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A Physiologically-Based Pharmaco-Kinetic Model For Disposition Of Dioxins And Furans In Fish

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A PHYSIOLOGICALLY-BASED PHARMACO-KINETIC MODEL FOR DISPOSITION OF DIOXINS AND FURANS IN FISH

By

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Author's declaration

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ABSTRACT

A PHYSIOLOGICALLY-BASED PHARMACO-KINETIC MODEL FOR DISPOSITION OF DIOXINS AND FURANS IN FISH

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Master of Applied Science in: Environmental Applied Science and Management

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A Physiologically Based Pharmacokinetic (PBPK) model was developed for the disposition of dioxins in various fish species. The model was developed based on available information on the mechanisms of uptake, distribution, storage and elimination of dioxins in various species (other than fish) and empirical data on disposition of dioxins in the fish tissues.

Two versions of the model were implemented: one for exposure to dioxins in water through the gill and the other one for exposure through food. Model compartments included the gill, kidney, liver and other richly-perfused tissues, as well as fat and other slowly-perfused tissues. In the food exposure version, the gut was also included as a richly-perfused tissue.

The water exposure model was calibrated using two independent data sets for exposure of fathead minnow and medaka to 2,3,7,8-TCDD in water. The estimated parameter values in the two data sets were comparable and the predictions agreed with the observations very well. The results were compared to those produced by the default methods (bioconcentration factors). Uncertainty in the model prediction as a result of variability in input parameters was also discussed for the parameters with the highest impacts on the model outcome.

The predictions of the food pathway exposure model were compared to data for rainbow trout liver.

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- Miss Sara Parhizgari

Dedication

I would like to dedicate this work to my husband Sasan Parhizgari, without his patience and support, I would not have been able to withstand graduate school.

Acronyms

AhR	Aryl hydrocarbon Receptor
ANOVA	Analysis of Variance
BCF	Bioaccumulation Factor
BSAF	Biota-Sediment Accumulation Factor
BSR	Biota-to-Sediment Ratio
CCME	Canadian Council of Ministers of Environment
CYP1A	Cytochrome P4501A
EC	Environment Canada
EC(#)	Effect Level
EROD	Ethoxyresorufin O-deethylase
FAO	Food and Agriculture Organization (of the United Nations)
FAST	Fourier Amplitude Sensitivity Test
GLWQA	Great Lakes Water Quality Agreement
HPA	Health Protection Agency
HSL	Health and Safety Laboratory
IRIS	Integrated Risk Information System
LaMP	Lakewide Management Plan
LC	Lethal Concentration
LD	Lethal Dose
LOEL	Lowest Observable Effect Level
MC	Monte Carlo (Analysis)
MOE	Ontario Ministry of the Environment
NHMRC	(Australian) National Health and Medical Research Council
NOEL	No Observable Effect Level
PBPK	Physiologically-Based Pharmacokinetic
PCB	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-p-dioxins
PCDF	Polychlorinated Dibenzo-Furans
RfC	Reference Concentration
TBDD	Tetrabromodibenzo-p-dioxin
TCDD	Tetrachloro-dibenzo-p-dioxin
TDI	Tolerable Daily Intakes
TEC	Toxicity Equivalence Concentration
TEF	Toxic Equivalency Factor
TEQ_{mam}	Toxicity Equivalent (mammalian)
TK	ToxicoKinetic
TMI	Tolerable Monthly Intakes
TRG	Tissue Residue Guideline
US EPA	US Environmental Protection Agency
VBGF	Von Bertalanffy Growth Function
WHO	World Health Organization

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1 INTRODUCTION

1.1 BACKGROUND

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) commonly known as dioxins and furans, are toxic, persistent and bioaccumulative chemicals and predominantly result from human activities such as the production of chemicals and the metallurgical industry.

A Federal-Ontario assessment has estimated the average daily multimedia exposure (from air, water, food, soil, consumer products) of adult Canadians to dioxins and furans to be about 2 to 4 picograms of 2,3,7,8- tetrachloro-dibenzo-p-dioxin (TCDD) toxic equivalents per kilogram of body weight (MOE 1997). Because of their extraordinary environmental persistence and capacity to accumulate in biological tissue, dioxins and furans are scheduled for elimination under the Canadian Environmental Protection Act, the Federal Toxic Substances Management Policy, and the Canadian Council of Ministers of Environment (CCME) Policy for the Management of Toxic Substances (CCME 2001).

Contaminated fish is one of the major ingestion-based exposure sources for dioxins and furans, whereas other pathways such as drinking water and dermal absorption (from physical contact with contaminated soils or consumer products) account for far less exposure (MOE 1997). Based on measurements of dioxins and furans, mostly at freshwater sites with a history of contamination or with a known source of dioxins and furans, fish and invertebrates had contaminant levels ranging from undetectable to 112 nanograms of dioxins and furans TEQ_{mam} per kilogram of wet weight (EC 2005).

Fish consumption advisories are therefore issued by the Ontario Ministry of the Environment in order to advise consumption restrictions on sport fish to limit the uptake of dioxins and furans (and a number of other chemicals). Based on the 2008. Guide to Eating Ontario Sport Fish (Government of Ontario 2008), of the total number of fish consumption advisories issued for sport fish caught in the four Ontario Great Lakes, their connecting channels and inland locations, the following percentages were related to dioxin, furan, and other related compounds (dioxin-like PCBs):

- Lake Superior: 91%
- Lake St. Clair and St. Clair and Detroit rivers: 79%
- Lake Erie: 98%

- in Lake Ontario (including Niagara and St. Lawrence rivers): 92%
- Inland locations: 85%

The advisories issued by the MOE are based on guidelines developed by Health Canada which in turn, through research and review of toxicological data, have determined safe dosages for an extensive list of contaminants. These amounts are referred to as tolerable daily intakes (TDI) or tolerable monthly intakes (TMI). The TMI adopted for dioxins is 70 pg/kg body weight/month, and is based on the World Health Organization's guidelines. This TMI is the mid-point of a range of TMIs for PCDDs, PCDFs and coplanar compounds and has been derived based on scaling the doses from animals to humans. The animal dose used in this derivation was established based on observations of animal threshold response to the administered chemical. The scaling was based on body burdens and application of a safety factor of 9.6 to account for interspecies differences and the conversion of the Lowest Observable Effect Level (LOEL) to the No Observable Effect Level (NOEL).

Derivation of a NOEL is highly dependent on dose selection and number of test animals, and does not include the range of dose-response data (Kim et al. 2002). Another challenge in using NOEL is that in toxicological studies, animals are usually exposed to extremely high concentrations of test chemicals by unusual routes of exposure for their entire lifetime. Extrapolation to another species (e.g. human) therefore, entails four conversion steps: from high to low does, one species to another, one route of exposure to another, and from constant concentration to a discontinuous exposure (or vice versa) (Andersen 2003). In addition, environmental factors (e.g. temperature, oxygen, pH), which can have significant effects on the disposition of chemicals in animals are not incorporated in the use of NOEL (Barron et al. 1990).

These extrapolation methods also underestimate uncertainty as they use a large number of policy decisions or professional judgments incorporated into the methodology as exact values with no estimate of error (Fredrick 1993).

Evaluation of effective or tissue dose under a variety of conditions is possible using understanding of modes of action of xenobiotics, which provides insight into both the sites/mechanisms of action and the form of the xenobiotics causing the response. Uncertainties can also be decreased by improvement in the scientific basis used to describe the toxic mechanisms and impacts.

Physiologically-Based Pharmacokinetic (PBPK) models can describe the chemical disposition in the body on the basis of fundamental information about physicochemical properties, transport, metabolism, and various excretory mechanisms (US EPA 2006b). They

support the low-dose and interspecies extrapolations, which are important components of risk assessment. PBPK models also provide better methods for calculating target tissue dose metrics for use in risk assessment. Therefore, using the PBPK model for tissue dose calculations provides a direct link between contaminant exposure and hazard.

The predictive capacity of PBPK models makes it possible to estimate the time course behavior of chemicals in species without direct experimentation, by using the knowledge of more fundamental physiological and biochemical processes. These predictions are testable. If model predictions do not fit the experimental data, the biological or kinetic basis of the model can be revised (Andersen 2003). PBPK models incorporate metabolism data from in vitro and in vivo studies and in contrast to the whole-animal accumulation models, PBPK models consider metabolisms localized to tissues and organs where the reactions occur (Nichols et al. 2004).

The application of PBPK models has been increasing in the scientific community since their first introduction in the 1970's. As of 2001, 700 papers were identified on the application of PBPK models in toxicology and risk assessment. The 2000 U.S. EPA reference concentration (RfC) documentation for vinyl chloride in the Integrated Risk Information System (IRIS) describes and uses a PBPK/PT model for standard setting and dose route extrapolation. In 1996, the U.S. Hazardous Air Pollutant Rule encouraged use of validated PBPK models for extrapolation across dose routes instead of new tests (Andersen 2003).

Although most PBPK models have been developed for mammals, progress has been made towards models for non-mammalian invertebrates including fish. Simplified models were adapted for small fish, which contribute to the aquatic toxicology databases. Improvements in the understanding of chemical exchange across fish gills supported development of PBPK models for disposition of waterborne chemicals in fish (Nichols et al. 1994).

Several authors have developed PBPK models for disposition of dioxins and furans in rodents (Leung et al. 1988, Pland et et. 1989, Kohn et al. 1993, Anderson et al. 1993 and 1997, Wang et al. 1997, Wang et al. 2000 and Kohn et al. 2001). One of the main concerns in risk assessment for exposure to dioxins and furans is their interactions with hepatic cytochrome P450 enzymes. PBPK models have successfully explained these interactions for a number of test animals (mostly rodents), by modeling dioxin binding to the Ah receptor and binding of the Ah receptor-dioxin complex to promoter sites on DNA, which can enhance the rate of gene transcription. These findings have been consistent across species, which increase the confidence in extrapolating the model to other species (Wang et al. 2000). The response to the binding mechanism is especially important in low doses and implication of this response in

should be more carefully examined and considered in regulatory decision making (Anderson 2003).

Assessment of exposure of human to dioxins through fish consumption can be enhanced by the understanding of the distribution and transfer of dioxins in fish and accumulation through the food chain. In addition, the application of PBPK model for aquatic species in a manner consistent with that used for mammalian species will allow for a better understanding of inter-species differences in disposition of these chemicals. Models developed for aquatic species have the potential to be adapted for human modes and environmental risk assessment (Barron et al. 1990).

1.2 Objectives and Scope

This thesis proposes a model for toxicity mechanisms of dioxins in fish in the form of a PBPK model. The model developed in this study was based on available information on the mechanisms of uptake, distribution, storage and elimination of dioxins in various species (other than fish). Model parameters not available in the literature were calibrated using available measurement data and limited published data.

Sensitivity of the model to input parameters was addressed, and parameters with the largest impacts on the results were identified. The model results, which are time-course of concentrations in whole body fish and in individual tissues, were also compared with results using alternative default approaches. Uncertainty in model predictions as a result of variability in input parameters was also discussed.

1.3 Thesis Structure

This research covers a variety of disciplines that needed to come together for analysis of this subject. The disciplines included chemistry (of dioxins and furans), biology (of fish), chemical and biological pathways, toxicity assessment (default approaches, PBPK models), model development, and uncertainty analysis. Each one of these subjects is discussed in proper detail in a section. Figure 1-1 provides an outline of the thesis structure.

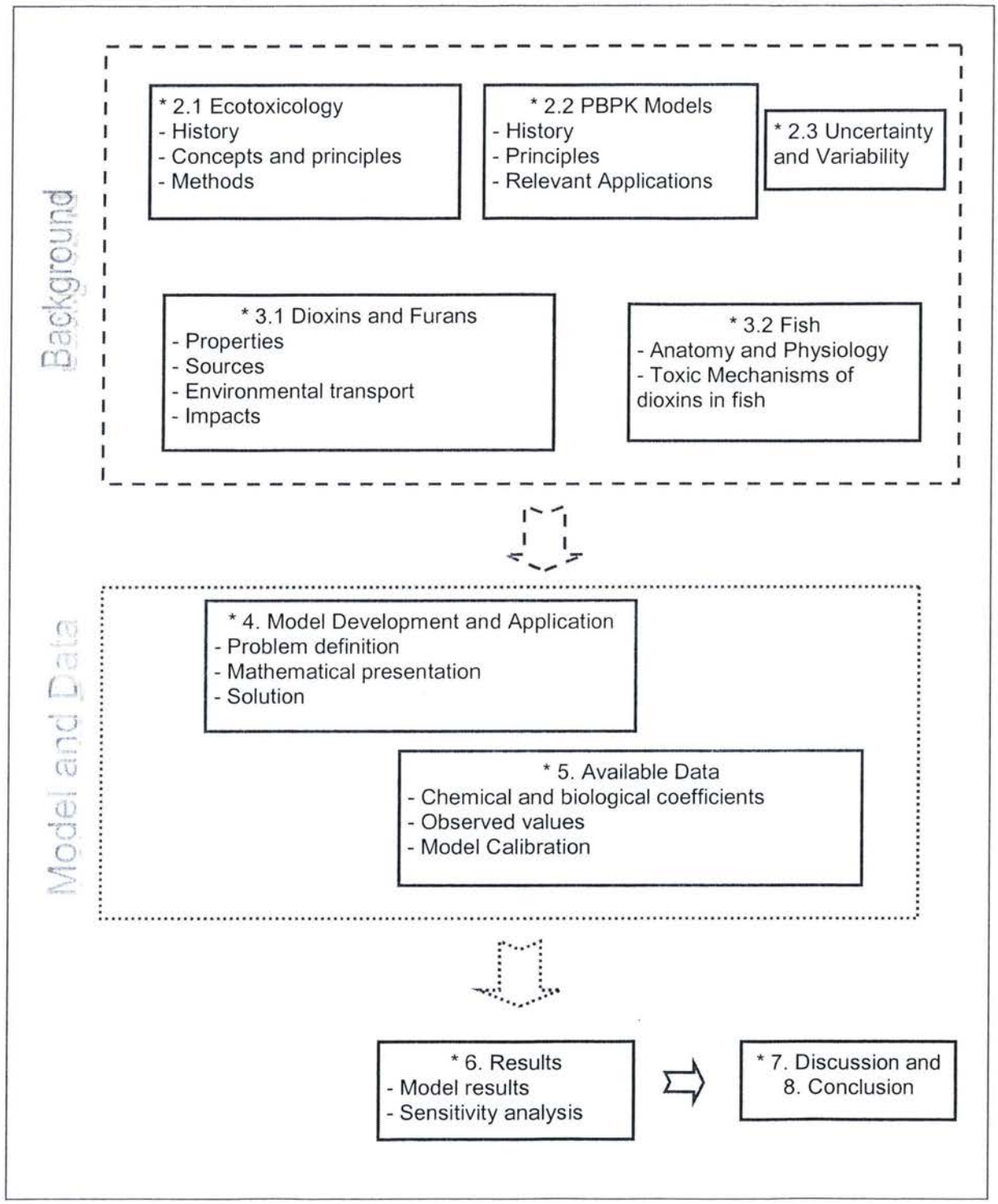

Figure 1-1: Thesis Structure

2 CONTEXT

This section provides a background for the discipline of ecotoxicology and its methods especially that of Physiologically-Based Pharmaco-Kinetic Modeling. Other background materials provided to complement and support the application of such modeling include a brief discussion of the policy making process (how the knowledge is applied), and of the uncertainty and variability analysis (how well the model is performing).

2.1 ECOTOXICOLOTY

2.1.1 History

Ecotoxicology originates from ecology and toxicology and it involves many other disciplines such as chemistry (inorganic, organic, physical, analytical, bio), sedimentology, hydrology, biogeochemistry, geography, climatology, geology, oceanography, molecular genetics, oncology, immunology, pharmacology, anatomy, population biology, microbiology and neurology (McCarthy 2007).

The discipline of ecotoxicology was first defined in 1969 by Rene Truhaut as "the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context "(Truhaut, 1977). Carins and Mount (1990) define it as "the study of the fate and effect of toxic agents in ecosystems".

The first indication of development of ecotoxicology is a book published in 1815 by Spaniard M.J.B. Orfila, *Traité des Poisons Tirés des Règnes Minéral, Végétal, et Animal, ou, Toxicologie Générale Considérée sous les Rapports de la Physiologie, de la Pathologie, et de la Médicine Légale*[1], on harmful effects of chemicals on organisms. In his book, Orfila examined the relationships between chemicals present in the body and observed poisoning symptoms. As a formal discipline, toxicology was introduced in 1880s when the organic chemistry began to develop. By the 1920s, toxic effects of food additives, drugs and pesticides were studied systematically in lab animals (McCarthy 2007). These studies were designed to protect humans using surrogate species (e.g. monkeys, dogs, mice, rats). Ecotoxicology, on the other hand,

[1] Which can be translated to English as "Treaty of poisons in the areas of mineral, plants and animal studies, or general toxicology considered under the relationships between physiology, pathology and legal medicine"

deals with many diverse species where experiments are conducted on the species of concern (except for human).

2.1.2 Basic Concepts and Principles

A toxicant is an agent that can produce an adverse response in a biological system. Toxicity, which is the agent's potential to harm the living organism, can only be measured using the living organism. Toxicity is a function of the organism (exclusion mechanisms, life stage, body size, etc), concentration and properties of the agent and condition of exposure.

2.1.2.1 Fate of Pollutant

The properties of an agent affect how it moves in the environment and in individuals. Physiochemical factors such as polarity and water solubility, hydrophobicity, vapour pressure, Henry's Law constant and molecular stability are very important (McCarthy 2007). Dioxin, a non-polar organic solid, is not very water soluble and is partitioned out of water. Its octanol-water partition coefficient (K_{ow}), which is a measure of polarity, is therefore very high. Based on Oost et al. (1996) the logarithm of 1-octanol-water partition coefficients for dioxins and furans is between 6 and 8. Zheng et al. (2003) observed log K_{ow} for various dioxins ranges from 4.3 to 8.2 with an average of 6.5. Molecular stability of dioxins is also high and it does not readily undergo chemical transformation.

While exposed to a chemical, a species can uptake, store or distribute, metabolize and/or excrete the chemical. The uptake is defined as movement into/onto the organism and it may be through dermis, gills, pulmonary surfaces or gut. If a xenobiotic is inert, it may be stored in the organism without further action. Storage occurs mostly in fat depots, membranes and lipoprotein. After uptake, the xenobiotic can be transported to different sites by blood or lymph. Movement into tissues can be through diffusion across membrane for highly lipophilic chemicals (with high K_{ow}). Active chemicals may be metabolized by enzymes and be detoxified and also may cause activation of the biological system. A chemical can interact with endogenous macromolecules or structures and lead to toxic manifestations. Excretion may involve the original chemical or the biotransformation product, the water-soluble metabolite or conjugate (McCarthy 2007).

2.1.2.2 Toxic Action

In general terms, the mode of toxic action is a set of biochemical, physiological and behavioral indicators that characterize an adverse biological effect. These are specifically

important in characterizing impacts of mixtures of chemical stressors, where a classification scheme for modes/mechanisms can help define a mixture's dose and/or response (McCarty 2002).

After a chemical enters a biota, two major mechanisms may activate; protective and non-protective. The protective mechanisms can reduce concentration of the chemical through enzymatic activity (e.g. oxidation by mixed function oxydase), binding to another molecule for excretion or storage and release of stress proteins that repair the damage. The non-protective mechanisms may or may not lead to toxic manifestations. Examples include inhibition of acetlcholinesterase and formation of DNA adducts[1]. Other mechanisms also exist such as those involving mitochondrial poison and vitamin K antagonists (McCarthy 2007).

2.1.2.3 Dose- response Relationship

A causal relationship must be reasonably established between the observed effect and the presence of toxic agent in order for an agent to be considered toxicant. This is known as the principle of causality (McCarthy 2007), and can be depicted in ways such as a dose-response relationship.

Dose-response assessment determines the relationship between the amount of exposure and the probability of the adverse effect (Willms & Shier Env. 2008).

The measurement of dose is different at different levels of biological organization. At the top (the ecosystem), the dose is estimated from total amount emitted. In the middle (individual organisms), the dose can be an exposure or a received dose. Exposure can be defined by common metrics such as LC50 (concentration at which 50% of individuals react) for a specific duration, exposure medium, route of exposure, and whether it relates to total or bioavailable fraction of the compound. The received dose relates to the body residue, which can be the level at which the effect occurred or the area under the residue-time curve, both of which may be corrected for lipid levels. At the bottom of the organization level (site of toxic action), the dose can be defined as concentration at site, degree of binding or percent occupation (McCarty 2002).

The response measurement also varies within different levels of biological organization. The response can be characterized from various perspectives, such as acute or chronic, lethal or non-lethal, effects on mechanisms (such as affecting reproduction, mutagen, teratogen,

---

[1] A DNA adduct is an abnormal piece of DNA which is covalently bonded to a cancer causing chemical.

carcinogen, enzyme inhibitor, hormone mimic), epidemiology (such as behavior, IQ) and ecology (such as diversity, integrity, growth, stress, energy flow). The challenge is that these cannot be readily converted or compared to each other (McCarty 2002).

Challenges in application of this method are (i) selection of a dose metric which is both easy to measure and useful and valid and (ii) selection of a response metric that is detectable and quantifiable in terms of significance of adversity (McCarty 2002).

2.1.2.4 Aquatic Ecotoxicology

Aquatic biota have been used for many decades to investigate the impact of chemical agents. Fish, often as the top predator of the aquatic ecosystem and as a source of high quality protein for many humans, has been of special interest. However, ecotoxicological testing of fish can be complicated by the chemical route of exposure (water, sediment, food chain) and variation in behavior, reproduction, etc. between species.

Fish experiments can be conducted in the field or in a lab. Experiments in field microcosms have been conducted to discover the reason for fish decline or to assess the impact of known stressors present in water. A prominent example of field studies is the one conducted by Schindler et al. (1974) on eutrophication of pristine lakes in Northwestern Ontario. These experiments, although more realistic, have many limitations such as controlling variables and monitoring and replication difficulties. Lab-oriented toxicity studies focus on direct toxic effects on individual species under controlled conditions. These are very important for testing new chemicals (McCarthy 2007).

Environmental conditions can affect the transfer of organic contaminants to organisms and include water temperature, pH, salinity, oxygenation, exposure concentration and duration, dissolved organic matter and particulates and water quality (Nowell et al. 1999).

2.1.3 Methods of Toxicity Assessment

Toxicological processes can be studied at various levels of detail.

The first level is when the organism is exposed to a (bioavailable) toxicant and endpoints such as LD/LC50 are used to assess the toxicity. The conventional or default toxicity assessment methods focus on this level, where lab-oriented studies are conducted and toxic impacts are defined using individual experiments or by integration of a range of studies (McCarthy 2007). Tests such as LD/LC50 and inter or intra-species extrapolation are extensively used in regulatory activities.

Bioaccumulation models have also been useful in exposure assessment through establishing correlations between a measure of bioaccumulation (e.g. Bioaccumulation Factor or BCF in fish) and physical and chemical properties of the xenobiotic agent (e.g. its K_{ow}, molecular connectivity index, molecular weight and soil sorption coefficient). Such correlations are useful but have limitations, e.g. they do not include metabolism of chemicals (Nowell et al. 1999).

Toxicokinetic models study the uptake, distribution, metabolism and elimination of the toxic agent by the organism. In a dynamic sense, they can predict the time course of biological response when the agent reaches the toxic action site, interacts with receptors and produces an effect (McCarthy 2007).

Kinetic models can be compartment or physiologically-based. Compartmental models help understand the chemical accumulation in tissues and organs. They are, however, limited in application as a new empirical set of parameters needs to be fitted for each new compound, species and exposure route. Physiologically-based kinetic models on the other hand, define the organism in terms of its anatomy, physiology and biochemistry, which leads to a better understanding of the disposition and toxicity of a chemical. This can provide a sound basis for extrapolation between species and over a wide range of conditions (Nichols et al. 1990).

2.2 Physiologically-Based Pharmaco-Kinetic Modeling

2.2.1 History

Pharmacokinetics (PK) is the study of the biological processes that affect the absorption, distribution, metabolism and excretion of a substance, such as a drug or a toxicant in an organism (US EPA 2006b).

Pharmacokinetic modeling in general has a rich history. Data-based PK compartmental models were first developed in the 1930's. They were expanded in the 1960's and 1970's in order to accommodate new observations of dose-dependent elimination and flow-limited metabolism. In the 1970's PBPK models were developed to evaluate the metabolism of volatile compounds of occupational importance. At this time, dose-dependent processes in toxicology were included in PBPK models in order to evaluate the conditions where metabolic and elimination processes resulted in non-linear dose response relationships. In the 1980's, insights from chemical engineers and occupational toxicology were combined to develop PBPK models to support risk assessment with methylene chloride and other solvents. In the 1990's a significant increase occurred in risk assessment applications of PBPK models and in applying

sensitivity and variability methods to evaluate model performance. Some of the compounds examined in detail include butadiene, styrene, glycol ethers, dioxins and organic esters/aids (Andersen 2003).

Although the PBPK models have been successfully implemented to extrapolate kinetic data between mammals (e.g. by King et al. 1983, Lutz et al. 1984, Ramsey and Anderson 1984, all three cited by Nichols et al. 1991), until early 1990's the applications to fish were limited to models adapted from mouse models describing the kinetics of compounds injected intravenously (Nichols et al. 1990). Gill description and elimination mechanisms were included in later models. In 1990, Nichols et al. developed a PB-TK model for uptake and disposition of waterborne organic chemicals in fish. The model was parameterized using information from the published data, and it accurately simulated the uptake of pentachloroethane in rainbow trout. Nichols and his co-authors further evaluated this model using two other compounds in 1991.

PBPK models are designed to estimate an internal dose of a proposed toxic compound to a target tissue(s) or to define some appropriate surrogate dose metric for a target tissue dose. The choice of an internal dose metric is based on the understanding of the chemical's mode of action. The internal dose metric (sometimes called the biologically effective dose) is used instead of the administered dose, which is usually used in the derivation of the quantitative dose-response relationship. Replacing the administered dose by the internal dose metric reduces the uncertainty inherent in risk assessments based on an applied dose. This reduction in uncertainty and the improved scientific basis for the dose-response value are the main advantages of PBPK models and for the growing interest in their use. PBPK models also can simulate an internal dose from postulated exposure conditions where no data are available. In other words, the PBPK models can extrapolate to conditions beyond those of the data set used to develop the model (US EPA 2006a).

2.2.2 Principles

In PBPK models, the compartments correspond to discrete tissues or groups of tissues with defined volumes, blood flow rates, and pathways of metabolism of the test chemical. Relevant biochemical and physical chemical constants for metabolism and distribution are incorporated directly in the descriptions of each tissue compartment. Routes of exposure are included in their relationship to the overall physiological structure. The exposure scenarios are accounted for in the time sequence of the dose input terms. Each compartment (i.e. organ or tissue) in the model is described by a mass-balance differential equation, and the set of equations is solved by

numerical integration to predict time-course concentrations of the test chemical and its metabolites in the tissue (Andersen 2003).

Figure 2-1 shows an idealized approach to PBPK modeling, where tissue compartments are defined, equations and constants are identified and predictions are compared against observed values.

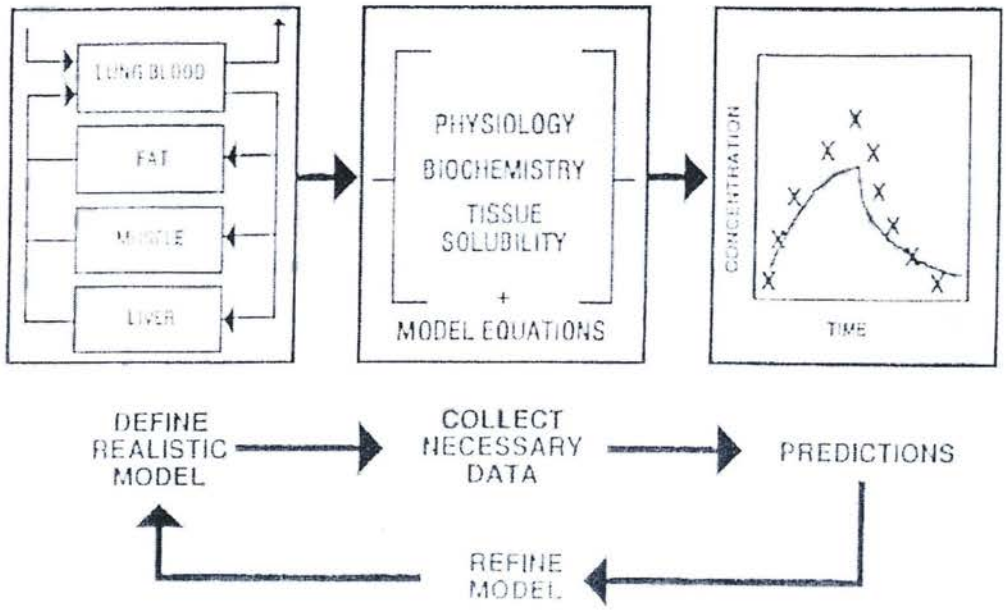

Figure 2-1: Idealized Approach to PBPK Modeling

Reference: Andersen (2003)

2.2.3 Applications

PBPK models have been developed for a wide range of chemicals. Most of these chemicals distribute systemically within the body and cause systemic effects (US PA 2006a).

Since the physiologically- based pharmacokinetic models quantitatively describe the chemical disposition in the body on the basis of fundamental information on physicochemical properties, transport, metabolism, and various excretory mechanisms, these models can aid in scaling across species and doses (Wang et al. 1997). Other applications of PBPK models include route-to-route extrapolation, an estimate of the response from unsteady exposure conditions, an estimate for population variability and high-to-low dose extrapolation. PBPK models are also useful for converting applied dose to tissue dose, estimation of tissue dose

from multi-route and multi-media exposure, and relating biomarker data[1] to tissue dose (US PA 2006a).

For dioxin-furan risk assessment, the use of a PBPK model reduces the reliance on default assumptions and methodologies. The model can reliably predict dioxin distributions throughout the body, and incorporate pharmacokinetic and pharmacodynamic events subsequent to the absorption and distribution of dioxins (Kim et al 2002).

Several PBPK models for TCDD and related compounds have been reported since 1980. Early PBPK models examined the disposition of PCBs and PCDFs by taking into account the role of lipid solubility in tissue uptake. In the early 1990's models were expanded by including induction of hepatic binding protein in response to interactions of TCDD, the Aryl Hydrocarbon (Ah) receptor and DNA binding sites. In addition, membrane transfer resistance was taken into account (Wang et al 1997).

Leung et al. in 1988 used an alternative approach to examine liver and fat dioxin concentrations in mice. Quantitative physiological models were used to examine the role of the Ah receptor in accounting for the accumulation of dioxins in the liver. The results showed that despite its very high affinity, Ah receptor binding could not account for dioxins sequestration in the liver at dosages used in the earlier studies. In 1996, a generic toxicokinetic model for TCDD was proposed based upon the assumption of pseudoequilibrium between tissue and blood in humans, with the distribution of TCDD in a specific tissue depending on the fraction of lipid in that tissue. Wang et al (1997) improved the previous models with the time-course tissue distribution data obtained from studies on rats. They developed parameter values such as permeability (Wang et al 1997).

Only one study was located in which a PBPK model was developed for disposition of dioxins in a fish species. Nichols et al. (1998) proposed a model for maternal transfer of 2,3,7,8-TCDD in brook trout. This model considered dietary uptake and was evaluated by comparing results to measured data. The comparison suggested that measured whole body residues could be used to estimate residues in developing ovaries within a factor of two.

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[1] Biomarkers are defined as "anatomic, physiologic, biochemical, or molecular parameters associated with the presence and severity of specific disease states... [which are] detectable and measurable by a variety of methods including physical examination, laboratory assays, and medical imaging" (Jenkins 2008)

2.3 Uncertainty and Variability Analysis

2.3.1 Need for Analysis

In order for a model to be applicable in the real world, one should substantiate that it behaves with satisfactory accuracy. In addition, in risk management based on decisions informed by a model, information is needed on uncertainty and variability. Uncertainty analysis is required to reflect the level of confidence in model predictions. It is particularly important to assess the uncertainty associated with the aspects that are untested (US EPA 2006a). Variability analysis is required to represent the degree to which predictions may differ across a population (Barton et al. 2007). In models of biological systems, there is some variance in the estimated model parameter values due to both biological variation, or experimental and model error.

In default approaches for toxicity assessment, uncertainty is usually dealt with by using an uncertainty factor such as 10 fold or another arbitrary number to account for a variable parameter. PBPK models, on the other hand, have the ability to use diverse data sets from multiple sources to make predictions and characterize uncertainty and variability for model parameters and predictions (ibid). With the PBPK approach, uncertainties in the model structure, knowledge of parameter values, and their impact on risk estimates can be evaluated quantitatively. Such evaluations are very difficult to interpret when default approaches to risk assessment are applied.

The international workshop on Uncertainty and Variability in PBPK Models (Research Triangle Park, NC, 2006) emphasized characterizing uncertainty and variability should become a standard practice in PBPK modeling and risk assessment. Key scientific issues were organized into the topical areas of model specification, calibration and prediction, as discussed below.

2.3.2 Uncertainty in Model Specification

The model specification is defined as "the process for determining the structure and level of complexity needed in a particular model application" (Barton et al. (2007). If discrepancies appear between available data and model predictions, then better biological understanding may be needed to improve model performance by changing the model structure. The structural change in the model can be related to the route of exposure, fate of the compound in the organism (in various compartments), the organisms life stage(s) and the time scale.

The initial structure can be determined using existing understanding of a system. The structure can be modified to reduce discrepancies between empirical data and the model predictions.

DeWoskin (2007) summarized the considerations that Clark et al. (2004) suggested for structuring a model, including the appropriate tissue compartments, relevant physiological and biochemical parameters, presence of cross-chemical analogies and chemical-specific characterization, life-stage considerations, and the appropriate exposure scenarios.

2.3.3 Model Calibration and Error

Calibration is defined by as "the process of obtaining values for model parameters that allow the model to reasonably approximate the data" (Barton et al. 2007). Optimization methods are used to fit the data and include iterative processes of adjusting parameter values to minimize the error, where error is defined as the "distance" between model predictions and empirical data. Typically only a selected few parameters are adjusted since the larger the number of parameters, the more difficult the calibration becomes.

Calibration results can be compared with predictions for a surrogate dose metric based on experimental data (e.g., blood concentration), which are less uncertain, or with predictions based on default approaches. However, those approaches (e.g. allometric scaling or uncertainty factors) provide little information about the uncertainty and variability.

Some authors suggest the adjustment (or evaluation) process should be completed in two stages: calibration (or validation) with some of the available data, and verification with the rest. Balci (1997) makes the distinction that "model validation deals with building the right model, whereas model verification deals with building the model right"

Whether available data should be separated into two data sets may depend on the availability of sufficient data and the evaluation approach chosen. In principle, statistical tools (e.g., measures of goodness-of-fit, cross-validation) exist to determine if a model adequately describes data sets, while taking into account that parameters may have been estimated from those data. US EPA (2006a) suggests that a multivariate analysis of variance represents the most appropriate classical statistical test for comparing PBPK model predictions with experimental data.

2.3.4 Sensitivity to Input and Uncertainty in Predictions

Sensitivity analysis can measure the sensitivity of a model output to model input parameters and how much of the total model uncertainty can be attributed to each parameter. It can also help identify knowledge gaps and where additional research is needed.

Sensitivity analysis can be local or global. In univariate or local sensitivity analyses, parameters are varied individually by a small amount from "baseline" values, and the impact of each is assessed on the model result. Local sensitivity analyses are widely used in the field of pharmacokinetics. Kohn and Melnick (2000) conducted such an analysis on a six-compartmental PBTK model for 1,3 butadiene and concluded that the model outputs were more sensitive to physiological parameters compared to biochemical parameters. Sweeney et al. (2003) conducted a similar analysis for a model of acrylonitrile exposure to humans and found similar results to those of Kohn and Melnick.

However, covariance or interactions between parameters cannot be assessed in such analyses. Global sensitivity analysis, on the other hand, examines the impact of varying all parameters simultaneously throughout an expected range of values. In such analyses, a sensitivity index is obtained for each parameter. The index shows the direct impact of the parameter on the output and its interactions with other parameters (Brochot et al. 2007). In other words, global sensitivity analysis determines how the uncertainty in the model output can be apportioned to various sources of uncertainty in the model inputs (Salettli 2005).

Methods developed for global sensitivity analysis include variance-based techniques such as Analysis of Variance (ANOVA), Fourier Amplitude Sensitivity Test (FAST) and Extended FAST, Bootstrap and non-linear methods. Brochot et al. (2007) used an Extended FAST for a PBPK model of butadiene and four major metabolites in humans. They concluded that their model was sensitive to metabolism processes.

The uncertainty in model predictions has to be estimated since the model may be used to make predictions which are sensitive to parameters without experimental basis (Barton et al. 2007). It should be considered to evaluate the predictive capacity and complexity of the model, and to identify parameters that contribute significantly to variability in model output (sensitivity analysis). Large uncertainties in a sensitive model parameter can result in misleading estimates of target tissue doses. In addition, although PBPK models describe the biological and kinetic processes with some degree of accuracy, describing the behavior of the chemical in an average member of the population will not capture the inter-individual variability in the model input or output (Thomas et al. 1995).

The most popular methods for analyzing total model uncertainty are Monte Carlo (MC) simulations. These have been used in uncertainty analysis of PBPK models by some authors such as Thomas et al. (1995), Sweeney et al. (2001) and Cronin et al. (2006). Monte Carlo simulations involve selection of parameter values using a random or stochastic selection scheme, which runs for a very large number of interactions and produces values to be used in later analysis. Therefore, the distribution of model outputs and their uncertainty can be estimated and related to combined uncertainties of model inputs.

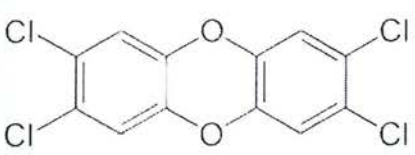
3 DIOXINS AND FISH

As discussed in section 2.1.2, toxicity is a function of properties of the agent and the organism. Therefore, it is important to have a good understanding of the characteristic of dioxins and furans, the biology of fish and the relation and interactions of these chemicals with fish.

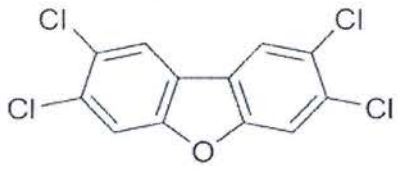
3.1 DIOXINS AND FURANS

3.1.1 Properties

The chemical structure of polychlorinated dibenzodioxins (PCDDs or dioxins) and polychlorinated dibenzofurans (PCDFs or furans) consists of two benzene rings joined by two or one oxygen bridge(s), respectively, with different arrangements of chlorine groups (see Figure 3-1).



Dioxins



Furans

Figure 3-1: Chemical Structure of Dioxins and Furans

Dioxins and furans, when found in the environment, biological tissues, and industrial sources, are usually present as complex mixtures. Homologs of dioxins and furans are shown in Table 3-1. There are 210 different dioxins and furans in existence, of which only 17 are toxic enough to be of concern. The potency of different dioxins can be ranked relative to 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD), the most toxic member of the class. Some of physiochemical properties of 2,3,7,8-TCDDs are shown in Table 3-2 (MOE 1997). The focus of this thesis will be on the 2,3,7,8-TCDD.

Table 3-1: Homologs of Dixons and Furans

Dioxins	Abbreviation	Furans	Abbreviation
4 chlorine	TCDD	4 chlorine	TCDF
5 chlorine	PeCDD	5 chlorine	PeCDF
6 chlorine	HxCDD	6 chlorine	HxCDF
7 chlorine	HpCDD	7 chlorine	HpCDF
8 chlorine	OCDD	8 chlorine	OCDF

Table 3-2: Physiochemical Properties of 2,3,7,8-TCDD

Parameter	Value
Empirical formulae	$C_{12}H_4Cl_4O_2$
Molecular weight	322
State at room temperature	Solid
Volatility	Vapor pressure negligible at 25 C
Specific gravity	1.8 at 20 C
Water solubility	Not soluble at 25 C. Low solubility in organic solvents
Reactivity	Decomposes when exposed to UV light

Reference: HPA (2008)

The toxicity rankings for dioxins and furans are known as Toxic Equivalency Factors (TEFs). To be included in the TEF scheme, a compound must be structurally related to PCDDs and PCDFs, bind to the cellular aryl hydrocarbon (Ah) receptor, elicit Ah receptor-mediated biochemical and toxic responses, be persistent, and accumulate in the food chain (NHMRC 2002). These characteristics are further described in section 3.3.6.

To express the overall toxicity of a mixture of dioxins and furans, the concept of Toxic Equivalents (TEQ) has been developed. This scheme weighs the toxicity of the less toxic compounds as fractions of the toxicity of the most toxic TCDD. Each compound is attributed a specific "Toxic Equivalency Factor" (TEF), which has a reference value of 1 for 2,3,7,8-TCDD.

The most recent review of TEFs was that of the WHO in 1998. Under the WHO TEF scheme, PCDDs, PCDFs and PCBs have TEF values ranging from 1.0 to 0.00001. To estimate the toxic potency of a given dioxin mixture, the mass concentration of each individual component is multiplied by its respective TEF, and the products are summed to represent the dioxin toxic equivalence (TEQ) of the mixture (NHMRC 2002).

Studies have shown that there are also 12 forms of Polychlorinated biphenyls (PCBs), usually referred to as dioxin-like PCBs which have toxicological properties similar to toxic forms of dioxins (Government of Ontario 2008).

3.1.2 Sources

Dioxins are formed as by-products from a number of human activities including certain industrial processes (e.g. production of chemicals, metallurgical industry) and combustion processes (e.g. waste incineration). Accidents at chemical factories have also resulted in high emissions and the contamination of local areas. Other dioxin sources include domestic heaters, agricultural and backyard burning of household wastes. Some natural processes such as volcanic eruptions and forest fires are also known to produce dioxins. Certain commercial PCBs are known to be contaminated with dioxins and therefore could be regarded as a source for dioxins.

Based on FAO and WHO (2005), current sources of dioxins entering the food chain include new emissions and re-mobilization of deposits in the environment. New emissions are mainly via the air route. They decompose in the environment very slowly; therefore, a large part of current exposure is due to releases of dioxins that occurred in the past.

Their presence in the Canadian environment can be attributed to three principle sources: point source discharges (to water, air and soil), contamination from in situ dioxins and furans, and loadings from the long-range transportation of air pollutants. Contamination of soil, water, sediments, and tissues (in situ contamination) are the subject of national guidelines, which outline benchmark ambient or "alert levels". These levels may be used by jurisdictions for the management and monitoring of dioxins and furans present in the environment.

Point source discharges to aquatic environments have been the target of aggressive federal and provincial regulation, as well as industry innovation and change. These types of discharges reached non-measurable levels in 1995 (CCME 2001).

3.1.3 Transport in the Environment

When released into the air, dioxins can deposit locally on plants and soil, contaminating the food and they can also be widely distributed by long-range atmospheric transport. The amount of deposition varies with proximity to the source, plant species, weather conditions and other conditions (e.g. altitude, latitude, temperature).

The entry of waste water or contaminated effluents from certain processes, such as chlorine bleaching of pulp and paper or metallurgical processes, can lead to contamination of water and sediment of coastal ocean areas, lakes and rivers.

Dioxins are poorly soluble in water. In water, they are adsorbed onto suspended mineral and organic particles. The surfaces of oceans, lakes and rivers are exposed to aerial deposition of these compounds which are consequently concentrated along the aquatic food chain.

Sources of dioxins in soil include deposition from atmospheric dioxins, application of sewage sludge to farm land, flooding of pastures with contaminated sludge, and prior use of contaminated pesticides (e.g. 2.4.5-trichlorophenoxy acetic acid) and fertilizers (e.g. certain compost).

3.1.4 Levels in Aquatic Environment

Several studies have been conducted on the levels of dioxins and furans in the Canadian inland waters especially in Great Lakes and their connecting channels and inland waterbodies. In addition, dioxins levels in Lake Ontario have been the subject of several studies by the U.S. governmental agencies.

The Ontario Ministry of the Environment (MOE) collects and maintains data on environmental contaminants in Ontario sport fish. This database is called the Sport Fish Contaminant Monitoring Program Database (FISHBASE), and contains data on contaminant levels in media (water), bio-monitoring, toxicological, chemical and geographic data. Bhavsar et al. (2008) used this database to study the temporal trends and spatial distribution of dioxins and furans in lake trout and lake whitefish of the Canadian Great Lakes. They used measurements of the 17 most toxic congeners during 1989 and 2003 and calculated the TEQ. The highest TEQ was found for Lake Ontario lake trout at 22-54 pg/g.

Based on measurements of dioxins and furans, mostly at sites with a history of contamination or with a known source of dioxins and furans, at freshwater sites, fish and invertebrates had contaminant levels ranging from undetectable to 112 nanograms of dioxins and furans TEQ per kilogram of wet weight (EC 2005).

In 1987, the governments of Canada and the United States made a commitment, as part of the Great Lakes Water Quality Agreement (GLWQA), to develop a Lakewide Management Plan (LaMP) for each of the five Great Lakes. Dioxins and furans were identified as LaMP critical pollutants since levels of these contaminants exceeded human health standards in some Lake Ontario fish, and because these chemicals were considered to limit the full recovery of the Lake Ontario bald eagle, mink, and otter populations by reducing the overall fitness and reproductive health of these species (LaMP 2006). The 2002 Lake Ontario LaMP report identified about 20 pg/g wet weight of dioxins in the herring gulls eggs collected from Toronto harbor. The trend between 1987 and 2001 followed a slow decline in this location.

Eighteenmile and Hyde Park have been known as areas of concern in Lake Ontario for high concentrations of dioxins. Woodfield and Estabrooks (1999) have summarized some of the known studies of dioxins and furans levels in the Lake Ontario drainage basin.

Concentration of dioxins in natural waters is usually below the detection limit of current analytical techniques. The detection limit is approximately two orders of magnitude greater than EPA's water quality standard of 0.013 ppq[4]. However, methods have been devised for collection and concentration of large volumes of water. Dinkins and Heath (1998) used such a method and measured the concentration of 2,3,7,8-TCDD in Ohio River at 0.001 to 0.02 ppq.

3.1.5 Impacts

A MOE scientific criteria document for standard development (MOE1985) summarized available studies on dioxins toxicity for fish and other aquatic biota and concluded that fish exposed to 2,3,7,8-TCDD in water react to very low concentrations (about 1 ng/L), usually with an extended latency period, and that embryonic and larval forms of fish appeared to be the most sensitive.

Extensive laboratory animal testing has shown that 2,3,7,8-TCDD, even at low doses, can cause a number of serious health problems: weight loss, skin disorders, immune system damage, impaired liver function, altered blood function, impaired, reproduction/birth defects, increased incidence of tumors and increased enzyme production. These effects are not limited to 2,3,7,8-TCDD. They can also be caused by high doses of the 16 other dioxins and furans of concern depending on their toxicity relative to 2,3,7,8-TCDD (MOE 1997).

Cook et al. (2003) attributed lake trout extinction in Lake Ontario by the 1960s to dioxins and dioxin-like pollution in the Lake and its high toxicity to embryos and very young trout just after hatching. According to them, transfer of TCDD from the mother trout to her eggs kills the fry at dioxin levels above 30 picograms/gram (ppt). By 100 ppt, all fry die. Adult female lake trout showed no overt sign of toxicity to TCDD levels three times that sufficient to cause 100% mortality in their offspring, however these females failed to ovulate.

A more recent study by Environment Canada (EC 2005) shows that in mammals, birds, and fish, dioxins and furans bind to a specific protein in their cells and this results in many different effects on the animal. Sensitivity to dioxin and furan exposures varies widely among species: some experience minimal effects when exposed to very high levels while others die when exposed to low levels. Effects on mammals include decreased food consumption, less weight

[4] Part per quadrillion (10E-15)

gain, changes in the size and shape of the liver and other organs, and changes in heart rate and blood pressure. Other problems are hair loss, a suppressed immune system, and changes to the number of blood cells. The fetus and nursing offspring can also have growth and developmental problems. Long-term exposure to dioxins and furans in the diet has been linked to a high incidence of tumors in mammals. In birds, lower food consumption and lower body weight have been reported as effects of dioxins and furans. Other effects include producing fewer eggs and higher death rates of embryos within the eggs. Some bird species, notably bald eagles, seem to be quite tolerant of dioxin's toxicity. In fish, reduced survival and growth rates, reproductive failure, and death have all been found as a result of dioxin and furan exposure.

Cook et al. (2003) conducted research on the impact of dioxins and dioxin-like pollution in Lake Ontario and their impacts on fish decline in the Lake in the 20th century. They reconstructed dioxin levels in Lake Ontario from sediment cores and from fish samples and predicted lake trout toxicity equivalence concentrations (TEC) in egg for a 90-year period (1910-2000) and indentified a 40-year period during which the toxicity of TCDD would cause sac fry mortalities.

3.2 Fish Anatomy and Physiology

A typical bony fish, such as trout, has the following systems (SeaWorld 2002) and components:

- Respiratory system (gills)
- Digestive and excretory system (including stomach, pyloric caeca, liver, intestines and kidney)
- Cardiovascular system (heart, arteries and veins)
- Skeletal system (spine)
- Muscular system
- Nervous system (brain, spinal cord and lateral line)
- Swim bladder
- Reproductive system (gonads)

A schematic of fish anatomy is presented in Figure 3-2.

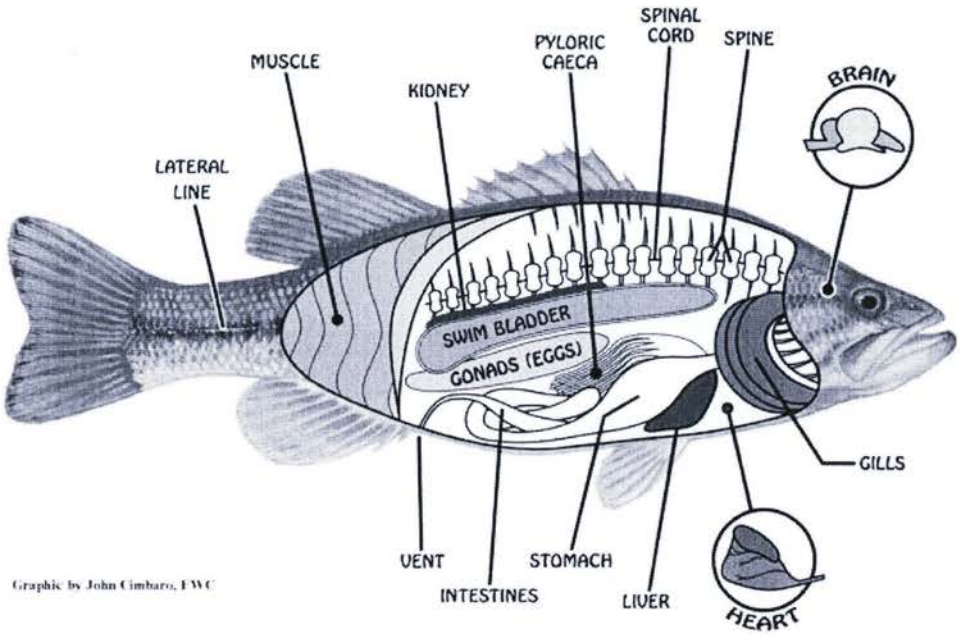


Figure 3-2: Schematic of Fish Anatomy

As discussed in section 2.1.2, toxicity depends on the organism uptake, storage and distribution, metabolism and excretion mechanisms. The systems and mechanisms important in toxic impact of dioxins and furans are discussed in here.

3.2.1 Respiratory system

Both marine and freshwater fish must regulate the movement of water across their body surfaces. Water enters the body of a freshwater fish continually through its skin and gills. Freshwater fish do not drink water, and produce large amounts of dilute urine.

The gill comprises rows of filaments, which are arranged to maintain the flow of water countercurrent to the subepithelial blood flow in the lamellae. The volume of water flowing through the gill is called the ventilation volume. The ventilation volume depends on breathing rate, stroke volume and bronchial shunting of water away from the lamellae. In general, the ventilation rate increases in response to hypoxia, increased temperature and exercise. The outer surface of the lamellae is covered with a thin layer of mucus. Because water flow across the lamellae is linear, a stationary layer of water exists adjacent to the mucus. The mucus and stagnant layer make up a diffusion barrier. The rate of blood perfusion of the gill is nearly equal to the cardiac output, both of which generally increase with temperature and exercise. Some species of bony fishes can absorb considerable amounts of oxygen through their skin (Hayton and Baron 1990).

The movement of water and blood through the gill results in a counter-current exchange between the two, as depicted on Figure 3-3. Mathematical representation of this exchange was used to explain uptake through gills (see section 4.2.2).

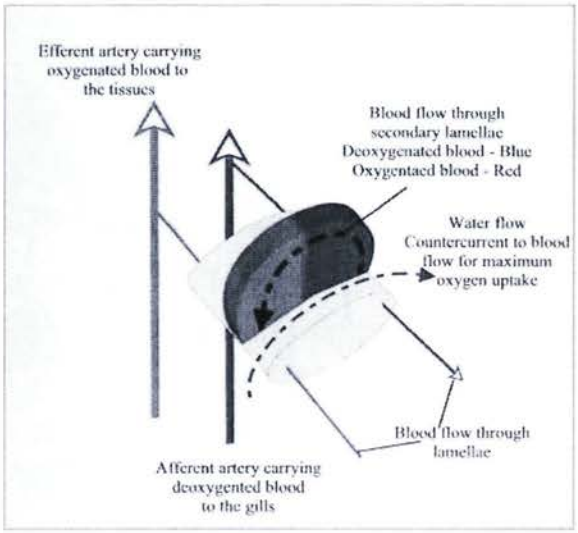


Figure 3-3: Counter-Current Flow of Water and Blood in Fish Gills
Reference: Fishdoc.co.uk (2009); Blue is the darker color in a black and white copy

3.2.2 Circulatory System

Fish have a closed loop circulatory system which includes a heart pumping blood into a single loop throughout the body. The blood flow starts from the heart to gills and from there to the rest of the body. It then flows back to the heart. It is in the gill that oxygen is absorbed and carbon dioxide is expelled.

Contaminants absorbed by the gill (or intestinal epithelia) pass the external mucus layer, the outer and inner epithelial membrane, and the blood vessel cell, and then are transferred via the blood stream to the various organs. The contaminants undergo sorption and desorption mechanisms in various binding sites in blood and tissues.

Fish blood consists of suspended blood cells and a solution for transport of materials. This solution contains plasma proteins, which are responsible for the osmotic pressure of blood facilitating transport of lipids and lipophilic chemicals. In fish, the most important plasma proteins are albumin-like proteins and lipoproteins, transporting free fatty acids and uptake of lipophilic contaminants from water into blood. The amount of blood in the fish circulation system has been estimated at three to four percent of fish fresh weight. Overall blood circulation takes

about 60 seconds in a rainbow trout (Braunbeck et al. 1998). Blood circulation is represented mathematically in section 4.2.2).

3.2.3 Digestive System

The fish digestive system consists of esophagus, stomach, pyloric caeca, liver, pancreas and intestine.

The esophagus in bony fishes is short and expandable so that large objects can be swallowed and transferred to the stomach. Gastric glands release enzymes that break down the food to prepare it for digestion. At the end of the stomach, many bony fishes have blind sacs called pyloric caeca. The pyloric caeca is believed to secrete digestive enzymes and absorb nutrients from the digested food.

The liver and pancreas secrete enzymes into the digestive tract. The liver secretes enzymes that break down fats; it also serves as a storage area for fats and carbohydrates. The liver is important in the destruction of old blood cells and in maintaining proper blood chemistry, as well as playing a role in nitrogen (waste) excretion.

The intestine is where the majority of food absorption takes place. The length of the intestine in bony fishes varies greatly. Herbivorous bony fishes generally have long, coiled intestines, whereas carnivorous bony fishes have short intestines. Wastes are diffused through gills or kidneys. Kidneys also help with regulating ammonia level in fish. In freshwater fish, the kidneys pump large amount of dilute urine.

Species with high fat levels, such as salmon, trout, carp and catfish, tend to accumulate organic chemicals in their fatty tissue (Government of Ontario 2008).

This information was used to build the overall structure of the model as shown in section 4.1).

3.2.4 Mechanism of Cytotoxicity

It has been suggested (e.g. by Courtenay et al. 1999, Buckley 1995, Giesy et al. 2002) that toxic effects of dioxins in fish are mediated through increase in the amount of some proteins.

These proteins belong to the Cytochrome P4501A (CYP1A) subfamily of the Cytochrome P450 family of enzymes, which function in the metabolism of many endogenous and exogenous substrates. In fish, the cytochrome P450 is mainly located in the endoplasmic reticulum and mitochondria of liver and other tissues. It acts as a catalyst in oxidation of a number of organic chemicals to more soluble and excretable metabolites (Arinç et al. 2000).

The subfamily CYP1A contains three known proteins, which are responsive to some of the more common and harmful anthropogenic organic contaminants including dioxins and furans. All of the three proteins including CYP1A1, 2 and 3 have been identified in fish and are suggested to interact with dioxins (Courtenay et al. 1999). In fish, CYP1A exists as a hybrid of both CYP1A1 and CYP1A2 (Arinç et al. 2000). CYP1A1 has been identified in rainbow trout, plaice, red sea bream, scup, toadfish, killifish, European sea bass, and Atlantic tomcod whereas CYP1A3 has been identified in rainbow trout (Courtenay et al. 1999).

The induction of CYP1A is regulated through a specific cellular receptor, the hydrocarbon receptor (AhR). The AhR is a transcription factor, which is a protein that works with other proteins to either increase or decrease the transcription of genes. CYP1A1 and CYP1A2 are regulated by the same AhR.

Activity of the CYP1A-associated enzymes can be determined using one of two approaches. The first one is by using 7-ethoxyresorufin as a substrate, and measuring the 7-ethoxyresorufin O-deethylase (EROD) activity. This is a very sensitive way for determining induction response of CYP1A in fish. The other approach is measuring CYP1A mRNA using Northern Bolt analysis (Arinç et al. 2000).

Ethoxyresorufin O Deethylase (EROD) is a cytochrome P-450 enzyme capable of activating procarcinogenic polycyclic hydrocarbons and halogenated aromatic hydrocarbons into mutagenic compounds. Ethoxyresorufin acts as a substrate for CYP1A1 and measurement of ethoxyresorufin O-deethylase provides a more direct method of detection for this enzyme (Medical Dictionary Online 2008).

These mechanisms are represented mathematically in section 4.2.5.

3.2.5 Biological Factors

Many biological factors may affect the contaminant concentration in an aquatic biota. Some of these vary between species (e.g. uptake, metabolism and enzyme induction), whereas others vary within individuals in one species (e.g. age, body weight and length, lipid content, blood flow, reproductive state and sex) (Nowell et al. 1999).

Gill ventilation volume is one of the biological factors affecting uptake through controlling water movement across gills.

Metabolism or transformation contributes to elimination significantly. Hydrophobic compounds are usually metabolized to more water-soluble compounds that can be excreted. But, biotransformation may also produce metabolites which are more toxic.

Many field observations have suggested that accumulation of hydrophobic chemicals in aquatic biota is impacted by body weight and length, as well as by age, which in turn correlates positively with lipid content. Other studies have suggested more accumulation in average-sized biota and decline in longer ones. Dioxins residues measured in fish liver downstream of a paper mill were not found to correlate with fish age. Several explanations have been offered for the effects of age and body size on accumulation. Some of these include: larger, older fish have higher lipid content, that elimination is faster in smaller fish, or larger fish take longer to reach steady state concentrations, and that larger fish have smaller surface-to-volume ratio. Growth can also be important in contaminant elimination through dilution.

Organic compounds have a tendency to accumulate in organs with higher lipid content. However, the relationship is not straightforward and factors such as lipid composition, seasonality in lipid content, body size, age and reproductive state can affect the relationship.

Reproduction can decrease the concentration of hydrophobic contaminants through transfer to juveniles. Sex hormones can influence the enzyme activity and metabolism of contaminants, and changes in feeding habit and lipid content during reproductive state can also impact the accumulation pattern.

Sex can also be a deciding factor in accumulation of contaminants as it impacts biological factors such as lipid distribution, reproductive cycle, enzyme activity, and metabolic capability.

3.3 Toxic Mechanisms of Dioxins and Furans

Bioaccumulation of persistent organic chemicals in fish are the results of a complex set of physical and biochemical processes: partitioning between sediment, water, food and fish, uptake from water (bioconcentration) and/or sediments and contaminated food (biomagnifications), clearance by non-metabolic routes (e.g. fiscal or respiratory excretion) and by metabolic routes (binding to particulate and dissolved fractions) among others (Oost et al. 1996). The relevant processes for dioxins and furans are described in this section.

3.3.1 Exposure Pathways

Contradictory ideas have been postulated about the relative importance of various pathways (water, sediment, food) in the exposure of fish to dioxins and furans.

The relative contributions of uptake from food and uptake from water to the total bioaccumulation of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in guppies was studied by Loonen et al. (1993) when they conducted a bioconcentration (with contaminated water) and a biomagnification (with contaminated food)

experiment. The concentrations of the 73 PCDD and PCDF congeners in food varied from 32 ± 1 to 753 ± 44 μg/kg. Fish (guppies) were fed at a rate of 0.02 g food/g fish/day (wet weight) for 180 days. Concentrations in water varied from non detectable to 1.47 ± 0.28 ng/L for an exposure duration of 21 days. For congeners with chlorine atoms at the lateral 2,3,7,8 positions, C_{biota}/C_{food} on a lipid weight basis varied from 0.01 to 0.1, implying that uptake from food was not a partitioning process between fish lipid and food lipid. However the C_{biota}/C_{water} ratio exceeded 103.9, meaning the uptake from water was in fact a water-to-lipid partitioning process. It was concluded that the uptake from food was small compared to the uptake from water (from 0.9 to 4.9%).

Rifkin and Lakind (1991; cited in Oost et al. 1996) reported that fish accumulate dioxins by ingestion (biomagnification) rather than by bioconcentration. Muir et al. (1992; cited in Oost et al. 1996) demonstrated that PCDF/Ds in the water phase were either almost entirely associated with small sized particles or with dissolved organic carbon. They suggested that since only the fraction dissolved in water is directly bioavailable for uptake through the gills, sorption behaviour limits the total bioavailability of PCDF/Ds.

Karl et al. (2003) conducted experiments to quantify the transfer of PCDDs and PCDFs from commercial fish feed into the edible part of rainbow trout under normal rearing conditions. Trout were fed with high-energy feed for salmon (fat content 26 to 30%) over a period of 19 months. The average weight of the fish increased from 10 g to more than 2092 g. Dioxin concentrations increased during the time of feeding from 0.054 up to 0.914 ng WHO-PCDD/F-TEQs kg^{-1} wet weight and from 4.991 to 15.815 ngWHO-PCDD/F-TEQs kg^{-1} fat. A correlation was found between the dioxin concentration of the feed and the resulting concentration in the fat in muscle tissue. Transfer rates (uptake%) of dioxins (WHO-PCDD/F-TEQs) in muscle tissue of farmed trout fed on a high energy diet was between 11% after 6 months, and 34% after 19 months of feeding.

Based on the Loonen et al. (1993) study, increasing concentrations in the higher levels of the food chain seem to indicate accumulation through the food chain. They argued that much of the concentration difference can be explained by the increasing lipid contents of the organisms higher in the food chain. The observed concentrations in the food chain, however, cannot be completely predicted by lipid-based bioconcentration factors.

On the importance of the sediment pathway, bottom dwelling fish species and bottom feeders are more exposed to contaminated sediments than pelagic fish species. However, levels of dioxins in bottom dwelling fish are not necessarily higher than those in pelagic fish

considering the size, diet and hysiological characteristics of the fish. In general, fish shows an age-dependent accumulation of dioxins (FAO and WHO 2005).

Oost et al. (1996) investigated the behavior of persistent organic trace pollutants (including TCDF and TCDD compounds) in sediment and eel from six different freshwater sites. They took the ratio between the lipid weight standardized concentrations in eel and the concentrations in the organic matter of the sediment as the biota-sediment accumulation factor (BSAF). The result showed that BASF values for PCDF/Ds are generally lower than those for other persistent organic chemicals (such as PCBs). This was also reported by Loonen et al. (1991, cited in Oost 1996). Virtually no accumulation of hepta- and octachlorinated PCDFs and PCDDs was observed in the eel muscle tissue. This was explained by a reduced uptake or an increased metabolic clearance (e.g. due to the larger effective cross diameter of the molecules which reduced membrane permeability).

Randall et al. (1998) analyze the relative importance of water and food pathways from a theoretical and experimental standpoint. From a theoretical standpoint, bioconcentration (process of partitioning of the chemical between the fish and the water involving a bidirectional flux across gills) was compared to biomagnification (uptake from food and loss of chemical in faeces). In the experimental section of the analysis, 1,2,4,5-tetrachlorobenzene was used as the test toxicant and rainbow trout as the test species. They concluded that the uptake of persistent lipophilic toxicants in fish does not occur via the food but by transfer across the body surface, notably in the gills. They argue that flux across the gills is rapid and in order for feeding to have a significant effect on toxicant concentration in the body, the fish must eat at a very high rate.

Nichols et al. (1996) investigated the importance of dermal absorption of organic chemicals. They developed a PBPK model to describe the absorption mechanism and represented the skin as a discrete compartment. The model was evaluated by exposing rainbow trout and channel catfish to hexachloroethane, pentachloroethane and 1,1,2,2-tetrachloroethane. Modeling results for a hypothetical combined dermal and branchial exposure indicated that dermal uptake could contribute between 1.6 to 8.3 percent to the total uptake.

The review of available studies on the relative importance of contaminant uptake from water versus food by Nowell et al. (1999) revealed that most of the studies observed a greater accumulation from water than from food. Based on the above-mentioned literature, this study considers intake of dioxins from water through gills as the base case and the food pathway was studied as an extension to the water model.

3.3.2 Uptake

Several authors (e.g. Hamelink et al. 1971 and Murphy and Murphy 1971; cited in Nowell et al. 1999) suggested that the uptake of chlorinated hydrocarbons by fish occurs through transfer from water to blood through gills.

Uptake from water through gill consists of four steps (Hayton and Barron 1990):

- Transport by water flow to close proximity of the gill epithelium
- Diffusion from water across the aqueous stagnant layer into the epithelial surface
- Diffusion across the gill epithelium to blood
- Distribution through the body by blood

These steps are consecutive and therefore, the slowest step controls the overall rate of uptake. The operative barrier will depend upon the anatomy and physiology of the animal, as well as the properties of the chemical. Further, the rate-limiting barrier is a function of environmental factors, size, species, and the aquatic animal's activity level.

The proportionality constant that relates the rate of uptake of a chemical by the gill to the concentration difference between the external water and the blood plasma water is called uptake clearance. It can be envisioned as the volume of water totally cleared of chemical per unit time when concentration in fish is significantly lower than concentration in water.

A few authors (e.g. Rifkin and Lakind 1991 and Karl et al. 2003) have suggested that the uptake of dioxins takes place mainly through food (in addition to water). In this scenario, uptake occurs in the gastrointestinal (GI) tract, which includes stomach, pyrolic ceca, upper intestine and lower intestine. The important parameter in this process is nutrient uptake (Nichols et al. 2004).

Since dioxins do not undergo major metabolic biotransformation processes in the gut, the remaining processes involved in storage, distribution and metabolism of dioxins (in liver) are the same regardless of the uptake mechanism (from water through gills or from food).

3.3.3 Storage

The fate of dioxins in the body is unusual, primarily because most of the congeners are highly fat soluble but practically water insoluble. Dioxins are distributed via the blood to all organs, but are preferentially retained in adipose (fatty) tissue (NHMRC 2002) as well as the liver, where high concentration of dioxins has been observed. It has been suggested that at low doses, dioxins partition according to its lipophilic nature (i.e. more in adipose tissues), but at

higher doses, it causes protein induction (discussed later) and accumulates in the liver. At very high doses, where the specific binding sites are saturated, the dioxin concentration in the liver decreases and it again accumulates in adipose tissues (Buckley 1995).

3.3.4 Distribution

Dioxins are distributed via blood flowing from gills to all organs. Portal blood flows to the liver and kidney also impact the distribution pattern.

In terms of flow of arterial blood into the organ, fish tissues can be classified into richly-perfused and slowly-perfused tissues. Richly-perfused tissues include stomach, pyloric ceca, intestine, spleen and gonads, whereas slowly-perfused tissues include white muscle, fins, skin and bones (Nichols et al. 1990).

Chemical flux in the tissue can be blood-flow-limited or diffusion-limited. When the flux is limited by blood-flow, a chemical equilibrium exists between the tissue in the compartment and the blood exiting it (Nichols et al. 1990). Tissues with blood-flow-limited fluxes include gills, liver, kidney and other richly perfused tissues (red muscle, digestive tract and spleen).

The diffusion-limited flux can be represented by a tissue blood subcompartment, where transport of dioxins from tissue blood to tissue is proportional to a mass transfer coefficient (Buckley 1995). Tissues with diffusion-limited flux include fat and other slowly perfused tissues (white muscle, fins, skin and bones).

3.3.5 Elimination

Chemical elimination occurs across the gills and through metabolism and excretion. The latter can occur to a limited extent from all tissues (mostly through gills, skin, urine or feces); however, for simplicity the elimination can usually be assumed to be localized to one compartment. This compartment and the kinetics of elimination depend on the chemical properties, the species and other variables (Nichols et al. 1990).

Growth is also considered an elimination process because the contaminant concentration in an organism decreases as the body mass of the organism increases (Nowell et al. 1999).

Metabolism of dioxins occurs primarily in liver. The metabolite is excreted into the feces via the bile (Buckley 1995). Other chlorinated organic chemicals have been observed to be eliminated via both bile and urine (Nichols et al. 1991). The excretion rate is limited by the metabolism and the elimination is a first order function of dioxin concentration in liver (Buckley 1995).

The release of stored dioxins from adipose tissue into the circulation is extremely slow, limiting the rate of metabolism by the liver and subsequent excretion. Dioxins are composed of a mixture of compounds and each has a different half-life, but the commonly quoted average in fish ranges between 64 and 105 days (Oost et al. 1996). The long half-life of dioxins means that, over time, even a low rate of exposure leads to accumulation of dioxins in the body. Continual exposure might lead in the long term to an extremely high body burden (i.e. the total amount of dioxins in the body). The biochemical and toxicological effects of dioxins, furans and PCBs relate more closely to their concentration in the target tissue than to the daily dose (NHMRC 2002).

3.3.6 Molecular Toxicology

Fish response to dioxins (and other halogenated hydrocarbons) in the form of induction of specific cytochrome P450 (CYP1A) izozymes has been shown in various reports. It has also been shown that this response is regulated by a particular cellular receptor (the AhR) that binds dioxins and related planar. This binding has a variety of affinities for different chemicals which result in different induction potencies (Giesy et al. 2002).

CYP1A encodes monooxygenases, which metabolize environmental procarcinogens to reactive metabolites. These metabolites adduct to cellular DNA and proteins or alternatively are conjugated with carrier molecules (such as glutathione and glucuronic acid) and expedite their elimination from the body. It has been suggested that induction of CYP1A in fish may serve as a sensitive marker of xenobiotic exposure and early biological response. Correlations have been found between CYP1A enzyme activity and body burdens of contaminants such as coplanar PCBs. Some authors have extended their description of this binding mechanism to a ternary binding between dioxins, the Ah receptor and sites on DNA (Buckley 1995). A simplified schematic of binding of dioxins to AhR is shown in Figure 3-4.

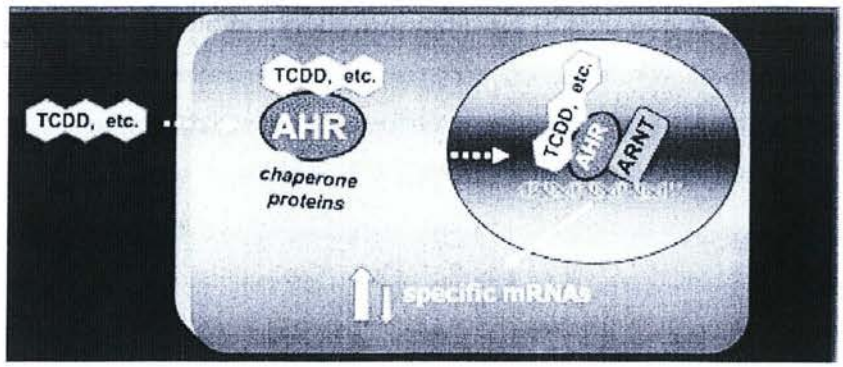


Figure 3-4: A Schematic of TCDD interaction with AhR

Source: Cover of the Toxicological Sciences journal (2007: 98(1))

Courtenay et al. (1999) conducted experiments to quantify levels of hepatic CYP1A1 mRNA levels in Atlantic tomcod. The fish were injected with various concentrations of halogenated compounds including of 0.5 µg/kg fish of TCDD. The results varied among experiments and time of measurement, with induction folds between 0.4 after 9 hrs of injection in one experiment to 32 fold after 24 days in another experiment. These authors suggested that the hepatic CYP1A1 mRNA concentration can be a useful bioindicator of exposure to some aromatic hydrocarbon compounds in the aquatic environment, and that the profiles of gene induction and disappearance may help identify environmental inducers if the gene responsiveness is also evaluated under controlled laboratory conditions.

A mounting body of evidence suggests a concurrence of elevated CYP1A in fish and a variety of toxicological effects including: mortality, growth inhibition, liver damage and lymphocyte depletion in rainbow trout, oxidative stress in lake trout, reduced innate immune responses in channel catfish, reproductive impairment in starry flounder and white sucker, increased overall DNA damage, K-ras oncogene activation in winter flounder and tomcod, and neoplasia in English sole and Atlantic tomcod (Courtenay et al. 1999).

The interaction of dioxins with a cytosolic protein, the Ah receptor causes a wide range of biological effects (Andersen 2003). Although the precise chain of molecular events is not fully understood, alterations in key biochemical and cellular functions are probably responsible for dioxin toxicity. Activation of the Ah receptor has two major consequences: increased transcription of various genes (e.g. coding for drug-metabolising enzymes) and immediate activation of tyrosine kinases. The Ah receptor may also directly or indirectly regulate expression of other networks of genes. Activation of the Ah receptor can result in endocrine and paracrine disturbances, as well as in alterations in cell functions (including growth and

differentiation). Some of these effects have been observed in both humans and animals, which indicates the existence of common mechanisms of action in different species. Some of the ligands that bind the Ah receptor may block its activation by behaving as weak agonists or antagonists (NHMRC 2002).

All these time-dependent processes, including synthesis of specific messenger-RNA or specific proteins, can be modeled with approaches that realistically incorporate the knowledge of the chemistry and biology of the overall response of the tissue or organism (Andersen 2003).

4 MATERIALS AND METHODS

The information provided in section 2 (ecotoxicology and PBPK modeling) and in section 3 (dioxins and fish) were used to develop a PBPK model for dioxin and furan toxicity in fish. Details of this model are provided here.

4.1 PROBLEM DEFINITION

This model was prepared for analysis of toxicity of dioxins and furans (and specifically 2,3,7,8-TCDD) in various fish species. A schematic representation of the model components is shown in Figure 4-1. In this model, the pathway of exposure is via water to fish, and the uptake is considered to be primarily through gills to blood. The food exposure pathway was constructed with addition of a gut compartment to the water model (see Figure 4-2 for the relevant addition and changes).

The water-exposure model components include gills, blood, richly-perfused tissues (including red muscle, digestive tract and spleen all represented as Rich-p and kidney as a separate component) and slowly-perfused tissues (including white muscle, fins, skin and bones all represented as Slow-p and fat and liver as separate components). The blood flows from the gills to various tissues; it also flows from the richly-perfused tissues to the liver (100%) and from the slowly-perfused tissues to the kidney (60%). Chemical flux in the kidney, liver and the Rich-p tissues is blood-flow limited, whereas it is diffusion limited in fat and the Slow-p tissues. The liver compartment is special because it eliminates dioxins through metabolism and accumulates it through binding to AhR and protein. The food pathway model includes an additional compartment for absorption of food and excretion of feces.

The time scale for exposure was considered from the juvenile stages to the age where the fish was exposed to dioxins (depending on the experiments used for model calibration) and beyond. Fish growth during this period was taken into account. The model was implemented in daily time steps since most available data (observations used for model calibration) had been collected in intervals of a few days.

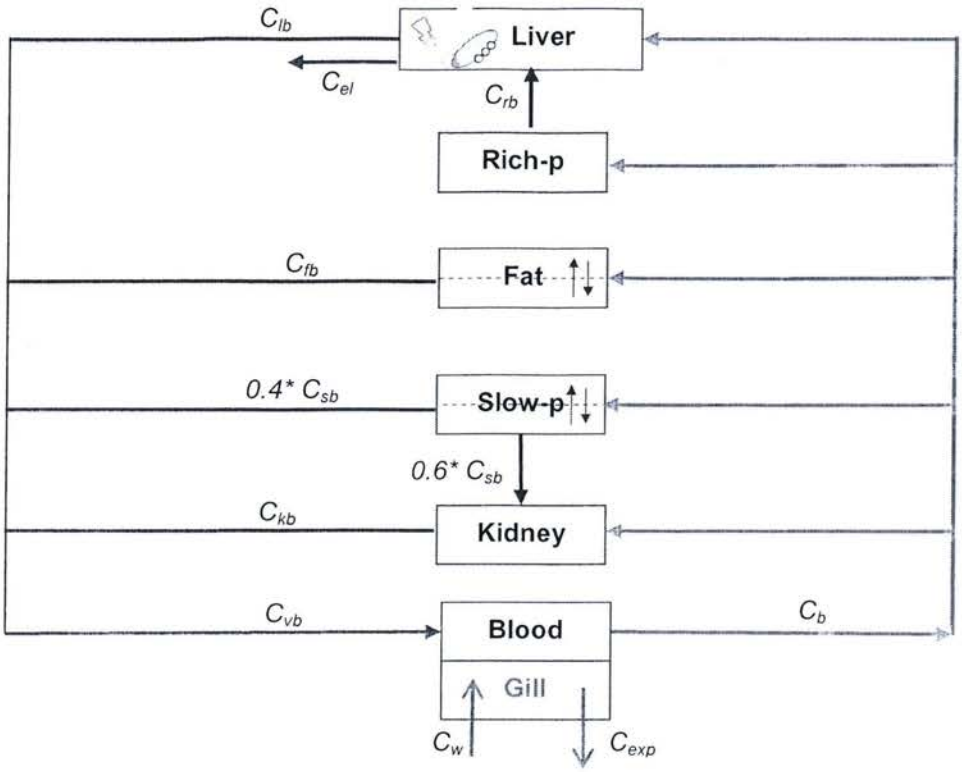


Figure 4-1: Schematic Representation of the PBPK Model for Exposure to Water

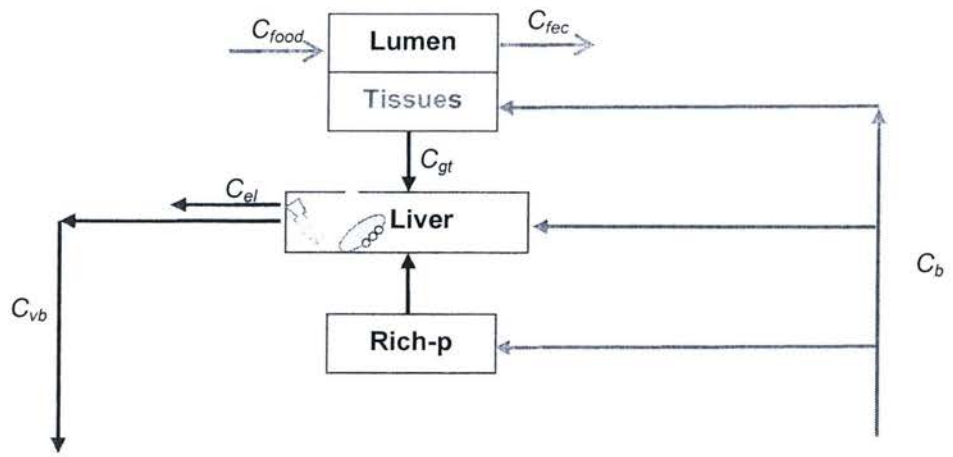


Figure 4-2: Schematic Representation of the Food Exposure-Specific Components

Note: Both figures were adapted from Nichols et al. (1998)

4.2 REVIEW OF MATHEMATICAL PRESENTATIONS

Each tissue compartment is represented by one or more mass-balance differential equation(s) that describe changes in the amount of chemical in the tissue over time. Mathematical equations reviewed to represent the tissue and processes are provided in here. The next section shows the equations selected for use in the model developed in this study.

4.2.1 Growth Model

Fish growth was modeled using the Von Bertalanffy growth function (VBGF) (as used by Luk 2000). VBGF defines the wet weight (W) as a function of age (t), the asymptotic weight (W_∞), growth coefficient (k) and a power term in the weight-length function (b), as follows:

$$W(t)=W_\infty(1-e^{-kt})^b$$ **Equation 4-1**

The weight-length function relates the weight to length (L) through coefficients of "a" and "b", as follows:

$$W(t)= a\,L(t)^b$$ **Equation 4-2**

Many physiological parameters change with body weight. Alometric equations were used to describe some of these dependencies. For example, the value of a parameter (A) for a fish weighing BW is related to an allometric coefficient (A0) and a scaling factor (SF), as follows:

$$A= A0\times BW^{SF}$$ **Equation 4-3**

Valued of allometric coefficients and scaling parameter values are provided in Section 5.

4.2.2 Uptake

This study considers uptake through two routes: water to gills and food to mouth. Mathematical representations of both of these routes were reviewed and are discussed here.

4.2.2.1 Flux Across Gills

Nichols et al. (1990) in their PBTK model for uptake of organic chemicals by fish, used a general expression for chemical flux across gills (F_g) and related it to an exchange coefficient (k_{x-g}), the concentration gradient between the inspired water (C_w) and the venous blood (C_{vb}) and a blood to water partition coefficient (P_{bw}), as follows:

$$F_g=k_{x-g}\times(C_w-C_{vb}/P_{bw})$$ **Equation 4-4**

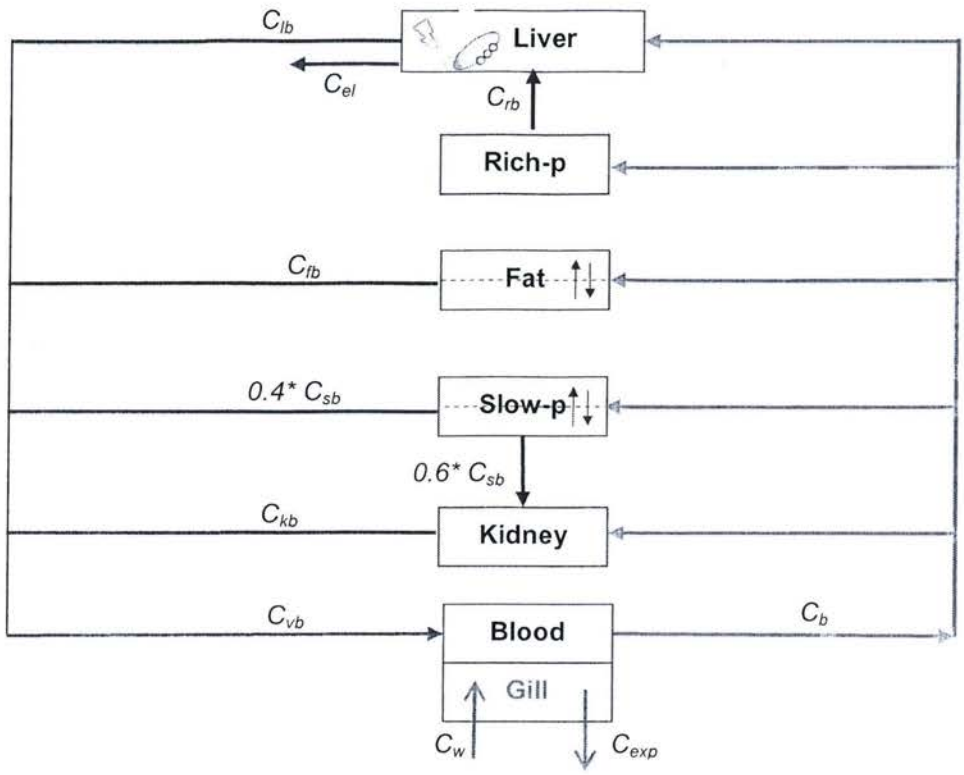
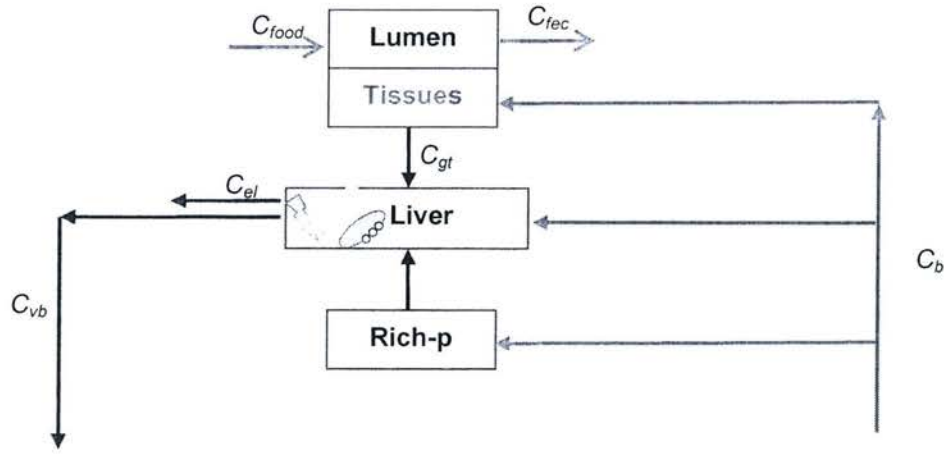


Figure 4-1: Schematic Representation of the PBPK Model for Exposure to Water



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$$F_g=k_{x-g}\times(C_w-C_{vb}/P_{bw})$$ **Equation 4-4**

Chemical flux was also shown as a simple mass balance which related the exchanged inspired water (Q_w) and the concentration gradient in water (incoming versus expired water) to the cardiac output (Q_T) and the concentration gradient in blood, as follows:

$$F_g = Q_w \times (C_w - C_{\exp}) = Q_T \times (C_b - C_{vb}) \qquad \textbf{Equation 4-5}$$

The exchanged inspired water is in fact the product of ventilation volume, (Q_v) and effective respiratory volume, (V_{ER}).

By re-arrangement of these two equations, the exchange coefficient can be expressed in terms of blood flow, water flow and chemical concentrations, as follows:

$$k_{x-g} = \frac{Q_w \times (C_w - C_{\exp})}{C_w - C_{vb} / P_{bw}} = \frac{Q_T \times (C_b - C_{vb})}{C_w - C_{vb} / P_{bw}} \qquad \textbf{Equation 4-6}$$

Lien and McKim (1993) incorporated the diffusion limitation into a more detailed gill model and suggested a numerical solution for the exchange coefficient. Their equation relates the exchange coefficient to the blood flow (through K_{bf}, which equals Q_T x P_{bw}), water flow (through K_{wf}, which equals Q_w), and diffusion limitation (K_{dl}). The overall exchange coefficient is calculated as follows:

$$K_{x-g} = \frac{e^{-K_{dl}/K_{bf}} - e^{-K_{dl}/K_{wf}}}{\frac{e^{-K_{dl}/K_{bf}}}{K_{wf}} - \frac{e^{-K_{dl}/K_{wf}}}{K_{bf}}} \qquad \textbf{Equation 4-7}$$

K_{dl} was defined as a function of molecular diffusivity or the gill permeability coefficient for a specific chemical (Perm), total lamellar surface area (A_{gill}), and the average thickness of the diffusion path length (T_{path}), as follows:

$$K_{dl} = Perm \times A_{gills} / T_{path} \qquad \textbf{Equation 4-8}$$

Blood concentration was then calculated using the exchange coefficient, as follows:

$$C_b = C_{vb} + \frac{K_{x-g}}{Q_T} \times (C_w - \frac{C_{vb}}{P_{bw}}) \qquad \textbf{Equation 4-9}$$

Lien et al. (2001) proposed a model for countercurrent exchange of chemicals at the gill surface. The flux of chemical is described using an exchange coefficient (K_{x-g}), the ratio of free chemical concentration to total concentration in water (f_w), total concentration in water (C_w), the ratio of free chemical concentration to total concentration in blood (f_b) and the concentration of chemical in the blood into the gill (C_{vb}), as follows:

$$F_g = K_{x-g} \times (f_d \times C_w - f_b \times C_{vb})$$ **Equation 4-10**

They calculated the exchange coefficient for three chloroethanes in a way that represented limitations of three barriers including flow of water and blood, diffusion barriers and blood-water partitioning.

Hayton and Barron (1990) proposed a model for transfer of chemicals across gill to blood considering the following barriers: water flow across gill, a stagnant water layer over the gill epithelium, the gill epithelium, and blood flow through the gill. The rate of transfer of chemical to fish (dX/dt or F_g as used by Nichols et al. 1990) was calculated based on a concentration gradient between water (C_w) and blood (C_b), and absorption clearance (P) as follows:

$$F_g = P(C_w - C_b)$$ **Equation 4-11**

According to these authors, depending on the properties of the chemical and the fish physiological properties, any of the listed barriers can be rate limiting. If the permeabilities of the four barriers are added together, P can be defined as below:

$$P = [(d / D_m \cdot A \cdot K_m) + (h / D_a \cdot A) + 1 / K_b \cdot \dot{V}_b + 1 / \dot{V}_w]$$ **Equation 4-12**

Where d is the thickness of the epithelium (length), D_m is the diffusion coefficient for chemical in the epithelium (length^2/time), A is the effective absorbing surface of the epithelium (length^2), K_m is the epithelium to water distribution coefficient for the chemical (unitless), h is the length of the stagnant film (length), D_a is the diffusion coefficient of the chemical in water (length^2/time), K_b is the blood to water distribution coefficient (unitless), $\dot{V}_b$ is the blood flow through the gill (volume/time) and $\dot{V}_w$ is the effective ventilation volume. This model, however, does not account for the counter-current structure of the gills, which requires the use of venous blood concentration in describing the blood:water equilibrium.

The concentration in blood returning to the gill from tissues, C_{vb}, was defined by Lien et al. (2001) as a weighted average of blood concentrations exiting each tissue (C_{tb}), as follows:

$$C_{vb} = \frac{\sum (C_{tb} \times Q_t)}{Q_T}$$ **Equation 4-13**

where Q_t is the tissue blood flow rate and Q_T is the cardiac output.

The rate of change for the venous blood concentration (C_{vb}) is shown by others (e.g. Parham and Portier 1998) as the difference between sum of product of tissue blood flow (Q_t) and tissue venous blood concentration (C_{tb}), and the product of cardiac output (Q_T) and venous blood concentration, as follows:

$$\frac{dC_{vb}}{dt}=\frac{1}{V_b}\left[\left(\sum Q_t C_{tb}\right)-Q_T C_{vb}\right]$$ **Equation 4-14**

However this equation seems to consider accumulation of dioxins in blood, and therefore was not applied in the proposed model.

4.2.2.2 Bioavailability

Binding to organic carbon in water reduces the free chemical concentration that is available to diffuse across the gills. Arnot and Gobas (2004; cited in Nichols et al. 2004) related the dissolved (bioavailable) concentration of chemicals in water (C_{wd}) to the dissolved organic carbon (DOC), particulate organic carbon (POC), and the octanol:water partition coefficient (K_{ow}) of the chemical, as follows:

$$C_{wd}=\frac{1}{1+0.35\times POC\times K_{ow}+0.08\times DOC\times K_{ow}}$$ **Equation 4-15**

4.2.2.3 Uptake through Diet

Nichols et al. (1998) developed a PBPK model for dietary uptake of 2,3,7,8-TCDD by female brook trout. The model was tested against data collected during a 6-month feeding and depuration study. The relationship suggested for dietary flux of dioxins involved mass balance differential equations for the contents of the intestinal tract and the tissue. Intake of dioxins through food (Q_{food} x C_{food}) was related to amount of TCDD in feces (A_{fec}) and partitioning of dioxins between gut tissues (C_{gt}) and the feces (C_{fec}) , through a feces:gut partitioning coefficient (P_{fgt}) and a first order rate constant (PA_{gt}) representing the capacity of chemical diffusion across the guts, as follows:

$$\frac{dA_{fec}}{dt}=(Q_{food}\times C_{food})-(Q_{fec}\times C_{fec})-PA_{gt}\times(C_{fec}-C_{gt}\times P_{fgt})$$ **Equation 4-16**

The gut was also considered to follow a diffusion-limited mechanism, and the concentrations in gut tissue (C_{gt}) and blood (C_{gtb}) were related to the flux through the guts, as follows:

$$\frac{dA_{gt}}{dt}=Q_{gt}\times(C_b-C_{gtb})+PA_{gt}\times(C_{fec}-C_{gt}\times P_{fgt})$$ **Equation 4-17**

The concentration in gut tissue and gut blood (blood draining the gut) was related through a gut:blood partition coefficient (P_{gt}), as follows:

$$C_{gtb} = \frac{C_{gt}}{P_{gt}}$$ **Equation 4-18**

Nichols et al. (2004) developed a model for dietary uptake of hydrophobic organic compounds by fish. They modeled the gastrointestinal (GI) tract as four compartments corresponding to stomach, pyrolic ceca, upper intestine and lower intestine. The luminal volume of each compartment changed in time as a function of bulk flow down the GI tract and nutrient uptake.

4.2.3 Concentrations in Richly-Perfused Tissues

Chemical flux to the richly -perfused tissues such as kidney, liver, red muscle, digestive tract and spleen is limited by the blood flow. A general equation (based on Buckley 1995) relates the tissue concentration (C_t) to the blood flow to the tissue (Q_t) and the tissue volume (V_t) as well as the concentration gradient between the afferent blood and the venous blood exiting the tissue (C_{tb}), as follows:

$$\frac{dC_t}{dt} = \frac{Q_t}{V_t} \times (C_b - C_{tb})$$ **Equation 4-19**

The venous blood concentration is related to the tissue concentration through a blood:tissue partition coefficient (P_t) as follows:

$$C_{tb} = \frac{C_t}{P_t}$$ **Equation 4-20**

Kidney is also a richly-perfused tissue; however, different equations are proposed for it as discussed below.

Kidney needs a special treatment as it receives blood not only from the gills, but also from the slowly-perfused tissues. Sixty percents of blood from these tissues (with a flow of Q_s and a concentration of C_{sb}) are directed to the kidney (Nichols et al. 1990). The rate of change in the concentration of dioxins (C_k) is defined using a total flow of $Q_{m\text{-}k}$ and combined concentration of $C_{m\text{-}k}$ (adopted from Nichols et al. 1990), as follows:

$$\frac{dC_k}{dt} = \frac{Q_{m-k}}{V_k} \times (C_{m-k} - C_{kb})$$ **Equation 4-21**

$$Q_{m-k} = Q_k + 0.6 \times Q_s$$ **Equation 4-22**

$$C_{m-k}=(\frac{Q_k}{Q_{m-k}}\times C_b)+(\frac{0.6\times Q_s}{Q_{m-k}}\times C_{sb})$$ **Equation 4-23**

The venous blood concentration is related to the tissue concentration through a blood:tissue partition coefficient (P_k) as follows:

$$C_{kb}=\frac{C_k}{P_k}$$ **Equation 4-24**

4.2.4 Concentrations in Slowly-Perfused Tissues

Chemical flux between the slowly -perfused tissues such as fat, white muscle and skin is limited by diffusion across the tissue lipid/aqueous biological media. In slowly-perfused tissues, distribution is limited by penetration of the cellular membrane by the chemical and therefore an additional term for permeability needs to be added. As shown in Figure 4-3, a slowly-perfused tissue is shown in sub-compartments with exchange between tissue and tissue blood.

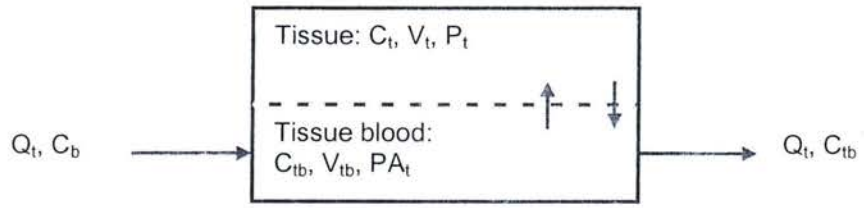


Figure 4-3: Sub-compartments of a Slowly-perfused Tissue

The rate of change of chemical concentration in tissue is described as a function of the concentration gradient between tissue and tissue blood and a mass transfer coefficient (PA_t), as follows (based on Buckley 1995):

$$\frac{dC_t}{dt}=\frac{PA_t}{V_t}\times(C_{tb}-\frac{C_t}{P_t})$$ **Equation 4-25**

The rate of change of chemical concentration in tissue blood is described as a function of the concentration gradient between blood afferent to the tissue and the tissue blood, as well as the concentration gradient between the tissue and the issue blood (based on Buckley 1995):

$$\frac{dC_{tb}}{dt}=\frac{1}{V_{tb}}[Q_t\times(C_b-C_{tb})+PA_t\times(\frac{C_t}{P_t}-C_{tb})]$$ **Equation 4-26**

4.2.5 Concentration in Liver

Liver was modeled as a flow-limited tissue considering the high level of blood perfusion that it receives. Liver receives blood from the richly-perfused tissues (that is mainly the guts), and it also has additional mechanisms for metabolism and elimination of dioxins.

Dioxins concentration in tissue (C_l) was defined in terms of free (or diffused) concentration (C_{lf}), concentration eliminated through metabolism (C_{el}) and the dioxins bound to the liver protein (C_{BM}), as follows:

$$C_l = C_{lf} + C_{BM} - C_{el}$$ **Equation 4-27**

Liver receives arterial blood as well as all portal blood from other richly-perfused tissues with a total flow of Q_{m-l} and combined concentration of C_{m-l} (Nichols et al. 1990).

$$Q_{m-l} = Q_l + Q_r$$ **Equation 4-28**

$$C_{m-l} = \left(\frac{Q_l}{Q_{m-l}} \times C_b\right) + \left(\frac{Q_r}{Q_{m-l}} \times C_{rb}\right)$$ **Equation 4-29**

The free tissue concentration (C_{lf}) is related to the blood flow to the tissue (Q_{m-l}) and the tissue volume (V_l) as well as the concentration gradient between the afferent blood (C_{m-l}) and the venous blood exiting the tissue (C_{lb}), as follows:

$$\frac{dC_{lf}}{dt} = \frac{Q_{m-l}}{V_l} \times (C_{m-l} - C_{lb})$$ **Equation 4-30**

The rate of change in the dioxins concentration in tissue blood (C_{lb}) is related to the tissue concentration through a blood:tissue partition coefficient (P_l) as follows:

$$C_{lb} = \frac{C_{lf}}{P_l}$$ **Equation 4-31**

The term C_{BM} represents the dioxins bound to the liver proteins at Ah receptor (AhR) and to the plasma protein. As discussed in section 3.3.6, dioxins bind to AhR with a binding affinity of KB1, as follows (Buckley 1995):

$$AhR + TCDD \xleftrightarrow{KB1} [AhR - TCDD]$$ **Equation 4-32**

The ternary binding between TCDD, Ah receptor and DNA sites has been shown using a Hill coefficient (n) to represent interaction between multiple DNA binding sites with a binding affinity of K_d , as follows (Buckley 1995):

$$[AhR - TCDD]^n + DNA^n \xleftrightarrow{K_d} [AhR - TCDD - DNA]^n$$ **Equation 4-33**

The Hill coefficient is used in biochemistry to describe the concept of cooperative binding, which is the effect of existing ligands on a macromolecule in enhancing binding of other ligands on the same macromolecule.

$[AhR-TCDD]$ is a function of the amount of basal proteins occupied by dioxins at Ah receptors. The change in the total (occupied and un-occupied) basal protein has been shown in terms of the basal synthesis rate, induced rate and degradation rate (Kedderis et al. 1993, Buckley 1995 and Wang et al. 2000).

Kedderis et al. (1993) studied the disposition and enzyme induction properties of 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) in rats through development of a PBPK model. In their model, they included a ternary interaction between TBDD, the Ah receptor and specific DNA-binding sites, as well as induction of a TBDD-binding protein specific to the liver (assumed to be CYP1A2). Wang et al. (2000) developed a PBPK model for dioxins in rats. In their model, they included the AhR concentrations, CYP1A2 concentrations and binding affinities to the AhR receptor.

The proposed equations relate the basal protein ($BM2_t$) to the synthesis rate at its basal (K_0) and maximum (K_{0max}) values, concentration of Ah receptors occupied by TCDD ($[AhR-TCDD]$), binding affinity with DNA (K_d), degradation rate (K_1) and the Hill coefficient (n), as follows:

$$\frac{dBM2_t}{dt}=K_0\times(1+K_{0\max}\times\frac{[AhR-TCDD]^n}{[AhR-TCDD]^n+K_d{}^n})-K_1\times BM2_t \qquad \textbf{Equation 4-34}$$

Kedderis et al. (1993) and Wang et al. (2000) defined the concentration bound to the Ah receptor (A1) as a function of free tissue concentration (C_{lf}), concentration of Ah receptor (BM1), and binding affinity (KB1), as follows:

$$A1=\frac{BM1\times C_{lf}}{KB1+C_{lf}} \qquad \textbf{Equation 4-35}$$

The concentration bound to the CYP1A2 (A2) was similarly defined as a function of free tissue concentration (C_{lf}), amount of basal protein (BM2T), and binding affinity (KB2), as follows:

$$A2=\frac{BM2T\times C_{lf}}{KB2+C_{lf}} \qquad \textbf{Equation 4-36}$$

Liver metabolizes dioxins and eliminates it. For a first-order elimination rate of K_m (/time), the eliminated concentration (C_{el}) is defined as a function of the free concentration of dioxins in the tissue (C_{lf}), as follows (adopted from Parham and Portier 1998):

$$\frac{dC_{el}}{dt} = K_m \times C_{lf}$$ **Equation 4-37**

4.2.6 Concentration in Whole-Body Fish

The mass balance equation for the whole-body fish implies that the total amount of dioxins that entered fish equals the amount accumulated in tissues plus the amount eliminated.

The concentration in whole-body fish at any point in time is calculated as average of concentrations in various tissues, and their respective blood, as follows:

$$C_{fish} = \frac{\sum C_t \times V_t}{\sum V_t} + \frac{\sum C_{tl} \times V_{tl}}{\sum V_{tl}}$$ **Equation 4-38**

4.3 Equations Used in Model

A list of equations selected for use in the model is given below. Notations are defined previously where the equations were first introduced.

Fish growth was modeled using Equation 4-1, as repeated here:

$$W(t) = W_\infty (1 - e^{-kt})^b$$ **Equation 4-39**

Dissolved water concentration was calculated using Equation 4-15, as repeated here:

$$C_{wd} = \frac{C_w}{1 + 0.35 \times POC \times K_{ow} + 0.08 \times DOC \times K_{ow}}$$ **Equation 4-40**

Flux across gills was estimated using Equation 4-9 , as repeated here:

$$C_b = C_{vb} + \frac{K_{x-g}}{Q_T} \times (C_{wd} - \frac{C_{vb}}{P_{bw}})$$ **Equation 4-41**

The overall exchange coefficient was estimated using Equation 4-7, as repeated here:

$$K_{x-g} = \frac{e^{-K_{dl}/K_{bf}} - e^{-K_{dl}/K_{wf}}}{\frac{e^{-K_{dl}/K_{bf}}}{K_{wf}} - \frac{e^{-K_{dl}/K_{wf}}}{K_{bf}}}$$ **Equation 4-42**

where the diffusion limitation exchange coefficient was calculated using Equation 4-8, as repeated here:

$$K_{dl}=Perm\times A_{gills}/T_{path}$$ **Equation 4-43**

and where K_{bf}, = Q_T x P_{bw} and K_{wf}= Q_w.

Uptake through diet was modeled using Equation 4-16 to Equation 4-18, as repeated here:

$$\frac{dA_{fec}}{dt}=(Q_{food}\times C_{food})-(Q_{fec}\times C_{fec})-PA_{gt}\times(C_{fec}-C_{gt}\times P_{fgt})$$ **Equation 4-44**

and

$$\frac{dA_{gt}}{dt}=Q_{gt}\times(C_b-C_{gtb})-PA_{gt}\times(C_{fec}-C_{gt}\times P_{fgt})$$ **Equation 4-45**

$$C_{gtb}=\frac{C_{gt}}{P_{gt}}$$ **Equation 4-46**

Concentration in richly-perfused tissues was calculated similar to Equation 4-19, as follows:

$$\frac{dC_r}{dt}=\frac{Q_r}{V_r}\times(C_b-C_{rb})$$ **Equation 4-47**

Concentration in the venous blood leaving the richly-perfused tissues is calculated as follows:

$$C_{rb}=\frac{C_r}{P_r}$$ **Equation 4-48**

Concentrations in slowly-p tissues and fat were calculated based on Equation 4-25 and Equation 4-26, as repeated here:

$$\frac{dC_s}{dt}=\frac{PA_s}{V_s}\times(C_{sb}-\frac{C_s}{P_s})$$ **Equation 4-49**

And, the following equation was used for blood concentration:

$$\frac{dC_{sb}}{dt}=\frac{1}{V_{sb}}[Q_s\times(C_b-C_{sb})+PA_s\times(\frac{C_s}{P_s}-C_{sb})]$$ **Equation 4-50**

For fat, the subscript "s: is replaced by "f".

Concentration in kidney was calculated based on Equation 4-47 and Equation 4-48, as repeated here:

$$Q_{m-k}=Q_k+0.6\times Q_s$$ **Equation 4-51**

$$C_{m-k}=(\frac{Q_k}{Q_{m-k}}\times C_b)+(\frac{0.6\times Q_s}{Q_{m-k}}\times C_{sb})$$ **Equation 4-52**

$$\frac{dC_k}{dt}=\frac{Q_{m-k}}{V_k}\times(C_{m-k}-C_{kb})$$ **Equation 4-53**

$$C_{kb}=\frac{C_k}{P_k}$$ **Equation 4-54**

Free concentration in liver tissue was calculated based on Equation 4-28, as repeated here:

$$Q_{m-l}=Q_l+Q_r$$ **Equation 4-55**

$$C_{m-l}=(\frac{Q_l}{Q_{m-l}}\times C_b)+(\frac{Q_r}{Q_{m-l}}\times C_{rb})$$ **Equation 4-56**

$$\frac{dC_{lf}}{dt}=\frac{Q_{m-l}}{V_l}\times(C_{m-l}-C_{lb})$$ **Equation 4-57**

Liver blood concentration was calculated using Equation 4-24, as repeated here:

$$C_{lb}=\frac{C_{lf}}{P_l}$$ **Equation 4-58**

Dioxin bound to the liver tissue was calculated as the sum of Equation 4-35 and Equation 4-36, as follows:

$$C_{BM}=\frac{BM1\times C_{lf}}{KB1+C_{lf}}+\frac{BM2_t\times C_{lf}}{KB2+C_{lf}}$$ **Equation 4-59**

The first term represents the binding of TCDD to AhR, and therefore, it was considered equal to $[AhR-TCDD]$.

$BM2_t$ was calculated based on Equation 4-34, as follows:

$$\frac{dBM2_t}{dt}=K_0\times(1+K_{0\max}\times\frac{[AhR-TCDD]^n}{[AhR-TCDD]^n+K_d{}^n})-K_1\times BM2_t$$ **Equation 4-60**

Elimination in liver was defined based on free concentration in liver tissue, as shown in Equation 4-37:

$$\frac{dC_{el}}{dt}=K_m\times C_{lf}$$ **Equation 4-61**

And the total concentration in liver tissue was calculated as shown here:

$$\frac{dC_l}{dt}=\frac{dC_{lf}}{dt}+\frac{dC_{BM}}{dt}-\frac{dC_{el}}{dt}$$ **Equation 4-62**

The rate of change for the venous blood concentration was calculated using Equation 4-14, which has the following form:

$$\frac{dC_{vb}}{dt}=\frac{1}{V_b}\left[\left(\sum Q_t C_{tb}\right)-Q_T C_{vb}\right]$$ **Equation 4-63**

Concentration in whole-body fish was calculated following Equation 4-38, as repeated here:

$$C_{fish}=\frac{\sum C_t \times V_t}{\sum V_t}+\frac{\sum C_{tl} \times V_{tl}}{\sum V_{tl}}$$ **Equation 4-64**

4.4 Solution of Equations

Computer implementation of the model requires the use of numerical simulation models to solve the differential equations. There are a number of integration algorithms and programming languages available for coding and solving PBPK model equations, such as Acslx (which is the most commonly used for PBPK modeling in the toxicology community), Matlab (mathematical software with matrix-related computations and numerical integration) and Microsoft Excel (US EPA 2006a). Acslx (AEgis 2008) was used in this study due to relative ease of use and popularity in the toxicology community.

5 DATA COLLECTION

PBPK models are often used to estimate target tissue dose when there is little data on exposure conditions. In the absence of an optimal data set (including experimental kinetic data for the chemical in the target tissue), data on the toxic mechanisms, physiological constants of the animal and biochemical or metabolic coefficients are used to parameterize a PBPK model.

A detailed literature review was conducted in order to obtain values for model parameters introduced in section 4. Some of the values were used only as initial estimates and were refined later through parameter estimation using observed values.

5.1 PHYSIOLOGICAL AND BIOCHEMICAL/METABOLIC COEFFICIENTS

5.1.1 Physiological Constants

Published data on physiology of various fish species are plentiful; however, the model can only be calibrated for the fish species with existing dioxin experiments. These species include rainbow trout, fathead minnow and medaka (see section 5.2 for studies in which the results are expressed in terms of fish/tissue concentrations).

In addition, each study provides values for a different set of parameters, which needed to be interpreted to represent the dataset required by the specific PBPK model (see section 4.24.3).

The values of parameters involved in fish growth model were obtained from published data and are presented in Table 5-1.

Table 5-1: Parameter Values for Growth Model

Parameter	Notation	Unit	Rainbow trout	Fathead minnow	Medaka
Asymptotic Length	$L\infty$	cm	-	6	-
Asymptotic Weight	$W\infty$	g	6670	-	208
Growth Coefficient	k	/d	0.00076	0.00194	0.0016
Power Term	b	-	2.911	3	3
Reference	-	-	Pidgeon 1981 cited in Fishbase	Page and Burr (1991) cited in Fishbase	k and b: Roberts, T.R. (1998) cited in Fishbase
Others	-	-	-	W= 2.11* L- 9.92 (Verreycken 2006)	$W\infty$ calculated based on a weight of 175 mg at 62 days

Example usage in growth model: for rainbow trout, the weight-age function was defined as $W(t)=6670\times(1-e^{-0.00076\,t})^{2.911}$, where weight is in g and time is in days.

The cardiac output of fish (Q_T) for rainbow trout was estimated as a function of temperature (Temp in °C) and body weight (BW in kg) as per Nichols et al. (1998):

$$Q_T = ((5.69 \times Temp) - 18.6) \times BW$$ **Equation 5-1**

The cardiac output for fathead minnow was estimated using an allometric equation, as follows:

$$Q_T = Q_{T0} \times (BW)^{0.75}$$ **Equation 5-2**

Q_{T0} was estimated using one data point from Freidig (2000) where cardiac output was 21 mL/h for a 5 g fish.

For medaka, a relationship suggested by Erickson and McKim (1990, cited in Lien and McKim 1993) was used to relate the cardiac output (Q_T in L/kg.h) to temperature (Temp in °C; average of 15 °C) and body weight (BW in g) as follows:

$$Q_T = (0.23 \times Temp - 0.78) \times \left(\frac{BW}{500}\right)^{-0.1}$$ **Equation 5-3**

The published values for volume and blood flow for each organ of rainbow trout were summarized (Low et al. 1991, Nichols et al. 1990 and Nichols et al. 2004) and ratios were calculated as presented in Table 5-2. The physiological values for fathead minnow and medaka based on Freidig (2000), as shown also in Table 5-2.

Blood volume in each tissue was considered 3% of the tissue volume.

Table 5-2: Physiological Parameter Values

Compartment	Tissue Blood Flow / Cardiac Output (%)			Tissue Volume/ Body Weight (%)		
	Rainbow Trout	Fathead Minnow	Medaka	Rainbow Trout	Fathead Minnow	Medaka
Blood	n/a	n/a	n/a	3	3	3
Gills	n/a	n/a	n/a	2	3	3
Fat	8	8	8	7	10	10
Kidney	8	6	6	1	1	1
Liver	3	3	3	2	2	2
Richly perfused	19	23	23	7	6	6
Slowly perfused	56	60	60	65	74	74
Guts	6	n/a	n/a	17	n/a	n/a

References:

- Rainbow trout: Calculated based on Low et al. 1991, Nichols et al. 1990 and Nichols et al. 2004
- Fathead Minnow: Freidig (2000)
- Medaka: assumed same as minnow

n/a : not required

Ventilation volume (Q_v), effective respiratory (Erv), and effective ventilation volume (Q_w, = Q_v x Erv) were taken from available published data for the three species.

For rainbow trout, the ventilation volume was estimated using the equation suggested by Nichols et al. (1998), which used oxygen consumption rate (VO_2 in mg/kg.d, a function of body weight and temperature), dissolved oxygen content of water (DO, mg/L), oxygen uptake efficiency from expired water (U%) and body weight (BW in g), as follows:

$$Q_v = \frac{VO_2}{DO \times U\%} \times \frac{BW}{1000}$$ **Equation 5-4**

And

$$VO_2 = (12.43 \times BW^{-0.165}) \times Temp^{1.2} \times e^{-0.0314 \times Temp}$$ **Equation 5-5**

An average temperature (Temp) of 15 °C, a U% of 0.58 (Nichols et al. 1998) and an oxygen content of 7 mg/L were used in calculation of VO_2.

For ventilation volume for fathead minnow and medaka, an alometric equation was used similar to that used for cardiac output. The reference effective ventilation volume for fathead minnow was 0.2 L/h for a 5 g fish (Freidig 2000). For medaka, the reference volume was 0.014 L/h for a 202 mg fish (Lien and McKim 1993) and the allometric equation had the following form:

$$Q_v = Q_{v0} \times (BW)^{0.75}$$ **Equation 5-6**

Other physiological parameters, which impact uptake through the gill, are the gill surface area (A_{gill}) and the average thickness of the diffusion path length (T_{path}). The gill surface area was estimated through an alometric equation proposed for trout, with a coefficient of 0.0006 m^2, as follows (Nichols et al. 1998):

$$A_{gill} = 0.0006 \times (BW)^{0.8}$$ **Equation 5-7**

For fathead minnow and medaka, the equation suggested by Lien and McKim (1993) with a coefficient of 0.000865 m^2 was used, which is as follows:

$$A_{gill} = 0.000865 \times (BW)^{0.785}$$ **Equation 5-8**

The average thickness of the diffusion path was considered 14 μm for the rainbow trout and 8 μm for fathead minnow and medaka (Lien and McKim 1993).

5.1.2 Biochemical Constants for Dioxins in Fish

Absorption, distribution, metabolism and elimination of dioxins in fish are controlled not only by physiological characteristics of the fish, but also by how the chemicals interact with and within each compartment. The biochemical parameter values collected from published data are

presented here. Due to lack of comprehensive datasets, the values found were used for all fish species and then refined through model calibration.

5.1.2.1 Absorption

Uptake through the gill is controlled by physiologic parameters (discussed above), partitioning between blood and water (P_{bw}), and the gill permeability for dioxin, also called the molecular diffusivity of dioxins (Perm).

Molecular diffusivity of 2,3,7,8-TCDD in water has been estimated at 5.6E-06 cm^2/s (Thibodeaux 1996). The initial value for diffusion rate constant for branchial flux was considered the same as that in water.

Initial values for parameters involved in uptake through diet were mostly based on Nichols et al. (1998). The parameters include the diffusion capacity from the gut lumen to the gut tissue, food consumption and the feces egestion rate. Nichols et al. (1998) calibrated their model with an egestion rate of 1.6 times the food consumption rate. However, in order to maintain the overall mass balance, these two rates were considered equal in the model proposed in this study. The parameter values are shown in Table 5-3.

Table 5-3: Initial Parameter Values for Uptake through Diet for Rainbow Trout

Parameter	Notation	Unit	Value
Diffusion capacity	PAgt	L/d	α * BW ^0.75
Gut diffusion constant	α	L/kg.d	0.07
Food consumption	Qfood	L/d	0.0056 * BW
Feces egestion rate	Qfec	L/d	ff * Qfood
Feces to Food ratio	ff	-	1

Reference: Nichols et al. (1998), except for ff.

5.1.2.2 Distribution

Distribution of chemical in blood and tissues can be advective transport via blood flow or passive diffusion across the membrane. Parameters that need to be quantified include partition coefficients (tissue: blood) for all compartments, and the mass transfer coefficient (or permeability) for tissues with a diffusion-limited distribution.

Tissue:blood partition coefficients are usually determined in vitro using the vial equilibrium method (US EPA 2006); however, in the absence of experimental data, published data were used as initial estimates for the purpose of this study.

Nichols et al. (1991) proposed a relationship between the blood:water (P_{bw}) and tissue:water (P_{tw}) partition coefficients and log K_{ow}, the sample's nonpolar lipid content (F_f) and the sample's water content (F_w), as follows:

$$P_{bw} or P_{tw} = (10^{\log K_{ow}} \times \frac{F_f}{100}) + (1 + \frac{F_w}{100}) \qquad \text{Equation 5-9}$$

The tissue: blood partition coefficient (P_t) can then be calculated using tissue:water and blood:water coefficients, as follows:

$$P_t = \frac{P_{tw}}{P_{bw}} \qquad \text{Equation 5-10}$$

Data on nonpolar lipid content and water content of tissues are provided in Bertelsen et al. (1998) and Lien et al. (2001) for various fish species. A summary of these data and the calculated tissue:blood partition coefficients for rainbow trout is shown in Table 5-4.

The partition coefficient (P_{bw}) between total blood (plasma water and cells) concentration (C_b) and plasma water concentration (C_f) was calculated as follows:

$$P_{bw} = \left(10^{Log\,6.75} \times \frac{0.7}{100}\right) + \left(1 + \frac{83.9}{100}\right) = 39{,}366$$

Table 5-4: Estimation of Partition Coefficients for Dioxins and Furans in Rainbow Trout

a) Lipid and water content of tissues for trout(Bertelsen 1998 and Lien et al. 2001):

Tissue	Nonpolar lipid content (%)	Water content (%)
Blood	0.7	83.9
Fat	93.4	5
Kidney	3.7	78.9
Liver	1.8	74.6
Richly-p *	10.3	69.4
Slowly-p *	2.7	76.3

* Richly-p and slowly-p tissue values are from Lien et al. (2001) for total lipid, which were assumed same as non-polar lipid.

b) Tissue:water and tissue:blood partition coefficients (calculated; dimensionless):

Tissue	Tissue: water	Tissue:blood
Fat	5,252,268	133
Kidney	208,067	5.3
Liver	101,222	2.6
Richly-p	579,515	15
Slowly-p	151,660	3.9

Example for fat: P_{tw} was calculated similar to P_b (above) at 5,252,268, P_{tb} was calculated as 5,252,268/ 39,366= 133.

b) Other Tissue:water and tissue:blood partition coefficients (based on Nichols et al. 1998):

Parameter	Value
Feces to gut partition coefficient	5 (median of 0.1-10)
Feces to gastrointestinal tract partition coefficient	2

In tissues with diffusion-limited distribution (fat, liver and other slowly perfused tissues), the dioxins transport from tissue blood to the tissue is explained using a mass transfer coefficient (PA_t) (Buckley 1995). This is called a permeability or diffusion constant by some authors (e.g. Parham and Portier 1998). PA_t data for dioxins in fish are not well documented in the current literature. Therefore, scarce available data were used to establish initial values for these parameters.

Nichols et al. (1998) developed a PBPK model for maternal transfer of 2,3,7,8-TCDD in brook trout. They called the permeability constant for fat, the 'capacity for chemical diffusion from blood to fat tissue' and suggested a value of 0.25 times the fat blood flow rate.

The initial value for other tissues was selected using the same ratios suggested by Wang et al. (2000) for a PBPK model for dioxins in rodents, where the permeability constant for the slowly-perfused tissues and fat were considered 0.25 of the tissue blood flow.

5.1.2.3 Elimination

The chemical overall elimination rate (including elimination across the gills and through metabolism) has been related to hydrophobicity by several authors. Nowell et al. (1999) compiled data from several other authors and developed a relationship between a logarithm of elimination (k_2) and the log K_{ow} for selected halogenated compounds. These data (with different symbols for each study) are shown in Figure 5-1.

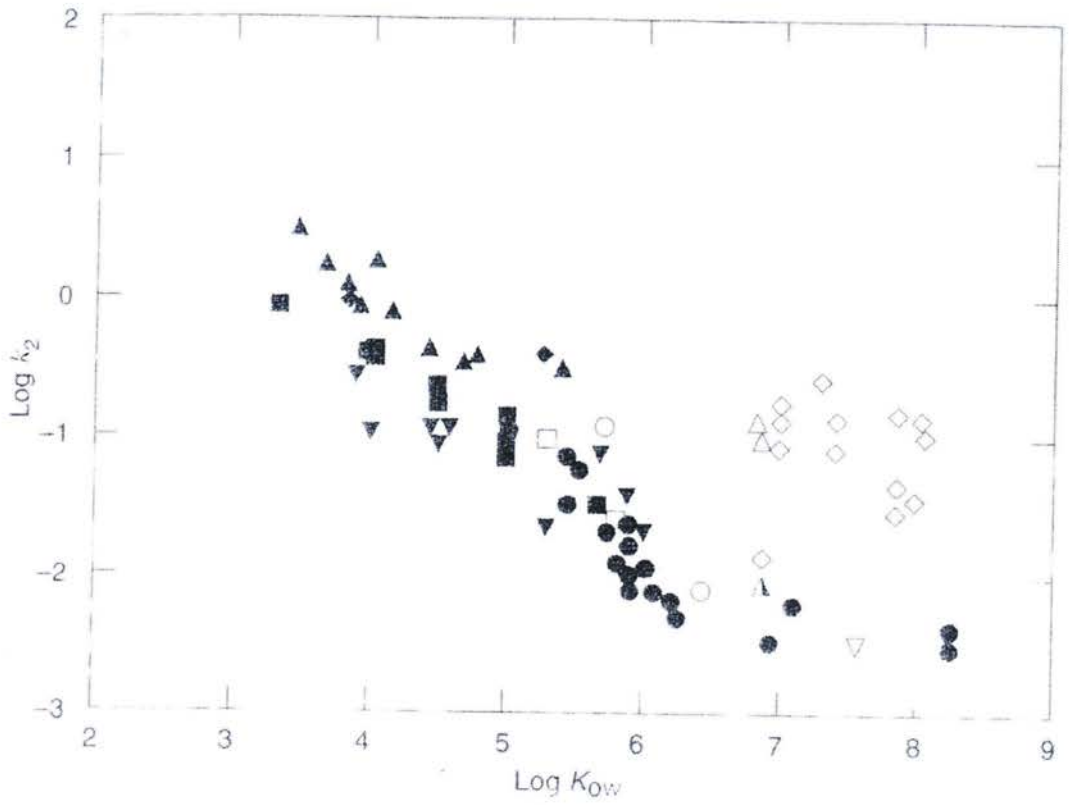


Figure 5-1: Relationship between Log of Elimination Rate Constant (log k_2) and Log of Octanol-Water Partition Coefficient (log k_{ow})

Reference: Howell et al. (1999); PCDDs are shown by a ◊, and PCDFs are shown by a Δ.

Based on Figure 5-1, the log k_2 for dioxins and furans is in the range of -0.7 to -1.5, resulting in k_2 values of 0.032 to 0.2 /d.

Schmieder et al. (1995) estimated an elimination rate constant for medaka exposed to 2,3,7,8-TCDD in flowing water. They exposed the fish to contaminated water (101 ± 26 pg/L) for 12 days and then quantified the elimination after 28, 90 and 175 days in uncontaminated water. The elimination rate was estimated using a one-compartment, linear mass balance model with adjustment for growth at 0.0045/d.

Adams et al. (1986) evaluated TCDD toxicity in fathead minnows using 28 days of exposure to 0.85-1.39 ng/L followed by a 20- day period of observation and estimated an elimination rate constant of 0.12/d for 2,3,7,8-TCDD. This value was used as the initial value for elimination through metabolism (used in section 4.24.3).

A summary of biochemical constants discussed so far and their initial values are shown in Table 5-5.

Table 5-5: Initial Values for Biochemical Constants (Except for Binding and Induction)

Parameter	Notation	Initial Value	Unit
Partition coefficients:			
Blood- water	Pbw	39366	-
Fat- blood	Pf	133	-
Kidney- blood	Pk	5.3	-
Liver- blood	Pl	2.6	-
Richly-perfused tissue- blood	Pr	15	-
Slowly-perfused tissue- blood	Ps	3.9	-
Guts	Pgt	2	-
Permeability:			
Fat	PAf	0.25* Qf	L/d
Gut	PAgt	0.25 * Qgt	L/d
Slowly-perfused tissue	PAs	0.25 * Qs	L/d
Elimination rate through metabolism	Km	0.12	/d

References:

- Partition coefficients: calculated based on data in Bertelsen (1998), Lien et al. (2001), method by Nichols et al. (1991), and Nichols et al. (1998)
- Permeability: for fat based on Nichols et al. (1998); proportions for other based on Wang et al. (2000)
- Elimination: Adams et al. (1986)

5.1.2.4 Molecular Toxicity

In order to characterize the binding of dioxins to the fish liver, values need to be assigned to the Ah receptor and CYP1A2 characteristics. Therefore, published data were reviewed to establish initial values for these parameters.

Lorenzen and Okey (1990) undertook a study to detect and characterize the binding of 2,3,7,8-TCDD to Ah receptor in a rainbow trout hepatoma cell line in rainbow trout. They suggested that the binding affinity of the cytosolic Ah receptor was about 0.4 nM. The concentration of Ah receptor was about 20 fmol/mg cytosolic protein or approximately 4400 receptors per cell. This is about half to one fifteenth of the concentrations observed in rodents.

Giesy et al. (2002) exposed adult female rainbow trout to dietary 2,3,7,8-TCDD (1.8, 18 and 90 ng TCDD/kg wet weight food) for up to 300 days. They measured the liver EROD activity and EROD induction fold resulting from exposure to various doses and periods (see Table 5-6). The maximum observed induction fold was 24.8 for exposure to 1.8 ng TCDD/kg food for 200 days. The results are in concert with findings of other studies suggesting that CYP1A1 activity varies seasonally. It also varies based on health, reproductive development status (decreases with the approach of spawning), conditions of fish and environmental temperature.

Table 5-6: EROD Activity and EROD Induction Fold Measured in Female Rainbow Trout Exposed to Dietary 2,3,7,8-TCDD

a) Liver EROD Activity (pmol/min/mg)

Days	Dietary Concentration (ng TCDD/kg food)			
	0	1.8	18	90
50	29.7	45.23	67.58	57.9
100	5.193	12.46	11.21	24.09
150	32.27	17.75	33.67	60.12
200	1.41	34.59	16.46	1.912

b) Induction Fold

Days	Dietary Concentration (ng TCDD/kg food)			
	0	1.8	18	90
50	1	1.5	2.3	1.9
100	1	2.4	2.2	4.6
150	1	0.6	1.1	1.9
200	1	24.8	11.7	1.4

Reference: Giesy et al. (2002)

Liver EROD represents the catalytic activity associated with the CYP1A1 synthesis rate (Braunbeck and Hinton 1998). Based on data presented in Table 5-6, the basal synthesis rate was set at 17 pmol/min/mg and the induction fold was set to 4.7. These values were used in Equation 4-60.

For the remaining parameters where no data were found for fish, data used or estimated by Wang et al. (1997) for rats were used as the initial estimates.

Initial estimates for parameters identified in binding process are presented in Table 5-7.

Table 5-7: Initial Parameter Values for Binding and Induction of Dioxins

Parameter	Notation	Initial Value	Unit	Reference
Total Ah receptor in liver †*	AhR0	0.012	nM	Wang e al. (1997) and Lorenzen and Okey (1990)
Basal CYP1A level	BM2T0	1.6	nmol/g	Wang e al. (1997)
TCDD-Ah binding affinity*	KB1	0.4	nM	Lorenzen and Okey (1990)
TCDD- CYP1A binding affinity	KB2	9	nM	Kedderis et al (2002)
TCDD-Ah-DNA binding affinity	Kd	130	nM	Wang e al. (1997)
Basal synthesis rate *	K0	17	pmol/min/mg	Giesy et al. (2002)
Maximum fold in synthesis rate *	K0max	4.7	-	Giesy et al. (2002)
Hill coefficient	n	0.6	-	Wang e al. (1997)
Degradation rate	K1	0.035	/hr	Kedderis et al (2002)

† 0.12 based on Wang e al. (1997); divided by 10 for fish (based on Lorenzen and Okey 1990)

* For a fish species; other values are for rodents

5.2 Data for Model Evaluation and Result Interpretation

Since no experiment was conducted in support of this modeling effort, published data were reviewed for calibration of the developed PBPK model.

Studies on the accumulation of chemicals in aquatic organisms are conducted either on the organisms collected from the actual environment (field studies), or they can be conducted under controlled conditions in laboratories. Some studies are conducted on the live animal (in vivo) or on organs removed from the animal (in vitro). Laboratory studies usually provide a description of the pathways and other conditions involved and are conducted under controlled conditions. They are therefore more relevant for calibration of the PBPK model developed in this study. However, field studies are also briefly described here as they can be used for comparison purposes.

5.2.1 Laboratory Studies

Several laboratory studies have been conducted on the accumulation and disposition, as well as toxic mechanisms of dioxins in fish. In these studies, species have been exposed to dioxins through water, food or injection. These studies are listed in Table 5-8. For each study, a brief description of the experiment conditions and the relevant results are presented below.

Table 5-8: Experiments on Dioxin (2,3,7,8-TCDD) in Fish

Exposure Route	Fish Species	Life Stage	Result Types	Reference
Water	Fathead minnow	Juvenile	Fish concentration	Adams et al. (1986)
Water	Medaka	Juvenile	Fish concentration	Schmieder et al. (1995)
Water	Zebrafish	Early life stage	Response	Henry et al. (1997)
Food and injection	Rainbow trout	Fingerling	Tissue concentration and response	Kleeman et al. (1986)
Food	Rainbow trout	Female adult	Tissue concentration and response	Walter et al. (2000)
Food	Rainbow trout	Female adult	Fish and egg concentration and response	Giesy et al. (2002)
Food	Zebrafish	Female adult	Tissue concentration and response	Heiden et al. (2005)

Adams et al. (1986) evaluated 2,3,7,8-TCDD toxicity in juvenile fathead minnows (0.5-1 g) using 28 days of exposure to 0.85-1.39 ng/L (in water) followed by a 20- day period of observation. The results of this study are provided in Table 5-9.

Table 5-9: Water and Whole Body Fish Concentration of 2,3,7,8-TCDD in Juvenile Fathead Minnow Exposed to ,3,7,8-TCDD in Water

Time (day)	Water Concentration (ng/L)	Fish Concentration (µg/kg dry weight)
6	1.39	15
14	1.01	18.3
22	0.9	20.2
26	0.85	22.4
28	0.87	25.4
32	n/a	21.2
40	n/a	15.8
48	n/a	11.7

Reference: Adams et al. (1986)
n/a: depuration period; concentration was not measured

Schmieder et al. (1995) exposed juvenile medaka (62-day old weighing an average of 175 mg) to ,3,7,8-TCDD (101 ± 26 pg/L) in flowing water. They exposed the fish to contaminated water for 12 days followed by 175 days in uncontaminated water and measured TCDD accumulation in fish from water at various times. Their findings are summarized in Table 5-10. They also noted that approximately 90% of the measured TCDD was in the extracted lipid.

Table 5-10: Residue Levels of 2,3,7,8-TCDD in Juvenile Medaka Exposed in Water

Time (day)	Water Concentration (pg/L)	Fish Concentration (pg/g wet weight)*
2	101	572
4	101	932
6	101	1328
10	101	2207
12	101	2408
40	0	1480
102	0	1002
187	0	757

Reference: Schmieder et al. (1995)

* The wet weight basis was implied from the paper.

Henry et al. (1997) characterized toxicity and histopathology of 2,3,7,8-TCDD in zebrafish early life stages from 12 to 240 hr postfertilization (hpf) following waterborne exposure of newly fertilized eggs. Egg dose of 1.5 ng TCDD/g or greater produced toxic responses in zebrafish larvae. The LD50 was determined at 240 hpf at 2.5 ng TCDD/g egg. Histological examination of the test animals revealed various epithelial lesions including arrested gill development and ballooning degeneration and/or narcosis of the renal tubules, hepatocytes, pancreas and all major brain regions.

Kleeman et al. (1986) studied accumulation, tissue distribution and depuration of 2,3,7,8-TCDD in fingerling rainbow trout (initial weight 7-14 g) by feeding it a diet containing 494 ppt TCDD for 13 weeks. This was followed by 13 weeks of feeding with the same diet without TCDD, and analysis of TCDD concentration and lipid concentration in various organs of fish (see Table 5-11). For unknown reasons, the liver was a minor site for TCDD accumulation, and the gill accumulated more TCDD than liver and kidney. The whole-body depuration half time of TCDD was estimated at 15 weeks.

Table 5-11: 2,3,7,8-TCDD Concentration and Lipid Content in Various Organs of Rainbow Trout Fed 494 ppt TCDD

Organ	TCDD Concentration (pg/g)		Lipid Content (g lipid/g)	
Visceral fat	3269	± 667	0.98	± 0.01
Pyloric caeca	355	± 79	0.48	± 0.06
Carcass	315	± 25	0.55	± 0.02
Gill	244	± 19		
Skin	201	± 40	0.29	± 0.01
Gastrointestinal tract	102	± 14		
Kidney	92	± 11		
Spleen	85	± 10		
Liver	72	± 9		
Heart	70	± 3		
Skeletal Muscle	29	± 3	0.14	± 0.01

Reference: Kleeman et al. (1986)

Walter et al. (2000) studied pathologic alterations in adult rainbow trout exposed to dietary 2,3,7,8-TCDD at 0, 1.8, 18 and 90 ng/kg food for up to 320 days. Effects ranged from a slight increase in the incidence of lesions of the caudal fins to a decrease in peripheral leukocyte counts after 50 and 100 days. The LOAEL for these effects was 5.69 ng TCDD/kg in diet and 0.90 ng TCDD/kg liver.

Giesy et al. (2002) exposed adult female rainbow trout to dietary 2,3,7,8-TCDD (1.8, 18 and 90 ng TCDD/kg wet weight food) for up to 300 days. At the end of the exposure, TCDD had been accumulated into tissues and eggs in a dose-dependent manner with a steady state starting after 50 to 100 days. Liver concentrations measured in this study are shown in Table 5-12. Giesy et al. also cite a study by Mehrle et al. (1988), where exposure of swim-up fry of rainbow trout to water concentrations of 0.038-0.789 ng dioxins/L for 28 days resulted in a whole body concentration of 0.99 ng/g (units are not shown and were implied).

Table 5-12: Liver Concentrations (ng /kg) Measured in Female Rainbow Trout Exposed to Dietary 2,3,7,8-TCDD

Days	Dietary Concentration (ng TCDD/kg food)		
	1.8	18	90
50	0.26	1.65	-
100	0.31	2.88	12.92
150	0.21	1.72	9.93
200	0.2	2.85	16.24

Reference: Giesy et al. (2002)

Heiden et al. (2005) studied accumulation, tissue distribution and maternal transfer of dietary 2,3,7,8-TCDD in adult female zebrafish (0.08-2.16 ng TCDD/fish.day). TCDDs accumulated in fish tissue in a time-dependent and dose-dependent manner, except in the

brain. The ovosomatic index was impacted by accumulation of 0.6 ng TCCD /g fish. Ten percent of test animals showed signs of ovarian necrosis following accumulation of about 3 ng/g. Offspring health was impacted with an accumulation of about 1.1 ng/g. Maternal transfer resulted in accumulation of 0.094-1.2 ng/g and it was sufficient to induce the blue sac syndrome, which is a typical endpoint of larval TCDD toxicity.

5.2.2 Field Studies

A number of field studies have been conducted in the region of the Great Lakes and its tributaries on contamination of the aquatic environment by dioxins.

In 1987, the governments of Canada and the United States made a commitment, as part of the Great Lakes Water Quality Agreement (GLWQA), to develop a Lakewide Management Plan for the Great Lakes (LaMP 2008). The data collected under this plan are maintained in a database called the FISHBASE. FISHBASE contains data on fish tissue concentrations in a fish fillet. It does not however, contain water concentrations. The LaMP status reports (which present analysis of the FISHBASE data) provide information on the critical pollutant indicators among others. The 2008 LaMP report, prepared based on 2004 data, indicated that the dioxin concentration in young-of-the-fish was below the respective guideline.

In a 1995-1997 sampling program from Tributaries to Lake Ontario, Woodfield and Estabrooks (1999) established a database of dioxin and furan concentrations in the various media (water, tissue and sediment) in the tributaries to Lake Ontario, 23 locations within Lake Ontario and several tributaries to Lake Erie. However, only two of the fish sampling sites corresponded to locations were water and sediment samples were collected. The analysis of data showed that there were elevated levels of dioxins or furans is a number of sediment samples.

In 2005, the US EPA prepared a report on Bioaccumulative Contaminants in Lake Ontario Surface Water using concentrations of PCBs, pesticides and other bioaccumulative contaminants measured in various locations in the Lake in waters greater than 100 m deep. Congeners detected in the suspended soil phase are summarized in Table 5-13. Dissolved phase dioxin and furan results were judged to be unreliable as for example, the suspended solids in the same location (with much lower detection limits than those achieved for the dissolved phase) did not confirm detectable levels of the contaminants.

Table 5-13: Dioxins and Furans Detected in Lake Ontario Surface Water Suspended Solids

Congener	Concentration (pg/L)
Octochlorodibenzodioxin (OCDD)	0.034 to 0.064
1,2,3,4,6,7,8 heptachlorinated dibenzodioxin (1,2,3,4,6,7,8-HpDD)	0.003 to 0.006
Octochlorodibenzofuran (OCDF)	0.001 to 0.004
1,2,3,4,6,7,8 heptachlorinated dibenzofuran (1,2,3,4,6,7,8-HpDF)	0.001 to 0.004
Tetradibenzofuran	0.0007 to 0.001

Reference: US EPA (2005) using 1999 data

The Ontario Ministry of the Environment (MOE) collects fish samples, including lake trout, from the Canadian Great Lakes and measures concentrations of 17 PCDDs and PCDFs in fish skinless, boneless fillet.

Data for lake trout in Lake Ontario (east of Scarborough) are summarized for 2,3,7,8 TCDD in Table 5-15. A total of five samples (four male and one female) fish was taken from this location. The length and weight of each fish was also measured. It can be seen from the data that the concentrations increase with increase in length and weight, except in one sample. This trend is seen in 10 other congeners measured; however, there is a decreasing trend for the six congeners. Water samples in these locations did not contain detectable concentrations of dioxins.

Table 5-14: 2,3,7,8 TCDD in Fillet of Lake Trout in a Site in Lake Ontario

Fish Sample	Sex	Length (cm)	Weight (g)	Concentration (pg/g wet weight)
1	M	57.2	2188	0.52
2	M	64.4	2936	1.4
3	M	65.7	3529	0.74
4	M	71.8	4910	0.86
5	F	77.4	6556	1.7

Reference: Personal communication with MOE staff (2008)

Several researchers have studied the receiving waters downstream of pulp and paper mills for the impact of dioxins (and other halogenated hydrocarbons) on fish. Servos et al. (1994) measured PCDDs and PCDFs in white sucker liver and fillet samples from seven pulp and paper mill sites and three reference sites in Ontario. Concentrations in liver were several fold higher than those in fillet. This difference was smaller with lipid normalization. Table 5-15 shows the 2,3,7,8-TCDD concentrations and lipid content of liver and fillet of female fish.

Table 5-15: 2,3,7,8-TCDD Concentration and Lipid Content in Fish Liver and Fillet Downstream of Pulp and Paper Mills

Sampling site	Liver concentration (pg/g wet weight)		Liver Lipid Content (%)		Fillet Concentration (pg/g wet weight)		Fillet Lipid Content (%)	
#1	6.09	± 2.23	21.24	± 5.65	1.75	± 0.5	5.41	± 1.18
#2	35.3	± 8.56	11.36	± 5.47	1.8	± 0.74	1.07	± 0.23
#3	14.9	± 11.94	35.17	± 4.72	1.85	± 1.32	1.36	± 0.17

Reference: Servos et al. (1994)

Wu et al. (2000) monitored 10-year-old common carp from contaminated sites in China for PCDD/F in various tissues. The concentrations were found to be the highest in liver, egg and intestine, followed by kidney and heart, followed by gill and bladder and lowest in muscle and brain (see Table 5-16). The results demonstrated less variation if normalized by lipid content.

Table 5-16: Concentration of 2,3,7,8-TCDD in Various Tissues of Common Carp in Contaminated Sites in China

Tissue	Concentration (ng/kg)	Tissue	Concentration (ng/kg)
Egg	0.44	Muscle	0.46
Gill	0.15	Brain	0.03
Bladder	0.95	Skin	0.93
Heart	0.14	Intestine	0.62
Kidney	0.46	Spleen	0.23
Liver	0.56		

Reference: Wu et al. (2000)

Wu et al. (2001) monitored PCDD/F in muscle and liver of common carp and big head from contaminated sites in China. They found out that muscle concentrations in different locations were comparable when normalized by lipid content, and that liver concentrations were higher than muscle concentrations. Table 5-17 shows the concentration of 2,3,7,8-TCDD and lipid content in muscle and liver of common carp of various ages caught in different locations.

Table 5-17: Concentration of ,3,7,8-TCDD and Lipid Content in Muscle and Liver of Common Carp in Contaminated Sites in China

Location	Fish Age (y)	Muscle Lipid Content (%)	Muscle Concentration (ng/kg lipid)	Liver Lipid Content (%)	Liver Concentration (ng/kg lipid)
#2	2	2.121	18.35	7.22	33.06
#3	1	1.509	19.02	6.52	107.5
#3	2	2.147	19.13	7.67	27.95
#3	3	3.336	13.79	8.78	68.83
#4	1	1.193	25.42	6.90	44.81
#4	2	2.02	14.06	7.63	188.3
#5	3	3.282	6.06	8.90	26.90

Reference: Wu et al. (2001)

5.3 PARAMETER ESTIMATION

The behavior of a PBPK model is governed by fundamental physiological and biochemical processes. While values for physiological parameters can usually be found in published literature, values for biochemical parameters are harder to locate by species and contaminant. In this model, such parameter values were established using published data for fish or other species. These values were used with caution and only as initial values.

The model predictions were compared to actual measurements (published data) to estimate the values of the most uncertain parameters values. These parameters include:

- Gill area and the thickness of the diffusion path
- Gut diffusion constant (in food model only)
- Blood: tissue partition coefficients
- Elimination rate
- Parameters used in binding equations

Since two relevant data sets were available for water exposure (see section 5.2), it was possible to calibrate the model with the two and compare the fitted parameters for consistency. For the food exposure route, one data set was used for calibration, but the other data set with tissue concentrations was not useful as it did not show the time course of concentrations. A summary of these data sets and their application is shown in Table 5-18.

Table 5-18: Use of Available Dataset for Model Calibration

Dataset	Description
Adams et al. (1986) Table 5-9	- Juvenile Medaka - Water exposure - C fish
Schmieder et al. (1995) Table 5-10	- Juvenile Fathead minnow - Water exposure - C fish
Giesy et al. (2002) Table 5-12	- Female adult rainbow trout - Food - C tissue

As discussed in section 2.3.3, the parameter estimation problem is usually formulated as an optimization problem which, based on the selected criteria, can be solved in many different ways. Selection of the criteria influences the accuracy of the estimated parameters, the efficiency of computations and the robustness of the errors. The Nelder-Mead parameter estimation algorithm provided in AcsIX software was used in this study. Based on communication with the software provider, the Nelder-Mead algorithm is the most reliable

algorithm and is frequently used by PBPK modelers. Based on the AcslX Optimum User's Guide, The Nelder-Mead "constructs a multidimensional shape called a simplex, which is defined by four vertices whose coordinates are trial values for the parameters. The optimization algorithm "walks" through the parameter space in search of an optimum by iteratively morphing the simplex using a simple heuristic for finding a better trial point".

A common practice in parameter estimation is to compare the model results with the observations and directly minimize the algebraic distance between each two corresponding points. The least square technique minimizes the sum of squared residuals (difference between an observed value and the value given by the model). For non-linear equations, numerical algorithms are used to find the parameter values that converge. However, complete convergence is often difficult to reach in practice.

The Chi square was calculated as a measure of goodness of the fit. The Chi square is one of the most robust non-parametric statistical techniques (Montgomery et al. 2005). An attractive feature of the Chi square is that it can be applied to any univariate data even if there is time dependence. It is also relatively distribution-free and the usual definition of normality is not required. However, this test is most appropriate when the errors are not too small. The concentrations were therefore converted to units of µg/kg before completing the test.

6 RESULTS

This section provides the solution of equations selected for this PBPK model (section 4) using the parameter values selected from published data or fitted to the observed data (section 5). The initial parameter values are summarized in Table 6-1 for medaka and minnow (water pathway), and rainbow trout (food pathway). The model results are presented for daily time steps, and start around the age in which the fish was exposed to dioxins.

Table 6-1: Initial Values for Model Parameter

Parameter	Notation	Unit	Fathead Minnow	Medaka	Rainbow Trout
Growth/physiological					
Growth Coefficient	K	/d	0.00076	0.00194	0.0016
Power Term in Growth	b	-	2.911	3	3
Cardiac output coefficient	QT0	L/d	0.151	n/a	n/a
Ventilation volume coefficient	Qv0	L/d	2.4	1.15	n/a
Effective ventilation	Erv	-	0.6	0.6	0.7
Gill surface area coefficient	Agills0	cm2	8.65	8.65	6
Average thickness of diffusion path	Tpath	m	8E-06	8E-06	1.4E-05
Uptake					
Blood-water partitioning (gills)	Pbw	L/kg.d	39366	39366	39366
Gill permeability for dioxin	Perm	cm2/s	5.6E-6	5.6E-6	5.6E-6
Diffusion capacity coefficient	α	-	n/a	n/a	1E-04
Feces to food ratio	ff	-	n/a	n/a	1
Partitioning					
Richly-perfused tissue: blood	Pr	-	15	15	15
Slowly-perfused tissue: blood	Ps	-	3.9	3.9	3.9
Fat: blood	Pf	-	133	133	133
Kidney: blood	Pk	-	5.3	5.3	5.3
Liver: blood	Pl	-	2.6	2.6	2.6
Gut: blood	Pgt		n/a	n/a	
Feces: gut	Pfgt	-	n/a	n/a	5
Feces: gastrointestinal tract	Pgt	-	n/a	n/a	2
Permeability					
Slowly-perfused tissue	PAsC	* Qs	0.25	0.25	0.25
Fat	PAfC	* Qf	0.25	0.25	0.25
Gut	PAgtC	* Qgt	n/a	n/a	0.25
Elimination					
Elimination rate	km	/d	0.12	0.12	0.12
Molecular toxicity					
Total AhR in liver	AhR	nM	0.012	0.012	0.012
Basal CYP1A in liver	BM2T0	nmol/g	1.6	1.6	1.6
AhR-dioxin binding affinity	KB1	nM	0.4	0.4	0.4
CYP1A-dioxin binding affinity	KB2	nM	9	9	9
DNA-dioxin binding affinity	kd	nM	130	130	130
Basal synthesis rate	K0	pmol/min/mg	17	17	17
Maximum fold in synthesis rate	K0max	-	4.7	4.7	4.7
Hill coefficient	n	-	0.6	0.6	0.6
Degradation rate	K1	/hr	0.035	0.035	0.035

** References for are shown in Table 5-2, Table 5-5 and Table 5-7 and throughout the text in section 5.

6.1 Model Results for Water-Exposure of Medaka/Fathead Minnow

The model was implemented for exposure of juvenile fathead minnow to 0.85-1.39 ng/L in water (2.6E-12 to 4.3-12 molar) of 2,3,7,8-TCDD in water (see Table 5-9). The predictions were made using parameter values shown in Table 6-1 and the concentrations observed in the species were compared to the model results. This comparison showed that the model underestimated the concentrations by an order of magnitude.

A detailed sensitivity analysis was completed, and the impacts of 50%, 100% and 200% change in parameter values on the whole body concentration were reviewed.

The parameters with the highest impact on the final result (whole body fish concentrations) were identified as follows:

- Gill surface area
- Average thickness of diffusion path
- Gill permeability for dioxin
- Tissue: blood partition coefficient for fat, liver and slowly-p tissues
- Elimination rate
- Binding affinity between CYP1A and dioxin

Less effective parameters include growth coefficient, blood:richly-p tissues partition coefficient, basal synthesis rate, and the Hill coefficients.

Other parameters which did not impose any noticeable impact even at 200% change include the power term in the growth model, effective ventilation, blood:water and blood: kidney partition coefficients, total AhR in liver, AhR-dioxin binding affinity, maximum fold in synthesis rate, DNA-dioxin binding affinity, degradation rate and basal CYP1A in liver.

Other settings such as environmental conditions were not taken into account in the model's calibration.

Figure 6-1 presents the results of the sensitivity analysis for the parameters showing some impact on the final results (i.e. the first two groups of parameters discussed above).

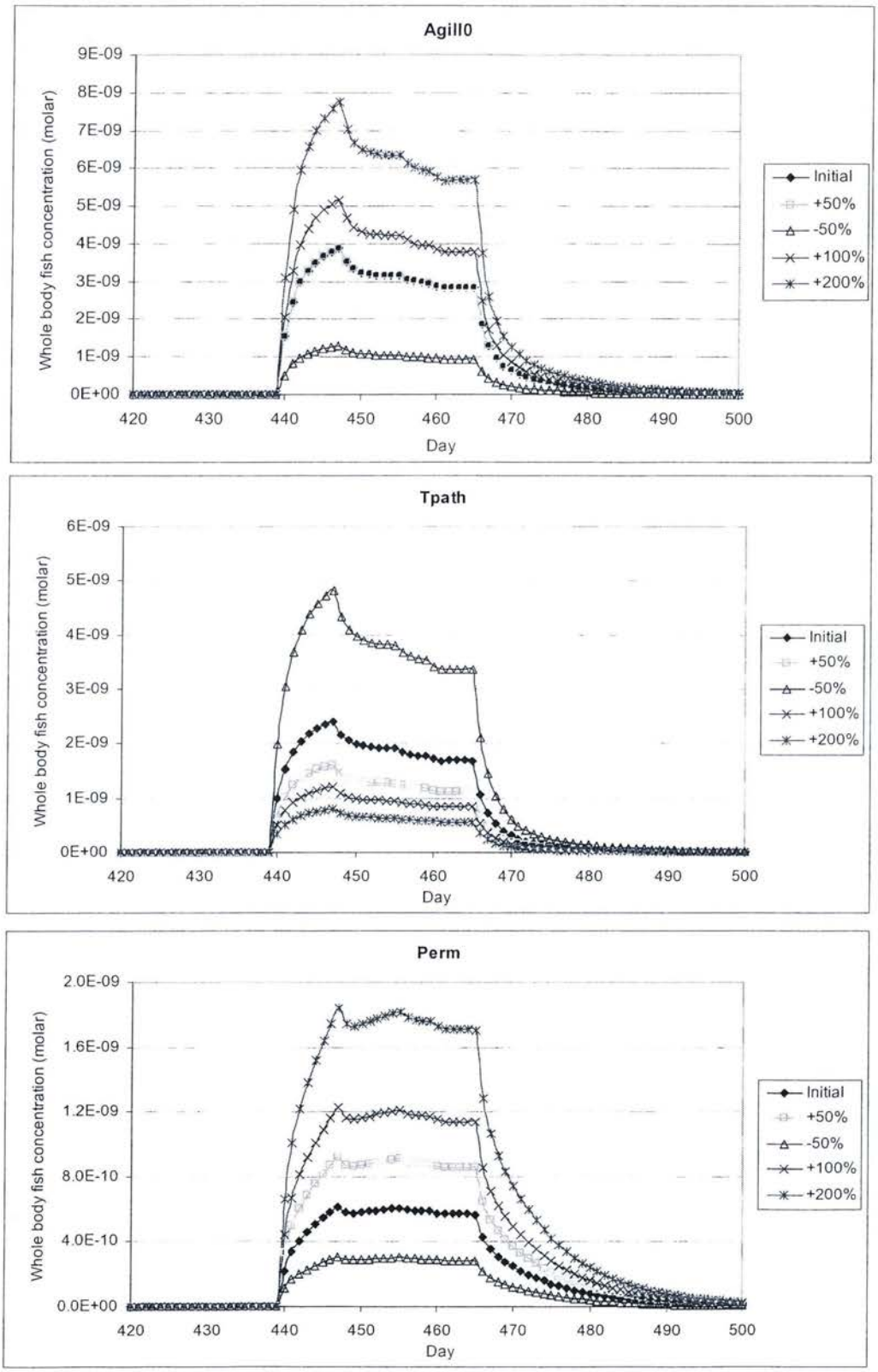
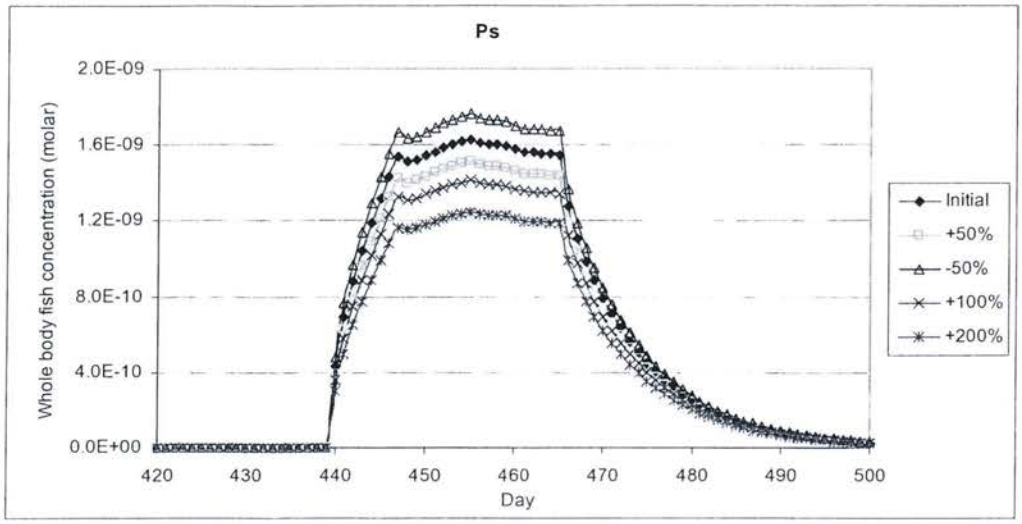
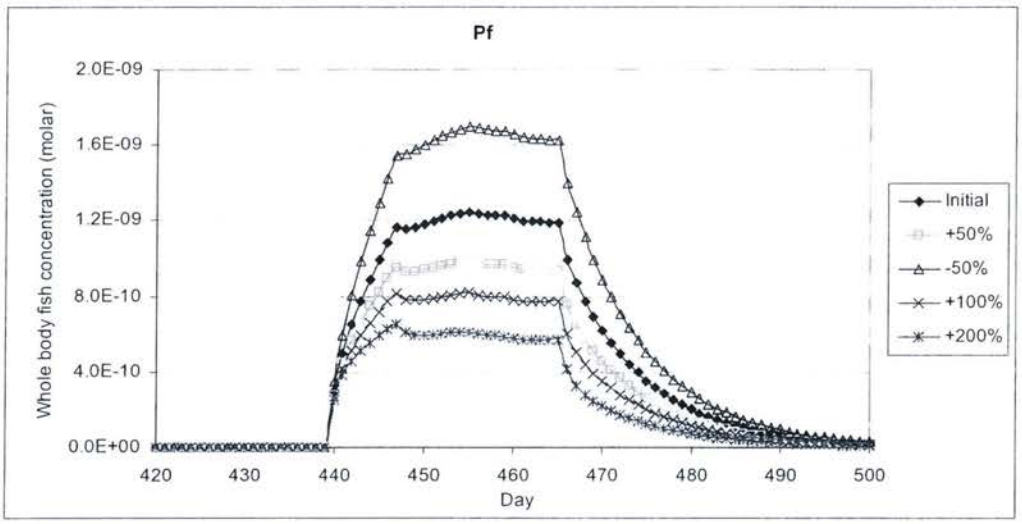


Figure 6-1: Impact of Changing the most Sensitive Parameters on Whole Body Concentration in Fathead Minnow Exposed to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water





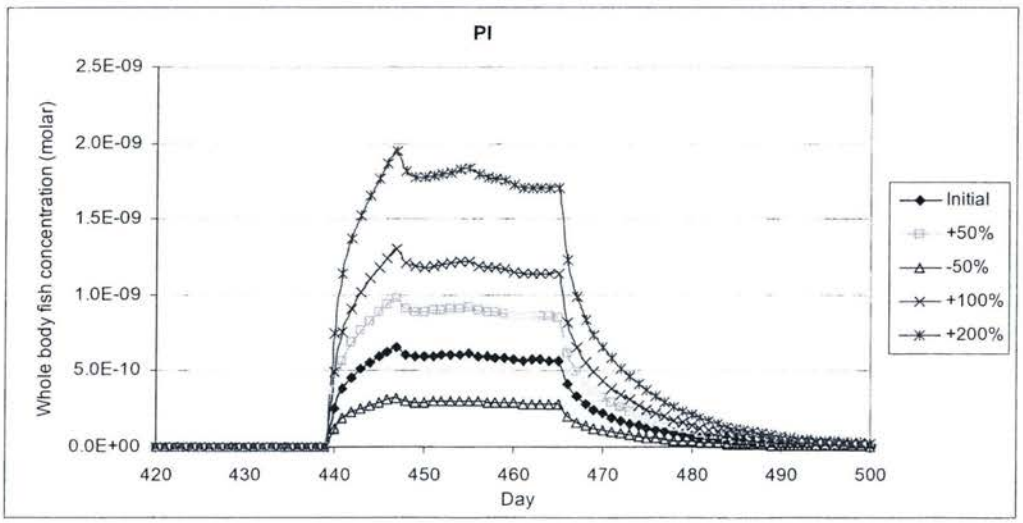


Figure 6-1: Impact of Changing the most Sensitive Parameters on Whole Body Concentration in Fathead Minnow Exposed to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water, Cont'd

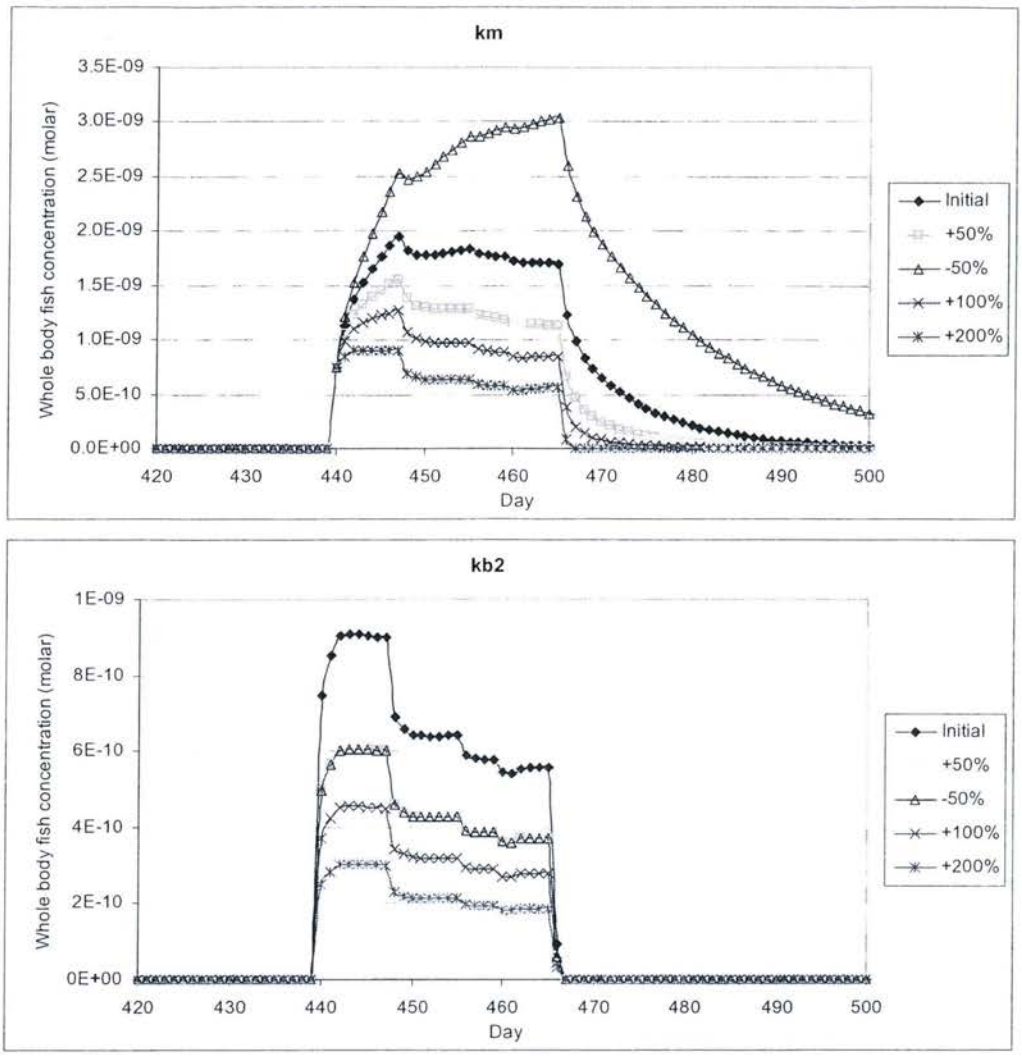


Figure 6-1: Impact of Changing the most Sensitive Parameters on Whole Body Concentration in Fathead Minnow Exposed to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water, Cont'd

Figure 6-2 shows the model result for exposure of juvenile fathead minnow to 0.85-1.39 ng/L in water (2.6E-12 to 4.3-12 molar) of 2,3,7,8-TCDD in water using initial parameter values. The concentrations observed in the species (shown in Table 5-9) are also depicted on the figure. The model predictions using the fitted parameters are shown on Figure 6-3 and fitted parameters are presented in Table 6-2. The concentrations were converted to µg/kg and a Chi square of 0.0001 was calculated for this model fit.

The same process was repeated for medaka exposed to 101 pg/L (3.1E-13 molar) of 23,3,7,8-TCDD in water (shown in Table 5-10). Figure 6-4 and Figure 6-5 show the predictions using initial values and the fitted parameter values for medaka and fitted parameters for this run also are shown in Table 6-2.

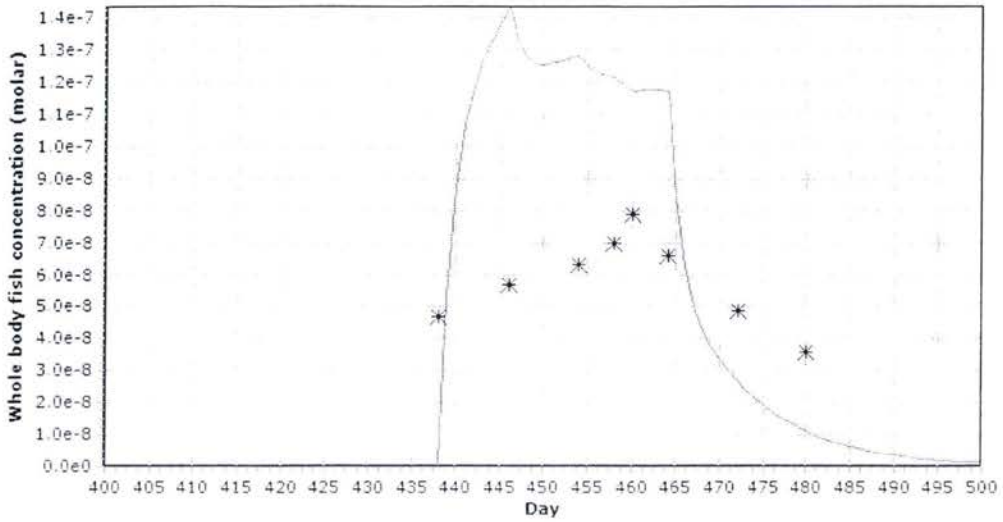


Figure 6-2: Model Results and Observed Concentrations for Exposure of Fathead Minnow to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water using Initial Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

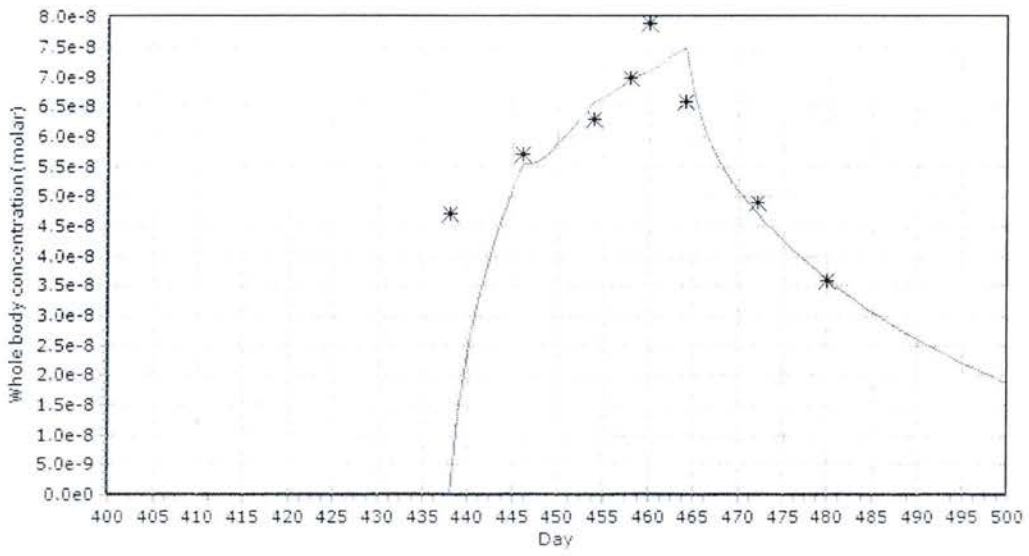


Figure 6-3: Model Results and Observed Concentrations for Exposure of Fathead Minnow to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water using Fitted Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

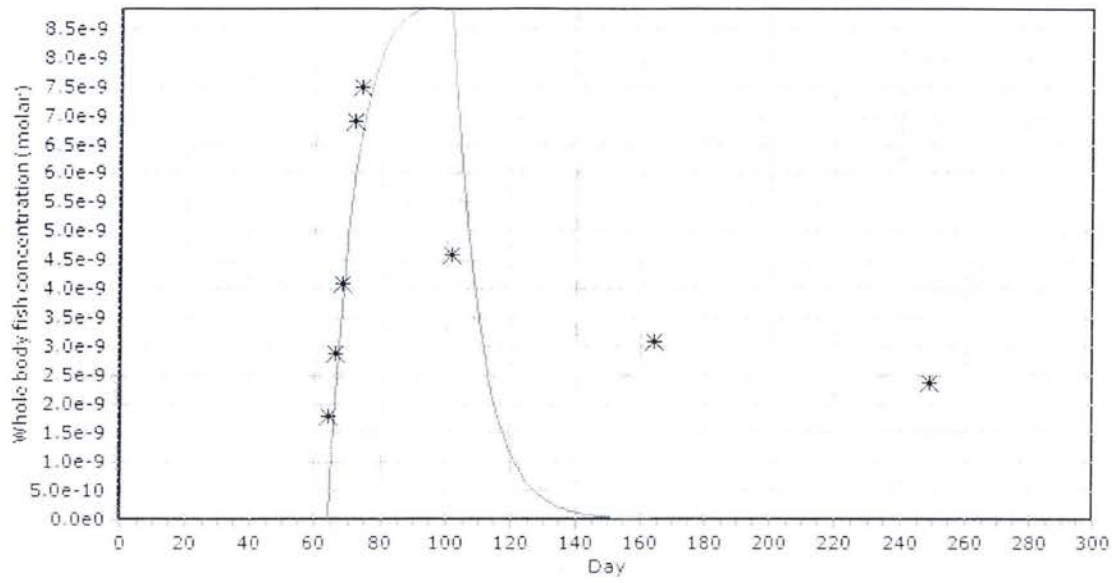


Figure 6-4: Model Results and Observed Concentrations for Exposure of Medaka to 3.1E-13 Molar of 2,3,7,8-TCDD in Water using Initial Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

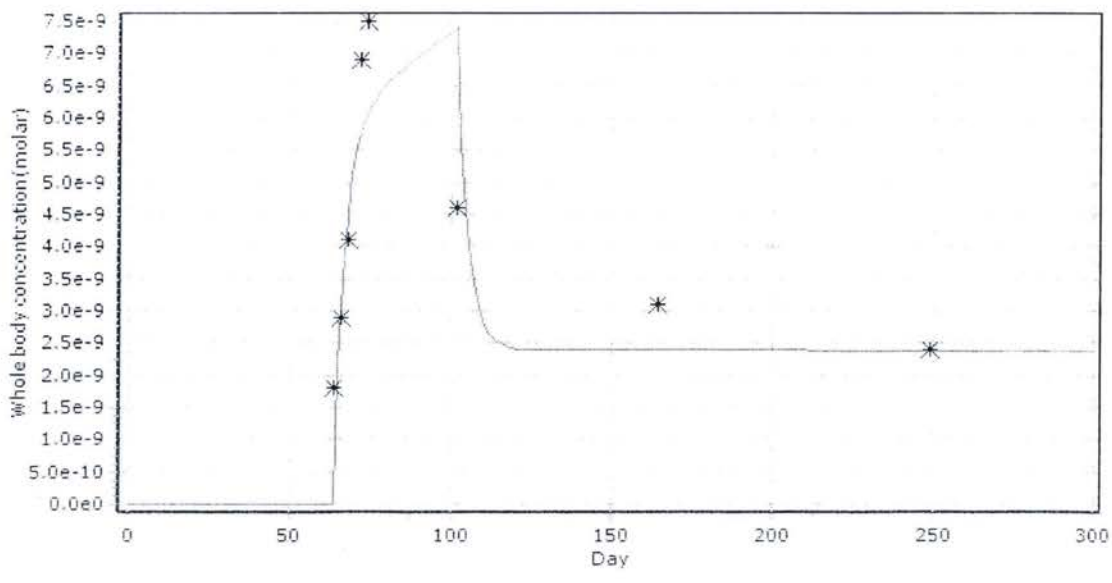


Figure 6-5: Model Results and Observed Concentrations for Exposure of Medaka to 3.1E-13 Molar of 2,3,7,8-TCDD in Water using Fitted Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

Table 6-2: Fitted Values for Model Parameters for Water Exposure Model

Parameter	Notation	Unit	Fathead Minnow	Medaka
Growth Coefficient	K	/d	0.0012	8.5E-06
Gill surface area coefficient	Agill0	m^2	0.0015	0.0096
Gill permeability for dioxin	Perm	cm^2/s	5.45E-06	7.26E-06
Richly-perfused tissue: blood	Pr	-	16.5	10.2
Slowly-perfused tissue: blood	Ps	-	6.8	9.5
Fat: blood	Pf	-	139	332
Liver: blood	Pl	-	0.9	1.1
Basal CYP1A in liver	BM2T0	nmol/g	1.24	1.20
CYP1A-dioxin binding affinity	KB2	nM	0.443	15.39
Degradation rate	K1	/hr	1.1	0.13
DNA-dioxin binding affinity	kd	nM	166	227
Hill coefficient	n	-	0.73	0.58
Basal synthesis rate	K0	pmol/min/mg	12.6	0.1
Elimination rate	km	/d	0.034	0.36

The only parameters which did not converge similarly between the minnow and medaka models were the growth coefficient, CYP1A-dioxin binding affinity, basal synthesis rate, and the elimination rate. The relatively large difference between these values may be due to physiological differences between the two species. The elimination rate was found to have the most influence in model calibration.

6.2 Model Results for Food-Exposure of Rainbow Trout

Figure 6-6 shows the predicted liver total concentrations for exposure of rainbow trout to 18 ng of 2,3,7,8-TCDD in kilograms of food (see Table 5-12). The model was implemented using the parameter values shown in Table 6-1. The observations made in the respective experiment (Table 5-12) are also shown. Attempts made to calibrate the predicted liver concentrations with these observations were not successful; however, the observations were comparable to the predicted free liver concentrations (see Figure 6-8) and Observed Concentrations.

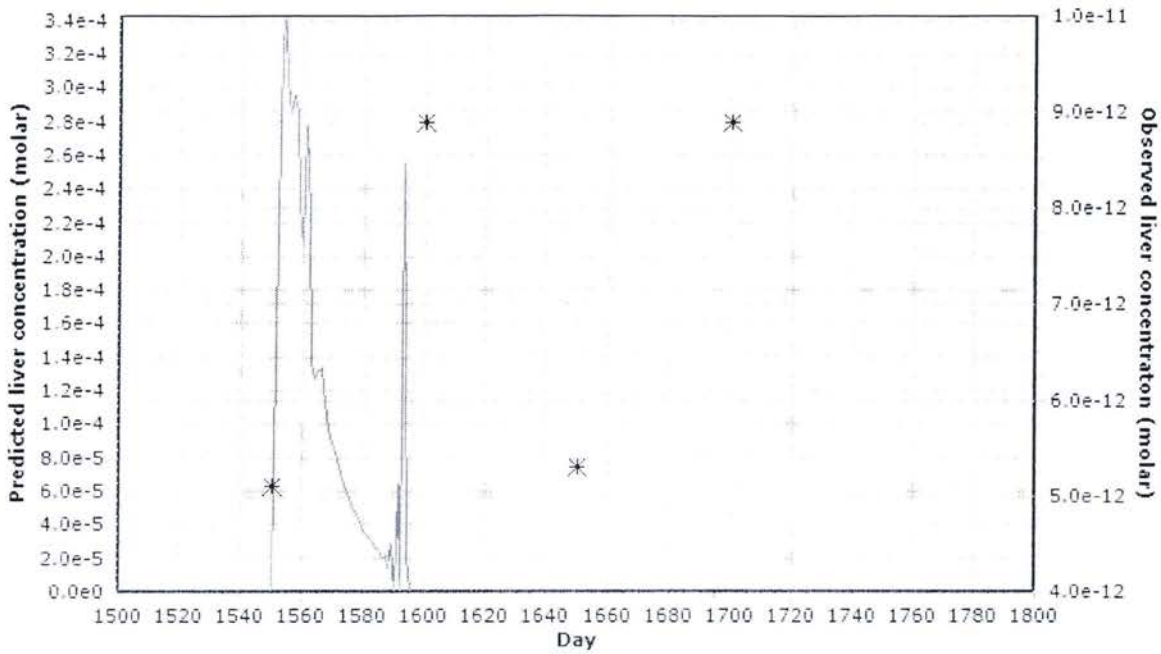


Figure 6-6: Predicted Total Concentrations and Observed Concentrations in Liver after Exposure of Rainbow Trout to 18ng of 2,3,7,8-TCDD in kg Food Using Initial Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

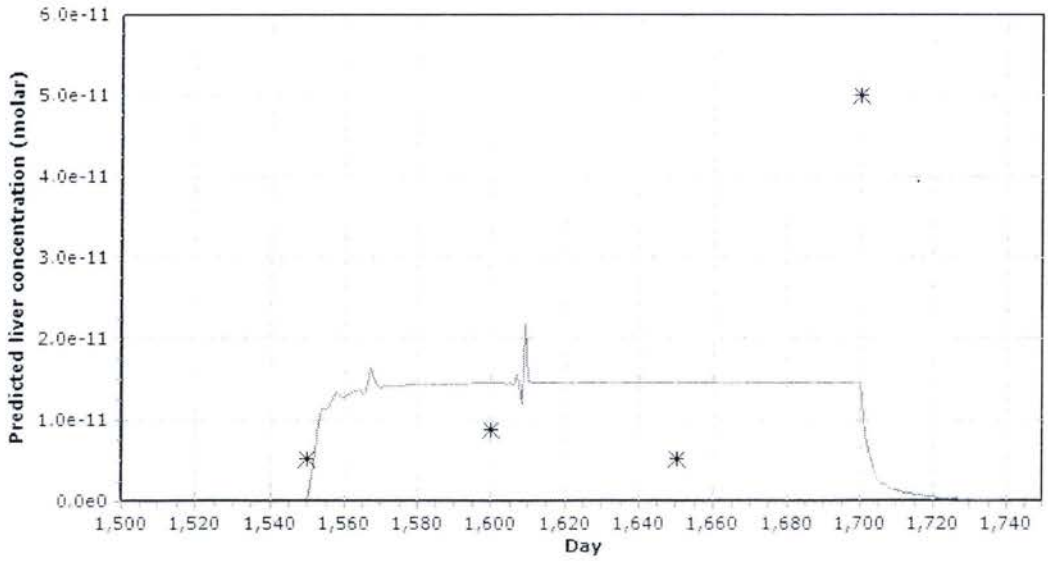


Figure 6-7: Predicted Free Concentrations and Observed Concentrations in Liver after Exposure of Rainbow Trout to 18ng of 2,3,7,8-TCDD in kg Food

Observed values are shown as stars, and the model prediction is shown as a solid line

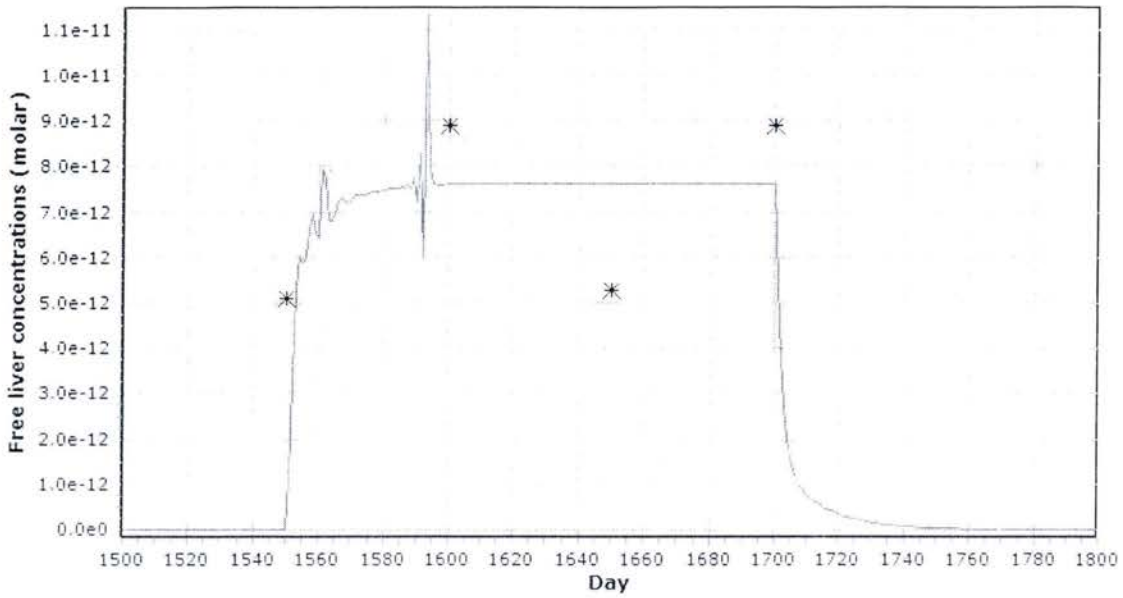


Figure 6-8: Predicted Free Concentrations and Observed Concentrations in Liver after Exposure of Rainbow Trout to 18ng of 2,3,7,8-TCDD in kg Food Using Fitted Parameter Values

Observed values are shown as stars, and the model prediction is shown as a solid line

7 DISCUSSION

7.1 MODEL PERFORMANCE

In this study, a physiologically-based pharmacokinetic model (PBPK) was developed to describe disposition of dioxins in the tissue of various fish species. Water and food pathways of exposure were considered in separate versions of the model and each model was calibrated using observations provided in the literature.

The water-pathway model was calibrated using two independent sets of data for two species. The model structure was proposed based on a variety of previous studies and is potentially applicable to other hydrophobic chemicals in water and, at a minimum, to the same or similar species of fish.

The estimated parameters were comparable between the two species and experiments, narrowing down the range of parameter values.

The food-pathway model did not predict the expected liver concentrations. One reason that can be envisaged is that, based on the fluctuations in the predictions, there could be a problem in the numerical solution of the equations, which can be due to one or more parameter values being out of an acceptable range. Another reason could be that the gut model, which suggests a first order kinetic between the gut lumen and gut tissue, is not the actual mechanism happening during food uptake. In the original paper suggesting this mechanism for food uptake (Nichols et al. 1998), an agreement was established between the predictions and observations; however, some parameter values had to be changed beyond their physical meaningfulness (namely, the egestation rate was fitted at 1.6 times the feeding rate, while experiments show that the ratio is at about 0.3). Another consideration relates to the seemingly seasonal variation in the data set used to calibrate the food model. This variation can be due to spawning, or other physiological changes, such as change in the CYP1A1 activity. This was not accounted in the model developed in this study.

7.2 UNCERTAINTY ANALYSIS

As discussed in section 2.3.4, the uncertainty in model predictions has to be estimated since the model may be used to make predictions which are sensitive to parameters for which no experimental data exist. In the minnow water-exposure model, the parameters with the most uncertain values include:

- Gill area and thickness of diffusion path

- Gut diffusion constant (in food model only)
- Blood: tissue partition coefficients
- Elimination rate
- Parameters used in binding equations

The sensitivity analysis in section 6.1 suggested that the parameters with the highest impact on the model results included:

- Gill area and thickness of diffusion path
- Gill permeability for dioxin
- Tissue: blood partition coefficient for fat, liver and slowly-p tissues
- Elimination rate
- Binding affinity between CYP1A and dioxin

Since large uncertainties in a sensitive model parameter can result in misleading estimates of target tissue doses, an uncertainty analysis was completed for parameters with the most uncertain initial values and the highest impact on the model results. These parameters and their assumed distributions are shown in Table 7-1. The mean values are the same as the values fitted for the fathead minnow model (see Table 6-2). Minimum values were set at zero and maximum values were selected between about one to three orders of magnitudes of the means (selection was limited by the numerical solution's stability). The ambient water concentration was considered at 0.02 ppq (6.2E-17 molar).

Table 7-1: Parameter Distributions for Monte Carlo Analysis of Water Exposure Model

Parameter	Notation	Unit	Min, Mean, Max
Gill area coefficient	Agill0	m2	0, 0.0015, 1
Thickness of diffusion path	Tpath	m	0, 8E-6, 0.001
Fat: blood	Pf	-	0, 139, 1000
Liver:blood	Pl	-	0, 0.9, 100
Slowly-p:blood	Ps	-	0, 6.8, 100
Elimination rate	km	/d	0, 0.034, 10
Binding affinity between CYP1A and dioxin	KB2	nM	0, 0.443, 10

* normal distributions were considered for all parameters.

After running the model for 1000 times with random sampling of parameter values from the ranges shown in Table 7-1, the results in fish concentrations were between 4.4E-18 and 1.4E-12 molar. The time course of these ranges is shown in Figure 7-1 for the selected mean values and ±3 standard deviations.

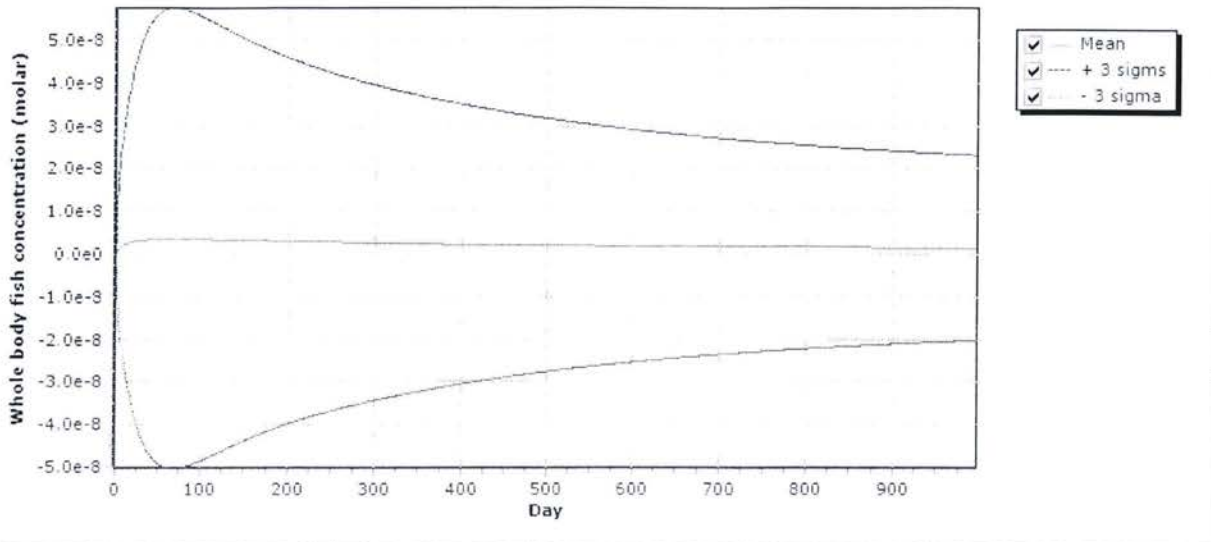


Figure 7-1: Model Response to Variation in Input Parameter Values for Exposure of Fathead Minnow to 6.2E-17 molar 2,3,7,8-TCDD in Water

* See Table 7-1 for ranges of input parameter values

7.3 MODEL COMPLEXITY

One argument that is usually brought up about PBPK models is their complexity and need for large amount of input data. Therefore, simpler alternatives were tested for comparison of the results to the proposed PBPK model.

A simple model, which is conventionally used for ecological hazard assessment is the use of bioconcentration factors (for water-fish pathway) or bioaccumulation factors (for the sediment/ food-fish pathway).

Loonen et al. (1994) exposed guppies to a complex mixture of PCDDs and PCDFs in water for 21 days. They calculated bioconcentration factors (BCFs) from the ratio of concentration in fish to water, as well as from the ratio of uptake rate to elimination rate constants that they fitted to data. The log BCF values for the 2,3,7,8-substituted PCDDs and PCDFs ranged from 3.90 to 5.27. These were considered to be lower than what would be predicted based on hydrophobicity of the compounds. This could be due to the limited lipid solubility of PCDDs and PCDFs, reduced membrane permeability and reduced bioavailability.

Based on LaKind and Naiman (1993), the most widely used BCF value for regulatory purposes is 5,000.

Endicott and Cook (1994) used lake trout and sediment data to define the biota-to-sediment ratio (BSR) for PCDDs and PCDFs in Lake Ontario. They derived the BSR using a modeling approach which combined bioaccumulation, partitioning and sediment-water chemical distribution; the BSR calculated for 2,3,7,8-TCDD was 0.41.

Oost et al. (1996) investigated the behavior of persistent organic trace pollutants (including TCDF and TCDD compounds) in sediment and eel from six different freshwater sites. They took the ratio between the lipid weight standardized concentrations in eel and the concentrations in the organic matter of the sediment as the biota-sediment accumulation factor (BSAF). They suggested that the biological half-life of PCDF/Ds in lake trout is between 64 and 105 days, and that the biota-sediment accumulation factor is in the range of 0.00074 to 0.27. They estimated a BSAF of 0.155 for 2,3,7,8 TCDD.

Loonen et al. (1995) conducted an ecological hazard assessment of 2,3,7,8-TCDD in fish and other aquatic biota. For transfer between water and fish, they used a bioconcentration factor (BCF) of about $10^{4.3}$ for rainbow trout and $10^{3.9}$ for fathead minnow.

For a water concentration of 0.001 to 0.02 ppq [5] measured in the Ohio River (Dinkins and Heath 1998), the fish concentrations calculated using such BCFs will be 20 pg/g to 0.4 ng/g for rainbow trout and 8 pg/g to 0.16 ng/g for fathead minnow, respectively. Based on an MOE publication (MOE1985), water concentrations as low as 1 ng dioxin /L (ppt [6]) water result in a reaction by fish. Loonen et al. (1995) derived no effect concentrations for exposure of lake trout embryo and rainbow trout at 0.17 and 0.46 pg/L water, respectively. Based on Heiden et al. (2005), accumulation of 0.6 ng dioxin /g fish impacts the fish (zebrafish in their experiment). These values show that a water concentration of 0.02 ppq (2E-17 g/g) is lower than the effect water concentration of 1 ppt (1E-12 g/g), but the resulting fish concentration of 0.4 ng/g is close to an effect tissue concentration of 0.6 ng/g. Two questions arise here: is the suggested BCF predicting fish concentration accurate; and are the endpoints valid and/or comparable? The first question can be tested using the model developed in this study.

The model developed in this study was implemented for exposure of fathead minnow to 0.001 ppq of 2,3,7,8-TCDD over its lifetime. Other parameter values were also changed to reflect the ambient water conditions (comparing to the laboratory experiments). This included the role of organic material in adsorbing dioxins and thus reducing the free concentration

[5] Part per quadrillion (10E-15)

[6] Part per trillion (10E-12)

available to fish. In this case, the dissolved and particulate organic carbon concentrations were considered at typical values of 4 mg/L and 1 mg/L, respectively.

Figure 7-2 shows the results of this modeling in comparison with the outcome of the BCF model. It can be seen from this figure that the BCF model is very conservative in comparison with the PBPK model, and excludes any variation in the disposition of the chemical.

Another aspect assessed regarding model complexity was whether the exclusion of binding mechanisms in liver impacted the model results and calibration. To test this, the model was simplified by excluding these mechanisms. However, no fit could be found between the model and observed data for fathead minnow when the data did not include accumulation through binding to the liver protein.

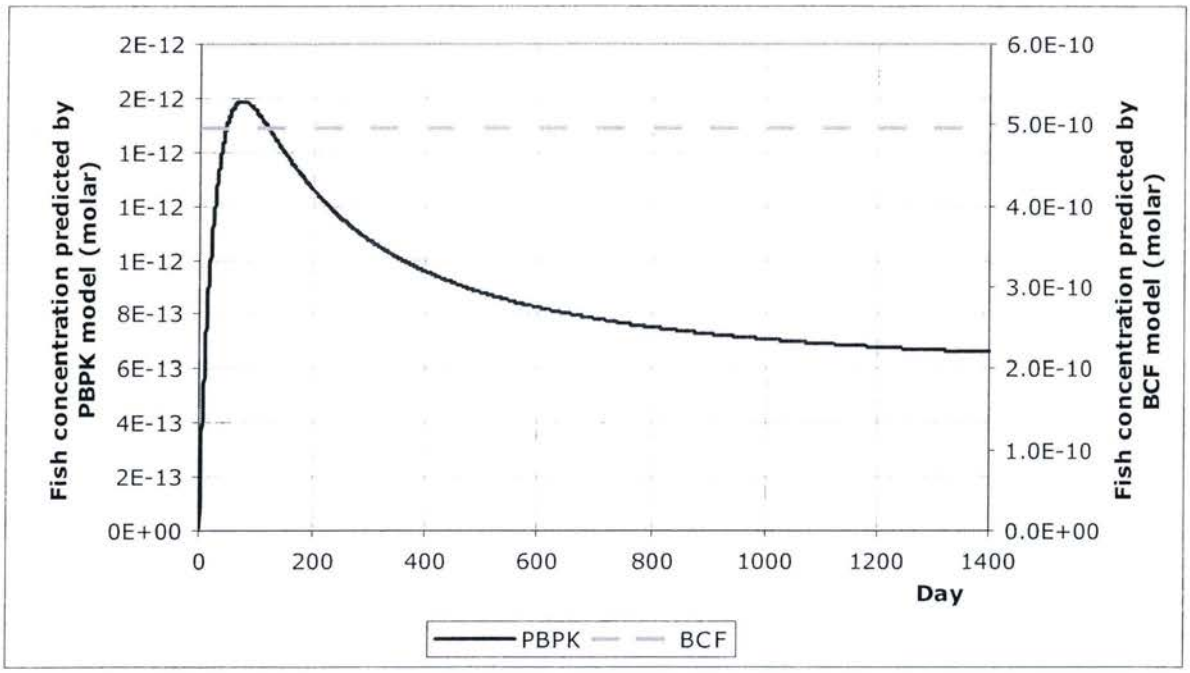


Figure 7-2: Predicted 2,3,7,8-TCDD Concentrations in Fathead Minnow Continuously Exposed to 0.02 ppq in Water

7.4 PREDICTIONS OF TISSUE CONCENTRATIONS

The developed model predicts a time-course of concentration in various tissue compartments. This aspect was not tested due to the lack of observed data; however, the results for fathead minnow are shown here for perspective and discussion (see Figure 7-3).

Based on these results, dioxins accumulate mostly in liver and adipose tissues. This can have implications for definition of exposure limits when the endpoints related to fish health are considered.

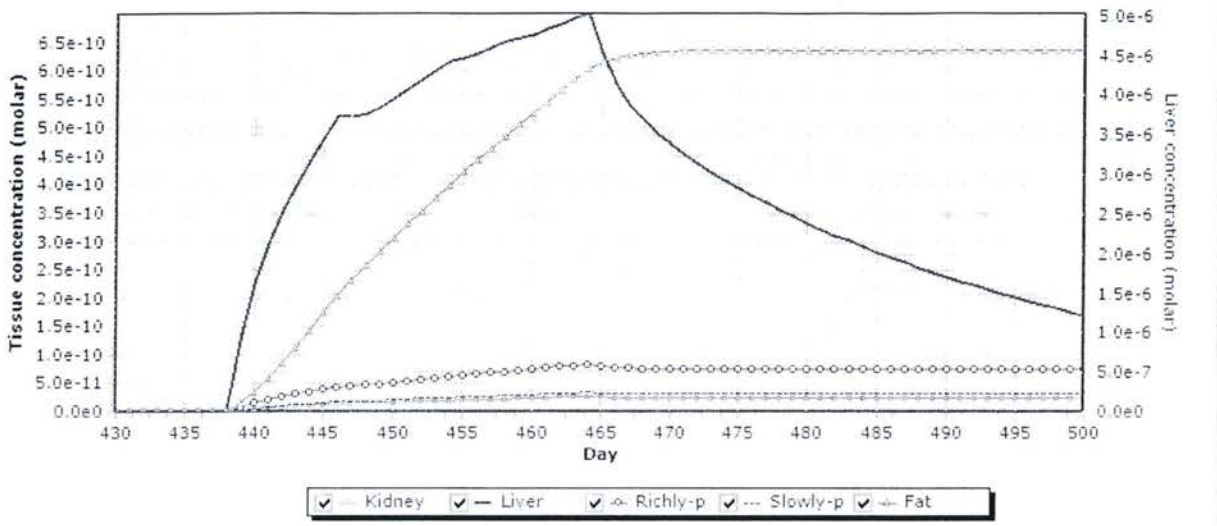


Figure 7-3: Model Results for various Compartments for Exposure of Fathead Minnow to 2.6E-12 to 4.3-12 molar of 2,3,7,8-TCDD in Water using Parameter Values Fitted for 2 Experiments

7.5 POLICY MAKING PROCESS

The policy making process in Canada in relation to establishment of water quality guidelines for the protection of aquatic life was reviewed to see how PBPK modeling can help strengthen the scientific basis of policy decisions.

Regulatory agencies derive dose-response values based on the current understanding of the relevant dose-response relationships. In deriving Canadian water quality guidelines for aquatic life, all components of the aquatic ecosystem (e.g., algae, macrophytes, invertebrates and fish) are considered if the data are available and where data are limited, interim guidelines are prepared. The guideline derivation includes: selection of variable (e.g. from the Priority Substances List), literature search, data set requirements, evaluation of toxicological data and guideline derivation. During the guideline derivation step, the most sensitive Lowest-Observable Effects Level (LOEL) from a chronic exposure study on a native Canadian species is multiplied by a safety factor of 0.1 to arrive at the final guideline concentration. Alternatively, the most sensitive LC50 or EC50 from an acute exposure study is multiplied by an acute/chronic

ratio or appropriate application factor to determine the final guideline concentration (CCME 1999).

The Canadian Council of Ministers of the Environment (CCME) task group develops Tissue Residue Guidelines (TRGs), which establish maximum acceptable levels of contaminants in aquatic organisms so that the birds and wildlife which feed upon them are protected from the adverse effects of toxins in the environment. TRGs are aimed particularly at persistent bioaccumulative substances which accumulate in biological tissues but may be undetectable in surface waters, rendering water quality guidelines irrelevant. Development of these guidelines follows the Protocol for the Derivation of Canadian Tissue Residue Guidelines for the Protection of Wildlife that Consume Aquatic Biota in 1998. The TRGs are used to assess whole fish monitoring data but are not used in sport fish guidelines which are human health-related (Willms & Shier Env. 2008).

The surface water standard for Ontario is under preparation and will be based on the Canadian Water Quality Guideline, which is under preparation, as well. This water quality guideline will protect aquatic life. The approach that the MOE used in developing standards for the regulation of dioxins and furans in the environment (for human protection) was based upon a review of technical literature, where relevant reports and literature were reviewed and the following steps were taken (MOE 1985):

- Estimation and identification of potential or actual concentrations and sources in the province based on data collected in Ontario
- Review and analysis of available toxicological data on the effects of dioxins and furans
- Identification of source/input, distribution, and fate of dioxins and furans in the environment, especially in Ontario
- Evaluation of the exposure risk to the population based on measured and estimated concentrations of dioxins and furans using the toxic equivalent approach in light of the recommended maximum allowable daily intake

The underlying assumption in deriving these values is that the exposure concentration of a chemical results in an internal dose that does not cause an adverse response during a lifetime. However, the actual toxic moiety level in the target tissue is difficult to obtain. Therefore, pharmacokinetic models, which estimate the tissue concentration of toxic substance, are useful in extrapolations needed to derive reference values.

8 CONCLUSION AND RECOMMENDATIONS

This study proposed a Physiologically Based PharamcoKinetic (PBPK) model to describe the mechanisms of disposition of dioxins in fish. It used the existing knowledge about the disposition of other hydrophobic chemicals in fish, as well as the disposition of dioxins in species other than fish. The contribution of this work to the science of ecotoxicology and risk assessment is a mathematical model which can describe the toxicity mechanisms of dioxins in a range of fish species, as well as parameter values for such a model.

It has been suggested that in rodents, the main mechanism contributing to disposition of dioxin is the binding of the chemical to CYP1A protein and its Aryl Hydrocarbon (Ah) receptors. This theory has been successfully modeled for rodents. A number of studies have suggested that such mechanisms apply to fish, as well; however, this is the first research to study such mechanisms in detail and through simulation.

In this research, the model was developed in two modes: uptake of dioxins from water through the gill and uptake through food. The water model was calibrated using two independent data sets. The parameter values estimated for the two independent data sets were comparable and the model could produce predictions which were very close to the respective observations.

A sensitivity analysis was conducted to identify parameters with the highest impacts on the model results. The results of this exercise were used to estimate parameter values for the most uncertain parameters in order to calibrate the model for the two data sets.

The food model, however, did not produce the expected concentrations in the fish liver. Further research is needed to refine the structure and/or parameter values for this model. Potential path forwards include considering seasonal variation in relevant physiologic parameters (e.g. the CYP1A activity), and modification of the mathematical representation of food intake/egestion mechanisms (e.g. relevant mechanisms established for other species/other contaminants in fish). In order to further refine and validate the model, experiments should be designed and conducted for exposure of fish to dioxins through food (and water), and concentrations in tissues and fish responses be monitored over time.

This model identified the parameters having the highest impact on the water-exposure model as the gill surface area, average thickness of diffusion path, gill permeability for dioxin, tissue: blood partition coefficient for fat, liver and slowly-p tissues, elimination rate and binding affinity between CYP1A and dioxin.

Collection of physiologic and kinetics data was a very time-consuming task. Establishment of a database of such parameters for various fish species and chemicals (similar to an existing database for human, HSL 2009) can reduce the amount of efforts spent on data collection, and expand the development and use of these models.

Several studies have been conducted on the accumulation of dioxins and furans on various fish species and other aquatic biota. These studies, however, do not usually report the respective values in water or prey. For example, Ontario Ministry of the Environment collects data on dioxins in the Great Lakes. While a valuable source of information on fish concentrations, these data do not provide information on the water and sediment concentrations and other environmental conditions, and therefore cannot be used to establish a causal relationship. Expanding the scope of such monitoring efforts to include all relevant parameters, such as sampling of water, sediment, prey and predators at the same location and time period, can enhance the applicability of the collected data significantly. One of its applications includes providing a basis for policy making and projection of future conditions.

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