD on aquatic life), a "wasting" syndrome (Hawkes and Norris, 1977; Miller et al., 1979; Kleeman et al., 1988), reproductive toxicity (Walker, 1991; Walker et al., 1993), histopathologic alterations (Helder, 1980; Johnson et al., 1986; Spitsbergen et al., 1988a; Cook et al., 1991), possible immunosuppression (Spitsbergen et al., 1988c) and induction of cytochrome P450-dependent monooxygenases (Pohl et al., 1975; Vodicnik et al., 1981; Janz and Metcalfe, 1991; van der Weiden et al., 1992). Conversely, long-term exposures of a number of invertebrate species (snails, worms, daphnids and mosquito larvae) and aquatic plants to TCDD failed to cause discernable toxicity (Miller et al., 1973; Isensee and Jones, 1975; Isensee, 1978; Yockim et al., 1978; Adams et al., 1986). In other instances, exposure of invertebrates to 3,3',4,4'-tetrachlorobiphenyl, a PCB congener with a MOA similar to TCDD, also did not cause toxicity, even in relatively long-term exposures (Borgmann et al., 1990; Dillon et al., 1990). More extensive studies, however, are required to fully evaluate the potential for TCDD to elicit adverse toxicological responses in aquatic invertebrates, either via protein(s) with properties similar to the Ah receptor or via another MOA.

The Ah receptor has been detected in most vertebrates and preliminary studies indicate a high degree of structural similarity among species (Denison et al., 1991; Bank et al., 1992). However, because of other factors (e.g., metabolism, distribution, differential gene expression, etc.) which may influence the toxicity of TCDD and related compounds, interspecies extrapolations of potential toxicity based only on presence of the Ah receptor should be made with caution. For example, although certain mono-ortho substituted PCBs (e.g., 2,3,3',4,4'-pentachlorobiphenyl, 2,3',4,4',5-pentachlorobiphenyl) strongly bind to the Ah receptor and induce cytochrome P4501A1 in mammals, these PCB congeners were found not to be potent inducers of cytochrome P450 in fishes (Gooch et al., 1989). Similarly, some evidence suggests that planar PCBs may be less potent in fishes than in mammals (Walker and Peterson, 1991).

The planar PCBs and 2,3,7,8-substituted PCDFs and PCDDs appear to act via the same MOA as TCDD, suggesting that their toxicities would be additive. In fact, a number of studies suggest that for certain endpoints, the effects of mixtures of PCBs, PCDFs and PCDDs are additive (Sawyer and Safe, 1985; Weber et al., 1985; Vecchi et al., 1985; Eadon et al., 1986; Bimbaum et al., 1987; Pluess et al., 1988). This is of concern because in most instances complex mixtures of the chlorinated hydrocarbons are simultaneously present in environmental samples. Thus, although an individual PCB, PCDF or PCDD congener may not be present at a toxic concentration, the combination of these compounds could result in toxicity. For this reason, the potential toxicity of mixtures of PCBs, PCDFs and PCDDs in environmental samples has been evaluated using different methods to determine sample "toxic equivalents" relative to TCDD. Two basic methods have been used for this. In the first, concentrations of individual PCB, PCDF and PCDD congeners are determined and multiplied by TEFs which then are summed to express potential toxicity in TCDD-equivalents (TCDD-EQ) (Sawyer and Safe, 1985; Eadon et al., 1986; Kannan et al., 1988; 1989; Niimi and Oliver, 1989; van Zorge et al., 1989; Bellward et al., 1990; Olafson et al., 1990; Safe, 1990; Ankley et al., 1992a). The TEFs may be derived from *in vitro* or *in vivo* studies evaluating the potency of individual congeners relative to TCDD. It should be noted that many of the TEFs currently employed for risk assessments were derived from induction of cytochrome P4501A1 in laboratory mammals or mammalian cell lines, and there remains not only some question as to exact relationships between P450 induction potency and *in vivo* toxicity of PCBs, PCDFs and PCDDs, but also uncertainty as to the validity of extrapolations from mammalian systems to nonmammalian species.

The second method for determining sample TCDD-EQ employs techniques to extract the total PCB/PCDF/PCDD mixture from environmental samples. The extract then is tested for potency, relative to TCDD, using a standard biological response as an endpoint. A commonly used biological system for the latter approach to generating TCDD-EQ is induction of cytochrome P4501A1 (and associated monoxygenase activities) in the H4IIE rat hepatoma cell line (Bradlaw and Casterline,

1979; Bradlaw et al., 1980; Casterline et al., 1983; Safe et al., 1987; 1989; Zacharewski et al., 1989; Ankley et al., 1991; Hanberg et al., 1991; Tillitt et al., 1991a; 1991b; 1992). Other biological systems also are potentially capable of measuring the overall potency (or toxicity) of complex mixtures of polychlorinated hydrocarbons. For example, the salmonid egg injection system described by Walker et al. (1992) may prove to be a useful method for this type of analysis. Overall, a biological approach to generating TCDD-EQ may in some instances be superior to the analytical technique described above, because the assumption of an additive model of toxicity for the complex mixtures of planar and nonplanar PCBs, PCDFs and PCDDs found in environmental samples may not always be appropriate (Birnbaum et al., 1985; Bannister et al., 1987; Bannister and Safe, 1987; Biegel et al., 1989). However, if a biological approach to measuring TCDD-EQ is to be used for quantitative risk assessment, it is important to calibrate the biological system used with specific toxicological endpoints in the species of concern.

4.2 EFFECTS OF TCDD ON AQUATIC LIFE

4.2.1 Toxicological Information

A review of the literature concerning the toxic effects of TCDD to aquatic life was obtained from both computerized and manual searches. Aquatic toxicity and accumulation data for freshwater and saltwater organisms from both laboratory tests and aquatic model ecosystem studies were included in the review. Most of the studies contained in previous reviews (Kenaga and Norris, 1983; U.S. EPA, 1984; U.S. Department of Interior, 1986; Cooper, 1989; and U.S. EPA, 1990) and in the present literature review, involved short-term exposure periods, where organisms were exposed to TCDD through several exposure routes followed by a depuration period where the organisms were held in clean water to observe delayed effects. The following sections provide both a narrative description of each of these studies and a summary of the toxicity results for some of the species tested (Table 4-1).

4.2.1.1 Freshwater Plants

The few data that are available for the effects of TCDD on freshwater aquatic plants are from aquatic model ecosystem studies and indicate that plants are less sensitive to this chemical than most species of aquatic animals after similar periods of exposure (Table 4-1). Measured water concentrations (as determined from initially exposing the bottom soils with TCDD) up to 1,330 ng/L had no observable effect on algae (*Oedogonium cardiacum*) and duckweed (*Lemna minor*) during 33 days of exposure (Isensee and Jones, 1975). TCDD residues in algae were 2,295,000 pg/g after the 33 day period. TCDD residues measured in algae exposed to up to 4.2 ng/L in other studies by these authors (Isensee, 1978; Yockim et al., 1978) ranged from 1,300 to 5,000 pg/g during a 32-day period. Residues of TCDD in duckweed exposed to 0.05 to 7.13 ng/L ranged from 200 to 30,700 pg/g (Isensee and Jones, 1975).

4.2.1.2 Freshwater Invertebrates

Only a limited number of experiments have been conducted to determine the effects of TCDD on freshwater aquatic invertebrates (Table 4-1). Isensee and Jones (1975), Isensee (1978) and Yockim et al. (1978) exposed invertebrates to measured water concentrations of TCDD in aquatic model ecosystems. After approximately 33 days of exposure, these authors found that concentrations ranging from 0.05 to 1,330 ng/L were not toxic to snails (*Physa* sp.) or daphnids (*Daphnia magna*), as determined by reproductive activity, feeding and growth. TCDD residues measured in snails and daphnids exposed to 1,330 ng/L were 502,000 and 1,570,000 pg/g, respectively, after the test period (Isensee and Jones, 1975; Isensee, 1978). In other studies by Isensee (1978) and Yockim et al. (1978), TCDD residues in snails and daphnids exposed to lower water concentrations of 2.4 to 4.2 ng/L ranged from 2,500 to 9,700 and 6,800 to 17,100 pg/g for these species, respectively, during the 32-day test period. In another study, Adams et al. (1986) found no effect on different age groups of *Daphnia magna* during a 7-day period following exposure for 48 hours to TCDD concentrations of 0.2 to 1,030 ng/L. Residue concentrations in daphnids were not measured in this study.

In other experiments, Miller et al. (1973) indicated that an initial nominal concentration of 200 ng/L of TCDD did not cause mortality to snails (*Physa* sp.), worms (*Paranais* sp.) or mosquito larvae (*Aedes aegypti*) during 36, 55 and 17 days of exposure in static tests, respectively. Although this concentration appeared to decrease reproductive success in snails after 48 days of observation and the total number of worms produced after 55 days, these decreases were not statistically significant. In addition, no effect on the growth of worms or the pupation rate of mosquitos were seen at this concentration after 55 and 40 day observation periods with these organisms. Residue concentrations were not measured in these studies.

4.2.1.3 Freshwater Fish

Because of the large number and diversity of fish studies, the following text is organized into subsections dealing with the route through which TCDD was administered to the test species. Fish were exposed to TCDD via water, egg injection, intraperitoneal (i.p.) injection and the diet. The studies included all life stages (i.e., eggs, larvae, juveniles and adult). A summary of the toxic effects of TCDD to some of these species is provided in Table 4-1.

Waterborne Exposure

Isensee and Jones (1975), Isensee (1978) and Yockim et al. (1978) found that mosquito fish (*Gambusia affinis*) and channel catfish (*Ictalurus punctatus*) exposed to continuous measured water concentrations of 2.4-4.2 ng TCDD/L died after 15-20 days of exposure in aquatic model ecosystem studies. Death was accompanied by nasal hemorrhaging, fin necrosis and listless swimming. Measured residues of

[^{14}C]TCDD in fish that died ranged from 4,400 to 7,200 pg/g wet weight. Other studies by Miller et al. (1973), indicated that initial mortality of coho salmon (*Oncorhynchus kisutch*) did not occur until 5 to 10 days after the beginning of the exposure period and that mortality often extended over a 2 month period. Fish exposed to 56 to 100 ng/L did not feed which resulted in significant decreases in growth after 80 days. Most fish exposed to 100 ng/L for 24 hours died after 60 days. After a 24-hour exposure period, the lowest test concentration of 0.056 ng/L caused 12% mortality to young coho salmon in 60 days compared to a 2% control mortality rate. However, the lowest concentration did not appear to cause significant effects in later studies (Miller et al. 1979). As with other fish species, signs of toxicity in salmon included skin discoloration, fin necrosis, and lethargy prior to death.

Later experiments (Miller et al., 1979) showed that an initial, measured concentration of 0.56 ng/L had no effect on food consumption, weight gain, or survival of young coho salmon but 5.6 ng/L reduced survival and growth 114 days after an exposure of 12 hours and caused 50% mortality to salmon in 56 days after exposure for 96 hours. Percent survival at 114 days decreased with increased exposure duration beyond 12 hours, indicating to the authors that a critical exposure period for long-term survival in these studies was between 12 and 24 hours. However, duration of exposure appeared to be less important than exposure concentrations in determining mean survival time. Based on these results, the reported waterborne, no-effect and effect TCDD concentration for young coho salmon (based on survival and growth) was between 0.56 and 5.6 ng/L. After exposures for 1.5, 3, 6, 12 and 96 hours, TCDD residues measured in whole body extracts of coho salmon selected at random from those surviving for 114 days indicated that residue levels in these fish increased with both water concentration and duration of exposure. Fish exposed to TCDD concentrations of 0.001 to 1.053 ng/L for 96 hours contained 0 to 125 pg/g and those exposed to 10.53 ng/L for 1.5 to 96 hours contained 68 to 2,170 pg/g after 114-days. Norris and Miller (1974) exposed different sized guppies (*Poecilia reticulata*) from 9 to 40 mm in static tests to nominal TCDD concentrations of 100, 1,000 and 10,000 ng/L for 120 hours. After exposure, fish were transferred to TCDD- free water and observed for up to 37 days. Eight percent of the fish exposed to 100 ng/L died during exposure, but all fish exposed to this and higher concentrations died during 37 days in clean water. Test results showed that smaller fish were more sensitive than larger fish. Similar results were observed for coho salmon in previous studies by Miller et al. (1973). Later tests by Miller et al. (1979) also showed that a 24-hour exposure to a much lower nominal water concentration of 0.1 ng/L caused a significant increase in fin necrosis during 42 days in guppies 8 to 12 mm in length. The severity of this effect was lessened after 69 days indicating that sublethal effects of TCDD may be reversible in fish. No fin disease was observed in fish exposed to 0.01 ng/L.

Eggs of northern pike (*Esox lucius*) and eggs, yolk sac fry and juvenile rainbow trout were exposed to four nominal concentrations of TCDD (0.1, 1, 10 and 100 ng/L)

Table 4-1. Summary of the toxic effects of TCDD to aquatic life and wildlife.

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
AQUATIC LIFE							
Freshwater Species							
Algae, *Oedogonium cardiacum*	Model ecosystem	1,330	2,295,000	33-d		No toxic effect	Isensee and Jones, 1975; Isensee, 1978
Vascular plant Duckweed, *Lemna minor*	Model ecosystem	1,330		33-d		No toxic effect	Isensee and Jones, 1975; Isensee, 1978
Duckweed *Lemna minor*	Model ecosystem	7.13	30,700	33-d		No toxic effect	Isensee and Jones, 1975
Annelid Worm, *Paranais* sp.	Water (static)	200[c]		55-d		No decrease in reproductive success	Miller et al., 1973
Mollusc Snail (adult), *Physa* sp.	Model ecosystem	1,330	502,000	33-d		No toxic effect	Isensee and Jones, 1975; Isensee, 1978
Snail (adult), *Physa* sp.	Water (static)	200[c]		36-d	12-d	No decrease in reproductive success	Miller et al., 1973
Arthropod Mosquito (larvae), *Aedes aegypti*	Water (static)	200[c]		17-d	23-d	No effect on pupation	Miller et al., 1973
Cladoceran (adult), *Daphnia magna*	Model ecosystem	1,330	1,570,000	33-d		No toxic effect	Isensee and Jones, 1975

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Cladoceran (1-21 d), *Daphnia magna*	Water (renewal)	1,030		48-h	7-d	No toxic effect	Adams et al., 1986
Fish							
Coho salmon, *Oncorhynchus kisutch*							
Juvenile (3.5 g)	Water (static)	0.56		96-h	114-d	No toxic effect	Miller et al., 1979
Juvenile (3.5 g)	Water (static)	5.60		96-h	56-d	50% mortality	Miller et al., 1973, 1979
Rainbow trout, *Oncorhynchus mykiss*							
Eggs	Water (renewal)	0.10[c]		96-h	160-d	Delayed development, reduced growth of fry	Helder, 1981; 1982a,b
Eggs	Water (renewal)	1[c]		96-h	160-d	Reduced growth, mortality in sac fry	Helder, 1981; 1982a,b
Eggs	Water (renewal)	10[c]		96-h	40-d	100% mortality in sac fry	Helder, 1982a,b
Eggs	Egg injection		230 (in eggs)	Single injection	Fertilized egg to swim-up	LR50[d] (sac fry of McConaughy strain)	Walker and Peterson, 1991
Eggs	Egg injection		240 (in eggs)	Single injection	Fertilized egg to swim-up	LR50[d] (sac fry of Erwin strain)	Walker and Peterson, 1991
Eggs	Egg injection		374 (in eggs)	Single injection	Fertilized egg to swim-up	LR50[d] (sac fry of Arlee strain)	Walker and Peterson, 1991
Eggs	Egg injection		488 (in eggs)	Single injection	Fertilized egg to swim-up	LR50[d] (sac fry of Eagle Lake strain)	Walker and Peterson, 1991

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Rainbow trout (cont.)							
Eggs	Egg injection		421 (in eggs)	Single injection	>48-h to post swim-up	LR50[d] (sac fry of Fish Lake strain)	Walker et al., 1992
Eggs	Water (renewal)		279 (in eggs)	48-h	>48-h to post swim-up	Significant mortality in sac fry	Walker et al., 1992
Eggs	Water (renewal)		439 (in eggs)	48-h	>48-h to post swim-up	LR50[d] (sac fry)	Walker et al., 1992
Sac fry	Water (renewal)	1[c]		96-h	160-d	Reduced growth, mortality	Helder 1981; 1982a,b
Sac fry	Water (renewal)	10[c]		96-h	10-d	100% mortality	Helder 1982a,b
Sac fry	Water (renewal)	12.2[c]		96-h	16-d	100% mortality	Helder and Selnen, 1985
Sac fry	Water (renewal)	1.83[c]		96-h	21-d	LC50	Bol et al., 1989
Swim-up fry (0.38 g)	Water (flow-thru)	0.176	3,220	28-d	28-d	95% mortality	Mehrle et al., 1988
Swim-up fry (0.38 g)	Water (flow-thru)	0.0011	21[g]	28-d	28-d	NOAEL[e]	Mehrle et al., 1988
Swim-up fry (0.38 g)	Water (flow-thru)	0.038	765[g]	28-d	28-d	LOAEL[f] (45% mortality)	Mehrle et al., 1988
Swim-up fry (0.38 g)	Water (flow-thru)	0.046		28-d	28-d	LC50	Mehrle et al., 1988
Juvenile (0.85 g)	Water (renewal)	10		96-h	68-d	Reduced growth, mortality	Helder 1981; 1982a,b

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Rainbow trout (cont.)							
Juvenile (0.85 g)	Water (renewal)	100		96-h	23-d	100% mortality	Helder 1981; 1982a,b
Fingerling (25-45 g)	i.p. injection		1,000[c]	Single injection	25-d	Significant hematological changes	Spitsbergen et al., 1988a
Fingerling (25-45 g)	i.p. injection		5,000[c]	Single injection	20-d	20% mortality	Spitsbergen et al., 1988a
Fingerling (25-55 g)	i.p. injection		5,000[c]	Single injection	11-12-wk	20% mortality, increased liver weight	van der Weiden et al., 1990
Fingerling (25-45 g)	i.p. injection		10,000[c]	Single injection	80-d	LD50	Spitsbergen et al., 1988a; Kleeman et al., 1988
Fingerling (35 g)	Water (static)	107	650-2,580	6-h	42-139-d	Mortality, fin rot, increased liver weight	Branson et al., 1985
Fingerling (7.8 cm)	Diet (3.29 ng/g)		314	71-d		No effect on survival and growth	Hawkes and Norris, 1977
Fingerling (7.8 cm)	Diet (1,700 ng/g)		276,000	71-d		100% mortality	Hawkes and Norris, 1977
Fingerling (3-7 g)	Diet (0.494 ng/g)		250	13-wk	13-wk	No toxic effect	Kleeman et al., 1986a
Fingerling (8 g) Yearling (100-150 g)	i.p. injection		10,000[c]	Single injection	2-4-wk post exposure	Fin necrosis, no effect on immune suppression	Spitsbergen et al., 1986; 1988c

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Rainbow trout (cont.)							
Juvenile (46 g)	i.p. injection		300-3,060	Single injection	6-12-wk	Fin hemorrhage, spleen histopathology, EROD induction, P4501A1 induction	van der Weiden et al., 1992
Juvenile (46 g)	i.p. injection		790	Single injection	3-wk	ED50 for EROD induction	van der Weiden et al., 1992
Immature Adult (300-400 g)	i.p. injection		640[c]	Single injection	72-h	ED50 for AHH induction	Janz and Metcalfe, 1991
Lake trout, *Salvelinus namaycush*							
Eggs	Water (renewal)		34 (in eggs)	48-h	>48-h to post swim-up	NOAEL[e]	Walker et al., 1991
Eggs	Water (renewal)		40 (in eggs)	48-h	>48-h to post swim-up	23% mortality in sac fry	Spitsbergen et al., 1991
Eggs	Water (renewal)		55 (in eggs)	48-h	>48-h to post swim-up	LOAEL[f] (sac fry mortality)	Walker et al., 1991
Eggs	Water (renewal)		65 (in eggs)	48-h	>48-h to post swim-up	LR50[d] (sac fry)	Walker et al., 1991
Eggs	Egg injection		47 (in eggs)	Single injection	Fertilized egg to swim-up fry	LR50[d] (sac fry)	Walker et al., 1992
Adult	Diet[h]		59 (in eggs)	90-d	Eggs to swim-up fry	LR50[d] (sac fry)	Walker, 1991; Walker et al., 1993
Adult	Diet[h]		104 (in eggs)	90-d	Eggs to swim-up fry	100% mortality to sac fry	Walker, 1991; Walker et al., 1993

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Northern pike, *Esox lucius*							
Eggs	Water (renewal)	0.1[c]		96-h	72-d	Delayed hatch, reduced growth of fry	Helder, 1980; 1982a,b
Eggs	Water (renewal)	1.0		96-h		53% mortality to fry	Helder, 1980; 1982a,b
Eggs	Water (renewal)	10.0		96-h		99% mortality to fry	Helder, 1980; 1982a,b
Carp, *Cyprinus carpio*							
Juvenile (20 g)	i.p. injection		3,000[c]	Single injection	80-d	LD50	Kleeman et al., 1988
Adult	Water (flow-thru)	0.060	2,200	71-d	61-d	Mortality and pathology	Cook et al., 1991
Zebrafish, *Brachydanio revio*							
Adult	Diet (1.7 ng/g)			Single dose	22-d	No effect	Wannemacher et al., 1992
Adult	Diet (≥8.3 ng/g)			Single dose	1-2 Spawnings	Reduced eggs per spawn, 100% larval mortality	Wannemacher et al., 1992
Fathead minnow, *Pimephales promelas*							
Juvenile (1.0 g)	Water (flow-thru)	0.049-0.067		71-d	61-d	Mortality and pathology	Cook et al., 1991
Juvenile (0.5-1.0 g)	Water (renewal)	1.7		28-d		LC50	Adams et al., 1986

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Fathead minnow (cont.)							
Juvenile (1.0-2.0 g)	Water (static)	7.1		24-h	60-d	40% mortality	Adams et al., 1986
Bullhead, *Ictalurus melas*							
Juvenile (6 g)	i.p. injection		5,000[c]	Single injection	80-d	LD50	Kleeman et al., 1988
Channel catfish, *Ictalurus punctatus*							
Fingerling	Model ecosystem	2.4-4.2		15-20-d		100% mortality	Yockim et al., 1978
Japanese medaka, *Oryzias latipes*							
Eggs	S,M	3.5-6.0		Fertilized egg to 3-d post hatch		EC50 (embryos with lesions)	Wisk and Cooper, 1990a,b
Japanese medaka (cont.)							
Eggs	S,M	9.0-13.0		Fertilized egg to 3-d post hatch		LC50	Wisk and Cooper, 1990a,b
Eggs	S,M	14.0-15.0		Fertilized egg to 3-d post hatch		EC50 (embryos with severe lesions)	Wisk and Cooper, 1990a,b
Eggs	S,M	14.0-17.0		Fertilized egg to 3-d post hatch		EC50 (prevent hatch)	Wisk and Cooper, 1990a,b

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Japanese medaka (cont.)							
Eggs	Water (static)		240 (in embryos)	Fertilized egg to 3-d post hatch		ER50[i] (embryos with lesions)	Wisk and Cooper, 1990b
Mosquito fish, *Gambusia affinis*	Model ecosystem	2.4-4.2		15-d		100% mortality	Yockim et al., 1978
Guppy, *Poecilia reticulata*							
Juvenile (8-12)	Water (static)	0.1[c]		24-h	42-d	Significant increase in fin necrosis	Miller et al., 1979
Juvenile (9-40 mm)	Water (static)	100[c]		120-h	37-d	100% mortality	Norris and Miller, 1974
Bluegill, *Lepomis macrochirus*							
Juvenile (30 g)	i.p. injection		16,000[c]	Single injection	80-d	LD50	Kleeman et al., 1988
Largemouth bass, *Micropterus salmoides*							
Juvenile (7 g)	i.p. injection		11,000[c]	Single injection	80-d	LD50	Kleeman et al., 1988
Yellow perch, *Perca flavescens*							
Juvenile (3-6 g)	Diet (0.494 ng/g)		143	13-wk	13-wk	No toxic effect	Kleeman et al., 1986b
Juvenile	i.p. injection		3,000[c]	Single injection	80-d	LD50	Spitsbergen et al., 1988b; Kleeman et al., 1988

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Amphibian							
Bullfrog, *Rana catesbeiana*							
Tadpole	i.p. injection		1,000,000[c]	Single injection	50-d	No effect on metamorphosis	Beatty et al., 1976
Adult	i.p. injection		500,000[c]	Single injection	35-d	No toxic effect	Beatty et al., 1976
			Saltwater Species				
Rays							
Little skate, *Raja erinacea*							
500-1,100 g	i.p. injection		1,000[c]	Single injection	10-d	Increased enzyme activity	Bend et al., 1974
500-1,000 g	i.p. injection		4,500[c]	Two injections	7-12-d	10-fold increase in enzyme activity	Pohl et al., 1974
Fish							
Mummichog, *Fundulus heteroclitus*							
Eggs	Water (static)	200[c]		Fertilized egg to hatch		20% mortality and 50% lesions in embryos	Cooper, 1989; Prince and Cooper, 1989
Winter founder, *Pleuronectes americanus*							
250 g	Oral dose		4,500[c]	Two doses	8-d	Increased enzyme activity	Pohl et al., 1974

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
			WILDLIFE				
			Mammals				
Mink, *Mustela vison*							
Newborn	i.p. injection		1,000[c]	Daily for 12 d	133-d	100% mortality after 14-d	Aulerich et al., 1988
Newborn	i.p. injection		1,000[c]	Daily for 12 d	133-d	62% mortality after 133-d	Aulerich et al., 1988
Adult	Oral dose		4,200[c]	Single dose	28-d	LD50	Hochstein et al., 1988
			Birds				
Bobwhite quail, *Colinus virginianus*	Oral dose		15,000[c]	Single dose	37-d	LD50	Hudson et al., 1984
Mallard, *Anus platyrynchos*	Oral dose		>108,000[c]	Single dose	37-d	LD50	Hudson et al., 1984
Ringed turtle dove, *Stretopelia risoria*	Oral dose		>810,000[c]	Single dose	37-d	LD50	Hudson et al., 1984

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Ring-necked pheasant, *Phasianus colchicus*							
Eggs	Egg injection, yolk		2,100[c]	Single dose	28-d post hatch	LD50	Nosek et al., 1992c
Eggs	Egg injection, yolk		10,000[c]	Single dose	28-d post hatch	LOAEL[f] mortality	Nosek et al., 1992c
Eggs	Egg injection, yolk		1,000[c]	Single dose	28-d post hatch	NOAEL	Nosek et al., 1992c
Eggs	Egg injection, albumin		1,400[c]	Single dose	28-d post hatch	LD50	Nosek et al., 1992c
Eggs	Egg injection, albumin		1,000[c]	Single dose	28-d post hatch	LOAEL[f] mortality	Nosek et al., 1992c
Eggs	Egg injection, albumin		100[c]	Single dose	28-d post hatch	NOAEL[e]	Nosek et al., 1992c
Adult hen	i.p. injection		100,000[c]	Single dose	77-d	100% mortality after 42-d	Nosek et al., 1992a
Adult hen	i.p. injection		25,000[c]	Single dose	77-d	80% mortality	Nosek et al., 1992a
Adult hen	i.p. injection		6,250[c]	Single dose	77-d	0% mortality	Nosek et al., 1992a

Test Species	Test Method	Water Conc. (ng/L)[a]	Organism Conc. (pg/g)[b]	Duration		Effect	Reference
				Exposure	Observation		
Ring-necked pheasant (cont.)							
Adult hen	i.p. injection		1,000[c]	Weekly injections for 10 wk	7-wk post dose	LOAEL[f] hen mortality hen weight egg production embryo mortality	Nosek et al., 1992a
Adult hen	i.p. injection		100[c]	Weekly injections for 10 wk	7-wk post dose	NOAEL[e]	Nosek et al., 1992a

[a] Measured TCDD concentration in water.
[b] Measured TCDD concentration in organism (wet weight).
[c] Unmeasured TCDD concentration in water or organism (wet weight).
[d] LR50 (corrected for control mortality) term defined in this report as the measured residue concentration in eggs that caused 50% mortality to sac fry.
[e] NOAEL = No observed adverse effect level.
[f] LOAEL = Lowest observed adverse effect level.
[g] NOAEL and LOAEL values (based on mean measured wet weight organism concentrations) were calculated for this report.
[h] Diet consisted of 22 ng/g pelletized feed followed by fathead minnows injected with 500 pg/fish.
[i] ER50 = Term defined in this report as the measured residue concentration in eggs that caused 50% effect.

that were renewed every 24 hours for a period of 96 hours (Helder, 1980; 1981; 1982a,b). After the exposures, eggs, fry and juveniles were held in TCDD-free water for extended periods of time. The lowest concentration of 0.1 ng TCDD/L caused embryo underdevelopment and delayed hatching in pike and caused significant growth retardation of fry of both species in 72 days. Larvae that hatched were also stunted and hemorrhaging and the onset of edema was observed. Higher concentrations increased the frequency of these effects. A concentration of 1 ng/L caused 53% mortality and significantly decreased the growth of rainbow trout fry exposed initially in the yolk sac stage. The next higher concentration of 10 ng/L killed all yolk sac fry and retarded growth of juvenile trout 68 days after the 96-hour exposure period. For juvenile trout, a 100 ng/L exposure for 96 hours was required to cause 100% mortality after 27 days indicating that older fish were less sensitive than yolk sac fry. TCDD-intoxication of pike and trout was generally characterized by growth retardation, delayed mortality and histopathological changes in the stomach, pancreas and liver. No TCDD residue information was obtained in these studies.

The toxicity and bioconcentration of [^{3}H]TCDD in fathead minnows were examined by Adams et al. (1986) using two exposure concentrations and duration regimes; concentrations of TCDD in both water and tissues were based on measured [^{3}H]TCDD equivalents. Toxicity tests included exposing juvenile fathead minnows to 0, 0.12, 0.72, 7.14 and 81.8 ng/L, each for 1, 2, 3 and 4 days, and to 0, 1.7, 6.7, 63 and 82 ng/L continuously for 28 days. At the end of each of these experiments, fish were transferred to clean water and observed for 150 and 20 days, respectively. Test results provided evidence that toxicity from acute exposure to TCDD was dose dependent and occurred as a function of body burden. TCDD was also found to be slow acting and to cause delayed mortality (up to 40%) for several weeks following a 1-day exposure to a concentration as low as 7.1 ng/L. Delayed mortality was typically complete 44 days after exposure which was consistent with studies by Helder (1980; 1981). The effect of longer exposure was a shorter time to 100% mortality; continuous exposure to 6.5 ng/L produced complete mortality in 23 days, whereas short exposures to similar concentrations produced 40 to 60% mortality 60 days after exposure. Continuous exposure to 1.7 ng/L produced 53% mortality in 28 days, whereas exposures to 0.1 and 0.7 ng/L for 4 days resulted in no significant mortality in fish during the 150-day observation period. Concentrations of 63 to 82 ng/L eventually produced complete mortality with exposures as short as 1 day. A 28-day LC50 of 1.7 ng/L for fathead minnows was calculated from the results.

Analysis of fathead minnows that died in these toxicity tests indicated that whole body residues were a function of both the length of exposure prior to death and exposure concentration. The minimum measured body burden observed in dead fish obtained from the toxicity studies was 16,700 pg/g and the maximum was 2,042,000 pg/g. Residue information from these experiments was not used by the authors to determine residue-based effect and no-effect concentrations for TCDD and this species.

Adult carp and fathead minnows were exposed continuously to TCDD for 71 days and then were placed in clean water for 61 days to determine the toxicity, uptake and elimination of TCDD over a period of 132 days (Cook et al., 1991). Mortality and other toxic symptoms occurred during the exposure and depuration period to both species. A water concentration of 0.06 ng/L (measured throughout the exposure phase) was toxic to carp causing mortality, fin erosion, hemorrhaging, cranial deformation, edema, exopthalmia, tumors and abnormal swimming behavior. Histological analysis of the liver, spleen, gill and gastrointestinal tract revealed extensive pathology in these fish (Johnson et al., 1986). Concentrations of 0.049 and 0.067 ng/L caused similar, but less pronounced, toxic effects in fathead minnows during this time period. Whole body analysis of carp resulted in a peak residue-effect concentration of 2,200 pg/g after 71 days and then declined to approximately 900 pg/g after the depuration period.

Rainbow trout sac fry were exposed to TCDD concentrations ranging from 0 to 50 ng/L and a fly ash extract for 96 hours using renewed water solutions (Helder and Seinen, 1985). After exposure, fish were held in clean water for 16 days to observe hemorrhages, edema or death. A comparison of TCDD toxicity with components of the incinerator fly ash showed that increased concentrations of TCDD caused increased mortality in less time to trout sac fry than lower concentrations. Cumulative mortalities ranged from 2% in the lowest concentration of 1.6 ng/L to 100% at 12.2 ng/L and higher concentrations in the test with TCDD alone. Residues in fish were not measured in these experiments.

Bol et al. (1989) also studied the interactive effects of PCDDs, PCDFs and PCBs using early life stage tests with rainbow trout. Newly hatched sac fry were exposed to TCDD and other compounds in water that was renewed every 24 hours for 96 hours. Fish were then held in a continuous clean water flow for three weeks for observation. Nominal concentrations of TCDD ranged from 0.61 to 20.09 ng/L. Results, similar to those found by Helder and Seinen (1985), indicated that higher concentrations caused increased mortality in less time to sac fry than lower concentrations. Observations on the interaction of TCDD with several compounds showed that additivity occurred, but that there was a lesser effect in fish exposed at higher temperatures. The authors also hypothesized that the synergistic effects were not sufficiently explained on the basis of using a single receptor model, but that a multiple receptor model consisting of an Ah receptor and a second receptor was more suitable for explaining the observed "dioxin-like" toxicity. The LC50 calculated for TCDD alone was 1.83 ng/L.

The toxicity and bioconcentration kinetics of TCDD in rainbow trout were studied by Branson et al. (1985). Fingerling trout were exposed for 6 hours to an initial, total measured [^{14}C]TCDD water concentration of 107 ng/L (dissolved TCDD based on filtration) and then placed in clean water for a period up to 139 days. TCDD water concentrations did not decline significantly over the 6-hour exposure period.

Trout appeared healthy for several weeks but delayed mortality was observed on days 78, 136, 137 and 139 after this period. No deaths were observed in the controls. Increased liver weights of fish were noted from day 42 through the end of the observation period. After day 64 of the observation period, clinical evidence of fin rot and the accompanying inflammatory reaction was also observed. Measured residue concentrations of [^{14}C]TCDD in whole fish decreased from 2,580 pg/g (wet weight) after the 6-hour exposure period to 780 pg/g after 64 days. Residues further decreased to 650 pg/g after the 139 day observation period.

Mehrle et al. (1988) exposed juvenile (post swim-up) rainbow trout continuously to measured [^{3}H]TCDD concentrations of 0.0011 (control), 0.038, 0.079, 0.176, 0.382 and 0.789 ng/L for 28 days in a flow-through system to determine toxicity and bioconcentration. After the exposure period, fish were transferred to clean water for another 28-day period for observation and residue analysis. Significant mortality in trout was observed during 14 days exposure to the highest concentration of 0.789 ng/L and there was a trend toward increased mortality in fish exposed to 0.176 and 0.382 ng/L. Significant mortality was not observed in the two lowest concentrations of 0.038 and 0.079 ng/L during the 28-day exposure although reduced weight and abnormal behavior (lethargic swimming, feeding inhibition and lack of response to external stimuli) were observed. However, a dose-dependent increase in mortality was observed throughout the depuration period. Significant mortality (45%) and fin erosion was recorded at 0.038 ng/L after 28 days of depuration. The next highest concentration of 0.079 ng/L caused 83% mortality and 95-100% of the fish were dead at concentrations of 0.176 ng/L and above after this time. Feeding inhibition and other behavioral changes were not reversed during the depuration period at these concentrations. A 56-day LC50 of 0.046 ng/L was calculated from the mortality data for the combined exposure and depuration periods. The no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) for TCDD and rainbow trout, based on mortality, growth and behavior, were 0.0011 (control) and 0.038 ng/L, respectively.

Tissue analysis of the rainbow trout showed that whole body residues in fish exposed to the lowest effect concentration of 0.038 ng/L were fairly constant throughout the exposure and depuration periods (380, 710, 960 and 930 pg/g wet weight in pooled fish after 7, 14, 21 and 28 days of exposure and 780 pg/g in fish after 28 days of depuration, respectively). Residue concentrations in control fish exposed to 0.0011 ng/L (likely due to volatilization and translocation of TCDD from treated aquaria) ranged from 12 to 27 pg/g (detection limit 6 pg/g) during the exposure period. If the mean values from these results were used to calculate no effect and effect values (based on tissue residues) for this study, the NOAEL and LOAEL for TCDD and rainbow trout using mortality, growth and behavior as the endpoints would be 21 and 765 pg/g, respectively.

Experiments were conducted by Spitsbergen et al. (1991) to study the pathological alterations in early life stages of the lake trout exposed to TCDD as fertilized eggs. Newly fertilized eggs of lake trout were exposed to graded nominal concentrations of [^{3}H]TCDD in water for 48 hours and were then transferred to clean water for up to 6 weeks. Water solutions were renewed during the exposure period to ensure stable solutions. Eggs exposed to 0.1, 1.0, 10, and 100 ng/L contained measured concentrations of 0, 0, 40 and 400 pg/g wet weight (detection limit for residue analysis was 0.7 pg/g), which remained constant over the 1- to 6-week post exposure period. Embryos developed normally in all groups until one week prior to hatch. At this time, a sharp increase in mortality occurred in embryos containing 400 pg/g where approximately 56% of the embryos died during the hatching process. Eggs containing 40 pg/g also showed an increase in embryo death during hatching. Cumulative mortality at swim-up of 23 and 100% was significant for embryos containing 40 and 400 pg/g, respectively. Retrobulbar, meningeal, subcutaneous and pericardial hemorrhages were evident in many live, morbid and dead embryos and sac fry containing 400 pg/g of TCDD. By two weeks after the onset of hatching, approximately 2% of the sac fry containing 40 pg/g developed lesions similar to those associated with the blue-sac disease syndrome (lesions with petechial hemorrhages, disruption of the vitelline circulation, cessation of circulation to the tail, head and gills, subcutaneous edema of the yolk sac, hypopigmentation and arrested development of skeletal and soft tissue). Mild yolk sac edema was observed in approximately 30% of this group 2 to 4 weeks after hatching but fry showed no gross lesions at the time of swim-up.

Newly fertilized lake trout eggs were exposed to nominal water concentrations of 10, 20, 40, 62 and 100 ng TCDD/L for 48 hours in another study (Walker et al., 1991). After exposure, the eggs were transferred to clean water to observe the toxic effects of TCDD on hatching and fry survival over a period of 180 days. Measured concentrations of [^{3}H]TCDD in eggs of 34, 55, 121, 226 and 302 pg/g (wet weight) remained constant through the egg and sac fry stages of development and were used as the basis for determining residue-effect levels. Cumulative mortality of embryos prior to the onset of hatching was unaffected by TCDD. However, hatchability was significantly less in eggs containing 226 and 302 pg/g. Toxicity was manifested in the sac fry stage where fry developed subcutaneous yolk sac edema, reflected by an increase in sac fry weight that preceded and paralleled fry mortality. Neither cumulative mortality nor wet weight gain in fry was affected by TCDD after swim-up. The second lowest concentration in eggs of 55 pg/g caused significant mortality to sac fry 120 days after the exposure period. Increased fry mortality occurred as concentrations increased. No adverse effects were observed in fry exposed to the lowest concentration of 34 pg/g in eggs. From these results, the NOAEL and LOAEL, based on residues, were 34 and 55 pg/g egg, respectively. The LR50 (term defined in this report as the measured residue concentration in eggs that caused 50 percent lethality to sac fry) for TCDD in lake trout was calculated to be 65 pg/g egg (corrected for control mortality) based on mortality of sac fry. Kinetic studies showed that residue

content in eggs and sac fry were constant during this period of life stage development, but in post swim-up fry, residue content of TCDD decreased in a first order manner (t½ was 35 and 37 days in eggs containing 34 and 55 pg/g, respectively). The authors noted that the decrease in TCDD concentration per fish, during the fry stage of development, was due to a combination of whole body TCDD elimination and dilution from growth.

Wisk and Cooper (1990a) compared the toxicities of several PCDDs (including TCDD) and TCDF and determined the stage specific toxicity of TCDD to different age embryos of the medaka. A time interval from fertilization to 3 days post hatch was used as the measure of survival in these studies. Exposure to graded, measured water concentrations of TCDD showed a concentration dependent increase in the percentage of embryos with lesions, embryos with severe, life-threatening lesions and embryos that were dead by 3 days post hatch. Serious hemorrhaging and pericardial edema were classified as severe lesions because animals with these lesions died prior to hatching or at hatch. Generally, the sequence of lesions began with hemorrhages in various areas including, the caudal region, periorbital, around the pectoral fins, in the vitelline veins and in the posterior brain area. Concomitantly, pericardial edema occurred which resulted in the collapse of the yolk sphere and prevented the heart from undergoing normal chamber formation.

The calculated EC50s and LC50s for the studied effects, in order of decreasing sensitivity, were: EC50 of 3.5-6.0 ng/L for embryos with lesions, LC50 of 9-13 ng/L for survival to 3 days post hatch, EC50 of 14-15 ng/L for embryos with severe, life-threatening lesions and an EC50 of 14-17 ng/L which prevented hatching. An ER50 (defined in this report as the measured residue concentration in embryos causing 50 percent effect) was calculated to be 240 pg/g based on concentrations analyzed in embryos that developed lesions (Wisk and Cooper, 1990b). Studies using embryos at different stages of development indicated that the most sensitive period for toxicity was during the time of liver formation on day 4 or 5 of development.

Egg Injection

A series of experiments were recently conducted to measure the effects of chemicals on early life stages of rainbow trout using an egg injection technique. Walker and Peterson (1991) determined the potencies of PCDDs, PCDFs and PCBs relative to TCDD for producing early life stage mortality in the trout to help assess the risk of these chemicals to early life stage mortality of lake trout in the Great Lakes. Fertilized rainbow trout eggs were injected with graded doses of TCDD or other PCDD, PCDF and PCB congeners and were then held in clean water until swim-up. Calculated LR50s (corrected for control mortality) for TCDD (the most potent in producing early life stage mortality) ranged from 230 to 488 pg/g and were determined based on the egg dose that caused mortality from hatching onset to swim-up. LR50s were dependent on the strain of rainbow trout studied (Table 4-1). Signs of toxicity

from TCDD exposure consisted of a low incidence of half-hatching, while the greatest TCDD-related mortality occurred in sac fry preceded by hemorrhages and severe fluid accumulation beneath the yolk sac epithelial membrane.

In a more recent publication, Walker et al. (1992) published the results of the egg injection technique used to expose fertilized rainbow trout eggs to TCDD (Walker and Peterson, 1991) and compared this method with waterborne exposure experiments. Egg injection data on lake trout were also compared to waterborne exposures with this species from previously published work (Spitsbergen et al., 1991; Walker et al., 1991). Eggs were either injected with graded doses of [^{14}C]TCDD or exposed in static tests for 48 hours to various concentrations of [^{3}H]TCDD and then held in clean water for post swim-up observation. Analysis showed that TCDD concentrations in rainbow trout eggs were constant through 22 days of development. Egg mortality of this species was not affected by TCDD following either route of exposure. Significant increases in mortality of rainbow trout occurred from hatching onset to swim-up and occurred at egg TCDD doses of 291 pg/g following injection and 279 pg/g following waterborne exposure. Egg mortality in controls in these studies was 46 and 35 percent for injected fish and those exposed in waterborne experiments, respectively. The LR50s (corrected for control mortality) for rainbow trout calculated from egg injection was 421 pg TCDD/g and from waterborne exposure was 439 pg TCDD/g (Walker et al., 1992).

Combined hatching and sac fry mortality was significantly increased at lake trout egg TCDD doses of ≥ 58 and 55 pg/g (Walker et al., 1991) following injection and waterborne exposure, respectively. Sac fry of lake trout and rainbow trout were the most sensitive stage tested, whereas no mortality occurred to post swim-up fry. As in previous studies, toxicity was characterized by half hatching and by sac fry mortality associated with pericardial edema, subcutaneous edema of the yolk sac, exopthalmia and subcutaneous hemorrhages, resembling blue-sac disease. LR50s (corrected for control mortality) for lake trout calculated from injection was 47 pg/g and from waterborne exposure was 65 pg/g (Walker et al., 1991); however, high mortality (73%) following injection of control lake trout eggs as compared to 15% for control fish in waterborne exposures somewhat obscured the interpretation of the egg injection results for this species.

I.P. Injection

Studies to determine the effects of TCDD on several species of fish using i.p. injection have also been conducted. Spitsbergen et al. (1988a) measured the effects of TCDD on survival, growth and morphological lesions in juvenile rainbow trout. Two strains of trout were anesthetized and injected i.p. with single, graded doses of TCDD; lethality was assessed daily for up to 80 days post treatment. A dose of 1,000 pg/g did not cause mortality or reduce growth, whereas, 5,000 pg/g caused 20 percent mortality and depressed body weight gain by week five after treatment. Doses of

25,000 and 125,000 pg/g caused 90 and 95 percent mortality after 80 days. An 80-day LD50 of TCDD for rainbow trout was calculated to be 10,000 pg/g (95% C.L. 7,000-15,000 pg/g). Gross and microscopic lesions were evident in trout treated with 10,000 pg/g but not in fish treated with 1,000 or 100 pg/g. However, significant ($p < 0.05$) hematological changes (leukopenia and thrombocytopenia) occurred in trout exposed to 1,000 pg/g after 25 days post treatment.

Other studies (Spitsbergen et al., 1986) showed that an injection of 10,000 pg TCDD/g did not significantly alter humoral immune responses of yearling rainbow trout (100 to 150 g) two weeks after treatment. Subsequent studies (Spitsbergen, 1988c) also showed that this dose did not significantly affect mortality or mean time to death of juvenile rainbow trout following challenge with Infectious Hematopoietic Necrosis Virus (IHNV). However, within 2 to 4 weeks post-treatment, both studies showed that fish with this dose became less active, exhibited necrosis of the fin margins and consumed less food than fish administered lower doses of TCDD. At early times following virus challenge, histopathologic lesions due to virus disease were more severe and occurred more frequently in virus-challenged fish which received TCDD than in virus-challenged control fish. Although TCDD exacerbated IHNV-induced disease in these fish, the authors suggested that it was not enough to overcome the battery of defense mechanisms to alter mortality. Spitsbergen et al. (1986) discussed that the general failure of TCDD to suppress immune responses in rainbow trout at doses below those causing toxicity parallels findings regarding the effects of a number of other pharmacologic immunomodulators in this species. Cyclophosphamide was indicated to suppress humoral immune responses in rainbow trout only at doses that approach those that caused lethality. Only corticosteroids were reported to have suppressed immune responses of rainbow trout at sublethal doses. Even *in vitro*, rainbow trout lymphocytes seem to be relatively more resistant to certain immunosuppressive agents than are lymphocytes of other animals such as mammals. Although it was suggested that the defense mechanisms against disease in rainbow trout are fundamentally similar to those of mammals, the processes involved in immunoregulation in this species seem to be somewhat different. It was suggested that additional work is needed to clarify immunoregulatory and effector processes in fish to elucidate the ways TCDD-like chemicals interact with these mechanisms.

van der Weiden et al. (1990) conducted TCDD dose-response studies with rainbow trout in an effort to correlate enzyme induction (cytochrome P450) with toxicological effects such as mortality, growth inhibition and histopathological changes in the liver and spleen. Trout received single TCDD i.p. injections of 10, 50, 100, 500, 1,000 or 5,000 pg/g. Growth inhibition and 20% mortality were observed 11 and 12 weeks after the administration of 5,000 pg/g. Fish exposed to this concentration were characterized by fin necrosis and abnormal behavior (head up swimming with hyperactivity followed by periods of immobility). Relative liver weight was also significantly increased after six weeks at 5,000 pg/g. Changes in spleen weight did not appear to be related to dose or exposure time, however, histopathological lesions

were observed. Increases in 7-ethoxyresorufin-O-deethylase (EROD) activity and total cytochrome P450 content in the liver were TCDD dose-related and persisted above control levels for 6 weeks at 500 pg/g and 12 weeks for the 1,000 and 5,000 pg/g dose levels. This EROD activity response pattern paralleled effects found on growth and survival. However, both EROD induction in the liver and histopathological changes in the spleen were observed at the lower dose of 500 pg/g, a dose that did not cause toxic effects to these animals. No TCDD residues in liver or whole fish were measured.

van der Weiden et al. (1992) conducted further studies to determine if a correlation or concurrence between cytochrome P4501A1 induction and toxic parameters could be established in fish. Juvenile rainbow trout (46 g) were given single i.p. injections of 6, 30, 60, 300, 600 or 3,060 pg/g body weight, respectively, and were observed for 1, 3, 6 and 12 weeks post treatment. Dose levels of 300 pg/g in trout caused hemorrhages in fins and the skin after 6 weeks. After 9 weeks, trout dosed with 3,060 pg/g showed discoloration, became lethargic and did not react to external stimuli. After 12 weeks, 20% mortality occurred at the highest dose but significant growth inhibition was not observed. Relative liver weights of trout did not show pronounced changes at any of the doses; however, histopathological evaluations revealed inflammations, necrosis and sinusoidal dilatation in the liver at a dose of 600 pg TCDD/g after 3 weeks. Examination of the spleen showed histopathological damage at the next lowest dose of 300 pg TCDD/g after this same time period.

The 300 pg/g dose of van der Weiden et al. (1992) was also the lowest dose which caused a significant induction of EROD activity in rainbow trout, although EROD activity due to this dose decreased to near control levels after 12 weeks post treatment. An ED50 of 790 pg TCDD/g body weight was calculated for rainbow trout based on EROD activity in the third week. The dose of 600 pg/g also significantly increased the total cytochrome P450 content in trout but decreases, similar to that found in trout EROD activity, were observed after 12 weeks. EROD activity for the 3,060 pg/g dose did not decline after 12 weeks suggesting that at this dose sufficient TCDD was available in the liver to maintain EROD activity. An overall correlation between EROD activity and cytochrome P450 content suggested that the increase in cytochrome P450 content might be caused by the increase in the cytochrome P4501A1 isoenzyme. Good correlations between enzyme induction and toxicological effects in rainbow trout showed that cytochrome P4501A1 induction may have applicability as a screening parameter for assessing aquatic pollution.

Janz and Metcalfe (1991) injected immature rainbow trout (300 to 400 g) with TCDD and 3,3',4,4'-tetrachlorobiphenyl (PCB 77) separately and in combination to investigate aryl hydrocarbon hydroxylase (AHH) induction potency in this species. Trout received nominal doses of 118, 704 and 1,440 pg TCDD/g by i.p. injection and were sacrificed 72 hours post injection for AHH determination. The ED50 calculated for AHH induction was 640 pg/g. Expected AHH activities were compared to observed

AHH activities from fish dosed with equivalent toxic units of the TCDD and PCB 77 mixtures. Observed activities were significantly greater than expected at the two lowest doses tested indicating that mixtures of TCDD and PCB 77 were greater than additive based on the AHH response.

Survival, growth and morphologic lesions in juvenile yellow perch were also studied after treatment with graded single i.p. injected doses of TCDD between 1,000 and 125,000 pg/g body weight (Spitsbergen et al., 1988b; Kleeman et al., 1988). Perch were then kept in clean water for the same 80-day post-treatment period. TCDD treatment caused a dose-dependent increase in cumulative mortality in perch. Doses of 25,000 or 125,000 pg/g caused over 95 percent mortality by the 28th day post treatment, whereas, approximately 80 percent mortality occurred 80 days after a single injection of 5,000 pg/g. Cessation of growth followed by loss of body weight began 2 to 3 weeks after perch received this dose. This dose affected only 20 percent of rainbow trout five weeks post treatment (Spitsbergen et al., 1988a) indicating that perch survival was more sensitive to TCDD than rainbow trout survival. As with the trout, no mortality was observed in perch at a dose of 1,000 pg/g or in the controls. The 80-day LD50 of TCDD in perch was calculated to be 3,000 pg/g (C.L. 2,000-4,000 pg/g). Although lesions in the liver tissues occurred in fish exposed to 1,000 pg/g, these and other histologic changes were not sufficient to explain mortality in perch. Residues of TCDD in these fish were not measured.

The toxicity and biotransformation of TCDD in carp, bullheads (*Ictalurus melas*), largemouth bass (*Micropterus salmoides*) and bluegills (*Lepomis macrochirus*) as well as in rainbow trout (Spitsbergen et al., 1988a) and yellow perch (Spitsbergen et al., 1988b) were evaluated by Kleeman et al. (1988). Fish were treated with a single nominal i.p. injection of 1,000, 5,000, 25,000 or 125,000 pg TCDD/g body weight and were again observed for the same 80-day post-treatment period. Cumulative mortality was more than 50 percent for all species at the highest doses of 25,000 and 125,000 pg/g. However, at 5,000 pg/g, mortality was greater than or equal to 50% for only the more sensitive species and no significant mortality was observed in any species at 1,000 pg/g. After an i.p. treatment of 125,000 pg/g, the time to produce 50% mortality in rainbow trout, largemouth bass, bluegill, and bullheads was 16 to 22 days. In contrast, yellow perch reached 50% mortality after this same dose in only 7 days, whereas carp required 44 days. In trout and bluegill, a decrease in body weight was observed at 1,000 and 5,000 pg/g, respectively, whereas in perch it was observed only at 5,000 pg/g. Perch treated with 25,000 or 125,000 pg/g exhibited the shortest delay period prior to death (about 7 to 21 days). The 80-day LD50s were: 3,000, 3,000, 5,000, 10,000, 11,000 and 16,000 pg/g for yellow perch, carp, bullheads, rainbow trout, largemouth bass and bluegills, respectively. Analysis of gall bladder bile following a 60,000 pg/g i.p. injection of [^{14}C]TCDD showed the presence in all species of TCDD metabolites in addition to the parent compound. However, bile samples for yellow perch indicated that the major metabolite was different from that of the other fish species. Although the authors did not define what the metabolites were in these

studies, further analysis suggested that two or more of the biliary TCDD metabolites were glucuronide conjugates. Because the retention times for the major metabolites were similar to those observed in mammals, it was suggested that fish and mammals may have similar pathways for TCDD biotransformation.

Dietary Exposure

Three species of fish in different experiments were fed diets containing TCDD to determine long-term adverse effects. Hawkes and Norris (1977) fed fingerling rainbow trout food containing nominal TCDD concentrations of 2.3, 2,300 and 2,300,000 pg/g for 6 days each week for 105 days. Measured values from a single sample of fish food for each dietary level were 10 (detection limit), 3,290 and 1,700,000 pg/g, respectively. Only the highest concentration in food caused adverse effects during the test. Feeding activity declined after 10 days and significant differences in growth were noted in fish after 30 days. After 33 days, the first death occurred at this concentration and by day 61, 50% of the fish had died. All but two fish had died by day 71. Fin erosion, the development of fungal growth and degenerative histological changes were also apparent in these fish during exposure. No effects on survival, feeding activity, growth or histopathology were noted in fish fed food containing lower TCDD concentrations or the control diet. Residue analysis of fish exposed to 3,290 and 1,700,000 pg/g indicated that TCDD was not concentrating significantly in body tissues after 65 and 105 days. The measured residue concentrations in single fish exposed to these no effect and effect concentrations, were 314 and 276,000 pg/g (wet weight), respectively, based on applying a dry to wet weight ratio of 0.2 to the author's dry weight values.

The effects of dietary TCDD on the reproduction and oogenesis of zebrafish (*Brachydanio rerio*) were studied by Wannemacher et al. (1992). After establishing a stable spawning cycle (10 weeks), female zebrafish weighing 0.6 g (mean weight) were exposed to nominal TCDD doses of 1,000, 5,000, 10,000 or 20,000 pg added to a chow diet of TetraMin^{R}. Single TCDD doses of 1,670 pg/g did not result in significant changes in the number of eggs/spawning, adult body weight, larval survival or histological development. In contrast, fish treated with TCDD at ≥8,300 pg/g showed a rapid decrease in the number of eggs per spawning, and after 1 to 2 spawning cycles, spawning was arrested completely. This dose also induced a significant loss of adult body weight and reduced active movements 20 to 24 days after exposure. This same dose caused all hatched larvae to develop cranial and thoracal edema, notochord malformations and killed all larvae 2 to 3 days after these initial symptoms occurred. Histological examinations of the ovaries revealed no differences between fish in the controls and those exposed to 1,670 pg TCDD/g. After TCDD treatment with 8,300 pg/g, the percentage of immature, previtellogenic oocytes (stages I-III) significantly increased while the number of mature, vitellogenic follicles (stages IV and V) decreased, and numerous atretic follicles were observed. Preliminary data from other feeding experiments with zebrafish using [^{3}H]TCDD

suggested that a single TCDD dose of 2,500 pg/g (which did not affect reproductive functions) led to a body concentration of approximately 670 and 330 pg/g (assuming a similar fish weight as above) after 15 and 45 days, respectively.

Kleeman et al. (1986a,b) studied the accumulation, tissue distribution, and depuration of TCDD in fingerling rainbow trout (3-7 g) and yellow perch (3-6 g) fed a diet containing [^{3}H]TCDD at 494 pg/g for 13 weeks followed by the same diet without TCDD for 13 weeks. This exposure, which resulted in an accumulation of about 250 and 143 pg TCDD/g expressed on a whole fish basis for trout and perch, respectively, did not cause effects such as fin necrosis, cutaneous hemorrhage or increase in mortality during the 26-week period. When dietary exposure was stopped, TCDD residues were slowly eliminated. Visceral fat, carcass, skin, liver, skeletal muscle, pyloric caeca and all fatty tissues, accounted for greater than 90% of the TCDD in fish after the 13 week exposure period.

Increased hepatic EROD activity in rainbow trout was reported to occur within two days following single doses of 60 - 2,000 pg TCDD/g delivered orally via gelatin in capsules (Parrott et al., 1992). EROD activity was estimated to increase significantly above controls at an oral dose of 100 pg TCDD/g, which corresponded to 1,000 pg TCDD/g of liver. However, no whole fish tissue concentrations of TCDD were reported. If 50% of the TCDD was assimilated by the trout, the oral dose threshold level for EROD induction corresponded to 50 pg TCDD/g of fish. Single i.p. injections of TCDD in rainbow trout required doses approximately five times greater to significantly raise EROD activity (van der Weiden et al., 1990) in comparison to the single oral dose threshold for EROD activity found by Parrott et al. (1992). Insufficient data exist to determine if greater concentrations of TCDD in the rainbow trout livers were associated with the apparent greater sensitivity for EROD activity as a result of single dietary exposures in comparison to single i.p. injections. The magnitude and duration of EROD responses for continuous dietary exposures are most relevant for assessing exposures and effects in the environment. Hepatic EROD activity in rainbow trout appeared to decline faster than whole fish TCDF residues (and probably liver residues) following cessation of a 30 day dietary exposure (Muir et al., 1992b). The same phenomenon appears to occur for i.p. injection exposure of rainbow trout with TCDD at dose below 3,060 pg/g, but at doses of 3,060 and 5,000 pg/g no change in EROD activity was observed for 12 weeks post-exposure (van der Weiden et al., 1990; van der Weiden et al., 1992).

Walker (1991) and Walker et al. (1993) fed adult lake trout TCDD contaminated food in an effort to determine adverse effects of TCDD on early life stages via maternal transfer. In the first phase of the study, adult female lake trout were fed pelletized food (controls), or graded amounts of pelletized food containing 22,000 pg TCDD/g food (same concentration of TCDD per dose) during the third month prior to spawning (early oocyte maturation). The diet was changed to live fathead minnows (controls), or graded numbers of fathead minnows injected with the same dose of

TCDD (500,000 pg/minnow) for the remaining period prior to spawning (late oocyte maturation). All oocytes (unfertilized eggs) produced were fertilized with sperm from unexposed males. Although high, medium and low TCDD exposures were attempted, females that were aggressive in feeding obtained more TCDD than less aggressive feeding females in each group. This resulted in sexually mature lake trout with a wide range of TCDD whole body burdens and associated TCDD egg burdens. In the second phase of this study, lake trout eggs were injected with graded amounts of TCDD to compare this route of exposure with effects resulting from maternal transfer and from waterborne exposure (Spitsbergen et al., 1991; Walker et al., 1991). Measured TCDD concentrations in the eggs from both phases of the study were used as the basis for determining relationships between residue and effect concentrations in early life stages.

TCDD concentrations in oocytes resulting from maternal transfer were: <1 pg/g (3 control egg samples), 1 to 23 pg/g (4 TCDD egg samples), 50 to 152 pg/g (3 TCDD egg samples) and 233 to 387 pg/g (3 TCDD egg samples). Because the egg TCDD dose following maternal transfer varied within and among the 3 exposure groups, no relationship could be determined between the targeted dietary exposure levels of TCDD fed to female lake trout and the egg TCDD dose spawned from these fish; however, TCDD concentrations in eggs were approximately 50% of the whole fish TCDD concentrations. TCDD doses ≥ 233 pg/g in the eggs resulted in inviable oocytes and failure of fertilization. Oocytes and ovarian fluid obtained from females exposed to the greatest TCDD doses were cloudy when released from the female. Following attempted fertilization, the eggs turned opaque and died when placed in water. However, eggs with 152 pg TCDD/g following maternal deposition were viable and successfully fertilized, but all sac fry that successfully hatched from these eggs died with a blue-sac disease syndrome (Spitsbergen et al., 1991) prior to swim-up. Therefore, egg TCDD doses lower than those that reduced oocyte viability and prevented fertilization caused 100% sac fry mortality. In contrast, egg TCDD doses ≥ 226 pg/g egg following waterborne exposure for newly fertilized lake trout eggs did not result in an increase in egg mortality (Spitsbergen et al., 1991; Walker et al., 1991). The difference in egg/oocyte viability between these routes of exposure suggested that reduced oocyte viability and failure of subsequent fertilization might be secondary to an effect of TCDD on maternal oocyte formation.

Mortality observed from maternally-derived TCDD, injected TCDD or waterborne TCDD exposure was similar and always associated with subcutaneous yolk sac edema and hemorrhages; the greatest TCDD-related mortality occurred during the sac fry stage. The TCDD LR50s for lake trout fry (based on egg residue concentrations) following maternal transfer, waterborne exposure and injection were 59, 65 and 47 pg/g, respectively, indicating that the route of exposure of TCDD to this life stage may not be as important as the delivered dose (Walker, 1991; Walker et al., 1991, 1992, 1993).

4.2.1.4 Amphibians

Only one study has been reported that examined the effects of TCDD on amphibians (Table 4-1). Beatty et al. (1976) injected (i.p.) tadpoles and adult bullfrogs (*Rana catesbeiana*) with TCDD at doses of 25,000-1,000,000 and 50,000-500,000 pg/g body weight, respectively. Through 50 days post-dose, no tadpole mortality was attributed to TCDD at any of the doses, indicating that these animals are more tolerant to TCDD than fish. All surviving tadpoles were observed to successfully complete metamorphosis with no observable morphological abnormalities. In addition, histopathological examination of the liver, heart, kidney, lung and reproductive organs of these animals, shortly after the completion of metamorphosis, revealed no lesions. No mortality occurred in any of the treatment groups of adult bullfrogs through 35 days post-injection. Although food intake was initially lessened in animals exposed to the highest dose of 500,000 pg/g, food consumption by these animals was not different from that of the controls. Histopathological examination of several organs revealed no significant lesions at any of the TCDD doses administered.

4.2.1.5 Saltwater Fish

Information concerning TCDD toxicity was found for only three saltwater species (Table 4-1). As with freshwater species, some delayed adverse effects occurred in these studies after short-term exposure (oral dose or i.p. injection). No information was found on the long-term exposure of TCDD to saltwater species.

Bend et al. (1974) exposed little skates (*Raja erinacea*) to TCDD (at an oral dose of 1,000 pg/g) and reported increases in renal and hepatic microsomal benzo(a)pyrene hydroxylase activity 10 days after treatment. Pohl et al. (1975) repeated these experiments with the little skate at higher doses (two doses at 4,500 pg/g wet weight administered by i.p. injection) and noted a ten-fold increase in hepatic benzo(a)pyrene hydroxylase activity 7 to 12 days after treatment. The same study also reported that winter flounder (*Pleuronectes americanus*), given two administrations of TCDD at 4,500 pg/g by stomach intubation, did not show an increase in the activity of benzo(a)pyrene hydroxylase; however, a significant increase in EROD activity was observed 8 days after treatment. Residue concentrations of TCDD in these organisms were not measured.

Studies have also been conducted to determine the effects of TCDD on killifish (*Fundulus heteroclitus*) embryos (Cooper, 1989; Prince and Cooper, 1989). Eggs and sperm were stripped from adult feral fish collected from a site known to be contaminated with TCDD (Newark Bay, NJ) and a site not impacted by TCDD (Long Island, NY). Eggs and sperm from fish collected at these two locations were exposed from fertilization through hatch to solutions containing various concentrations of TCDD. Major lesions (tubed heart/collapsed yolk sphere) and mortality of the embryos were observed. At an exposure concentration of 200 ng/L, 55 and 5.5% of the Long Island

and Newark Bay embryos, respectively, had major lesions. Twenty percent of the embryos from the Long Island fish and 12% of those from the Newark Bay fish died. The results suggested that previously exposed fish were less sensitive than fish that had not been previously exposed to TCDD. Residues were not measured in embryos from these studies.

4.2.1.6 Toxicokinetics

It is generally understood that persistent, lipophilic organic chemicals accumulate in fish in proportion to the lipid content and age of each animal (Gutenmann et al., 1992). Aquatic ecological risk assessments for TCDD and related chemicals that bind to the Ah receptor will be strongly facilitated when more specific information concerning the relationships between chemical concentrations in tissues associated with toxic effects and whole organism exposure levels is available. TCDD distribution between organs in carp was found to be proportional to lipid content (Kuehl et al., 1987). With time to reach steady-state, TCDD appeared to distribute in lake trout tissues in proportion to the total lipid content of the tissues (Cook et al., 1993b). Exceptions were the testes and ovaries with double and half, respectively, the lipid normalized TCDD concentration of other organs.

Comparison of [^{14}C]TCDD distribution in rainbow trout to cod with whole-body autoradiography and liquid scintillation counting was reported to reveal a substantial interspecies difference in TCDD distribution (Hektoen et al., 1992). The TCDD in rainbow trout followed the expected organ lipid distribution pattern but in cod the TCDD was predominantly associated with the central nervous system and liver. The cod has its lipid reservoir in the liver but the cod brain has no more lipid than the brain of the rainbow trout. Insufficient data were presented to allow comparison of lipid-normalized TCDD concentrations. It is possible that the lipid distribution pattern in cod may explain the observed differences in TCDD distribution.

Although TCDD is eliminated slowly from fish, it is metabolized (biotransformed) to an extent that increases the rate of elimination. TCDD metabolites were measured in the bile of rainbow trout (Kleeman et al., 1986a) and yellow perch (Kleeman et al., 1986b) but were not detectable in tissues. Other PCDDs and PCDFs were found to have faster elimination rates than TCDD due to faster rates of metabolism (Opperhuizen and Sijm, 1990). Also it has been demonstrated that the influence of biotransformation on bioaccumulation increases as a function of the K_{ow} of the chemical (de Wolf et al., 1992). Measurements of the rate of TCDD metabolism in fish have not been reported; however, comparison of measured elimination rates to theoretical elimination rates with no metabolism could provide such estimates. An alternative approach utilized piperonylbutoxide exposure of rainbow trout to examine the effect of partial inhibition of biotransformation on the elimination and tissue distribution 1,2,3,7-TCDD, 1,2,3,4,7-PeCDD and 2,3,4,7,8-PeCDF (Sijm et al., 1990).

Some variation in the effect of metabolism on TCDD elimination rate may occur as a function of gill versus digestive tract uptake. For example, dietary preexposure of toadfish (*Opsanus tau*) to benzo[a]pyrene was shown to facilitate metabolism of the compound and was associated with induction of intestinal AHH activity (van Veld et al., 1988). Unless lymphatic transport of lipids from fish gastrointestinal tracts exists as an alternative route, TCDD absorbed by fish from food in the intestine must pass through the portal vein to the liver before entering general circulation and transport to other organs. First-pass metabolism of TCDD entering the fish through the digestive tract could result in relatively faster elimination of TCDD than observed in bioconcentration studies in which the chemical is entering general circulation through gill uptake. If this phenomenon is only present during the exposure phase of laboratory bioaccumulation studies, its primary impact would be to reduce the measured assimilation efficiency and uptake rate constant (k_1). As reported earlier, net assimilation efficiencies reported for TCDD were less than for PCB congeners of similar K_{ow}, but with lower rates of metabolism.

Additional metabolism-related uncertainties in bioaccumulation modeling and predictions of tissue distribution of PCDDs and PCDFs in fish involve the use of nonsteady-state measurements to predict steady-state conditions. The dependence of metabolism rate on TCDD dose and length of exposure is not well understood but time-course studies of P450 induction in rainbow trout by β-napthoflavone demonstrate that different responses can occur over time depending on the frequency and duration of exposure (Zhang et al., 1990). Conditions of exposure were shown in mice to alter the toxic effects of TCDD. TCDD-induced suppression of antibody response was enhanced approximately 10-fold following subchronic versus acute exposures to the same cumulative doses of TCDD by oral gavage (Morris et al., 1992). These exposure complexities indicate that residue-based aquatic toxicity hazard testing should involve long-term, continuous exposures to the maximum extent possible until more is known about long-term versus short-term exposure induced responses.

4.2.2 Epidemiological Information

There have been many efforts undertaken to assess responses in fish, mammal, and bird populations in relation to residue concentrations of TCDD and similar acting compounds. Clearly, a fundamental challenge in interpreting the results of such studies lies in the elucidation of plausible cause and effect relationships, given the variety of chemical and nonchemical stressors that can impact populations. Fox (1991) discussed criteria by which "ecoepidemiological" studies can be judged and these include: (a) probability, (b) chronological relationship, (c) strength of association, (d) specificity, (e) consistency of association upon replication, (f) predictive performance, and (g) coherence (i.e., biological plausibility, presence of a dose-response relationship). It should be stressed that the validity of epidemiologically-based associations increase when they can be supported by independent and controlled experiments. Epidemiological data are reviewed in this report to insure that

TCDD residue-toxic effect threshold levels established from toxicological studies are not contradicted by reports of the existence of fish or wildlife population declines associated with TCDD exposures. The epidemiological data are not used to establish prima facie cause and effect relationships for TCDD exposures.

Residues of TCDD and related polychlorinated aromatic chemicals in fish have not been reported to approach levels associated with lethality to adults, but population declines through loss of adult organisms may have occurred through undocumented exposures in small ponds, streams or embayments directly contaminated by nearby sources of TCDD and/or mixtures of related chemicals. The extreme sensitivity of trout sac fry (Walker et al., 1991) indicates that early life stage lethality is more likely to cause fish population declines as a result of TCDD exposure of spawning adult fish. Insufficient information exists concerning sublethal effects that could contribute to decreased survival. Immunosuppression and associated biomarkers are particularly deserving of future epidemiological investigation.

4.2.2.1 Biochemical Changes Observed in Native Fish Populations

Field investigations have been conducted concerning cytochrome P4501A1 induction in fish exposed to TCDD and related compounds. Complicating factors in these studies are the possible presence of other contaminants or biological factors that could have caused the effects (Monosson and Stegeman, 1991), uncertainty regarding the level of exposure to TCDD and related chemicals and lack of established correlations between these levels of enzyme induction and chemical dose or toxic effects in fish. Hepatic EROD activity in fish has been used as a general screening technique for exposure to chemicals which induce cytochrome P4501A1 (Vindimian et al., 1991). EROD activity has also been used in the rat hepatoma cell bioassay as a correlate of the overall TCDD toxic potency of complex mixtures of PCBs, PCDDs and PCDFs in extracts from environmental samples (Tillitt et al., 1991b). TEFs based on these data did not appear to predict mortality in one study of Lake Michigan chinook salmon (*Oncorhynchus tschawytscha*) eggs and fry following fertilization (Williams and Giesy, 1992) but an inverse correlation between total PCB concentration in Lake Michigan salmon eggs and hatching success was found in a second study (Ankley et al., 1991). The relatively larger range of reproductive success observed by Ankley et al. (1991) may explain why they found a correlation between chemical exposure and hatching success for salmon eggs that Williams and Giesy (1992) did not find.

AHH activity in sac fry hatched from eggs obtained from Lake Michigan lake trout was 3.5 to 8.6-fold greater than hatchery controls (Binder and Lech, 1984). Dose-response experiments and Lake Michigan trout PCB residue data indicated that the induction of hepatic mixed function oxidase (MFO) activity in trout embryos and fry was attributable to PCBs and possibly other xenobiotic organic chemicals.

EROD activity has been found to be a sensitive biomarker for complex mixtures of organochlorine chemicals in chlorine bleached kraft pulp mill effluents (BKME) (Andersson et al., 1988). Rainbow trout in cages located in waters receiving BKME effluents for three weeks had up to seven-fold greater EROD activity than controls (Lindstrom-Seppa and Oikari, 1990).

The sediments and biota of Jackfish Bay, Lake Superior are contaminated with PCDDs and PCDFs attributable to BKME (Sherman et al., 1990). Concentrations of TCDF in sediments are reported to reach 3,000 pg/g dry weight and white suckers (*Catostomus commersoni*) contained 44 pg TCDF/g wet weight and at 7 pg TCDD/g wet weight. Suckers collected in August, 1988 were found to have elevated hepatic MFO activity and altered steroid profiles (Munkittrick et al., 1991). Further study (McMaster et al., 1991) of Jackfish Bay suckers, in comparison to two non-BKME exposed control populations, indicated increased liversomatic indices, elevated MFO activity, lower gonadosomatic indices, increased age to maturity and severe reductions in plasma steroid levels in both males and females. The reduced plasma steroids included testosterone and 17, 20-dihydroxyprogesterone in both sexes as well as 11-ketotestosterone in males and 17-estradiol in females. The females contained fewer eggs at maturity and males were reported to have reduced development of secondary sexual characteristics. Study of the reproductive performance of the suckers, however, did not reveal effects on fertilization potential, hatchability of eggs, larval size at hatch or MFO activity in larval fish (McMaster et al., 1992), although BKME exposed male suckers had reduced spermatozoan motility.

Hepatic MFO activity was reduced while gonadal steroid reductions persisted in Jackfish Bay fish just two weeks after a mill shutdown in September, 1990 (Munkittrick et al., 1992). Munkittrick et al. (1992) concluded that MFO induction was not related to accumulation of persistent compounds such as TCDF, however cessation of exposure of juvenile rainbow trout to TCDF appears to result in rapid reduction of EROD activity while TCDF residues in the fish decline slowly (Muir et al., 1992b). Thus the contribution of PCDDs and PCDFs in BKME effluents to observed biochemical and physiological changes in exposed fish populations is unclear at this time.

4.2.2.2 Fish Population Declines in Lake Ontario

The largest freshwater body documented to have widespread TCDD contamination is Lake Ontario (U.S. EPA, 1990). Here the extinction of major fish populations such as the lake trout and burbot (*Lota lota*) occurred before 1950 (Hartman, 1988) after which anthropogenic organochlorine chemical inputs to Lake Ontario increased dramatically. Commercial over-fishing and sea lamprey (*Petromyzon marinus*) predation are thought to be the primary causes of lake trout and burbot disappearance (Christie, 1972), although lamprey existed in Lake Ontario since the 1830s. Habitat degradation including eutrophication may also have been a

factor in the lake trout decline (Christie, 1974). Low dissolved oxygen and potentially toxic levels of ammonia in interstitial water of spawning beds may have contributed to the reproductive failure of lake trout in Lake Ontario (Sly, 1988).

The loss of a third deep water species, the fourhorn, or deep water sculpin (*Myoxocephalus quadricornis*) from Lake Ontario can not be explained (Christie, 1974). These small, bottom-dwelling fish which range to extreme depths were important forage for lake trout and burbot but their decline occurred during the 1950s after their predators had been extirpated. Lack of predation on sculpins has been suggested as an explanation for the disappearance of the fourhorn sculpin (Brandt, 1986). The slimy sculpin, under this theory, out-competed the fourhorn sculpin, however the slimy sculpin has not extended its range to the deep water habitat of the fourhorn sculpin. Since the fourhorn sculpin population disappeared at a time when sediments in Lake Ontario depositional basins were rapidly approaching the severe anthropogenic organic chemical contamination of the 1960s (Cook et al., 1993a), a toxic chemical etiology is warranted as an alternative theory. Limited data indicate that slimy sculpins present in Lake Ontario tend to bioaccumulate TCDD and other organic chemicals in proportion to the contamination levels in the surface sediments where they reside rather than on a lakewide average exposure basis as found for pelagic species. The direct toxicity of sediment contaminants such as TCDD and related chemicals; reproductive failure due to chemical exposure of parents, eggs or fry; or loss of food supply due to sediment contamination effects on benthic invertebrates are possible explanations for fourhorn sculpin extinction.

More than two decades after the extirpation of lake trout in Lake Ontario and after a lamprey control program was initiated in 1971, the restoration of a naturally reproducing lake trout population became a Lake Ontario management objective under the 1978 Great Lakes Water Quality Agreement (Edwards et al., 1990). Stocking of lake trout began in 1973 but natural reproduction was not achieved. Eggs collected from Lake Ontario lake trout in 1981-1984 showed a very high incidence of blue-sac disease and associated mortality (Symula et al., 1990). Blue-sac disease in salmonid sac fry is known to be caused by different physical and chemical stressors. The partial reduction in blue-sac mortality with reduced water temperature during incubation was interpreted as evidence for involvement of a pathogen such as myxobacteria rather than simple toxicity from chemical contamination of eggs prior to spawning. Fry that did not succumb to blue-sac did not exhibit the DDT-associated "feeding fry syndrome" (Skea et al., 1985) after swim-up. Swim-up fry mortality in Lake Michigan lake trout appears to be different than blue-sac syndrome and has been associated with exposure to organic chemicals that have a similar mode of action to that of DDT (Mac and Edsall, 1991). Lake Ontario lake trout eggs collected after 1984 are reported to have been successfully reared in U.S. Fish and Wildlife Service hatcheries but the data on the percentage of blue-sac incidence are not reported (Marsden et al., 1988). Seventy-five lake trout fry were captured in Lake

Ontario in 1986 (ibid). This provided the first evidence of natural reproduction since stocking began in 1973.

The strong association now established between TCDD exposure of lake trout eggs, either through water exposure (Spitsbergen et al., 1991; Walker et al., 1991), injection (Walker et al., 1992) or maternal transfer (Walker, 1991; Walker et al., 1993), and blue-sac syndrome-associated mortality of sac fry compels consideration of TCDD-related blue-sac disease as a primary cause of the failure to reestablish natural reproduction of lake trout in Lake Ontario. The egg TCDD residue range for this effect is 30 to 100 pg/g wet tissue (0-100% blue-sac associated mortality of sac fry prior to swim-up). TCDD analysis of 1987 Lake Ontario lake trout eggs indicated 7 to 16 pg/g wet weight (Cook et al., 1993a). The addition of toxic contributions from other PCDD, PCDF and PCB congeners is estimated to give a TCDD toxicity equivalency concentration (TEC) in eggs of 14 to 32 pg/g wet weight. This is consistent with observation of some natural reproduction in 1986 (Marsden et al., 1988). Only a doubling of the TEC would be predicted to result in up to 50% mortality from blue-sac disease without consideration of environmental factors, chemical synergism or additional toxic chemical contributions that may increase the response. Older lake trout in Lake Ontario have up to three-fold greater whole body TCDD residues and probably have egg TECs of 42 to 96 pg/g wet weight, and therefore may be ineffective spawners.

Lake Ontario lake trout collected in 1978 had average TCDD concentrations of 78 pg/g wet weight whole fish and therefore could have produced eggs with sufficient TCDD and related chemical residues to cause the average 48% blue-sac disease incidence observed for Lake Ontario lake trout eggs collected in 1979 (Symula et al., 1990). During the period of 1978-1988 Lake Ontario lake trout TCDD residues decreased proportionally to decreases in TCDD concentrations in Lake Ontario surface sediment indicated by analysis of radionuclide-dated 1 cm increments of sediment cores from depositional basins of the lake (Cook et al., 1993a). The core analysis indicates that the greatest sediment TCDD contamination occurred around 1962 at approximately eight times the 1987 level. Since lake trout TCDD residues appear to be declining at least as fast as surface sediment TCDD levels and bioaccumulation of TCDD by lake trout relative to sediment contamination would be greater when peak TCDD loadings occurred, lake trout egg residues in 1960 (if lake trout had been stocked then) would have probably exceeded 70 to 160 pg/g wet tissue. This level of exposure to TCDD alone would be sufficient to cause complete sac fry mortality due to the blue-sac syndrome. Since other Ah-active PCDDs, PCDFs and co-planar PCBs appear to have decreased less in fish and sediments since 1960, the TEC was probably more than twice the TCDD concentration in lake trout eggs during the 1960s and 1970s. Thus reproductive failure of lake trout during the 1970s could well have occurred due to TCDD and related chemical bioaccumulation by lake trout.

Because of (1) the strong association of TCDD with blue-sac disease observed in laboratory exposures and the effect in sac-fry from eggs collected from Lake Ontario lake trout; (2) the historical record of Lake Ontario TCDD and related chemical exposure to lake trout; and (3) the consistency of the predicted toxicity with the field sampling record of no natural reproduction until 1986, the probability is high that TCDD toxicity alone could have prevented lake trout reproduction during the post-1950 period. Attainment of a self-sustaining population of lake trout in Lake Ontario must still depend on further reduction in TCDD and related chemicals, control of the sea lamprey, maintenance of adequate spawning beds and water quality, and introduction of appropriate lake trout strains for Lake Ontario conditions and food web. More information is needed on the effect of TCDD on other fish populations such as the deep water sculpin before similar conclusions for rehabilitation can be made.

4.2.3 Effects Profile

4.2.3.1 Use of Bioaccumulation to Characterize Risk

A major issue in using laboratory information to characterize risk to organisms in natural systems is extrapolating effects information among different exposure conditions. This is especially true for highly bioaccumulative chemicals such as TCDD. The information for TCDD effects on aquatic organisms presented above exemplifies a number of important considerations. Various routes of exposure were used, including waterborne, i.p. injection and diet. For waterborne exposures, the duration of exposure varied from six hours to several weeks. Because TCDD accumulates slowly, the exposure concentrations needed to elicit effects change greatly over this range of durations. Among those studies using exposure via water, bioavailability probably varied due to the effects of different amounts and types of solvent carriers and natural organic matter in the test systems. Buildup of organic matter would be of particular concern for static exposures, which also would have exhibited declining TCDD exposure concentrations with time. Finally, because of delays in responses to a toxic TCDD dose, it is sometimes unclear to what magnitude and duration of exposure an organism is actually responding.

As explained in earlier sections, such difficulties in relating effects among different exposure conditions can be partially addressed by using toxicant accumulation as a reference point for effects. Because exposure duration and bioavailability are manifested in terms of how much chemical is accumulated, expressing toxicity on the basis of accumulation should largely adjust for the effects of these factors. In particular, this should better account for the fact that stopping an exposure has little immediate impact on response, since the accumulated TCDD concentration at the site of action persists for a long time due to this chemical's slow elimination. Expressing effects on the basis of accumulation also allows for waterborne, injection and dietary exposures to be directly compared and for better interpretation of field data on accumulation by organisms.

Expressing effects on the basis of accumulation does not solve all difficulties in extrapolating among exposures. Under typical laboratory exposures, accumulation will change with time. To the extent that effects are delayed or are cumulative, there can be some uncertainty regarding the accumulation associated with a particular level of effect. Also, the relationship between toxicity and accumulation is not well established and is not necessarily simple. This is particularly true of accumulation on a whole body basis, which would not reflect the different internal distribution pathways associated with different routes of exposure and rates of accumulation, and how this might alter response. Nevertheless, expressing toxicity on the basis of accumulation will reduce the uncertainty in comparing and applying toxicity information and will be the basis for the discussion of risk here.

Expressing effects on the basis of accumulation does not eliminate the need to address issues of bioavailability and kinetics of accumulation. Rather, it simply removes the confounding influence of these factors from the interpretation of effects data. Bioaccumulation relationships then must be explicitly considered in relating accumulation-based effects assessments to environmental concentrations. This integration of exposure, bioaccumulation, and effects will be considered in a later section on risk characterization (see section 5). The remainder of this section will provide a summary of effects to aquatic life, emphasizing accumulation as the basis for describing effects.

4.2.3.2 Relationship of Risk To TCDD Accumulation

Effects information presented earlier (section 4.2.1) indicates that fish are substantially more sensitive to TCDD than are plants, aquatic invertebrates and amphibians. Fish populations will therefore be the focus here of the effects profile for aquatic life. Available data also indicate that early life stages of fish are substantially more sensitive than older fish. Therefore, this section will summarize TCDD accumulations associated with (1) no evidence for risk to fish populations, (2) impairment of early life stage fish development and (3) effects on growth and survival in juvenile and older fish.

Although no study has evaluated the effects of TCDD on the entire reproductive process in fish, several studies discussed in section 4.2.1 examined the effects of TCDD exposure of fish eggs and have shown fry survival and development to be an important, sensitive endpoint. Wannemacher et al. (1992) also examined effects on oogenesis by exposing actively spawning female zebrafish to a single oral dose. Effects on oogenesis were only evident at doses which also affected embryo survival. Thus, there is as yet no evidence for endpoints significantly more sensitive than early life stage development, but definitive studies with chronic exposures are still needed.

For lake trout, comparisons among three routes of TCDD exposure to eggs (waterborne, injection, and maternal transfer) have shown 50% mortality of fry to be

associated with similar egg residues, ranging from 47 to 65 pg TCDD/g wet weight (Walker, 1991; Walker et al., 1991, 1992, 1993). For the waterborne exposure, a residue of 34 pg/g did not exhibit significant effects relative to controls, suggesting that it is a level associated with no or limited risk. However, concentrations ≥ 40 pg/g caused significant mortality indicating that the dose/response curve is very steep, with the response ranging from nondetectable to complete mortality over a threefold range of accumulation. For rainbow trout, the median lethal accumulation was somewhat higher, ranging from 230 to 490 pg TCDD/g egg depending on the strain of trout (Walker and Peterson, 1991; Walker et al., 1992). Results again were similar for waterborne exposure (for 48 hour post fertilization) and injection of eggs.

Waterborne exposure of medaka eggs and fry to TCDD from fertilization through 3 days post hatch (approximately two weeks) resulted in LC50s for fry of 13 ng/L (Wisk and Cooper, 1990a) and 9 ng/L (Wisk and Cooper, 1990b). Based on measurements of TCDD in 11 day old embryos after similar exposures, accumulation on a whole egg basis was approximately 100 times the water concentration (Wisk and Cooper, 1990b). The median lethal accumulation therefore would be expected to be about 1,000 pg/g. However, this likely is an overestimate of the accumulation needed to elicit this effect, since accumulation was probably increasing throughout the test period. This is in contrast to the salmonid studies cited above, in which water exposure continued for only 48 hours after fertilization and TCDD concentrations in eggs and fry were constant after that until fry began feeding. For the medaka, the observed effects would have depended in large part on lower accumulations earlier in the developmental period, especially since Wisk and Cooper (1990b) did demonstrate that the sensitivity of newly hatched fry depended on exposure in the first few days after fertilization. Thus, fry mortality in this species would occur at lower egg accumulations, probably within the range of the salmonid studies discussed above.

Helder (1980; 1982a,b) exposed rainbow trout and northern pike eggs for 96 hours to TCDD in water, but did not report any data on accumulation. The rainbow trout showed a slight increase in fry mortality at 1 ng/L, up to about 25% mortality at 10 ng/L, and complete mortality at 100 ng/L. These results are in the range of, or slightly more sensitive than, those of Walker et al. (1992) for rainbow trout (e.g., the 48-hour LC50 of about 80 ng/L was estimated from the results for the present report). The northern pike showed reduced fry survival at 0.1 ng/L, 50% mortality at 1 ng/L, and almost no survival at 10 ng/L. This suggests even greater sensitivity than for lake trout (for which a 48-hour LC50 of about 25 ng/L was estimated from the results of Walker et al. (1991)). However, it is quite possible that pike eggs absorb TCDD faster than the salmonid eggs, as do medaka, so whether these results indicate greater sensitivity is unclear. Even if the northern pike eggs bioconcentrated as much TCDD in their 4 day exposure as medaka eggs do in 11 days, the median lethal accumulation would be only 100 pg/g. Therefore, this study still provides evidence that the early life stage of another fish is nearly as sensitive, if not more so, than that of lake trout. Miller et al. (1979) also demonstrated adverse effects (fin necrosis) on

young guppies from a 24-hour exposure at water concentrations as low as 0.1 ng/L. Helder (1982a) reported delays in development when rainbow trout eggs were exposed to 0.1 ng/L and 20% mortality when newly hatched fry were exposed to 1 ng/L. These studies provide direct evidence that even acute waterborne exposures of fish eggs to TCDD would need to be limited to much less than 1 ng/L to prevent adverse effects to early life stages of fish.

Wannemacher et al. (1992) reported no effects on oogenesis and fry development when female zebrafish received a single, nominal oral dose of 1,670 pg/g, whereas a dose of 8,300 pg/g severely reduced oogenesis and caused severe malformations and complete mortality in those fry that did hatch. Based on separate experiments that documented accumulation in the adult fish, the concentration of TCDD 15 days post dose would be about 670 pg/g in fish exposed to the no effect dose. If egg concentrations were similar or moderately lower, this species may be more resistant than the salmonids discussed above, but should still show substantial early life stage effects for egg concentrations <1,000 pg/g.

The accumulation of TCDD in eggs should largely reflect maternal transfer, except in the unlikely situation that eggs are laid where they have high exposures while the parents have negligible exposure. Effects based on the concentration of TCDD in eggs must therefore be related to concentrations in the parents, to in turn relate these concentrations to environmental concentrations. In laboratory tests with maternal transfer, eggs were determined to have about 40% of the TCDD concentration (on a wet weight basis) as parent fish. For fish collected from Lake Ontario, this percentage was about 30%. However, the Lake Ontario data were for adult fish with 18% lipid whereas the eggs had about 8% lipid (in the laboratory, adult fish and eggs both had about 8% lipid). On a lipid normalized basis, the field data indicated the TCDD concentration of eggs to be about 65% of that in the adults. The fact that this percentage is greater than that found in the laboratory probably reflects continuous exposure over a longer time. For the purpose of the present analysis, this latter factor will be used to relate TCDD concentrations in eggs to that in adults with similar lipid contents. Using this value, the NOAEL of 34 pg TCDD/g for eggs corresponds to about 50 pg TCDD/g in the parent fish. The median lethal accumulation for lake trout eggs reported above would correspond to about 75 pg TCDD/g in parent fish. If it is assumed that similar parental/egg relationships exist for rainbow trout as for lake trout, the egg concentrations resulting in 50% rainbow trout fry mortality would correspond to about 350 to 750 pg TCDD/g in parent fish.

The growth and survival of older fish appear to be less sensitive to TCDD than that of early life stages. As summarized in Table 4-1, significant effects do not appear to occur at accumulations less than 500 pg TCDD/g wet weight for any of the studies. Median lethal accumulations range from about 1,000 to 15,000 pg TCDD/g, depending on species, lifestage, and route of exposure. Effects on growth are generally evident from 1,000 to 3,000 pg/g.

Based on the above information, the effects and associated uncertainties of TCDD on aquatic life are summarized in Box 1.

Box 1. Effects Profile for Aquatic Life

- The most sensitive effects thus far established for aquatic organisms are in fish fry after exposure of eggs before or shortly after fertilization. There is no definite evidence of adverse effects in any of the fish species tested if accumulation in eggs is less than 34 pg TCDD/g, the highest no observed effect level for lake trout fry. This likely corresponds to an accumulation in parent fish, with lipid content similar to the eggs, of less than 50 pg TCDD/g. These values do not incorporate any uncertainty factors that address issues such as greater sensitivity of untested fish species and endpoints, the relationship of TCDD in eggs to parents or the effects of lipid content. Furthermore, there is no reason at this point to suppose that this number is low simply because lake trout are exceptionally sensitive. Tests on other species, both early life stage and older fish, suggest that some might be as, or more, sensitive than lake trout. Because of the importance of this endpoint and the severity of response at exposures not much higher than this exposure, this estimate for low risk should not be considered conservative or having limited applicability. There is also the possibility of the existence of more sensitive endpoints that have not yet been tested.

- Effects on fish fry survival are expected to be substantial when accumulations in the eggs are 50 to 500 pg TCDD/g. This likely corresponds to a range of about 75 to 750 pg TCDD/g in parent fish. This range does not represent uncertainties in the response of individuals, the relationship of accumulation in eggs to that in parents, the effects of lipid, untested species, etc. Nor does it reflect the difference between a severe effect (50% mortality) and threshold effects. Rather, it is the range for the median lethal accumulation among species actually tested. Thus, near the lower end of this range, mortality in fry of sensitive species is likely to occur and, near the upper end of this range, reproduction of many species will probably be severely impaired. Even lower concentrations might be needed to protect against sublethal effects that might still impact fish populations.

- Substantial mortality to older fish is expected to occur when total body accumulations are in the range of 1,000 to 15,000 pg TCDD/g. This range again primarily represents differences between sensitive and resistant species, not uncertainties within a species. Therefore, major effects on the sensitive species in an aquatic community are likely near the lower end of this range and the elimination of all or most fish species will probably occur near the upper end. It should also be noted that this risk category has limited utility. It is relevant only when protection of early life stages is not intended, both during the period of exposure and for a considerable time afterwards (due to the long retention of TCDD and transfer to the eggs via maternal transfer). In other words, protection of early life stages would require restricting accumulation by parent fish in all circumstances, not just at the time and place for reproduction.

- Available laboratory toxicity information indicate that aquatic invertebrates, plants and amphibians are substantially less sensitive to TCDD than fish. However, the database regarding this sensitivity is limited and the exposures are not always readily comparable to the data on fish. It is possible that aquatic ecosystem components other than fish are sensitive to TCDD but simply have not been adequately tested.

4.3 EFFECTS OF TCDD ON AQUATIC-ASSOCIATED WILDLIFE

4.3.1 Toxicological Information

Wildlife toxicology literature on TCDD was obtained, in part, through searches on a base set of mammals and birds listed in Table 4-2. Because very few bird studies were available, the literature search was expanded to include domestic fowl, such as the chicken, and upland game birds, such as the ring-necked pheasant (*Phasianus colchicus*) and bobwhite quail (*Colinus virginianus*). Attempts to obtain TCDD toxicology literature for marine mammals and reptiles were unsuccessful.

Table 4-2. The base set of mammals and birds included in the literature search for TCDD wildlife toxicity data.

Common Name	Scientific Name
Mammals	
Beaver	*Castor canadensis*
Fisher	*Martes pennanti*
Marten	*Martes americanus*
Mink	*Mustela vison*
Muskrat	*Ondatra zibethicus*
Racoon	*Procyon lotor*
River Otter	*Lutra canadensis*
Birds	
Black Duck	*Anas rubripes*
Mallard	*Anas platyrhynchos*
Northern Pintail	*Anas acuta*
Red-Breasted Merganser	*Mergus serrator*
Common Merganser	*Mergus merganser*
Canada Goose	*Branta canadensis*
Common Loon	*Gavia immer*
Double-Crested Cormorant	*Phalacrocorax auritus*
Great Blue Heron	*Ardea herodias*
Herring Gull	*Larus argentatus*
Ring-Billed Gull	*Larus delawarensis*
Caspian Tern	*Sterna caspia*
Common Tern	*Sterna hirunda*
Forster's Tern	*Sterna forsteri*
Brown Pelican	*Pelecanus occidentalis*
American White Pelican	*Pelecanus erythrorhynchos*
Red-Winged Blackbird	*Agelaius phoeniceus*
Bald Eagle	*Haliaeetus leucocephalus*
Peregrine Falcon	*Falco peregrinus*
Osprey	*Pandion haliaetus*

Mammalian and avian wildlife toxicity data were collected without regard to study duration, number of doses, or route of exposure. In general, however, only those studies that incorporated exposure durations of sufficient length to establish dose-response curves for reproductive and developmental effects were summarized for this report.

4.3.1.1 Mammals

No reproduction and developmental studies with mammalian wildlife were found in the literature; however, studies by Aulerich et al. (1988) and Hochstein et al. (1988) provided information on the toxicity of TCDD to mink following exposures of one to twelve days.

Hochstein et al. (1988) administered TCDD as a single oral dose (0, 2,500, 5,000, and 7,500 pg/g body weight) to adult male mink and observed the animals for 28 days. No animals died at the lowest two doses; however, between 14 and 17 days post-exposure, 100 and 75% mortality was observed at doses of 7,500 and 5,000 pg/g, respectively. A 28-day LD50 of 4,200 pg/g was calculated. Food consumption, body weight, and adipose tissue were significantly reduced in mink receiving doses of 5,000 and 7,500 pg/g. Food consumption in mink receiving TCDD at the low dose of 2,500 pg/g was depressed by approximately 25 to 40% during the first two weeks post-exposure (not statistically significant), but increased to control levels after three weeks. Body weights were significantly ($p < 0,05$) depressed 11.4% in mink exposed to TCDD at the lowest dose. In mink exposed to the two higher TCDD doses, gross necropsy revealed mottling and discoloration of the liver, spleen, and kidneys and enlarged brain, kidneys, heart, and thyroid and adrenal glands. Animals that survived TCDD exposure at the lowest dose showed no alterations in hematological and thyroid hormone measurements.

Aulerich et al. (1988) administered TCDD at doses of 100 and 1,000 pg/g by i.p. injection to newborn mink for 12 consecutive days. Newborn mink exposed to TCDD at a dose of 1,000 pg/g died within 14 days, whereas mortality reached 62% by 19 weeks in mink exposed to 100 pg/g. Body weight gains were initially reduced in mink exposed to the low dose; however, by 19 weeks of age there was no significant difference in body weights between survivors and control animals. No discernible effects on the time of eyelid opening, the time of tooth eruption, and hair growth were attributed to TCDD exposure in the survivors.

Studies by Hochstein et al. (1988) and Aulerich et al. (1988) indicated that the mink is among the mammalian species most sensitive to TCDD intoxication. Based on a 28-day LD50 of 4,200 pg/g (Hochstein et al., 1988), the mink seems to be less sensitive than the guinea pig, for which LD50s of 600 to 2,000 pg/g have been reported, but more sensitive than the rat (LD50s of 22,000 to 45,000 pg/g), rabbit (LD50 of 115,000 pg/g), mouse (LD50s of 114,000 to 284,000 pg/g), and hamster

(LD50s of 1,157,000 to 5,000,000 pg/g) (see reviews of Kociba 1982a,b; Schwetz et al., 1973).

4.3.1.2 Birds

Several studies have been undertaken to determine the lethal potency of TCDD to avian wildlife. Hudson et al. (1984) reported 37-day LD50s of 15,000, >108,000 and >810,000 pg/g for the bobwhite quail, mallard, and ringed turtle dove (*Stretopelia risoria*), respectively, following a single oral administration of TCDD. In comparison, Grieg et al. (1973) reported that chickens given single oral doses of TCDD at 25,000 to 50,000 pg/g died within 12 to 21 days post treatment. In a longer study Schwetz et al. (1973) orally administered TCDD to 3-day-old white leghorn chickens for 21 days at doses of 0, 10, 100, 1,000 and 10,000 pg/g/day and reported a NOAEL for mortality of 100 pg/g/day. Nosek et al. (1992a) treated ring-necked pheasant i.p. with single doses of TCDD at 0, 6,250, 25,000 and 100,000 pg/g and observed the animals for 11 weeks post treatment. All the birds treated at the high dose of 100,000 pg/g died within six weeks of exposure. Onset of mortality in birds exposed to 25,000 pg/g was observed six weeks post treatment and at 11 weeks post exposure, 80% mortality was observed. No birds died in the 6,250 pg/g exposure group during the course of the study. Common to all of the above mentioned studies, a dose-dependent decrease in food consumption and body weight were reported to precede death.

Nosek et al. (1992a) also investigated the effects of TCDD on reproduction in ring-necked pheasant. Hens were administered TCDD by i.p. injection once a week at doses of 0, 10, 100 or 1,000 pg/g for 10 weeks. During the final two weeks of exposure, hens were paired with roosters and kept in egg production for an additional 9 to 13 weeks. No deaths were observed in the control group or with hens exposed to TCDD at the lowest two doses; however, a 57% mortality rate occurred in the group administered TCDD at 1,000 pg/g/week. A significant decrease ($p < 0.05$) in adult body weight and egg production, compared to controls, was also associated with the highest dose, but not with the lowest two doses. For these two endpoints there was no indication of a dose-response relationship between 0, 10 and 100 pg/g/week. There was a trend towards increasing embryo mortality rates with increasing dose to the hens. At the highest dose, 100 $\pm$ 2% of the embryos died, whereas mortality rates of 15 $\pm$ 5 and 25 $\pm$ 15% were reported for hens dosed at 10 and 100 pg/g/week, respectively (percent mortality corrected for lethality in control eggs; values cited based on interpretation of Figure 6 in Nosek et al., 1992a). A dose of 450 pg/g/week was calculated to elicit a 50% increase in embryo mortality above the control rate. The embryo mortality rates at 10 and 100 pg/g/week were, however, not significantly different nor were they different from control mortality ($p < 0.05$).

Several studies have also been undertaken to assess the toxicity of TCDD to birds following *in ovo* treatment; however, there were only a limited number of studies available with wildlife species. Nosek et al. (1992c) injected fertile ring-necked

pheasant eggs with graded TCDD doses of 0.01 to 100,000 pg/g. Injections were administered into the albumin (0.01 through 100,000 pg/g) in one experiment and into the yolk (10 to 10,000 pg/g) in another. Mortality was assessed in chicks through 28 days post hatch. Embryo mortality rates of 50 and 37.5% were reported for control eggs injected with 1,4-dioxane, which served as a carrier, in the yolk and albumin, respectively. Nosek et al. (1992c) reported that mortality in the range of 30 to 50% is within the historical range for embryo mortality with uninjected fertile eggs obtained from the hatchery used in the study. A dose dependent increase in embryo mortality was observed following both albumin and yolk injections and LD50s of 1,400 (99 - 3,700 pg/g; 95% C.I.) and 2,100 (522 - 4,393 pg/g; 95% C.I.) pg/g, respectively, were reported. Based on injections into the yolk, the LOAEL for mortality was 10,000 pg/g, and the NOAEL was 1,000 pg/g ($p < 0.05$). At 10,000 pg/g, 97.5% mortality was observed, whereas at 1,000 pg/g a mortality rate of 60% was noted. At 10 and 100 pg/g, 52.5 and 47.5% mortality were recorded, respectively. Results from albumin injection studies indicated a LOAEL of 1,000 pg/g (57.7% mortality) and a NOAEL of 100 pg/g (38.7% mortality). Mortality rates of 35.0, 30.0, 32.5, and 31.2% were recorded for doses of 0.01, 0.1, 1, and 10 pg/g, respectively. At doses of both 10,000 and 100,000 pg/g, 97.5% mortality was observed. Martin et al. (1989), as cited by Nosek et al. (1992c), reported 100% embryo mortality in Eastern bluebirds (*Sialia sialis*) following injection into the albumin of TCDD at a dose of 10,000 pg/g and no mortality at 1,000 pg/g.

The chicken may be more sensitive than the pheasant or bluebird. A LD50 of 240 pg/g has been reported by Allred and Strange (1977) following injection into the airspace of fertile eggs. Cheung et al. (1981) reported up to approximately 30% mortality in chicken eggs injected at 0.05 to 450 pg/g (assuming a 55 g egg) in the albumin; however, a significant linear log dose-response relationship was not observed ($p > 0.05$; approximately 20% mortality in control eggs).

In the same egg injection study outlined previously, Nosek et al. (1992c) also assessed growth, histological effects and immune response in pheasant chicks through 28 days post hatch. Injections of TCDD into the albumin at 1, 10, 100, or 1,000 pg/g had no effect on growth, nor on carcass morphometrics, cardiac morphometrics, absolute organ weights, or relative organ weights in 1-day old hatchlings and 28-day old chicks compared to chicks exposed *in ovo* to the vehicle. There was also no significant TCDD effect on histology of the liver, spleen, heart, Bursa of Fabricius, or thymus. No TCDD related increase in the incidence of ascites or subcutaneous, pleural, or pericardial edema was observed. Immune response, as monitored by serum titers of IgG, IgM, and total antibody in 28-day old chicks injected with washed sheep erythrocytes, was not significantly affected by TCDD exposure.

At non-lethal, *in ovo*, doses both Nosek et al. (1992c) and Martin et al. (1989), as cited by Nosek et al. (1992c), did not report any evidence of TCDD related effects on chick growth, histopathological abnormalities, or the incidence of edema, ascites, or

hydropericardium in pheasants or Eastern bluebirds. The role of TCDD and related PCDDs, PCDFs, and PCBs in producing a syndrome of edema and histological alterations in chickens has been reviewed extensively (e.g., see review of Gilbertson et al., 1991). For example, Sawyer et al. (1986), Flick et al. (1972) and Cheung et al. (1981) reported edema, involution of the Bursa of Fabricius, and cardiovascular malformations in chickens following i.p., dietary or *in ovo* exposure, respectively, to TCDD. Cheung et al. (1981) reported concentrations of approximately 5.8 pg/g egg (assuming a 55 g egg) associated with the combined occurrence of four cardiovascular malformations (combined occurrences of ventricular septal defect, aortic arch anomaly, aortic arch anomaly plus ventricular septal defect, and conotruncal malformation) in 50% of chicken embryos. There was approximately a 30% incidence rate for combined malformations in control groups, for which a similar rate in uninjected, sham-injected, and vehicle injected eggs was observed. There were, however, no significant relationships between TCDD exposure and the percentage of embryos having cardiac malformations when each of the four defects were analyzed separately, rather than combined. These differences between the chicken, pheasant and bluebird might reflect variations in routes of exposure and/or species sensitivity.

Only limited studies are available on the toxicokinetics of TCDD in avian species. Nosek et al. (1992b) reported that the bioavailability of TCDD was 30, 33, 41, and 58%, respectively, when adult pheasants were orally administered suspensions of treated earthworm, soil, paper mill sludge, and cricket homogenates. Martin et al. (1989), as cited by Nosek et al. (1992b), found that the oral bioavailability of TCDD to European starlings (*Sturnus vulgaris*) was 14, 17, 37, and 44% from suspensions of earthworms, paper mill sludge, soft-bodied invertebrates, and hard-bodied invertebrates, respectively. These results were consistent with the longer gastrointestinal retention time for hard-bodied insects (Nosek et al., 1992b).

Elimination studies of [^{3}H]TCDD by adult non-egg producing ring-necked pheasant (Nosek et al., 1992b) and European starlings (Martin et al., 1989, as cited by Nosek, et al., 1992b) provided whole-body half-lives (total TCDD on a whole-body wet-weight basis) of 380 and 7.2 days, respectively. Nosek et al. (1992b) also derived a half-life of 13 days for pheasant chicks exposed *in ovo*. The differences in half-lives between adult pheasants and pheasant chicks and starlings were attributable, in part, to differences in mass-specific metabolic rates. Differences in species- or age-specific TCDD metabolism was also thought to contribute to the variability in elimination. In adult pheasant carcass samples, TCDD was detected but no metabolites of TCDD were recovered, indicating little or no biotransformation in adult hens. However, fecal material and chick carcass samples were not analyzed for TCDD or metabolites, thus it is not possible to assess the role of age-specific differences in metabolism to differences in TCDD elimination. Nosek et al. (1992b) also suggested that declining lipid content in developing chicks, as the yolk lipids are depleted, coupled with the

developing gastrointestinal tract and high lipid content of chick starter feed could result in a redistribution of TCDD leading to an increased elimination rate.

Nosek et al. (1992b) also assessed the elimination of TCDD in egg-producing pheasant hens. Birds were exposed by i.p. injection to [^{3}H]TCDD at a rate of 100 pg/g once a week for 10 weeks and it was reported that a mean of 1.1% of the cumulative TCDD dose was eliminated in each of the first 15 eggs laid by a hen. The tritium in pheasant eggs was exclusively associated with the parent compound in the yolk. Martin et al. (1989), as cited by Nosek et al. (1992b), estimated that approximately 5% of a TCDD body burden in an Eastern bluebird would be eliminated in each egg. Based on an average of 30.5 eggs laid per pheasant hen over a 7-week post treatment period, it was estimated that approximately 35% of the total administered dose was eliminated in the eggs (Nosek et al., 1992b). At the time of sacrifice, an average of 30% of the administered TCDD remained in the hens. Although not actually measured, it was speculated that the remaining 35% of the cumulative TCDD dose was excreted. The greatly increased rate of TCDD elimination in laying hens, as opposed to non-laying hens, suggested that egg laying is an important route of elimination and that changes in metabolism and/or lipid distribution in the adult bird during egg development and reproduction might be responsible for increased fecal elimination (Nosek et al., 1992b).

Consideration of the pheasant toxicokinetic information with the results of the toxicity studies provides some limited insights into the relationship between female exposure, egg residue levels and embryotoxic effects of TCDD. Based on *in ovo* exposures, Nosek et al. (1992c) reported that NOAELs for embryo mortality ranged from 100 to 1,000 pg/g egg. In an extended reproduction study with TCDD exposure to the hens, a NOAEL for embryotoxic effects was associated with a total accumulated body burden of 1,000,000 pg in a 1.0 kg hen, whereas a LOAEL was associated with a 10,000,000 pg body burden (Nosek et al., 1992a). Results from the toxicokinetic studies (Nosek et al. 1992b) suggest that approximately 1.0% of a hen's body burden is translocated to each egg laid. Therefore, assuming a pheasant egg weight of 30 g (Nosek et al., 1992c), a cumulative NOAEL body burden of 1,000,000 pg TCDD and a LOAEL of 10,000,000 pg TCDD in a hen would correspond to NOAEL and LOAEL TCDD egg concentrations of approximately 300 and 3,000 pg/g, respectively. These derived pheasant egg effect levels are within the range of those levels obtained empirically from *in ovo* pheasant studies. For several reasons, comparisons between maternal and *in ovo* exposures must certainly be viewed with caution before any definitive conclusions can be made on the defensibility of solely using *in ovo* exposures to assess the impact of TCDD on avian reproduction. First, the available dose-response curves are based on regimes that incorporate 10-fold exposure increments, which makes quantitative comparisons uncertain. Secondly, *in ovo* exposures fail to incorporate any possible adverse effects that may result from TCDD-mediated alterations in the adult female or male reproductive physiology and endocrinology.

4.3.2 Epidemiological Information

As was discussed in the context of fish populations (see section 4.2.2), the criteria discussed by Fox (1991), which include: (a) probability, (b) chronological relationship, (c) strength of association, (d) specificity, (e) consistency of association upon replication, (f) predictive performance, and (g) coherence (i.e., biological plausability, presence of a dose-response relationship), should be considered when assessing "ecoepidemiological" studies for wildlife populations. The elucidation of plausible cause and effect relationships, given the variety of chemical and nonchemical stressors that can impact populations, is obviously a fundamental challenge in interpreting the results of wildlife studies. Certainly the validity of epidemiologically-based associations increase when they can be supported by independent and controlled experiments.

Using the criteria of Fox (1991), by far the most convincing case linking a specific suite of adverse biological effects (embryonic and chick mortality, edema, growth retardation, deformities) to residue concentrations of TCDD-like chemicals can be made for fish-eating birds (herring gulls, Forster's terns, double-crested cormorants, Caspian terns) from the Great Lakes (Gilbertson et al., 1991 and references cited therein; Tillit et al., 1992). One confounding factor in the interpretation of studies with populations of Great Lakes birds is the co-occurrence of a number of other potentially toxic contaminants (e.g., DDT, dieldrin, mercury) with TCDD and toxicologically related PCDDs, PCDFs, and PCBs. However, because of reasonably consistent exposure profiles, studies with herring gulls in Lake Ontario (Mineau et al., 1984; Environment Canada, 1991a,b) and Great Blue herons in British Columbia (Bellward et al., 1990; Hart et al., 1991) provide some data relating reproductive success to egg concentrations of TCDD itself.

The productivity of herring gull colonies at several islands in Lake Ontario was monitored from 1972 through 1984, as summarized by Mineau et al. (1984) and Environment Canada (1991a). From 1971 through 1975, productivity, defined as the number of young reaching 21 days of age per nesting adult, ranged from 0.06 to 0.21, which was well below the range of 0.8 to 1.0 that is required for population stability. However, in 1977 productivity values exceeded 0.8 and through 1984 (the last year of reported data), ranged from 0.86 to 2.13. The population of gulls increased from 520 pairs in ten colonies in 1976 to 1,540 pairs in 15 colonies in 1987. During the period of poor reproductive performance, a suite of adverse effects that are commonly associated with TCDD and toxicologically-related compounds (Gilbertson et al., 1991), was reported. However, egg shell thinning of only 4 to 8% was noted in this DDE-resistant species, which strongly suggested that total DDT exposure was not associated with the low productivity (Environment Canada, 1991a).

During the period of time that productivity was assessed at gull colonies associated with Snake, Muggs and Scotch Bonnet Islands in Lake Ontario, TCDD levels were also monitored in gull eggs sampled from these sites (Environment Canada, 1991b). In 1971 and 1972, TCDD levels of approximately 2,000 to 2,400 pg/g in gull eggs were reported for Scotch Bonnet Islands (1972 productivity of 0.12); in 1974, levels were down to approximately 900 pg/g (1973 and 1975 productivities of 0.06 and 0.15) and in 1977 and 1978, were at 500 pg/g (productivities of 1.10 and 1.01). In 1982, levels were down to 204 pg/g (1981 productivity of 2.13). Concentrations of TCDD in gull eggs from Snake Island dropped from approximately 175 pg/g in 1981 (productivity of 1.73) to 90 pg/g in 1989. At Muggs Island, TCDD concentrations in gull eggs have remained relatively constant at approximately 25 to 50 pg/g from 1984 through 1989 (1981 and 1984 productivities of 1.40 and 1.17). The reduction in TCDD levels in gull eggs is consistent with discontinued TCDD inputs from waste sites and chemical manufacturing plants and declining concentrations in water, sediments and fish (Hallet and Brooksbank, 1986; Cook et al., 1993b; Environment Canada, 1991b). It should also be noted that during the period of 1974 to 1977/78, when productivity sharply improved, total PCB, mirex, hexachlorobenzene and dieldrin concentrations in gull eggs also dropped from approximately 100,000,000 to 35,000,000; 7,000,000 to 1,000,000; 500,000 to 100,000; and 500,000 to 100,000 pg/g, respectively (Environment Canada, 1991b).

In the studies by Bellward et al. (1990) and Hart et al. (1991), survival and growth of Great Blue heron chicks were monitored in colonies from three sites (Nicomekl, Vancouver and Crofton) in British Columbia which varied in PCDF and PCDD concentrations. The predominant congeners present in Great Blue heron eggs from these sites were TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin. However, based on mammalian TEF models (Safe, 1990), the most toxic of the congeners was TCDD. Mean TCDD concentrations (± SEM) in eggs from the Nicomekl, Vancouver, and Crofton sites were 10 ± 0.9, 135 ± 49.6 and 211 ± 33.7 pg/g, respectively. Hatching success did not differ significantly across sites but there was an apparent increase in the incidence of edema in chicks with increasing TCDD concentration (0 of 11, 2 of 13, and 4 of 12, from the Nicomekl, Vancouver and Crofton colonies, respectively). In addition, there was an inverse correlation between TCDD egg concentrations and various growth measurements made on the heron chicks. In a more recent study, preliminary results suggest there may also be an inverse association between TCDD egg concentrations and morphometric changes in the brains of these hatchlings (Henshel et al., 1992).

The herring gull and Great Blue heron data indicate that successful reproduction exists in wild colonies (i.e., maintenance of a stable population), even though TCDD is present at concentrations in eggs between 200 to 500 pg/g. In turn, toxic effects at the individual level are associated with concentrations above 100 pg/g. These data are consistent with the results of laboratory toxicity studies for birds described previously (see section 4.3.1.2), which indicated that a threshold for embryo

mortality would be expected in the range of 100 to 500 pg/g. The causal connection between TCDD and the observed field effects is, however, by no means proven, especially since a number of other contaminants were also present in the egg samples. Further, the generalizations concerning adverse effect levels of TCDD in eggs, drawn from the field studies, should be considered conservative because PCBs, PCDDs, PCDFs, and, in the case of the herring gulls in Lake Ontario, hexachlorobenzene, dieldrin and mirex, may also have contributed to the observed responses. As a consequence, it is impossible to quantitatively extrapolate these results to a more definitive TCDD-specific threshold value. These results clearly illustrate the need for validated methods to assess TCDD equivalents in avian models to establish defensible approaches for evaluating mixtures of PCBs, PCDFs and PCDDs, as well as the need to develop techniques to assess chemical mixtures in general.

4.3.3 Effects Profile

4.3.3.1 Approach for Summarizing Effects on Wildlife

In cases where studies have related TCDD effects to TCDD accumulation in wildlife, the approach for summarizing effects will be the same here as that used earlier for fish. However, much of the toxicity information for wildlife presented previously, especially for sensitive endpoints, cannot be expressed on this basis. This is due to the lack of wildlife accumulation measurements and because there is uncertainty about how the route and duration of exposure would affect accumulation. Rather wildlife-effect information is more conveniently expressed on the basis of TCDD dose via oral consumption. This dose metric has the added advantage of being able to relate effects on both wildlife and fish to TCDD accumulation in fish.

The approach used here to express wildlife effects profiles is similar to that in the proposed Great Lakes Water Quality Initiative procedure for deriving criteria for the protection of wildlife (U.S. EPA, 1991b) and the analysis of risk of selenium to wildlife (Peterson and Nebeker, 1992). These efforts were intended to identify the highest aqueous concentrations of toxicants that would not cause unacceptable reduction in the growth, reproduction, or viability of representative mammalian and avian species which ingest surface water or aquatic life taken from surface waters. For this analysis, concentration in the food, rather than water, will initially be the focus to allow comparisons of risk between wildlife and fish. Water as a route of TCDD exposure to wildlife can be ignored because the BAF for TCDD in food organisms is generally 10^4 or greater; therefore food consumption, which in wildlife is of similar magnitude to water consumption, would provide nearly all TCDD exposure.

For the analyses here, the following equation will therefore be used:

$$FC = \frac{EL}{EF_S \cdot EF_C} \cdot \frac{Wt_A}{F_A} = \frac{EL}{EF_S \cdot EF_C \cdot R_A}$$

where:

FC= Food concentration associated with an effect level (pg/g)
EL= Effect level from a toxicity study (pg/g/d)
Wt_A= Weight of the organism of interest (g)
EF_S= Extrapolation factor for relative species sensitivity
EF_C= Extrapolation factor for subchronic to chronic exposure
F_A= Food consumption for the organism of interest (g/d)
R_A= Food consumption as fraction of weight (1/d)

The following analyses will use the above equation and consider risk to mammalian and avian wildlife species that have a diet consisting solely of fish, or other aquatic macrofauna that would have TCDD levels comparable to fish. Representative mammalian wildlife species would include the river otter and mink. Representative avian wildlife would include bald eagle, osprey, kingfishers, terns, herons, diving ducks, mergansers, and loons. For the mammals, the food consumption rate (R_A) would be expected to be in the range of 10-20% of body weight per day (Aulerich et al., 1973; Lauhachinda, 1978; Bleavins and Aulerich, 1981; Linscombe et al., 1982; Toweill and Tabor, 1982; Newell et al., 1987). For birds, the value can range from 10 to 50% (Alexander, 1977; Fry, 1980; Stalmaster and Gessaman, 1982, 1984; Bortolotti, 1984; Newell et al., 1987; Nagy, 1987; Craig et al., 1988; Palmer, 1988; Poole, 1989).

4.3.3.2 Selection of Effect Levels - Mammals

As discussed in section 4.3.1.1, a 28-day LD50 for adult mink after a single oral dose was estimated to be 4,200 pg/g, with some effects on growth at 2,500 pg/g (Hochstein et al., 1988). The extent of absorption of this dose is not known, but assuming absorption is substantial but not necessarily complete (e.g., 25 to 75%), this suggests the median lethal accumulation would be 1,000 to 3,000 pg/g. For newborn mink, exposed via i.p. injection for 12 days, a dose of 100 pg/g/day (total dose of 1,200 pg/g) resulted in a mortality of 62% over an observation period of 19 weeks (Aulerich et al., 1988). The median lethal accumulation would be near 1,000 pg/g, assuming that the half life of this material is greater than the 12-day exposure period. These data indicate that mink are among the most sensitive mammals tested and that their sensitivity is within the range exhibited by fish. However, because of inadequate knowledge of the half life of TCDD in this species, this lethal accumulation data cannot be related to food or environmental concentrations over a more prolonged exposure.

As also discussed in section 4.3.1.1, there are no subchronic or chronic studies available for mammalian wildlife species. Numerous chronic studies are, however, available for other mammalian species. These investigations have been summarized in reports previously published by EPA in the mid-1980s (e.g., see U.S. EPA, 1984). Of these investigations, the study of Murray et al. (1979) with Sprague-Dawley rats was considered the most complete for the mammalian wildlife analysis. Subsequent to the investigation of Murray et al. (1979), two noteworthy studies with Rhesus monkeys (*Macaca mulatta*) have also been published (Bowman et al., 1989a,b).

Murray et al. (1979) maintained three generations of Sprague-Dawley rats on TCDD treated diets equivalent to doses of 0, 1, 10, and 100 pg/g/day. Decreases in F_0 generation fertility and F_1 generation litter size were reported for rats in the 100 pg/g/day treatment group. Furthermore, rats in the 10 pg/g/day group exhibited decreases in fertility in the F_1 and F_2 generations. Other effects observed included decreased litter size at birth, decreased gestational survival and decreased neonatal growth and survival. Murray et al. (1979) reported that the reproductive capacity of rats in the low dose group was not significantly affected in any generation and a NOAEL of 1 pg/g/day can be inferred. It should be noted that there has been debate in the literature regarding whether or not a statistically significant reduction in offspring survival occurred at the low dose (Nisbet and Paxton, 1982; Kimmel, 1988); however, this dose probably reasonably reflects the threshold for risk in this species.

Bowman et al. (1989a,b) assessed the reproductive success of Rhesus monkeys maintained on TCDD treated diets equivalent to doses of 0, 0.13, and 0.67 pg/g/day. Reproductive success was monitored in two cohorts produced during a four-year exposure period and in a third cohort produced ten months post-exposure. No reproductive effects were observed in the third cohort. At a dose of 0.13 pg/g/day, no significant effects of TCDD on pregnancy rate, abortion rate, still birth rate, and survival through one year were observed in the first two cohorts. At the higher dose, significant effects on reproduction were observed. Results from these studies suggest a NOAEL of 0.13 pg/g/day for reproductive success in the Rhesus monkey, which is approximately eight-fold lower than the value reported for rats.

For the present risk characterization, the study of Murray et al. (1979) was considered the most relevant for assessing adverse effects in wildlife populations because it incorporated a multigenerational exposure regime. However, as discussed previously, the mink appears to be one of the mammals most sensitive to TCDD intoxication and based on a comparison of LD50 values is about an order of magnitude more sensitive than the rat. An inter-species extrapolation factor (EF_S) of 10 will therefore be used. Applying this factor to the rat NOAEL results in a value roughly equivalent to using the NOAEL from the Rhesus monkey study with no inter-species extrapolation factor.

This analysis therefore suggests that TCDD poses no demonstrable risk to mammalian wildlife if daily intake does not exceed 0.1 pg/g/day, based on a NOAEL of 1 pg/g/day and a interspecies extrapolation factor (EF_S) of 10. For a consumption rate (R_A) of 10-20%, this daily intake corresponds to concentrations in fish of 0.5-1.0 pg/g. For sensitive organisms, substantial effects on reproduction would be expected at concentrations approximately ten-fold higher.

4.3.3.3 Selection of Effect Levels - Birds

As discussed in section 4.3.1.2, LD50s vary markedly among birds. For a single oral dose followed by 37 days of observation, an LD50 for quail was 15,000 pg/g, but was greater than 100,000 pg/g for mallards and greater than 800,000 pg/g for turtledoves. After a single i.p. dose, pheasants had an LD50 between 10,000 and 20,000 pg/g. Chickens that were orally dosed for 21 days died at a total dose of 21,000 pg/g, but not at 2,000 pg/g. These data suggest that gallinaceous birds may be the most sensitive to TCDD, and for acute lethality, this group of birds seems to be somewhat more resistant than sensitive mammals and fishes. As was the case for mammals, there is an absence of good toxicokinetic information (especially half life), so it is difficult to link these numbers to more chronic dietary exposure or to environmental concentrations. If it is assumed that these acute median lethal doses ($LD50^a$) can be approximately equated to a lethal body burden under chronic exposure (LR50), the median lethal chronic dose ($LD50^c$) can be estimated as $LD50^a \cdot k_2$. If the half life of ca. 1 year for non-egg laying adult pheasant is used ($k_2 = 0.693 \div t_{1/2} = 0.002$/day), an LR50 of around 15,000 pg/g corresponds to an chronic absorbed oral dose of about 30 pg/g/day. If the half life of the starling is used (7.2 days), the chronic dose would be about 1,400 pg/g/day.

Based on the summary of TCDD wildlife toxicity studies in section 4.3.1.2, the report of Nosek et al. (1992a) on reproductive effects in ring-necked pheasants was selected to establish effect profiles for birds. Ring-necked pheasants were dosed weekly (i.p.) for 10 weeks with TCDD at rates equivalent to 1.4, 14 and 140 pg/g/day. A significant decrease in egg production and 100% mortality in embryos was observed in hens treated at 140 pg/g/day. The numbers of eggs produced by the control hens and hens in the 14 and the 1.4 pg/g/day groups were not significantly different. There was a tendency for embryo mortality to increase with the cumulative TCDD dose to the hens; however, the dose/response slope was very shallow and mortality in the two lower dose groups was not significantly different from that observed in the control group. A value of 14 pg/g/day will be used here as being associated with a low level of risk for reproductive effects in this species. The steepness of the response curve above this level is uncertain, but complete failure of reproduction would occur at concentrations tenfold higher.

In applying these data to characterizing risk for birds, two considerations must be made regarding whether extrapolation factors are needed. First, although studies

using a route of exposure comparable to that employed by Nosek et al. (1992a) are not available, the results of egg injection studies described previously indicate that the chicken embryo is more sensitive than the pheasant, based on overt lethality. In addition, histopathological alterations have been observed in the chicken, but not in the pheasant. These data suggest that gallinaceous birds other than the pheasant could be more sensitive to TCDD intoxication. However, the pheasant is still one of the most sensitive birds tested thus far and there is no clear need for extrapolating to the chicken (which seems to be the most sensitive member of a sensitive group) in order to assess risk to piscivorous avian wildlife. Therefore, an EF_s of 1 will be used in this analysis. Second, based on the half life of nearly a year reported earlier for the elimination of TCDD from non-egg laying adult pheasant, the subchronic exposure (10 weeks) in the study of Nosek et al. (1992a) would have resulted in achieving only 13% of steady-state accumulation. A truly chronic exposure could presumably have had nearly an order of magnitude lower concentration in the food and still elicited the same tissue levels and effects. Thus, a subchronic to chronic extrapolation factor (EF_c) of 10 will be employed.

An exposure associated with low risk to avian wildlife of 1.4 pg/g/day is therefore calculated here using an EL of 14 pg/g/day based on the NOAEL derived from the pheasant study and an $EF_s \cdot EF_c$ of 10 based on considerations of inter-species sensitivity and exposure duration. For a food consumption rate of 10-50% of body weight per day, this daily intake would correspond to a TCDD concentration in fish of 3 to 14 pg/g. Substantial effects on the reproduction of sensitive birds would be expected at chronic exposures approximately ten times higher.

As discussed in section 4.3.2, a threshold TCDD concentration of 500 pg/g in gull eggs was associated with effects on herring gull production on Lake Ontario. Since Braune and Norstrom (1989) reported a biomagnification factor of 21 for TCDD in gull eggs relative to alewives in Lake Ontario, this threshold is associated with a concentration in alewives of approximately 24 pg TCDD/g. It should be noted that these wild populations are subject to a variety of other stressors, including other hydrophobic chemicals (PCDDs, PCDFs and PCBs) that could act jointly with TCDD. Therefore the value of 24 pg TCDD/g likely underestimates a no effect exposure if TCDD was the sole chemical stressor responsible for the adverse effects.

Based on the above information, the effects and associated uncertainties of TCDD on aquatic-associated wildlife are summarized in Box 2.

Box 2. Effects Profile for Aquatic-Associated Wildlife

- The most sensitive ecologically important endpoints established for both mammals and birds are associated with reproductive effects. For mammals, observed reproductive effects in tests on the rat, combined with the relatively high sensitivity of mink based on lethality tests, indicate that little adverse effects should be associated with a diet that provides up to 0.1 pg TCDD/g/day. For mammals which consume only fish and have an intake of 10-20% of body weight per day, this corresponds to fish that have a total TCDD concentration of 0.5-1.0 pg TCDD/g. For birds, a similar analysis based on a laboratory study with pheasants suggests that there is no evidence for significant risk associated with a diet providing up to 1.4 pg TCDD/g/day. For birds with a food consumption rate of 10-50% of body weight per day, this corresponds to fish with 3-14 pg TCDD/g. Based on epidemiological evidence for herring gulls in Lake Ontario, there is no evidence for significant risk associated with fish concentrations of up to 24 pg TCDD/g.

- For both birds and mammals, available data suggest that substantial effects on reproduction are expected at exposures ten times the exposures specified above for risk thresholds. Thus, for sensitive mammals, substantial effects would be expected with a diet of fish containing 5-10 pg TCDD/g and for sensitive birds with a diet containing 30-140 pg TCDD/g. The use of extrapolation factors for sensitive organisms makes these value somewhat uncertain. The range of tolerance among all species is also uncertain, but at least for birds might extend an order of magnitude or more.

- For the more sensitive mammals, including mink, mortality is observed at body burdens of 1,000-2,000 pg/g. For birds, mortality requires 10,000 pg/g or more and shows a broad range of species sensitivities. The relationship of these body burdens to food consumption is difficult to specify due to the lack of good toxicokinetic information. However, since these accumulations are 100-fold higher than the daily consumption that elicits significant reproductive effects, these numbers would be of limited value since substantial risk under virtually all exposure conditions would be dictated by other endpoints.

- Estimates of fish TCDD contamination that pose a risk to wildlife are for organisms which are essentially completely piscivorous; other dietary sources can alter the risk. For mink, this analysis may overestimate TCDD exposure for those individuals that are not primarily foraging for fish and aquatic invertebrates. In contrast, for bald eagles nesting on the shores of Lake Superior, a study by Kozie and Anderson (1991) suggests that fish comprise about 97% of an eagle's diet and that birds and mammals each comprise 1.5%. Braune and Norstrom (1989) reported a TCDD BMF for herring gulls in Lake Ontario of 32, so if even 1.5% of an eagle's diet was fish-eating birds with similar accumulation, exposure could be nearly 50% higher.

- The limited database concerning effects makes the values specified above uncertain. The use of effects data from relatively resistant species (i.e., the mammalian analysis) and/or subchronic exposures (i.e., the bird analysis) necessitated the use of extrapolation factors to estimate risk to more sensitive species and longer exposures. The summary numbers reported above for chronic effects would be tenfold higher for organisms with sensitivities or exposures comparable to those used in the cited experimental studies. These assessments are also based on studies with large differences between treatment levels, making the dose-response curves uncertain. Additionally, the general issue of differences among species in TCDD toxicokinetics and toxicodynamics is poorly resolved and contributes to uncertainty in this risk characterization, especially given the wide range of effect levels across wildlife species.

5. RISK CHARACTERIZATION METHODOLOGY

In an ecological risk assessment for a chemical stressor, risk characterization is the phase in which the results of exposure and effects analyses are integrated to evaluate the likelihood of adverse effects in exposed organisms, populations, communities, or ecosystems based on actual or projected exposures of organisms to the chemical, or suite of chemicals, in the environment (U.S. EPA, 1992c). The degree to which risk is characterized can vary markedly, but ideally involves a quantitative scale of effects and estimation of probabilities and uncertainties. Current information is insufficient to provide such a thorough description for TCDD risk to aquatic life and associated wildlife, with quantification of uncertainty being particularly difficult given the limited knowledge base. Furthermore, a thorough assessment of TCDD risk should consider its joint action with other contaminants and non-chemical stressors and the expression of effects on individual organisms at a population and community level; such techniques are even less well established and must await further development. However, the adequacy of a risk characterization depends on the nature of the specific problem of interest, so current information can be adequate to characterize TCDD risk to aquatic life and associated wildlife in some cases.

The principal goal of this report was to evaluate and summarize data and methods that are available for the assessment of TCDD risk to aquatic life and associated wildlife, and to identify the major uncertainties that currently limit how well risks can be characterized. A definitive risk assessment for a specific problem was not a goal of this report, and there was consequently no explicit risk assessment problem identified earlier in the report. However, it is necessary to discuss how exposure and effects information should be integrated into a description of risk and it is most effective to do this in the context of actual problems regarding the risk of TCDD to aquatic life and associated wildlife. Therefore, this section will summarize the exposure and effects information already presented and then offer examples of how this information can be applied to the characterization of risk. These examples are intended only to illustrate methods that can be used in risk characterization and to identify major uncertainties that should be of concern. The level of detail presented will be limited to that needed to accomplish this purpose and will be less than that which would be provided in complete risk characterizations.

5.1 SUMMARY OF EXPOSURE AND EFFECTS INFORMATION

5.1.1 Exposure

As discussed earlier in this report (section 2 and references cited therein), the high bioaccumulation potential and toxicity of TCDD result in water concentrations of concern that are below ordinary analytical detection limits. Thus, there are few measurements that reliably quantify the concentration of TCDD in natural waters. The

one example cited in Section 2.3 indicated that concentrations in the Baltic Sea are below a detection limit of 0.0002 pg/L in most samples, but some samples showed concentrations in filtrates of 0.0002 to 0.0003 pg/L and similar amounts associated with particulate matter. These data document the general magnitude of TCDD concentrations in this specific system and demonstrate the importance of particulate phases in TCDD distribution, even in waters with very low suspended solids. However, there are as yet no water measurements that will support risk characterization at the types of sites that are of concern to EPA regulatory activities.

In contrast, TCDD in sediments is more readily measured at exposure concentrations of concern and there are measurements of sediment contamination from several sites. Concentrations range from less than 1 pg TCDD/g dry sediment in relatively uncontaminated sites to several hundreds and thousands of pg/g in the highly contaminated Newark Bay, NJ. Concentrations above 10 pg/g commonly appear at sites that have some industrial impact, but the sites evaluated thus far are so limited and selective that firm conclusions about the general extent of sediment contamination are not possible. Concentrations within a particular system also tend to be variable, in part due to the variation in the organic content of sediments. Surficial sediments in Lake Ontario were found to contain an average of 70 pg TCDD/g dry weight in 1987, and appear to have been as high as 500 pg/g in the early 1960s. On an organic carbon normalized basis, the concentrations average approximately 2,500 pg TCDD/g organic carbon and tend to be similar throughout the depositional zones of the lake.

TCDD contamination in fish has been much more widely measured than in water or sediments, and results of systematic surveys of large number of sites in the U.S. have been published (U.S. EPA, 1987; 1992b). While such data do not define environmental exposure concentrations, they do provide information on the potential exposure of fish-eating wildlife and also can help assess risk to fish when compared to accumulation-based effects information. These data will be discussed at length in section 5.2.1 below.

TCDD risk characterizations are subject to a fundamental, and nonquantifiable, uncertainty in the aforementioned lack of essentially any data on water concentrations and only a handful of data on sediment concentrations. This precludes any characterization based on measured environmental concentrations except at a very few sites, and forces reliance on accumulation in organisms as a measure of exposure and/or on fate models to estimate exposure. Improvements in analytical methods and expanded efforts to measure TCDD in water and sediment are needed to address these uncertainties. The database of concentrations in fish can be used to assess a variety of sites or classes of sites, but there is considerable uncertainty when these concentrations must be related back to water and sediment concentrations or to loadings of TCDD to an ecosystem.

5.1.2 Bioaccumulation

As discussed in previous chapters, bioaccumulation relationships for TCDD in fish are an important tool for integrating exposure and effects information for highly hydrophobic, bioaccumulative chemicals. For TCDD, accumulation in fish is both the primary referent for exposure of fish-eating wildlife and a better basis for assessing effects in aquatic life than using water concentrations directly. Steady-state BCFs determined in the laboratory vary by over an order of magnitude (Section 3.2, Table 3-1). This variability is likely due largely to incomplete characterization of exposure concentrations or experimental shortcomings, including partitioning onto organic matter in test systems, oversaturation of TCDD, and time-varying concentrations in static systems. On a lipid normalized and total water concentration basis, steady-state bioconcentration factors ($ssBCF_\ell^t$) would appear to be at least 10^5 even for systems with uncertain exposures and would appear to more likely be of the order of 10^6. Because these factors do not reflect exposure via food, they should underestimate accumulation under more natural exposures. However, the relative bioavailability in laboratory versus natural systems and the estimation of steady-state through extrapolation results in uncertainties which might lead to either underestimation or overestimation of BAFs in the field.

BAF_ℓ^ts calculated for lake trout from Lake Ontario data are based on water concentrations from chemical mass balance model calculations, but also seem to suggest values of about 10^6 on a total water TCDD basis (Table 3-2). The BAF_ℓ^d for lake trout is estimated to be $2 \cdot 10^6$ to $3 \cdot 10^7$; uncertainty in the K_{ow} of TCDD results in this variability of predicted partitioning of TCDD between fish tissue and the freely dissolved fraction. In contrast, BAF_ℓ^ts do not vary much with K_{OW} because the partitioning both within the water (to organic matter) and the organism are roughly proportional to K_{OW}. As discussed in section 3, the best current estimate for extrapolating this Lake Ontario BAF to other situations is to equate BAF_ℓ^t to $3 \cdot 10^6 \cdot f_d$, which should be approximately $0.2 \cdot 10^6$/POC.

As discussed in section 3.4 of this report, BSAFs for TCDD appear to vary within the 0.03 to 0.3 range for a variety of systems and fish species. Although BSAFs for specific sites can always help to interpret problems and address remedial actions at those sites, a concern here is how well these TCDD BSAF measurements apply to other sites. A key issue in this extrapolation is whether TCDD BSAFs invariably are below the expected equilibrium value, which is 1 or slightly higher. Low BSAFs might reflect disequilibrium between water and sediment ($R_{ws}<1$), possibly due to the effects of decreasing anthropogenic inputs into the water, sediment diagenesis which increases the freely dissolved chemical concentration in the sediment, and/or loss processes from the water which keep the chemical concentration in water depressed relative to the sediment. The low BSAFs might also reflect effects of growth dilution and metabolism (in the entire food chain) which keep tissue concentrations below equilibrium values with the water ($R_{aw}<1$). Whatever the reason

for the lower than expected BSAFs for TCDD, based on appropriate measurements for some rather diverse sites and species, there is no evidence that these factors are greater than 0.3 or less than 0.03, with the best point estimate being on the order of 0.1. If these values are in some part due to water/sediment disequilibrium, then such an extrapolation would underestimate risk at sites with R_{ws} closer to 1. However, this error is unlikely to be more than two- to four-fold, given the observed range of BSAFs.

The paucity of exposure data from natural systems also affects understanding of bioaccumulation because there are no directly measured BAFs and only a limited number of measured BSAFs. For BAFs, it is difficult to quantify the uncertainty, but it could conceivably be several-fold. This uncertainty is associated in large part with the variation in estimates for K_{ow} and by a lack of a good empirical database on the effects of organic matter on the distribution of TCDD. BSAFs are somewhat better defined, but they do vary an order of magnitude among species and sites in a manner that is inadequately understood; extrapolation of these BSAFs to new situations could arguably be uncertain by two- to four-fold.

5.1.3 Effects

As discussed in section 4.2 and the references cited therein, fish appear to be much more sensitive than other aquatic organisms and early life stages of fish appear to be significantly more sensitive than older fish. Studies with lake trout currently provide the most useful information on an important, sensitive endpoint, with fry survival being little impacted for egg accumulations up to 34 pg TCDD/g, but being severely affected at concentrations only 1.5- to 2-fold higher. Current information suggests that accumulation is higher in maternal fish than in eggs, but only by about 50% on a lipid normalized basis. Thus, using these lake trout as a surrogate for sensitive fish species would suggest that low risk would be associated with accumulations of up to 50 pg/g in fish with the same lipid content (about 8%) as eggs (about 600 pg/g lipid) and high risk would be associated with accumulations above 80 pg/g (about 1000 pg/g lipid). Based on the variability among tested species discussed in section 4.2, more tolerant organisms would be at high risk at exposures approximately ten-fold higher than for sensitive organisms.

The most sensitive ecologically important endpoints established for both mammals and birds are associated with reproduction. For mammals, observed reproductive effects in tests on the rat and Rhesus monkey, combined with the higher sensitivity of mink than rats based on lethality tests, suggest the risk is low for mammalian wildlife which consume less than 0.1 pg TCDD/g/day. For a food consumption rate of 15% of body weight per day (a median value for the species reviewed) this low risk level corresponds to fish with a TCDD concentration of up to 0.7 pg/g. A similar analysis suggests that there is no evidence for significant risk to avian wildlife which consume less than 1.4 pg TCDD/g/day. For a food consumption rate of 25% of body weight per day, this corresponds to fish with up to 6 pg TCDD/g.

Based on epidemiological evidence for herring gulls in Lake Ontario, there is no evidence for significant risk to gulls associated with fish concentrations of up to 24 pg TCDD/g. Substantial effects on reproduction for both sensitive birds and mammals are likely at exposures 10-fold higher than specified above for low risk, but the dose-response curves are uncertain because available studies are limited and employed large differences between treatment levels. The range of tolerance among all species is also uncertain, but for birds probably exceeds an order of magnitude.

The limited database concerning wildlife effects makes these values uncertain. The use of effects data from relatively resistant species (e.g., the rat) and/or subchronic exposures (e.g., the pheasant study) necessitated the use of extrapolation factors to estimate risk to more sensitive species and longer exposures. In addition, the number of species studied is limited, TCDD accumulation was usually not monitored, and studies used widely-spaced exposure concentrations. The net result is very little information on variability among species and exposure concentrations and a poorly defined dose-response curve. Uncertainties again might be several-fold and the underlying deficiency is a limited database on reproductive effects of TCDD to organisms of interest.

Effects data for aquatic life provide somewhat more information on uncertainties. If the focus of a risk characterization is solely on the early life stage of lake trout as the receptor of interest, uncertainties in egg accumulation associated with different levels of risk are probably about a factor of 2 to 3. This uncertainty is small because of the steep response curve and the good agreement in LR50s among different experiments and routes of exposure. This uncertainty range also includes consideration of possible sensitivity differences in stocks of fish, which can have a range of about two based on experiments with different strains of rainbow trout. On the basis of accumulation in adult fish, the uncertainty would be slightly higher (e.g., a factor of 3 to 4) because of uncertainty in the relationship of TCDD in eggs to that in maternal fish.

However, the definition of lake trout early life stage survival as the measurement endpoint for aquatic life risk assessments is based on limited testing. Effects of TCDD on fish reproduction have not been evaluated after exposure of males and females through a complete reproductive cycle. On the basis of accumulations in eggs (which is necessary for application of these data), sensitivities of early life stages have been established for only two species in Salmonidae, with tests on other species providing indications that their sensitivities are not dissimilar to the salmonids, but may span a somewhat broader range. The range of aquatic species other than fish that have been tested is limited. Also, many tests do not document accumulation, so there is no ability to extrapolate to different exposure conditions. These factors add additional uncertainty to this analysis which is difficult to quantify.

5.2 APPLICATION OF INFORMATION TO RISK CHARACTERIZATION

5.2.1 Fish Contamination in the United States: Risk to Aquatic Life and Associated Wildlife

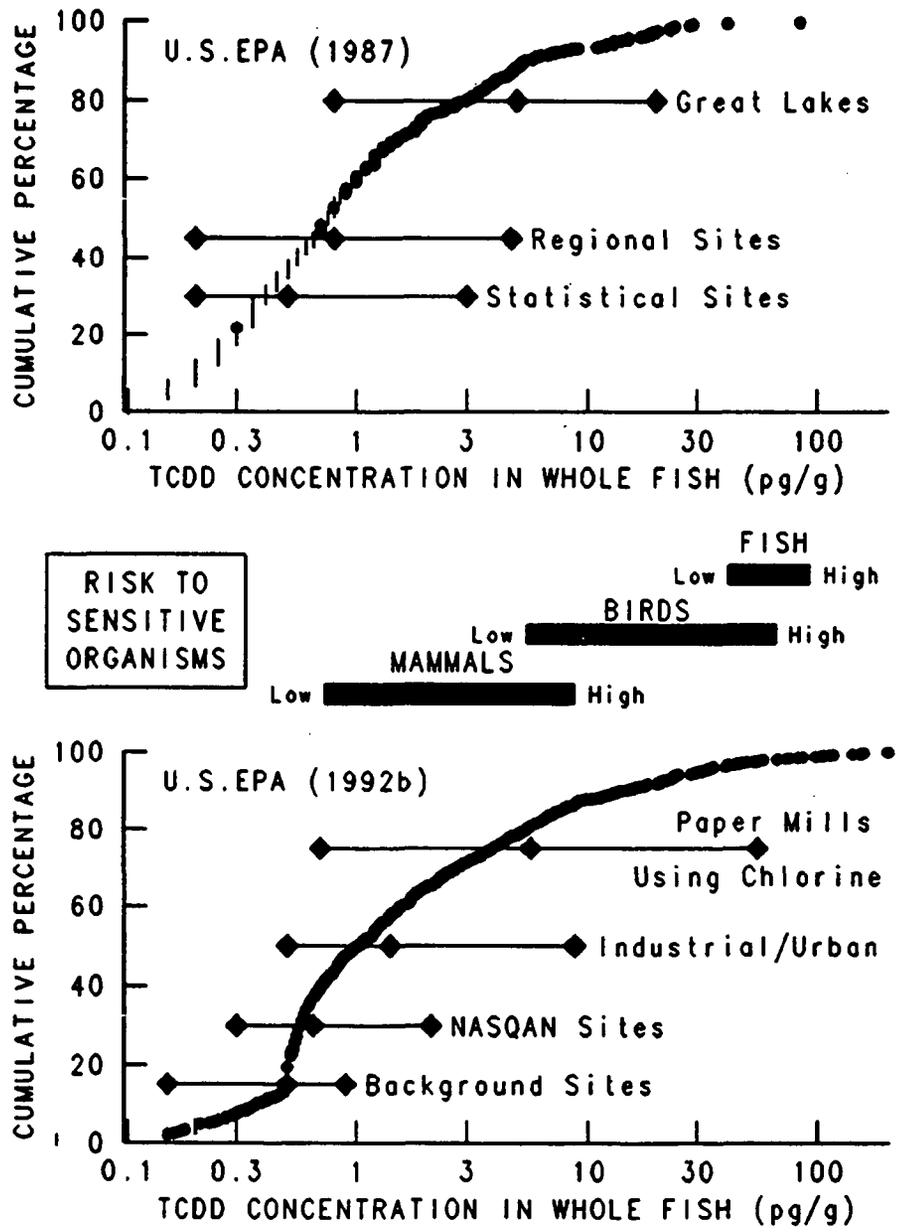
Two national surveys by the EPA (U.S.EPA, 1987; 1992b) analyzed fish from a large number of diverse sites for TCDD and other chemicals. These surveys provide the best set of data for consideration of risk on a national basis and at different classes of sites, although effects can only be considered on the basis of accumulation in fish and cannot be reliably related back to environmental concentrations and source loadings. This section will consider how this information can be applied to evaluations of the risk of TCDD to aquatic-life and associated wildlife.

The National Dioxin Survey (U.S.EPA, 1987) reported results from fish sampled at 395 sites. Ninety of these sites were randomly located throughout the United States at U.S. Geological Survey (USGS) National Stream Quality Accounting Network (NASQAN) and Benchmark Network sites. In these statistically located sites, TCDD was not detected in almost 85% of the samples (detection limits ranged from 0.2 to 4 pg TCDD/g and were typically about 1 pg/g) and exceeded 5 pg/g in only about 6% of the samples, with a maximum concentration of 19 pg/g. In sites selected based on regional concerns with certain discharges, 75% of the samples had no detectable TCDD, while only 10% were >5 pg/g and 2% were >25 pg/g. Greater contamination was generally observed for 29 sites sampled in the Great Lakes, where TCDD was detected in 80% of samples, exceeded 5 pg/g in 60% of the samples, and exceeded 25 pg/g in about 6% of the samples. The results for whole fish from all sites in this survey are plotted on the top portion of Figure 5.1 as cumulative percentile versus log concentration in whole fish. This plot also includes designation of the 10^{th}, 50^{th}, and 90^{th} percentiles for each of the three subsets of sites.

Fish analyses from 388 sites were reported as a result of the National Study of Chemical Residues in Fish (U.S.EPA, 1992b). This study emphasized sites influenced by point and nonpoint sources (314), but also included a sample of USGS NASQAN sites (39) and selected background sites (35). Concentrations in fish were less than 1 pg/g or not detectible in nearly 50% of the samples, exceeded 10 pg/g in about 13% of the samples, and reached a maximum of about 200 pg/g (bottom of Figure 5-1). This survey noted significant differences among classes of sites, and Figure 5-1 depicts the 10^{th}, 50^{th}, and 90^{th} percentiles for several of these classes.

U.S. EPA (1992a) summarizes results from several other studies which are largely consistent with these distributions. However, it should be noted that there are a variety of locations with contamination significantly higher than noted in Figure 5-1, which would not be expected to be included in general surveys. For example, U.S. EPA (1992a) noted concentrations reaching several hundred pg/g at some locations.

Figure 5-1. Whole fish TCDD concentration versus cumulative percentile from two national surveys (U.S.EPA, 1987; 1992b). Filled circles denote measurements on individual samples of fish. Vertical lines in cumulative plot depict one or more samples in which TCDD was below the detection limit. Diamonds on horizontal lines denote 10th, 50th, and 90th percentiles for subset of samples. Thick bars denote estimated range for low and high risk of TCDD to sensitive species in group of organisms.



Interpretation of this data must also be tempered by several considerations. First, the datasets in their entirety are not random samples of U.S. waters, but rather skewed to some extent to waters affected by anthropogenic activities. The percentiles on Figure 5-1 must be interpreted accordingly. Additionally, fish surveys inherently reflect the life history and sensitivity of fish, which can bias their use as exposure indicators for a particular site. At sites where TCDD contamination would preclude self-sustaining fish populations, fish might not be available or the sampled fish might be immigrants that do not completely reflect exposure at that site. At the more contaminated sites, fish would also be expected to reflect those species which are less sensitive and/or do not readily accumulate TCDD because of their life history and behavior. The database has not been evaluated for trends that would reflect such considerations and, in fact, the inadequacies of current understanding of species sensitivity and TCDD accumulation would make any definite conclusions difficult.

Nevertheless, these data can support some characterization of the risk of TCDD to aquatic life and associated-wildlife on a national and regional scale. Figure 5-1 depicts the TCDD concentrations in fish associated with low and high risk to sensitive fish and wildlife. The risk ranges are as developed in sections 4.2.3 and 4.3.3 and summarized in section 5.1.3.

Relative to these risk ranges, these survey data suggest that TCDD contamination is below levels of concern for aquatic life at all but a small percentage of the sites nationwide. However, there are a variety of sites in these surveys, and from other studies, where TCDD concentrations are high enough to pose significant risk to fish. This is especially true if joint action with other chemicals is considered.

For wildlife, Figure 5-1 suggests significant risk is more widespread than for aquatic life, which is expected since the effects profile developed in this report suggested piscivorous wildlife are more susceptible than the fish they consume. In particular, the low end of the risk range for mammals is exceeded in about half of the samples in these surveys. This characterization must be qualified by the uncertainties cited earlier for wildlife effects data. Furthermore, it is unknown whether sensitive piscivorous wildlife are actually or potentially present at these sites and if their diet is actually comprised primarily of contaminated fish. Such questions would need to be resolved in any specific risk assessment, but are beyond the scope of this discussion. Nevertheless, this comparison of fish survey results and wildlife effects data does raise significant concerns about the risk of TCDD to piscivorous wildlife. Research to address the uncertainties which limit current abilities to characterize risks to wildlife is clearly warranted.

5.2.2 Lake Trout Reproduction in Lake Ontario

A good example of TCDD risk characterization for a particular assessment endpoint and site is that of lake trout reproduction in Lake Ontario. This system has

been the subject of considerable study, both in documenting problems in fish reproduction and in measuring and modeling levels of TCDD and other organic contaminants. Also, lake trout have been the subject of laboratory studies on the effects of TCDD. The Lake Ontario lake trout problem has already been discussed soemwhat in section 4.2.2 and a more definitive review of this problem is the subject of a separate effort (Cook et al., 1993a). The following discussion is designed to briefly illustrate how the information and methodologies presented in this report can be applied and is not intended to be a complete discussion of this problem.

As discussed in section 4.2.2, lake trout populations in Lake Ontario declined in the early part of this century and were severely depleted by 1950, before significant contamination by TCDD and other organochlorine chemicals occurred. Commercial fishing, lamprey predation, and/or degradation of spawning habitats from conventional pollutants probably were largely responsible for the original decline. However, even after the reduction of these stresses, a stocking program failed to establish a natural-reproducing population, apparently at least in part due to blue-sac syndrome in lake trout sac fry. The association of blue-sac syndrome with chemical stress in laboratory studies raises the possibility that a chemical, or combination of chemicals, might have contributed to continued reproductive failure of Lake Ontario lake trout.

As summarized earlier, laboratory studies have established that lake trout sac fry survival is severely affected by TCDD, with a steep dose-response curve which ranges from little or no mortality at approximately 30 pg TCDD/g wet weight of egg to complete mortality at about 100 pg/g. In separate experiments with different routes of exposure, 50% mortality occurred at accumulations in the eggs of 47 to 65 pg/g. Parent fish from Lake Ontario have approximately three-fold higher TCDD concentrations than their eggs, presumably in large part due to a higher lipid content (18% in parent fish versus 8% in eggs), so the threshold concentration in eggs would correspond to about 90 pg/g wet weight in fish and the LR50s in eggs to about 140-200 pg/g in the adult fish. In addition to some experimental variability and some question on the egg/parent relationship, these numbers are also uncertain because they are based on a limited stock of fish. Experiments with different strains of rainbow trout showed over a two-fold variability in LR50s, so it is quite possible that some lake trout in natural systems are as much as twofold more or less sensitive.

Information on TCDD residues associated with toxic effects can be compared to TCDD concentrations observed in Lake Ontario lake trout and eggs. In 1987, eggs collected from Lake Ontario contained about 10 pg TCDD/g wet weight. This is about three-fold lower than the threshold cited above. Therefore, even given the uncertainties in the effect relationships, it is unlikely that lake trout reproduction is currently at risk in Lake Ontario due solely to TCDD effects on sac fry survival.

However, in the past, the impact of TCDD to Lake Ontario lake trout may have been significant. TCDD in surficial sediments and lake trout both declined two- to

three-fold from 1978 to 1988. In 1978, lake trout had average concentrations of 78 pg TCDD/g wet weight, near the threshold for effects from TCDD alone. Based on the sedimentary record, this concentration would have been several-fold higher in 1962, well above that which would have precluded successful reproduction.

Even based on the data from 1987-1988, TCDD might still be contributing to continuing Lake Ontario lake trout reproduction problems in concert with other PCDDs, PCDFs, and planar PCBs. TCDD concentrations in eggs are at about one-fifth of the LR50, which could be a significant contribution to total effects in a complex mixture. As mentioned throughout this report, risk of TCDD cannot be adequately evaluated in isolation from chemicals with which it often occurs. The joint toxicity of TCDD and related chemicals is a major uncertainty that needs to be addressed. There also is an issue of whether the measurement endpoint used here, sac fry survival in a laboratory environment, is an adequate surrogate for the assessment endpoint of interest, namely lake trout reproduction in a natural system. Other toxicological endpoints associated with reproductive physiology might be more relevant and sublethal effects on fry might also affect their survival in a natural environment. These uncertainties need to be addressed to adequately assess risk of TCDD to Lake Ontario lake trout, and to aquatic life in general.

5.2.3 Environmental Concentrations Associated with TCDD Effects

Some EPA regulatory activities, such as the establishment of water and sediment quality criteria, require the specification of environmental concentrations that are considered to represent acceptably low risk. These concentrations are set generically, and are applied to specific sites to calculate allowable discharges or set goals for remedial actions. Such procedures do not fit the risk paradigm in which effects and exposure information are combined into a statement of risk and uncertainty, after which decisions about managing risk are made (U.S.EPA, 1992c). Rather, water and proposed sediment quality criteria incorporate risk management decisions before specific exposure information is introduced into the process. Nevertheless, the setting of such criteria still involve major elements of a risk assessment, albeit an incomplete one, and the entire regulatory process includes all the elements of risk assessment, although somewhat intertwined with risk management.

In this section, the information presented earlier on effects and their relationship to exposure conditions will be used to associate concentrations in water and sediment with different levels of risk to aquatic life and aquatic-associated wildlife. This integration provides a simple, limited demonstration of how the information reviewed in this document can be used and is not intended to be a complete and definitive characterization.

Concentrations associated with two levels of risk will be calculated here. "Low risk" will be equated to the highest concentration that is unlikely to cause significant effects to sensitive organisms. "High risk to sensitive organisms" will be associated with the lowest exposure concentration that will likely cause severe effects (an EC50 or LC50, or a worse effect if a median effect concentration is not available). Risk to more tolerant organisms will not be explicitly included in this discussion. Where relevant, calculations will be based on fish with 8% lipid content and sediment with 3% organic carbon. Concentrations for other conditions can be estimated based on procedures outlined in Section 3.

Table 5-1 lists TCDD concentrations in fish associated with low and high risk to sensitive fish, mammals, and birds. These concentrations were developed in the effects profiles in section 4 and are summarized in section 5.1.3. For fish, the survival of lake trout sac fry exposed as eggs was used as the measurement endpoint. For

Table 5-1. Environmental concentrations associated with TCDD risk to aquatic life and associated wildlife.

Organism	Fish Concentration (pg/g)	Sediment Concentration (pg/g dry wt.)	Water Concentration (pg/L)	
			POC=0.2	POC=1.0
Low Risk				
Fish	50	60	0.6	3.1
Mammalian Wildlife	0.7	2.5	0.008	0.04
Avian Wildlife	6	21	0.07	0.35
High Risk to Sensitive Species				
Fish	80	100	1.0	5
Mammalian Wildlife	7	25	0.08	0.4
Avian Wildlife	60	210	0.7	3.5

mammals, the effect concentrations are based on rat reproduction studies, adjusted for the apparent greater sensitivity of the mink. For birds, the effects levels are based on a pheasant reproduction study, adjusted for the absence of a chronic exposure.

To translate fish concentrations to sediment concentrations, a BSAF of 0.3 was used for assessing risk to fish. This upper end of the BSAF range was used (rather than an explicit value for lake trout) because as far as is currently known, some sensitive fish may be at the high end of the accumulation range. For wildlife, a midrange BSAF value of 0.1 was used to reflect the fact that wildlife would consume a variety of fish with a range of accumulations. It should be recalled that BSAFs are based on a very limited database of field values and pertain to the surficial sediment layers that interact with the organisms and overlying water.

To translate risk based on fish accumulations to water accumulations, BAFs based on Lake Ontario data were used, adjusting for the possible effects of POC using the relationships developed in section 3.4 and summarized in section 5.1.2. Different BAFs were not used here for sensitive fish and for fish that are wildlife food sources because current information does not support specifying any differences. For a POC of 0.2 mg/L, equal to that in Lake Ontario, the numbers in Table 5.1 reflect a BAF_i^t of 10^6. For a POC of 1 mg/L, effect concentrations are five-fold higher to reflect the presumed greater binding by organic matter. As discussed previously, this relationship is tentative and needs further investigation.

Relative to the limited information on environmental exposures available as summarized in section 5.1.1, the environmental concentrations listed in Table 5-1 do suggest that significant risk of TCDD to aquatic life and associated wildlife can be expected in some situations. This table also exemplifies the higher risk expected to occur for piscivorous wildlife that are exposed to TCDD contaminated fish. To better characterize these risks, improvements in data and methodologies are needed to reduce the uncertainties discussed thus far in this report.

6. RESEARCH NEEDS FOR REDUCING UNCERTAINTIES

Throughout this report it has been emphasized that there are important and immediate uncertainties associated with characterizing the risks of TCDD to aquatic life and associated wildlife. As outlined below, these uncertainties lead to a number of major research needs regarding both TCDD exposures to and effects in aquatic ecosystems. Consistent with the objective of this interim assessment to focus on TCDD only, the following discussion does not address needed studies to evaluate the appropriateness of using existing TEF values, based on human health and other toxicological endpoints, for quantifying aquatic life and wildlife effects resulting from exposures to mixtures of PCDDs, PCDFs, and PCBs. The following discussion also does not address the development of population and community level models and their linkage to toxicological inputs. Clearly, the development of such models are needed for improving ecological risk assessments of chemical stressors in general.

6.1 EXPOSURE

6.1.1 Octanol/Water Partition Coefficient

The interpretation and application of data on the partitioning of TCDD in test systems and natural ecosystems depend in part on estimates of K_{ow} for TCDD. As discussed in section 2, uncertainty in this parameter leads to uncertainty in extrapolating bioaccumulation information and estimating the bioavailable fraction of TCDD in water. Current understanding of the role of organic carbon on the partitioning and bioavailability of TCDD from water and sediment is primitive. Improved estimates of K_{ow} and its relationship to TCDD partitioning onto natural organic matter would reduce these uncertainties.

6.1.2 Detection Limits and Water Concentrations in Natural Systems

Typical analytical procedures are inadequate to reliably measure TCDD in water at the low concentrations expected to elicit ecological effects. This analytical limitation is especially true for dissolved TCDD. As a consequence of this analytical deficiency, there are few reports which quantify TCDD in natural waters and the values reported are uncertain. A high priority should be given to applying new techniques and instrumentation to lower the detection limit for TCDD in water samples. With the improvement of analytical techniques, total and dissolved TCDD should be measured in a variety of aquatic systems to better establish current and future exposures.

6.2 BIOACCUMULATION

Reliable BAFs for TCDD based on measurements of water and biota in natural systems are essentially nonexistent, which makes TCDD concentrations in fish tissues difficult to predict in terms of TCDD concentrations in water or surficial sediments.

Measurements of TCDD in surficial sediment, biota and water are needed to improve the database on BAFs, BSAFs and BSSAFs. In gathering such data, particular attention should be given to organism attributes (e.g., lipid content) and water column properties (e.g., particulate and dissolve organic matter) which might alter accumulation.

6.3 EFFECTS

6.3.1 Occurrence of Ah Receptor

Available toxicity data suggest that aquatic invertebrates and amphibians are much less sensitive to TCDD than fish, perhaps due to the absence of the Ah receptor, or a comparably sensitive receptor. A more rigorous assessment is needed to determine if the Ah receptor is present in these taxa, as well as taxa not tested, such as reptiles. The tissue distribution and appearance of the Ah receptor during embryo and fry development must be determined in order to understand the TCDD mode of action in fish. Based on the results of such studies, specific toxicity testing on selected species could be undertaken to insure that current conclusions on interspecies differences in TCDD sensitivity are valid.

6.3.2 Aquatic Life

Current data suggest fry survival as the most critical endpoint for fish; however, the current threshold values are based on exposures of limited durations in a few species. As a consequence, there is uncertainty regarding the species sensitivity distribution within fish, as well as the impact of chronic exposures on reproduction. At a minimum, additional early life stage tests with several fish species are needed to establish a better sensitivity distribution for fish. There is also a need to determine whether there are more sensitive endpoints than larval fish survival. Partial life cycle tests should be conducted on at least two fish species in which chronic exposures substantially precede the onset of reproduction. Effects on reproduction, early life stage development and immune response should be examined in long-term exposures to identify more sensitive chronic endpoints. Associated with such testing, TCDD accumulation should be monitored to establish the basis to characterize risk with increased certainty. These efforts would provide the basis to establish biologically-based/dose-response models and thereby improve extrapolations across fish species and exposure scenarios.

There is little available TCDD toxicity data for aquatic invertebrates and virtually no information on the relationship of toxic effects on these organisms relative to TCDD accumulation. Before reaching a final conclusion regarding invertebrate sensitivity, additional long-term toxicity tests on a diverse set of aquatic invertebrate species should be conducted to establish their sensitivity relative to that of fish and to identify sensitive chronic endpoints. Additional studies will need to be conducted to define the

relationship of these endpoints to TCDD accumulation. As mentioned previously, such testing should be integrated with Ah receptor analyses.

6.3.3 Wildlife

With both the mammalian and bird risk characterizations, there was a lack of quality reproduction bioassays and toxicokinetic information to establish well-defined dose response relationships. For the mammalian assessment there were no reproduction bioassays available for a representative piscivorous wildlife species (e.g., the mink) and therefore this characterization was based on an extrapolation of bioassay results from rat and Rhesus monkey tests. For the bird assessment, a ring-necked pheasant reproduction bioassay of limited duration and incorporating an i.p. exposure regime was the only study available, although some limited toxicokinetic data and *in ovo* toxicity studies were incorporated in the analysis. Finally, there are apparently no data available to assess the toxicity of TCDD to reptiles.

A long-term (i.e., one generation) feeding study with mink would provide data to more adequately assess reproductive and developmental effects. This bioassay should be supported by toxicokinetic studies and Ah receptor investigations to develop a biologically-based/dose-response model to better establish critical parameters in species extrapolations and in linking TCDD accumulation to toxic effects. A long-term (i.e., one generation) feeding study with birds would also support a more certain assessment of reproductive and developmental effects. This bioassay must be supported by toxicokinetic studies and Ah receptor investigations to better establish the delivered dose to both the adults and developing embryos. Again, these studies would contribute to a biologically-based appreciation of species extrapolation and TCDD accumulation. These studies should also be designed in such a way to better quantify the uncertainties of using egg injection studies as a source of toxicological data in avian hazard assessments.

6.3.4 Epidemiology

Finally, there is a need to assess TCDD concentrations in aquatic life and wildlife in appropriately selected natural systems. Such studies would further refine and validate the reliability of relationships between TCDD accumulation and toxic effects that have been established from laboratory investigations and epidemiological studies in Lake Ontario and British Columbia. Future investigations should focus on TCDD as well as chemicals with a mode of action similar to TCDD.

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