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Factors Affecting Critical Toxicokinetic Parameters of Polychlorinated Biphenyls (PCBS) in Japanese Koi (CYPRINUS CARPIO): Effect of Diet on Chemical Assimilation and Influence of Feces to Whole Body Chemical Elimination

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CARPIO)*: EFFECT OF DIET ON CHEMICAL ASSIMILATION AND INFLUENCE
OF FECES TO WHOLE BODY CHEMICAL ELIMINATION

by
Jian Liu

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DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

Chapters 2 and 3 of this thesis will be published as co-authered, peer-reviewed journal articles. Ken G. Drouillard contributed to the experimental design, execution, and editing of both Chapters. Doug G. Haffner contributed resources for using the Aquatic Facilities located in the Great Lakes Institute for Environmental Research, and provided editorial assistance for manuscripts that have been (Chapter 2) or will be submitted (Chapter3). The experiment implementation, chemical analysis, and data interpretation of the studies described in Chapters 2 and 3 were done by Jian Liu.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

ABSTRACT

This thesis provides enhanced calibration of critical parameters, chemical uptake efficiency (AE), chemical whole body elimination rate coefficient (k_{tot}), fecal elimination rate coefficients (k_{ex}) and biotransformation rate coefficient (k_{met}), which are applied in a generalized fish bioaccumulation model applicable to persistent, hydrophobic organic compounds such as polychlorinated biphenyls. Chapter 2 presents measurements of chemical AE based on a mass balance method and shows that chemical hydrophobicity has a significant influence on the chemical AE, and between diets comparison shows that factors related to dietary properties also have a strong influence on the chemical AE. Lipid content in the diet was not shown to have significant effect on the chemical AE, indicating that other properties in the diet should be responsible for the large variation in AE between diets. Chapter 3 provides measurements of k_{tot} and k_{ex} for both labile and recalcitrant PCB congeners across three dose levels, and k_{met} for labile PCBs was also estimated by the difference of k_{tot} from recalcitrant PCBs. First order kinetics was confirmed in the study, which also showed that fecal elimination counted for only a small fiction of the whole body elimination. Gill elimination and metabolic biotransformation were shown to be equally important as elimination routes for labile congeners.

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CHAPTER 1 GENERAL INTRODUCTION

1.1 General introduction

Beginning with the publication of Rachel Carson's book "Silent Spring" in 1962, the concern for persistent environmental contaminants led to the discovery of a large number of anthropogenic contaminants in the environment that further stimulated research on the toxic effects and exposure dynamics of these environmental contaminants. Hydrophobic organic compounds (HOCs) are a class of chemicals that have low water solubility and high lipid solubility. These chemicals are useful to society because of their physicochemical properties but these same properties can also lead to elevated bioaccumulation in organisms and food webs (Stow 1995; Connolly and Pedersen 1988; Bentzen et al. 1999). Unfortunately, many of these contaminants, particularly those that also demonstrate high environmental persistence and resistance to metabolic biotransformation, also tend to be toxic and pose a particular threat to the health of wildlife and humans (Gilbertson et al. 1991; Leadly et al. 1998; Lonky et al. 1996). Although current concentrations of many HOCs in the environment have decreased dramatically since the 1970s (Huestis et al. 1996; de Vault et al. 1996; Scheider et al. 1998), the presence of persistent halogenated hydrophobic chemicals, such as polychlorinated biphenyls (PCBs) and other legacy organochlorine compounds in aquatic systems continues to be a matter of concern (Hebert et al. 1999; Stow et al. 2004).

Bioaccumulation reflects the net processes of chemical uptake and assimilation into the animal relative to various chemical elimination processes (Gobas and Morrison 2000). In order to better interpret the toxicological responses of organisms, it is important to understand the relationship between the concentrations of the chemical in environmental

media and the accumulation and timing/approach to steady state of chemical concentrations in the animal's tissues over time. In the toxicological literature, it is commonly assumed that there is a constant relationship between chemical concentrations in the environment and organism tissue concentrations. For example, most toxicity studies establish dose by referring to chemical concentrations in water or food provided to the assay organism during toxicity tests (Coimbra et al. 2007; Liu et al. 2009). This approach assumes that the animal achieves steady state quickly with its environment and that the tissue concentration in the animal can be adequately predicted using a bioaccumulation factor (BAF).

However, individual chemicals display large differences in the magnitude of BAFs (Gobas et al. 1988; Fisk et al. 1998) and characteristic time to steady state (Sijm et al. 1992; Fox et al. 1994) for a given bioassay organism. This can confound interpretation of toxicity using the external medium reference approach. For example, a chemical that achieves steady state quickly in the animal for a given toxicity bioassay will likely exhibit a very different chronic toxicity etiology compared to a contaminant that requires a longer period to reach steady state relative to the bioassay duration. By not measuring accumulated tissue residues in the animal during and at the end of the bioassay test, interpretation of toxicity (i.e., mode of action and relative sensitivity of a given bioassay animal) can easily become confounded by differences in chemical exposure dynamics. This type of misinterpretation is common when comparing toxicity across chemicals for a given bioassay species or across species for a given chemical.

Non-steady state bioaccumulation models are necessary in order to predict the time course of chemical exposures in animals (Drouillard et al. 2009) while steady state models predict the maximum bioaccumulation potential in an animal (Gobas et al 1989; 1999). Both

types of models are necessary in order to better interpret toxicity bioassays described above. Food web bioaccumulation models build on single species bioaccumulation models and simultaneously solve for chemical concentrations in multiple animals occupying a given food web (Harrison et al. 1970; Mackay and Fraser 2000). These latter models use individual bioaccumulation models as their base unit but have the advantage of linking contaminant concentrations in top predators to water and sediment chemical concentrations. Food web bioaccumulation models are particularly useful to provide risk assessments for humans exposed to organochlorine chemicals via consumed sport fish (Arnot and Gobas 2004). They are also used to assess the types of environmental remediation strategies, for example, pollutant load reductions or contaminated sediment removal, necessary to decrease bioaccumulation in sport fish (Thoman et al. 1992; Arnot and Gobas 2004; Morrison et al. 2002). While each of the bioaccumulation models described above reflect a progression in model complexity, they all have a basic toxicokinetic sub-model as a common model unit. This thesis provides focused examination on critical assumptions and parameters incorporated into the toxicokinetic sub-model utilized in more advanced individual based and food web bioaccumulation models.

1.2 Bioaccumulation

Bioaccumulation is a fundamental process in environmental toxicology and risk assessment, because it controls the internal dose of potential toxicants (Mackay and Fraser 2000). Information regarding chemical bioaccumulation is important for determining environmental quality guidelines, establishing total maximum daily loadings, categorizing substances that are potential hazards to wildlife and humans, and quantifying the risk of

chemicals on ecosystems and human health (CEPA 1999; US EPA 2000; Burkhard LP, 2003). Persistent HOCs are notable in that they often achieve higher concentrations in organisms relative to the concentrations present in the abiotic environment the organism inhabits.

There are two general approaches for quantifying bioaccumulation. These include empirical approaches and modeling approaches. In empirical approaches, the concentration ratio between the animal and its exposure medium (water or food) is obtained by sampling animals and environmental media from the natural environment. These approaches assume that the animal is in steady state with its environment. The bioaccumulation factor (BAF) is defined as the ratio of the concentration of chemical in an organism to that in the water and is provided by:

$$BAF = C_B/C_W \qquad (1)$$

When an organism is exposed to a chemical only through its respiratory exchange medium, as can be determined in a controlled laboratory study, the term bioconcentration factor (BCF) is used in place of the BAF. Biomagnification refers to the condition where the chemical concentration in the organism exceeds that in the organism's diet due to dietary exposures that occur in addition to bioconcentration processes. The biomagnification factor (BMF) is defined as the ratio of the concentration of chemical in the organism to the mean chemical concentration in its diet and is provided by:

$$BMF = C_B/C_D \qquad (2)$$

In empirical approaches, the BAF can be deduced from the concentration in the organism and measured experimental or field concentrations. Although these values may be realistic, they are premised on the assumption of steady state and are subject to high degree

of biological variabilities in terms of changes in partitioning capacity, animal movements and other factors.

1.3 One compartment bioaccumulation model

In the modeling approaches, bioaccumulation is treated as the net result of various uptake and elimination processes. This approach can include species, population, and age-specific growth dilution as well as metabolic activity for a given field situation. These approaches attempt to adjust uptake and elimination rate processes according to environmental conditions encountered in the field and as such can provide site specific predictions that have greater accuracy when conditions vary from the model calibration condition. After 30 years model development, there are primarily two mathematical bioaccumulation models, one based on rate constants and contaminant concentrations (Arnot and Gobas 2004; Thomann 1981; Sijm et al. 1992), and the other based on fugacity and mass transport parameters (Campfens and MacKay 1997; Morrison et al. 1996; Clark et al. 1990). These two models are algebraically equivalent and can be easily converted from one to the other. In this thesis, the rate constants and chemical concentration model is adopted since the parameters being measured can be easily adopted by both model frameworks.

Most models assume that the chemical of concern is homogeneously distributed in the organism (i.e., one compartment) and this assumption is suitable when the contaminant concentration in the entire organism is of interest (Arnot and Gobas 2004) or when the time to steady state greatly exceeds the inter-tissue distribution kinetics. In the one compartment rate constant model, the fish is treated as a single compartment in which the chemical is evenly distributed and its respiratory surface (e.g., gill) and digestive system (e.g.,

gastrointestinal tract) are treated as the main exposure pathways for chemical transfer from/to the environment (water and diet).

It is assumed that the transport of chemical between environment and the organism is a passive diffusion process and subject to first order kinetics (Gobas et al. 1988, Thomman et al. 1992). Based on this model, the chemical concentration in fish is governed by the rate of chemical uptake from the water and food and total elimination from the fish. The mathematical description of the uptake and elimination of chemicals in fish is given by the following model (Gobas et al. 1993):

$$dC_f/dt = (k_1 \times C_{wat} + k_{feed} \times C_{food}) - k_{tot} \times C_f \qquad (3)$$

Where C_f (ng/g fish), C_{wat} (ng/mL water), and C_{food} (ng/g diet) are the wet weight chemical concentration in fish, freely dissolved concentration in the water, and the concentration in the diet, respectively. The rate coefficients denoted by k_1 (mL/ g fish · d) and k_{feed} (g diet/ g fish · d) are the rate coefficients for uptake via the gills and from the diet, respectively. The rate coefficient k_{tot} is the whole body elimination rate coefficient (1/d) and considers chemical losses through all possible elimination routes.

At steady state, $dC_f /dt = 0$ and Eq. 3 can be solved as:

$$C_{f(ss)} = (k_1 \cdot C_{wat} + k_{feed} \cdot C_{food})/k_{tot} \qquad (4)$$

Eq. 4 can subsequently be re-arranged to provide a model derived estimate of BAF and BMF described empirically above such that:

$$BAF = C_f / C_w = (k_1 + k_{feed} \times C_{food}/ C_{wat}) / k_{tot} \qquad (5)$$

and

$$BMF = C_f / C_{food} = (k_1 \times C_{wat} / C_{food} + k_{feed}) / k_{tot} \qquad (6)$$

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In order to establish more accurate model derived estimates of $C_{B(SS)}$, BAF and BMF, it is important to identify and quantify the individual mechanisms contributing to chemical uptake and elimination and to provide site specific information about the magnitude of the key toxicokinetics parameters used by the one compartment model, k_1, k_{feed} and k_{tot}.

1.3.1 Uptake rate coefficients

There are two major routes of hydrophobic organic chemical uptake by fish. Uptake from water through the respiratory surface ($k_1 \times C_{wat}$), and uptake from food through the digestive system ($k_{feed} \times C_{food}$). The uptake rate coefficient through gill ventilation is determined by both the gill ventilation rate (G_v; mL/g fish·d) and the chemical gill absorption efficiency (E_w; unitless). Values of G_v can be obtained by experimental approaches, such as from respirometry measurements or by bioenergetic calculations. Similarly, E_w has been obtained primarily from experimental measurements (McKim et al. 1985) or inferred from model simulations (Drouillard et al. 2009). Empirically measured E_w values for hydrophobic chemicals (log K_{OW} > 3) typical -ly range from 0.45 to 0.8 (Norstrom et al. 1976; McKim et al. 1982; 1983; 1985). The uptake rate constant through food digestion is determined by the feeding rate (G_{feed}; g/g fish·d) and the chemical dietary assimilation efficiency (AE; unitless). Typically, G_{feed} is deduced from experiments or from bioenergetic calculations and AE is measured from experiments.

The relative importance of water and food as exposure routes has received considerable debate in the literature. Randall et al. (1998) argued that uptake from water dominates hydrophobic organic chemical exposure and came to such conclusions by comparing the magnitude of k_1 and k_{feed} for a selected hydrophobic compound. However, the

above analysis ignores total chemical flux, which is further modified by differences in the magnitude of C_W and C_{food}. With increasing chemical hydrophobicity, there appears to be a trend where chemical dietary uptake becomes more important than uptake from gills (Qiao et al. 2000). This occurs because the process of chemical biomagnification becomes more pronounced with increasing hydrophobicity and results in a higher C_{food}/C_{wat} ratio (see Eq. 5) than realized for less hydrophobic compounds (Gobas et al. 1993; 1999).

Heath (1995) suggested that chemicals with a log K_{OW} <3 are mainly taken up by gills, those with log K_{OW} 3-6 are taken up by both gills and gut, and those with a log K_{OW} > 6 are taken up mostly by the gut. Opperhiuzen et al (1991) argued that uptake through gills and gastrointestinal track GIT are equally important, however, empirical evidence suggests that there is a clear transition to dietary uptake at a log K_{OW} value somewhere between 5.0 and 7.5 (Muir et al. 1985; Gobas et al. 1988; Batterman et al 1989; Servos et al. 1992; Qiao et al. 2000). Thomann and Connolly (1984) estimated that more than 99% of the PCB concentration in Lake Michigan lake trout was due to exposure through food. Food was also considered the major exposure route of PCBs in an aquatic food web studied in Lake Erie (Koslowski et al. 1994). Overall, a majority of studies indicate that the role of dietary uptake to total chemical exposure becomes more pronounced with increasing chemical hydrophobicity (Qiao et al. 2000; Gobas et al. 1988) and the dietary route dominates when the log Kow of the chemical exceeds a value of approximately 6 (Qiao et al. 2000; Gobas and Morrison 2000). Therefore, it is necessary to predict uptake rate constants from the diet (k_{feed}) with a high degree of accuracy when modeling bioaccumulation of highly hydrophobic chemicals.

The dietary uptake coefficient is provided by the feeding rate and chemical assimilation efficiency according to:

$$k_{feed} = AE \times G_{feed} \qquad (7)$$

Where AE is the chemical assimilation efficiency from the diet (unitless) and G_{feed} is the animal feeding rate (g feed$\cdot$g^{-1} fish$\cdot$d^{-1}). For model applications, G_{feed} is obtained from a bioenergetics sub-model and may be variable over time (Arnot and Gobas 2004; Drouillard et al. 2009) The dietary assimilation efficiency (AE) is chemical specific and considered to be a constant under thermodynamic model formulations, which consider dietary uptake and fecal egestion as separate processes (Barber 2008; Drouillard and Norstrom 2003). This should not be confused with net chemical AEs, which adjust the assimilated fraction for chemical elimination occurring over a feeding event. As the animal approaches steady state with its diet, its net AE approaches a value of 0 while its absolute AE remains constant with time. While feeding rate, G_{feed}, is an ecologically and bioenergetically variable component of k_{feed}, AE is a fundamental toxicokinetic component and thus a primary parameter of interest to this thesis.

The chemical AE can be measured relatively easily from empirical approaches. To establish the absolute AE, experiments should be conducted with clean organisms (with negligible body chemical concentration) so as to reduce elimination as a negligible component to measurement error. Using a mass balance approach, the animal is fed a measured quantity of food containing a known concentration of the chemical of concern. After allowing sufficient time for digestion and assimilation of the meal, the animal is sacrificed and the chemical mass in the animal's tissues is divided by the total mass of chemical ingested from the meal:

$$AE = \text{mass assimilated / mass consumed} \qquad (8)$$

Alternatively, kinetics based approaches are often used to estimate chemical AE values (Nichols et al. 2001). Under the kinetics approach, the uptake rate coefficient is measured as the linear slope of C_f/C_{food} over time. The uptake rate coefficient is subsequently divided by the G_{feed} as per Eq. 7. Although the second approach is more commonly used to estimate chemical AE values in fish (Fisk et al. 1998; Dabrowska et al. 1999; Gobas et al. 1993), it suffers from several methodological difficulties. Animals are often held communally, thus preventing the ability to measure accurate feeding rates or to consider individual variation in feeding rates. The kinetic approach can also result in underestimates of absolute AEs since net chemical AEs are determined unless corrected for the chemical elimination that takes place over the duration of the uptake portion of the experiment.

The AE values measured for HOCs from past studies demonstrated high variability (20% to >90%) in the magnitude of this parameter. Several explanations have been proposed for the variation in AE, including differences among the sorption coefficient of chemicals in different components of the ingested dietary matrix, composition of dietary matrices, digestibility of the dietary matrix (Gobas et al. 1988; 1993; 1999), steric hindrance in gut membrane permeation for large chemicals (Opperhuizen et al. 1985; Tulp and Hutzinger 1978), species specific gut morphology (Gobas et al. 1999) and technique biases in how experimental chemicals are added to experimental diets (Sijm et al. 1993).

The relationship between a chemical's AE and its hydrophobicity (K_{OW}) has been extensively studied (Mackay 1982; Devillers et al. 1996; Fox et al 1994). Despite high variation in AE values, a trend of decreasing dietary AE with increasing chemical K_{OW} is often reported (Gobas et al. 1988; Tanabe et al. 1982). As such, most food web

bioaccumulation models include predictive algorithms to estimate chemical AE for different species of fish as a function of chemical hydrophobicity (Arnot and Gobas 2006; Thomann et al. 1992). These algorithms, however, do not capture the majority of variability commonly observed in empirical measurements of AE (Gobas et al. 1988; Burreau et al. 1997; Fisk et al. 1998). Few studies have compared chemical AEs in fish provisioned with different types of foods (Gobas et al. 1993; Clark et al. 1990; Dabrowska et al. 1999). Furthermore, a number of researchers have recommended that artificial pellet diets, which reflect the majority of diets used in fish bioaccumulation studies, may provide a poor surrogate for the chemical uptake that occurs from natural food items (Fisk et al. 1998; Bruggeman et al. 1981; Burreau et al. 1997). These arguments are based on the premise that: 1) artificial diets have different digestibility compared to natural diets, and 2) spiking of chemicals into artificial diets does not distribute the chemical into the dietary matrix in the same way as bioaccumulation processes would do under a natural field setting.

Chapter 2 of this thesis provides experimental measurements of hydrophobic organic chemical AE's determined in Japanese koi (*Cyprinus carpio*) fed 5 different diets using a mass balance approach. This research provides the first comparative study to contrast empirically measured AEs in fish fed either natural diets or chemicals administered to artificial fish pellets.

1.3.2 Elimination rate coefficients

The whole body elimination rate coefficient (k_{tot}) is an important parameter for initial calibration of toxicokinetic models because the magnitude of this term is used to estimate the time required for an animal to achieve steady state with its environment and/or diet (Gobas and Morrison 2000). The whole body elimination rate coefficient can be obtained using a

depuration study, where a contaminated animal is placed in a clean environment and fed clean food (Bruggeman et al. 1981; Bruggeman et al. 1984). Under depuration conditions the change in animal concentrations through time is modeled by:

$$dC_f/dt = - k_{tot} \times C_f \qquad (9)$$

The above approach measures elimination via all elimination routes. However, since there are several elimination processes operating independently, and some processes are modified by different conditions than others, it is more accurate to provide estimates of elimination route specific rate coefficients. Major elimination mechanisms for hydrophobic organic contaminants include diffusive losses of chemical via exchange across respiratory surfaces (k_2), between the animal and its feces (k_{ex}) and non-diffusion based mechanisms including maternal deposition of contaminant to reproductive tissues (k_{egg}), metabolic biotransformation (k_{met}) and pseudo-elimination due to growth dilution (k_g). The k_{tot} therefore reflects summation of these processes and follows:

$$k_{tot} = k_2 + k_{ex} + k_{egg} + k_{met} + k_g \qquad (10)$$

In the modeling approach, chemical elimination through different mechanisms is estimated separately. The two diffusion based mechanisms (k_2 and k_{ex}) are estimated as follows:

$$k_2 = G_v \cdot E_w / K_{BW} \qquad (11)$$

$$k_{ex} = G_{ex} \cdot E_{ex} / K_{BEX} \qquad (12)$$

In Equations 11 and 12, G_v and G_{ex} represent the flow of water across gills (mL/g fish·d) and the feces production rate (g feces/g fish·d). The transfer efficiency term E_W is considered identical to the E_W defined for k_1. The transfer efficiency term E_{ex} is sometimes defined to

be equivalent to the AE value (Gobas et al. 1989) but in some cases modeled as an independent parameter (Drouillard et al. 2003). The partition coefficients, K_{BW} and K_{BEX}, refer to the biota/water partition coefficient and the feces/biota partition coefficient, respectively. K_{BW} and K_{BEX} are dependent on the whole body lipid content (de Freitas and Norstrom 1974) and non-lipid organic matter (DeBruyn and Gobas 2007) content of the organism divided by the partitioning capacity of water and feces, respectively (Gobas et al. 1999; Arnot and Gobas 2004). The bioenergetic related parameters such as gill ventilation rate (G_v) and fecal egestion rate (G_{ex}) are dependent on the metabolic rate of the animal, which ultimately governs respiration and feeding. These latter processes are influenced by many factors including temperature (Paterson et al. 2007a), animal age (Sijm et al. 1992), body size (Paterson et al. 2007b), growth rate (DeBruyn and Gobas 2006) and sex/reproductive status (Russell et al 1999; Johnston et al. 2002).

To date, there have been few attempts to empirically determine the relative contribution of different elimination mechanisms to whole body losses of HOCs by fish. With respect to gill and fecal elimination, inferences regarding the relative roles these processes play to whole body elimination have largely been deduced from modeling exercises (Gobas et al. 1989; Qiao et al. 2000; Gobas et al 1993; Drouillard et al. 2009). Losses of negligibly biotransformed organic chemicals via respiratory surfaces to water is considered to be inversely related to chemical K_{OW} and predicted to dominate whole body elimination for low to moderately hydrophobic compounds (log Kow<6.5, Gobas 1989). Gobas et al. (1989) argued that for fish, fecal and gill elimination of hydrophobic organic chemicals become equally important for chemicals with a log K_{OW} value of 6.5, with fecal elimination dominating with further increases in chemical hydrophobicity. Empirical studies

on PCB fecal elimination in birds (Drouillard and Norstrom 2003) and rats (Rozman et al. 1985) demonstrated that fecal elimination is an important elimination route for non-biotransformed congeners in terrestrial vertebrates. To date, there have been no experiments conducted to directly measure fecal elimination rates of hydrophobic organic chemicals by fish and to express the relative importance of k_{ex} compared to k_{tot} or other passive diffusive elimination mechanisms such as k_2.

Chapter 3 of this thesis measured both k_{tot} and k_{ex} for a series of PCB congeners in Japanese koi held under long term depuration conditions. The purpose of this component of the thesis research was to establish if k_{ex} approaches k_{tot} for super hydrophobic chemicals (i.e. log K_{OW} >6) as predicted by current bioaccumulation models.

1.4 Study compounds

This thesis presents the results of research on the bioaccumulation of PCBs, which were chosen as model compounds for HOCs. Polychlorinated biphenyls are a group of chlorinated aromatic hydrocarbons that are classified as persistent HOCs. The commercial manufacturing of PCBs began in 1929 (Hutzinger et al. 1974). PCBs were manufactured mainly as commercial mixtures under different trade names (e.g. Aroclor, Clophen, Phenoclor, Kanechlor and Sovol), which are generally characterized by their percentage weight of chlorine (e.g. Aroclor 1246, 46% chlorine by weight). They were used in a broad array of industrial and electrical applications, such as dielectric fluids for transformers, organic diluents, pesticides, extenders, flame retardants etc. because of their high dielectric constants, low vapour pressure, low degradability, and low reactivity (Swackhamer 1996). In the early 1970s, PCBs were reported to demonstrate toxicity and were found globally

throughout the environment (Jensen 1972). Following these observations, the open use and production of PCBs were banned in 1977. However, the physical and chemical properties that made these compounds so useful to industrial applications also resulted in their incredible persistence and lack of degradation in the natural environment (Tanabe 1988; Vallack et al. 1998).

The basic structural unit of PCBs is a biphenyl ring (Figure 1.1). With different number of chlorines attached at different position, there are a total of 209 structurally different congeners and they are identified by the International Union of Pure and Applied Chemistry (IUPAC) numbers (Guitart et al. 1993). All PCB congeners are considered hydrophobic with log K_{OW} values ranging from 4.09 to 8.18 (Hawker and Connell 1988). The chemical hydrophobicity is positively correlated to the number of chlorine substituents, but also partly related to the position of chlorine substitutions.
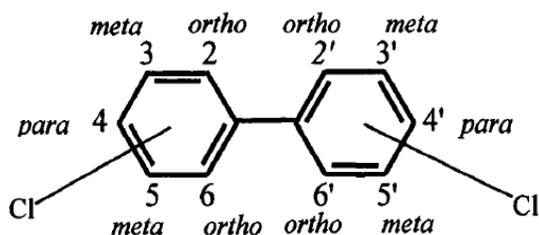
Figure 1.1. The general molecular structure and chlorine substitution position of PCBs

PCBs were initially detected in environmental samples including water and soil in 1966 (Jensen 1972) and have since been identified in nearly every matrix in the biosphere (Tanabe 1988; de March et al. 1998; de Wit et al. 2004). The global distribution of PCBs results from their physicochemical properties, such as their environmental persistence and

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ability to undergo long distance atmospheric transport (Meijer et al. 2003). Due to the highly

hydrophobic nature of PCBs, the concentration of these persistent contaminants are known to

biomagnify through food webs and are observed at higher concentration in top predators,

such as fish, birds and mammals compared to lower trophic animals. As early as the 1970s,

PCBs were reported to occur at high concentrations in Great Lakes fish (Veith 1975).

Although PCB concentrations declined dramatically in the years after they were banned in

the late 1970s (Huestis et al 1996; de Vault et al. 1996; Scheider et al. 1998), these decreases

have leveled off since the early 1990s in many environmental compartments (e.g., fish) of the

Great Lakes (Hebert et al. 1999). Concentrations of PCBs are likely to remain at detectable

levels in these matrices for some time due to their persistence in environmental biota and

abiotic samples.

The presence of PCBs in biota raises a number of concerns regarding the potential

impacts to organism and human health, particularly among people who consume

contaminated sport and commercial fish. Numerous toxicological effects such as

immunosupression, reproductive impairment, and endocrine disruption have been associated

with PCB exposure in wildlife (Safe 1994; Brouwer et al. 1999) as well as neurological and

behavioral impacts in human infants born to mothers with high PCB exposures (Chen and

Hsu, 1994; Masuda Y et al. 1985; Chen YC et al. 1994; Jacobson SW et al. 1985; Jacobson

JL et al. 1990a; 1990b). Among the Laurentian Great Lakes, between 80 to 99% of all sport

fish consumption advisories issued by the Government of Ontario are a result of high PCB

concentrations measured in sport fish (OMOE Sportfish Guidelines, 2009).

Polychlorinated biphenyls, therefore, offer an ideal candidate group of compounds for

this thesis research. First, PCBs exhibit a wide range of chemical hydrophobicities allowing

the testing of models across this important physical property. Second, PCBs are known to exhibit high resistance to metabolic biotransformation in fish and thus elimination associated with k_{met} is less of a confounding factor in interpreting the magnitude of k_{tot}. Finally, the improvement of PCB bioaccumulation models still has high relevance to society concerned with the human health impacts related to PCB exposures and attempting to reduce risks of such exposures by implementing environmental clean-up activities.

1.5 Objectives and hypotheses

The overall objective of this thesis is to increase our knowledge of the toxicokinetics of PCBs in fish with a special focus on PCB dietary uptake and the relative importance of fecal elimination to whole body elimination. PCB uptake from food is predicted to dominate chemical exposures for the majority of PCB congeners. Yet, databases on the critical toxicokinetic parameter, AE, are lacking particularly with respect to different types of diets. PCB elimination to feces is predicted to dominate only for highly hydrophobic PCB congeners and the transition from the dominance of gill elimination to fecal elimination is hypothesized to play a critical role in demarking which chemicals are capable of undergoing biomagnification. To date, no studies have validated the relative role of fecal elimination to whole body elimination of PCBs in fish.

Chapter 2 of the thesis investigates the influence of food properties on the dietary assimilation efficiency of individual PCB congeners by fish. Specifically the study design compares PCB assimilation efficiencies by Japanese koi fed two naturally contaminated diets and three artificial fish pellet diets, which vary in lipid contents. This chapter tests the following hypotheses:

1) Dietary assimilation efficiency of individual PCB congeners is controlled by chemical hydrophobicity

2) The relationship between dietary AE and chemical hydrophobicity is best characterized using a two-phase resistance model as recommended by Gobas et al (1989):

$$AE = (AK_{OW} + B)^{-1}$$

3) Dietary assimilation efficiency of individual PCB congeners is independent of dietary lipid content.

4) Dietary assimilation efficiency of individual PCB congeners is similar between natural foods collected from contaminated sites and PCB-spiked artificial pellet fish diets.

Chapter 3 of this thesis investigates the relative role of fecal elimination to whole body elimination of individual PCB congeners in Japanese koi. This chapter tests the following hypotheses:

1) Whole body PCB elimination by fish is a first order kinetic process and PCB elimination rate coefficients are independent of chemical concentration in animal tissues.

2) Whole body PCB elimination rate coefficients are dependent on chemical hydrophobicity.

3) Metabolic biotransformation of PCBs does not occur or occurs at a negligible extent as determined by a reference compound approach and structure activity relationships described for other vertebrates.

4) Fecal PCB elimination rate coefficients exhibit a lower magnitude than whole body elimination rate coefficients for congeners having log K_{OW} values < 6.5.

5) Fecal elimination PCB rate coefficients approach the magnitude of whole body elimination rate coefficients for congeners having log K_{OW} values > 6.5.

1.6 References

Arnot JA, Gobas FAPC. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ Toxicol Chem* 23: 2343–2355.

Arnot JA, Mackay D. 2008. Policies for Chemical Hazard and Risk Priority Setting: Can Persistence, Bioaccumulation, Toxicity, and Quantity Information Be Combined? *Environ Sci Technol* 42: 4648-4654.

Arnot JA, Gobas FAPC. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ Rev* 14:257–297.

Barber MC. 2008. Dietary uptake models used for modeling the bioaccumulation of organic contaminants in fish. *Environ Toxicol Chem* 27: 755–777.

Batterman AR, Cook PM, Lodge KB, Lothebbach DB, Butterworth BC 1989. Methodology used for a laboratory determination of relative contributions of water, sediment and food chain routs of uptake for 2,3,7,8-TCDD bioaccumulation by lake trout in Lake Ontario. *Chemosphere* 19: 451-458.

Bentzen E, MacKay D, Hickie BE, and Lean DRS. 1999. Temporal trends of polychlorinated biphenyls (PCBs) in Lake Ontario fish and invertebrates. *Environ Rev* 7: 203-223.

Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, Schantz S, and Winneke G. 1999. Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. *Environ Health Perspect* 107:639-649.

Bruggeman WA, Martron LBJM, Kooiman D, and Hutzinger O. 1981. Accumulation and elimination of di-, tri-, and tetra chlorobiphenyls by goldfish after dietary and aqueous exposure. *Chemosphere* 10:811-832.

Bruggeman WA, Opperhuizen A, Wijbenga A, and Hutzinger O. 1984. Bioaccumulation of super-lipophilic chemicals in fish. *Toxicol and Environ Chem* 7: 173-189.

Burkhand LP. 2003. Factors influencing the design of bioaccumulation factor and biota-sediment accumulation factor field studies. *Environ Toxicol Chem* 22: 351-360.

Burreau S, Axelman J, Broman D, and Jakobsson E. 1997. Dietary uptake in pike (*Esox lucius*) of some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers administered in natural diet. *Environ Toxicol Chem* 16: 2508–2513.

Campfens J, and MacKay D. 1997. Fugacity-based model of PCB bioaccumulation in complex aquatic food webs. *Environ Sci Technol* 31: 577-583.

Chen YC, Yu ML, Rogan WJ, Gladen BC, and Hsu CC. 1994. A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-cheng children. *Am J Public Health* 84: 415-421.

Chen YJ, and Hsu CC. 1994. Effect of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiology study. *Dev Med Child Neurol* 36: 312-320.

Clark KE, Gobas FAPC, Mackay D. 1990. Model of organic chemical uptake and clearance by fish from food and water. *Environ Sci Technol* 24:1203–1213.

Coimbra AM and Reis-Henriques MA. 2007. Tilapia Larvae Aroclor 1254 Exposure: Effects on Gonads and Circulating Thyroid Hormones During Adulthood. *Bull Environ Contam Toxicol* 79:488-493.

Connolly JP, and Pedersen CJ. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environ Sci Technol* 22: 99-103.

Dabrowska H, Fisher SW, Dabrowska K, Staubus AE. 1999. Dietary uptake efficiency of 2,2_,4,4_,5,5_-hexachlorobiphenyl in yellow perch and rainbow trout: Role of dietary and body lipids. *Environ Toxicol Chem* 18:938–945.

DeBruyn AMH and Gobas FAPC. 2007. The sorptive capacity of animal protein. *Environ Toxicol Chem* 26: 1803-1808.

DeBruyn AMH and Gobase FAPC. 2006. A Bioenergetic Biomagnification Model for the Animal Kingdom. *Environ Sci Technol* 40: 1581-1587.

de Freitas AS and Norstrom RJ. 1974. Turnover and netabolism of polychlorinated biphenyls in relation to their chemical structure and movement of lipids in the pigeon. *Can J Physiol Pharmacol* 52: 1080-1094.

Devillers J, Bintein S, Domine D 1996. Comparison of BCF models based on log P. *Chemosphere* 33: 1047-1065.

de March BGE, de Wit CA, Muir DCG, Braune BM, Gregor DJ, Norstrom RJ, Olsson M, Skaare JU, and Stange K. 1998. Persistent organic pollutants. *In* de March BGE, de Wit CA and Muir DCG (eds), AMAP Assessment Report: Arctic Pollution Issues, Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, p 183-317.

de Vault, D. S., Hesselberg, R., Rodgers, P. W., and Feist, T. J. 1996. Contaminant trends in lake trout and walleye from the Laurentian Great Lakes. *J Great Lakes Res* 22, 884-895.

de Wit CA, Fisk AT, Hobbs KE, Muir DCG, Gabrielsen GW, Kallenborn R, Krahn MM, Norstrom RJ, and Skaare JU. 2004. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, p -xvi + 310.

Drouillard KG, Paterson G, Haffner GD. 2009. A combined food web toxicokinetic and

    species bioenergetic model for prediction seasonal PCB elimination by yellow perch

    (*Perca flavescens*). *Environ Sci Technol* In press.

Drouillard KG, Norstrom RJ. 2003. The influence of diet properties and feeding rates on

    PCB toxicokinetics in the ring dove. *Arch Environ Contam Toxicol* 44:99–106.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG. 1998. Dietary accumulation and

    depuration of hydrophobic organochlorines: Bioaccumulation parameters and their

    relationship with the octanol/water partition coefficient. *Environ Toxicol Chem*

    17:951–961.

Fox K, Zauke GP, Butte W 1994. Kinetics of bioconcentration and blearance of 28

    polychlorinated biphenyls congeners in zebrafish (*Brachydanio rerio*). *Ecotoxicol*

    Environ Saf 28: 99-109.

Gilbertson M, Kukiak T, Ludwig J, and Fox G. 1991. Great Lakes embryo mortality, edema,

    and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to

    chick edema disease. *J Toxico Environ Health* 33: 455-520.

Government of Canada. 1999. Canadian Environmental protection Act, 1999. Canada Gazettt

    Part Three. Ottawa, ON.

Gobas FAPC, Morrison HA 2000. Bioconcentration and bioaccumulation in the aquatic

    environment. *In* Boethling R, Mackay D (eds) Handbook for environmental

    properties. CRC Press, Boca Raton, FL.

Gobas FAPC, Clark KE, Shu WY, Mackay D. 1989 Bioconcentration of polybrominated

    benzenes and biphenyls and related superhydrophobic chemicals in fish: role of

    bioavailability and elimination into the feces. *Environ Toxicol Chem* 8:231–245.

Gobas FAPC, Zhang X, Wells R. 1993. Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. *Environ Sci Technol* 27: 2855-2863.

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133–141.

Gobas FAPC, Muir DCG, Mackay D. 1988. Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.

Guitart R, Puig P, and Gomez-Catalan J. 1993. Requirement for a standardized nomenclature criterium for PCBs: computer-assisted assignment of correct congener denomination and numbering. *Chemosphere* 27: 1451-1459.

Harrison HL, Loucks OL, Mitchell JW, Parkhurs DF, Tracy CR, Watts DG, Yannacon VJ. 1970. Systems studies of DDT transport. *Science* 170: 503-508.

Hawker, D. W. and Connell, D. W. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ Sci Technol* 22: 382-387.

Heath AG. 1995. *Water pollution and fish physiology* CRC Press, Boca Raton, FL, USA

Hebert, C.E., Norstrom, R. J., and Weseloh, D. V. 1999. A quarter century of environmental surveillance: The Canadian Wildlife Service's Great Lakes herring gull monitoring program. *Environ Rev* 7: 147-166.

Huestis SY, Servos MR, Whittle DM, van den Heuvel M, and Dixon DG. 1997. Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in Lake Ontario lake trout, 1977 to 1993. *Environ Toxicol Chem* 16: 154-164.

Huestis, S. Y., Servos, M. R., Whittle, D. M., and Dixon, D. G. 1996. Temporal and age-related trends in levels of polychlorinated biphenyl congeners and organochlorine contaminants in Lake Ontario lake trout (*salvelinus namaycush*). *J Great Lakes Res* 22, 310-330.

Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, and Dowler JK. 1985. The effect of intrauterine PCB exposure on visual recognition memory. *Child Development* 56: 853-860.

Jacobson JL, Jacobson SW, and Humphrey HE. 1990a. Effects of utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J Pediatr* 116: 38-45.

Jacobson JL, Jacobson SW, and Humphrey HE. 1990b. Effect of exposure to PCBs and related compounds on growth and activity in children. *Neurotoxicology teratol* 12: 319-326.

Jensen S. 1972. The PCB story. *Ambio* 1: 123-131.

Jensen S, Sundstrom G 1974. Structures and levels of most chlorobiphenyls in two technical PCB products and in human adipose tissue. *Ambio* 3:70-76.

Johnston TA, Fisk AT, Whittle DM and Muir DCG. 2002. Variation in Organochlorine bioaccumulation by a predatory fish: Gender, geography and data analysis methods. *Environ Sci Technol* 36: 4238-4244.

Koslowski SE, Metcalfe CD, Lazar R, Haffner GD 1994. The distribution of 42 PCBs , including 3 coplanar congeners, in the food web of the western basin of Lake Erie. *J Great Lakes Res* 20: 260-270.

Leadley TA, Balch G, Metcalfe CD, Lazar R, Mazak E, Habowsky J, and Haffner GD. 1998. Chemical accumulation and toxicological stress in three brown bullhead populations in the Detroit River, Michigan, USA. *Environ Toxicol Chem* 17: 1756-1766.

Liu J, Pan LQ, Zhang L, Miao J, and Wang J. 2009. Immune responses, ROS generation and the haemocyte damage of scallop Chlamys farreri exposed to Aroclor 1254. *Fish Shellfish Immunol* 26: 422-428.

Lonky E, Reihman J, Darvill T, Mather SJ, and Daly H. 1996. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. *J Great Lakes Res* 22: 198-212.

MacKay D and Fraser A. 2000. Bioaccumulation of persistent organic chemicals: mechanisms and models. *Environ pollut* 110: 375-391.

MacKay D 1982. Correlation of bioconcentration factors. *Environ Sci Technol* 16: 274-278.

Masuda Y, Kuroki H, Haraguchi K, and Nagayam J. 1985 PCB and PCDF congeners in the blood and tissues of yusho and yu-cheng patients. *Environ Health Perspect* 59: 53-58.

McKim JM, and Goeden HM. 1982. A direct measure of the uptake efficiency of a xenobiotic chemical across the gills of brook trout (*Salvelinus fontinalis*) under normoxic and hypoxic conditions. *Comp Biochem Physiol C* 72: 65-74.

McKim JM and Heath EM. 1983. Dose determinations for waterborne 2,5,2',5',-[14C] tetrachlorobiphenyl and related pharmacokinetics in two species of trout (*Salmo gairdneri* and *Salvelinus fontinalis*): A mass-balance approach. *Toxicol Appl Pharmacol* 68: 177-187.

McKim, J. M.; Schmieder, P.K.; Veith, G.D. 1985. Absorption dynamics of organic chemical transport across trout gills as related to octanol-water partition coefficient. *Toxicol Appl Pharmacol* 77: 1-10.

Meijer SN, Ockenden WA, Sweetman A, Breicik K, Grimalt JO, and Jones KC. 2003. Global distribution and budget of PCBs and HCB in background surface soils: implications for sources and environmental processes. *Environ Sci Technol* 37: 667-672.

Morrison HA, Gobas FAPC, Lazar R, and Haffner GD. 1996. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environ Sci Technol* 30: 3377-3384.

Morrison HA, Whittle DM, and Haffner GD. 2002. A comparison of the transport and fate of polychlorinated biphenyl congeners in three Great Lakes food webs. *Environ Toxicol Chem* 21: 680-692.

Muir DCG, Marshall WK, Webster GRB. 1985. Bioconcentration of PCDDs by fish: effects of molecular structure and water chemistry. *Chemosphere* 14:829-833.

Nichols JW, Fitzsimmons PN, Whiteman FW, Kuehl DW, Butterworth BC, Jenson CT. 2001. Dietary uptake kinetics of 2,2_,5,5_-tetrachlorobiphenyl in rainbow trout. *Drug Metab Dispos* 29:1013–1022.

Norstrom RJ, McKinnon AE,and DeFreitas ASW. 1976. Bioenergetics-based model for pollutant accumulation by fish simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). *J Fish Res Bd Can* 33: 248-267.

OMOE (Ontario Ministry of the Environment). 2009. Guide to eating Ontario sport fish, 2009-2010, 25th, Rev., Queen's Printer for Ontario, ON, Canada.

Opperhuizen A, Van der Velde EW, Gobas FAPC, Liem DAK, Van der Steen JMD, and

Hutzinger O. 1985. Relationship between bioconcentration in fish and steric factors

of hydrophobic chemicals. *Chemosphere* 14:1871–1896.

Opperhuizen A 1991. Bioconcentration and biomagnifications: is a distinction necessary? *In*

Nagel R, Loskill R (eds) Bioaccumulation in aquatic systems: contributions to the

assessment. VCH, New York, NY, 43-66.

Paterson, G, Drouillard KG, and Haffner GD. 2007(a). PCB elimination by yellow perch

(*Perca flavescens*) during an annual temperature cycle. *Environ Sci Technol* 41: 824-

829.

Paterson G, Drouillard KG, Leadley TA, and Haffner GD. 2007(b). Long-term

polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca

flavescens*) *Can J Fish Aquat Sci* 64: 1222-1233.

Qiao P, Gobas FAPC, Farrell AP. 2000. Relative contributions of aqueous and dietary uptake

of hydrophobic chemicals to the body burden in juvenile rainbow trout. *Arch Environ

Contam Toxicol* 39:369–377.

Randall DJ, Connell DW, Yang R and Wu SS. 1998. Concentrations of persistent lipophilic

compounds in fish are determined by exchange across the gills, not through the food

chain. *Chemosphere* 37: 1263-1270.

Rozman T, Scheufler E, and Rozman K. 1985. Effect of partial jejunectomy and colectomy

on the disposition of hexachlorobenzene in rats treated or not treated with hexadecane.

Toxicol App Pharmacol 78: 421-427.

Russell RW, Gobas FAPC, and Haffner GD. 1999. Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: a model and field verification. *Environ Sci Technol* 33: 416-420.

Safe, S. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications of risk assessment. *Crit Rev in Toxicol* 24, 87-149.

Servos MR, Muir CG, Webster GRB 1992. Bioavailability of polychlorinated dibenzo-p-dioxin in lake enclosures. *Can J Fish Aquat Sci* 49: 435-742.

Scheider, W. A., Cox, C., Hayton, A., Hitchin, G., and Vaillancourt, A. 1998. Current status and temporal trends in concentrations of persistent toxic substances in sport fish and juvenile forage fish in the Canadian waters of the Great Lakes. *Enivorn Monit Assess* 53: 57-76.

Sijm DTHM, Seinen W, and Opperhulzen A. 1992. Life-cycle biomagnifications study in fish. *Environ Sci Technol* 26: 2162-2174.

Sijm DTHM, Bol J, Semen W, Opperhuizen A 1993. Ethyl maminobenzoate methanesulfonate dependent and carrier dependent pharmacokinetics of extremely lipophilic compounds in rainbow trout. *Arch Environ Contam Toxicol* 25: 102-109.

Stow. 1995. Great Lakes herring gull egg PCB concentrations indicate approximate steady-state conditions. *Environ Sci Technol* 29: 2893-2897.

Stow, C. A., Lamon, E. C., Qian, S. S., and Schrank, C. S. 2004. Will Lake Michigan lake trout meet the Great Lakes Strategy 2002 PCB reduction goal? *Environ Sci Technol* 38: 359-363.

Swackhamer DL. 1996. Studies of polychlorinated biphenyls in the Great Lakes. *In*: Hester RE, Harrison RM (eds) Issues in environmental science and technology, 6th,

Chlorinated organic micropollutants, Royal Society of Chemistry, Cambridge, UK, p137-153.

Tanabe S, Maruyama K, Tatsukawa R. 1982. Absorption efficiency and biological half-lives of individual chlorobiphenyls in carp (*Cyprinus carpio*) orally exposed to Kanechlor products. *Agric Biol Chem* 46:891–898.

Tanabe S. 1988. PCB problems in the future: Foresight from current knowledge. *Environ Pollut* 50: 5- 28.

Thomann RV. 1981. Equilibruim model of fate of microcontaminants in diverse aquatic food chains. *Can J Fish Aquat Sci* 38: 280-296.

Thomann RV, Connolly JP, Parkerton TF. 1992. A equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ Toxicol Chem* 11: 615-629

Thomann RV, Connolly JP 1984. Model of PCB in the Lake Michigan lake trout food chain. *Environ Sci Technol* 18:65-71.

Tulp MTM, and Hutzinger O. 1978. Some thoughts on aqueous solubilities and partition coefficients of PCB, and the mathematical correlation between bioaccumulation and physico-chemical properties. *Chemosphere* 7: 849–860.

U.S. Environmental Protection Agency. 2000. Methodology for deriving ambient water quality criteria for the protection of human health. EPA-822-B-00-004. Washington. DC.

Vallack HW, Bakker DJ, Brandt I, Brostrom-Lunden E, Brouwer A, Bull KR, Gough C, Guardans R, Holoubek I, Jansson B, Koch R, Kuylenstierna J, Lecloux A, MacKay D,

McCutcheon P, Mocarelli P, Taalman RDF. 1998. Controlling persistent organic pollutants- what's next? *Enivron toxicol pharmacol* 6: 143-175.

Veith GD. 1975. Baseline concentrations for polychlorinated biphenyls and DDT in Lake Michigan fish. *Pestic Monit J* 9: 21-29.

CHAPTER 2 - THE INFLUENCE OF DIET PROPERTIES ON THE ASSIMILATION EFFICIENCY (AE) OF 47 POLYCHLORINATED BIPHENYL (PCBs) CONGENERS IN JAPENES KOI (*CYPRINUS CARPIO*)

2.1 Introduction

Bioconcentration and food web biomagnification of persistent organic pollutants (POPs), such as PCBs and DDT, are wildly recognized in aquatic ecosystems. These combined processes lead to enriched tissue concentrations of priority pollutants that can cause toxic effects in wildlife (Giesy and Kannan 1998) as well as contribute to human health concerns related to the consumption of contaminated fish (Liem et al. 2000). The incentives for developing bioaccumulation models to predict bioaccumulation of POPs for regulation and risk assessment perspectives are well recognized and have a long history in POPs regulation (Harrison et al. 1970).

Bioaccumulation occurs due to the competing processes of chemical uptake and elimination to and from the organism. While empirical data on bioaccumulation factors (BAFs) have been developed (Arnot and Gobas, 2006), model-based approaches are necessary to determine the relative contributions of bioconcentration (i.e., chemical uptake from water via the gills) and biomagnification (i.e., chemical uptake from ingested food) to whole animal chemical exposures (Qiao et al. 2000; Gobas et al. 1989; Randall and Braunner 1990).

Toxicokinetic-based bioaccumulation models, including steady state food web bioaccumulation models (Arnot and Gobas 2006), are increasingly being used to aid in

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environmental management decisions. For example, models of this type are used as decision support tools to prioritize remediation activities such as sediment clean-up (Saloranta et al. 2008), forecasting the effects of pollutant abatement programs (Gobas et al. 1995) and to evaluate the risk of emerging chemicals of concern (Arnot and Mackay 2008).

Toxicokinetic parameters required by bioaccumulation models include chemical uptake rate coefficients from water and food and elimination rate coefficients. For aquatic organisms exposed to POPs, the role of dietary uptake to total chemical exposures becomes more pronounced with increasing chemical hydrophobicity (Gobas et al. 1989; Feldman and Titus 2001) and the dietary route dominates when the log K_{OW} of the chemical exceeds a value of approximately 6 (Connolly and Pedersen 1988; Heath 1995; Baber 2008). For these highly hydrophobic chemicals it is, therefore, necessary to predict uptake rate coefficients (k_D) from the diet with a high degree of accuracy.

The uptake rate coefficient (k_{feed}; g feed·g^{-1} BW·d^{-1}) is directly related to the animal feeding rate and chemical assimilation efficiency (AE) from the ingested diet of the animal:

$$k_{feed} = AE \cdot G_{feed} \qquad (1)$$

Where AE is obtained from the diet (unitless) and G_{feed} is the animal feeding rate (g feed·g^{-1} BW·d^{-1}). For model applications, G_{feed} is obtained from a bioenergetics sub-model and may be variable over time (Arnot and Gobas 2004; Drouillard et al. 2009). The dietary AE is chemical specific and considered to be a constant under thermodynamic model formulations, which consider dietary uptake and fecal egestion as separate processes (Barber 2008; Drouillard 2003). Empirical measurements demonstrate considerable variability in the magnitude of AE (20 to >90%) across different studies (Gobas et al. 1988; Burreau et al. 1997; Fisk et al. 1998).

Despite high variation in AE values, a trend of decreasing dietary AE with increasing chemical K_{OW} is often reported (Gobas et al. 1988; Tanabe et al. 1982). As such, most food web bioaccumulation models include predictive algorithms to estimate chemical AE for different species of fish as a function of chemical hydrophobicity (Arnot and Gobas 2006; Thomann et al. 1992). Mechanistic models describing chemical uptake from food, such as the gastrointestinal magnification model (Gobas et al. 1988; 1993a; 1999), predict that differences in digestive physiology as it relates to digestability of proximate diet components also influence AE (Gobas et al. 1999). Other studies have indicated that digesta residence times, animal feeding rate, body weight and growth rate could influence the magnitude of chemical AEs (Barber 2008; Clark et al. 1990; Drouillard and Norstrom 2000). Relatively few studies have directly tested the influence of diet composition on chemical AE (e.g., Gobas et al. 1993a; Clark et al. 1990; Dabrowska et al. 1999). Furthermore, many studies that have measured dietary AE in the laboratory used fish pellets or fish flakes (e.g., Fisk et al. 1998; Bruggeman et al. 1981), or have introduced the chemical to the diet by spiking chemical into food (e.g., Burreau et al. 1997). There is a need to determine whether dietary AE's are similar across natural relative to artificial/prepared diet formulations and whether differences in AE can be related to diet proximate content (Nichols et al. 2001).

In this study, Japanese koi (*Cyprinus carpio*) were used in laboratory feeding trials to determine PCB assimilation efficiencies following access to five experimental diets. Two diets consisted of natural food items (benthic invertebrates and contaminated forage fish) collected from contaminated areas of the Detroit River, Michigan, USA and western Lake Erie, Ontario, Canada. The remaining three experimental diets consisted of spiked commercial fish pellets that varied in lipid content. The data were used to test two major

hypotheses: 1) that PCB dietary AE's from natural food items are similar to those for fish pellet formulations, and 2) that dietary AEs are independent of dietary lipid content. In addition, the empirical measurements of AE's across PCB congeners and diet types were evaluated against published algorithms used to predict this parameter and included in food web bioaccumulation models.

2.2 Material and method

2.2.1 Experimental diets

Contaminated benthic invertebrates consisted of male and female emergent mayflies (*Hexagenia spp.*) collected from Middle Sister Island, Western Lake Erie, Ontario, Canada. Animals were collected during an overnight sampling trip to the island using light traps (Corkum et al. 1997). Mayflies spend approximately two years in contact with sediments during the nymph stage reaching steady state with the sediments they inhabit (Drouillard et al. 1996). The collection site was chosen because the island receives a plume of contaminated water and sediments from the Detroit River. Forage fish consisted of emerald shiners (*Notropis atherinoides*) collected from Trenton Channel, Detroit River using a boat electrofisher. This area of the Detroit River is known to have high sediment contamination (Drouillard et al. 1996). In order to ensure homogenous diet exposures, pooled mayflies or pooled forage fish were minced into 20mm length sizes suitable for the experimental fish to feed on. The food particles were well mixed prior to feeding experimental animals.

The commercial fish feed was obtained from Martin Mills Inc., Elmira, Ontario. Two commercial diets were obtained, one a koi-specific feed containing 6% lipids and the second a trout chow containing 11% lipids. The trout chow diet was amended with olive oil to

produce 2 lipid-enriched diet formulations. One diet was amended with oil to produce a nominal lipid content of 16% and the second diet amended to produce a nominal lipid content of 22%, respectively. The three pellet diets were spiked with PCBs by suspending 20 g pellet in 50 ml hexane solvent containing PCB Aroclor mixture (1242:1254:1256; 1:1:1 ratio). The excess solvent was evaporated to dryness in a fumehood under a gentle nitrogen gas stream. The nominal sum PCB concentration was 1 µg/g dry weight and was designed to be comparable with the chemical concentrations expected in the naturally contaminated experimental diets.

2.2.2 Feeding study

Thirty-five juvenile koi, averaging 10g, were obtained from Pro-Fish (a local pet fish store) located in Windsor, Ontario. Prior to the experiment, fish were acclimated for 5 days at 16°C in 4 aquaria (60L) receiving flow-through (1L/g fish/d) dechlorinated City of Windsor, Ontario municipal water. Activated carbon filters were installed in each tank to remove PCBs from the water. During the first three days of acclimation, fish were maintained on standard goldfish feed (Martin Mills Inc., Elmira, Ontario) at a feeding rate of 1% of the average body weight. All fish were observed to accept food given to them during the acclimation period. On days 4 to 5 of the acclimation, fish were fasted to ensure that they evacuated their gut contents and that they would actively feed on the experimental diets.

The thirty-five fish were randomly separated into five treatment groups plus two control groups (n=5 fish per treatment). Two control groups were sacrificed on the first and last day of the experiment, respectively, to determine the chemical residue in control fish and to make sure no other significant PCB sources other than the treatment diets occurred during the feeding trials. Each fish was placed in an individual glass aquarium (30 cm x 40 cm x 50

cm). The aquaria were maintained under static conditions and constantly aerated. Each fish was fed a pre-weighed amount of treatment or control (non-spiked gold fish pellets) food at time 0 and observed for 3 hours to ensure that all provided food was consumed. The fish were fed twice more at 3 h and 6 h from time 0. On the third feeding, fish became satiated and did not consume all of the food provided. Excess food was removed from each tank and the uneaten food was dried and weighed to determine the total amount of food consumed by each fish. After the last feeding event, fish were fasted for 48 h to permit digestion and assimilation of the consumed diet and the fish were subsequently sacrificed.

The wet body weights of the fish were recorded after blot drying and the animals were subsequently dissected to remove the gastrointestinal tracts. For those fish having undigested food in their stomachs, the digesta material was removed and dried in a drying oven (110℃ for 24 h). The undigested material was subtracted from the total food

consumption determined for the individual. The remaining carcass was homogenized using a solvent-rinsed stainless steel blender and the homogenate was stored in hexane-rinsed aluminum tins at $-20^{o}C$ until chemical analysis. This study was performed under ethics approval from the University of Windsor's Animal Care Committee according to Canadian Animal Care Guidelines.

2.2.3 Chemical analysis

Five samples of each diet were analyzed for lipid content and PCBs along with fish homogenate samples. Methods for PCB extraction and analysis followed the micro-extraction procedure described by Daley et al (2009). Briefly, sample homogenate (~1.5 g) was ground with 15 g activated Na_2SO_4 (ACS-grade, 10-60 mesh; activated by muffle furnace at 450 ºC overnight; Fisher Scientific; Ottawa, ON, Canada), and spiked with 250 ng

of tribromobenzene (TBB) and PCB30 as internal recovery standards. The glass columns consisted of 30 mL luer-lock glass syringes (plunger removed), affixed with 1 μm glass fiber syringe filters and connected to the valve of a 12 port solid phase extraction (SPE) vacuum manifold (Phenomenex, Torrance, CA, U.S.A). Eluents from each column were collected into custom glass reservoirs (70 mL) seated within the manifold. Initially, the valves were shut off and the homogenates were wet packed into a syringe column containing 20 mL hexane:dichloromethane (1:1 v/v; pesticide grade; VWR, St. Catherines, ON, Canada). The homogenate was allowed to extract in the solvent for 1 h, after which, the valve was opened up and the eluent was collected under gravity. Following elution of the first 20 mL of solvent, the homogenate was further extracted with 10 mL additional hexane:dichloromethane (1:1, v/v) for three times continuously. After elution was complete, remaining solvent in the syringes was collected by applying vacuum to the manifold. Extracts were concentrated to 10ml using a rotary evaporator. A 1 ml portion of the sample was removed for gravimetric lipid determination. Past studies have demonstrated that hexane:dichloromethane selectively extracts neutral, triglyceride lipids, and provides a better surrogate measure for sample partitioning capacity than total lipids determined by chloroform/methanol techniques (Drouillard et al. 2004).

The remaining extracts were cleaned up by activated Florisil (VWR, ON, Canada) as described in Lazar et al (1992). As only PCBs were quantified in the samples, only the first fraction (50 mL hexane) was collected from florisil columns. The cleaned-up eluent was then condensed to 1 ml by rotary evaporation. The extracts were sealed in 2 ml gas chromatograph (GC) vials, and stored at 4 ℃ until instrument analysis. Method blanks and

an in-house reference homogenate (Detroit River carp) were co-extracted for every batch of six samples analyzed.

All samples were analyzed on an Agilent 6890 Series Plus gas chromatograph with a ^{63}Ni micro electron capture detector (GC-µECD), which was equipped with an Agilent 7683 Autosampler. Compound separation was completed using 6m × 0.25mm (internal diameter) column with H_2 carrier gas (at a constant flow rate of 0.91 ml/ min) following the established methodology (Lazar et al. 1992). Nitrogen was used as the makeup gas for the electron capture detector (T = 325 °C). The oven program, description of standards and method recovery are described elsewhere (Lazar et al. 1992). Forty seven PCB congeners were identified by retention time and quantified using the standard response for equivalent peaks in the standard mixtures. Blanks and reference tissues, quantified during each batch of sample extractions, were in compliance with the normal quality assurance procedures instituted by the GLIER organic analytical laboratory. Sample recoveries for the two internal standards averaged 76± 3.36% and 91.3±2.76% for TBB and PCB30, respectively. Chemical concentrations were not corrected for recovery. Recovery of individual PCBs in the fish homogenates were within 2 SD of the control chart mean, meeting performance criteria of the accredited GLIER organic contaminant laboratory.

2.2.4 Data analysis

The differences in weight, lipid and moisture level of animals between treatments were determined by analysis of variance (ANOVA). The total PCB concentrations in two control groups were compared by student's t-test to determine if additional chemical bioaccumulation occurred during the feeding trial independent of treatment diet exposures. The concentration of PCB congeners in treatment fish was corrected by subtracting the

congener concentration in the control fish sacrificed at the end of experiment. The total PCB

concentrations (on the dry weight base) in five experimental diets were compared by

ANOVA.

The chemical AE was calculated as the ratio of assimilated chemical to the total

intake of chemical from the food:

$$AE = \frac{C_{org}}{C_{food}} \cdot \frac{BW_{Org}}{W_{Feed}} \qquad (2)$$

Where C_{org} is the chemical concentration (ng/g wet wt.) measured in the carcass homogenate

of a given fish, C_{food} is the chemical concentration measured in treatment food (ng/g dry

weight), BW is the body weight of the animal (g wet weight) and W_{Feed} is the total mass of

chemical fed by each individual (minus undigested material in its stomach content; g dry

weight).

Principle component analysis (PCA) was performed to reduce the number of

dependent variables (PCB AEs) and to increase statistical power. Prior to PCA analysis, the

data were tested for normality using normal probability plots. Missing values in the AE data

matrix were replaced by the mean AE from the same treatment groups of this specific PCB

congener. The PCA analysis was performed with PCBs as dependent variables and samples

as independent variables using a correlation matrix. In cases where the majority of variance

(75%) was explained by the first principle component axis, ANOVA was applied using

sample scores for the first component to test for differences among five treatment groups.

When more than one component axis was required to explain greater than 75% of the

variance, multivariate analysis of variance (MANOVA) was used. An individual PCBs was

considered to be strongly loaded onto a given PCA axis when its component loading was

greater than 0.7 (Drouillard and Norstrom 2000). The differences between treatments were

tested by ANOVA and post hoc comparisons were made using Tukey's honestly significant difference test.

The relation between AE and chemical K_{OW} was evaluated using two models, a non-linear regression based on a two-phase resistance model suggested by Gobas et al (1988) and a linear regression model:

$$\text{Model 1:} \qquad AE = \frac{1}{\left(A + B \cdot K_{OW}\right)} \qquad\qquad (3)$$

$$\text{Model 2:} \qquad AE = A + B \cdot \log K_{OW} \qquad\qquad (4)$$

The significance criterion for all the statistical tests was set at 0.05. All statistical analyses were performed using SPSS (ver. 16, Chicago, IL, USA).

2.3 Results

The lipid and moisture contents in the five experimental diets are presented in Table 2.1. Moisture contents of the different diets ranged from 3.4% to 81.3% across the diet treatments. Since the moisture content of the diets change after adding the food to fish tanks, all data on lipid and PCB concentrations in diets were expressed on a dry weight basis.

Dry weight lipid content across the diets ranged from 4.1±0.091 to 24.0±0.174 %. Each of the three pellet diets (low, medium and high fat) had significantly different (p<0.001; ANOVA; Tukey's HSD) lipid contents from one another. The two natural diets were also significantly different (p<0.001; ANOVA; Tukey's HSD) from one another. The benthic invertebrate diet treatment had the lowest lipid content and was significantly different (p<0.001; ANOVA; Tukey's HSD) from all other diet treatments. The forage fish diet treatment was not significantly different in its lipid content from the medium fat pellet diet (p>0.06; ANOVA). Congener specific PCB concentrations in the different diet treatments

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are presented in Table 1. Sum PCBs ranged from 553.8±62.3 to 696.7±69.0 ng/g dry wt. among the diets and were not significantly (p>0.24; ANOVA) different from one another.

Fish body weights and percent lipid from each diet are summarized in Table 2.2. There were no significant differences (p>0.4; ANOVA) in body weights across the experimental groups. Although there was large variation in body lipid content among individual fish (2% to 8% lipid/wet weight), no significant difference in body lipids occurred across the treatments (P>0.3; ANOVA).

Total amount of dry weight food consumed across the treatments is expressed on a per gram fish wet weight in Table 2.2. Out of 25 treatment fish, 2 fish from medium fat feeding group and 1 fish from high fat feeding group were found with a small amount of undigested food present in the GI tract following the 48 h fasting period. The amount of undigested food in the three fish was less than 10% of the total amount of feed consumed and provided a marginal adjustment to the measured feeding rate. The lack of food in the GI tract after visual confirmation of feeding activity in each fish provided confirmation that the 48 h post-feeding period was sufficient for all of the fish to complete or nearly complete digestion and assimilation of each diet.

The amount of food consumed by fish fed the natural foods was significantly different (P<0.001; ANOVA) from those fed fish pellets. The two groups fed natural food consumed similar amounts of food as one another, averaging 0.055±0.005 (benthic invertebrate) and 0.045g±0.006 (forage fish) g dry food/g fish wet weight, respectively (P>0.9, ANOVA Tukey's HSD). The groups fed pellet feed had slightly higher feeding rates that averaged 0.100±0.003 (low fat pellet), 0.124±0.008 (medium fat pellet) and 0.100±0.010 (high fat pellet) g dry food/g fish wet weight, respectively (P>0.3, ANOVA,

Tukey's HSD). For the pelleted food, there was no relationship between feeding rate and pellet lipid content.

Total PCB concentrations in control fish sacrificed at the beginning and the end of the feeding trials were 1.96±0.06 and 1.88±0.44 ng/g, respectively (Table 2.2) and were not significantly different (P<0.05, t-test) from one another. Experimental fish bioaccumulated PCBs from their contaminated diets and at the end of the study had significantly higher PCB burdens than control fish (P<0.001, ANOVA). Across the treatments, the forage fish diet group and high fat pellet diet group accumulated significantly (p<0.05, ANOVA, Tukey's HSD) lower PCB concentrations than the other three diet groups.

Dietary PCB assimilation efficiencies were determined for each fish according to Eq. 2. Estimates of dietary AE varied from 26 to 101% across the congeners and diet treatments. The median value and maximum/minimum AE values for all congeners in a given diet treatment are presented in Figure 2.1. PCA was performed on the congener specific AE values after testing for normality across the dependent variables. The first component axis in the PCA analysis explained 77% of the variation with all the congeners loading highly onto this component (0.73 to 0.96), except PCB 194, which has a lower loading of 0.55. An ANOVA and post-hoc comparison test indicated significant differences in the component 1 PCA sample scores between the benthic invertebrate diet group and the other diet groups (p<0.001) and no significant difference among the other four diet groups (p>0.135, Tukey's HSD). This indicates that the group fed benthic invertebrates had significantly higher PCB AEs across the individual PCB congeners compared to the other groups.

Figure 2.2 presents dietary AEs as a function of chemical K_{OW} across each of the diet treatments. For all diets, there was a highly significant relationship (p<0.001; ANOVA)

between chemical K_{OW} and dietary AE. Table 2.3 summarizes the non-linear regression fit to Eq. 3 and the linear regression fit to Eq. 4. High individual fish variation as well as congener specific differences in AE for a given K_{OW} resulted in the models describing relatively small amounts of total variation in the data, ranging from 7 to 44%. When comparing the two model fits, the linear model provided better fit to the data than the two phase resistance model. Across the different diet treatments, the linear model explained between 16.2% and 44% of the variability while the two phase resistance model explained between 7.3 and 39%.

2.4 Discussion

In this study, the observed PCB AE values varied from 23% to 101% across the diet treatments and PCB congeners. This high degree of variation in AEs is consistent with the wide range of AE values reported in the literature (Barber 2008; Hendriks et al. 2001). A number of arguments have been put forward to explain cross study and within study variation in PCB AEs for fish. These include AE estimation approaches (i.e., mass balance versus kinetic approaches, Nichols et al. 2001), dietary lipid content and composition (Gobas et al. 1993a; Dabrowska et al. 1999; Doi et al. 2000), food digestibility (Gobas et al. 1993a; 1999), fish feeding rate (Clark and Mackay 1991), fish age (Sijm et al. 1992) and the method used to dose contaminated food provisioned to animals (i.e., natural bioaccumulation versus food spiking, Nichols et al. 2001; Burreau et al. 1997).

Nichols et al (2001) argued that studies that used kinetic-based estimation approaches often produce lower AE estimates compared to those that used either mass balance from a single meal exposure or restricted short term feeding studies. In mass balance approaches, the quantity of food provided to individual animals is measured and dietary exposure is limited

to a single meal or relatively few meals to minimize chemical elimination and growth artifacts. In kinetic approaches, animals are often housed communally and the rate of food provisioning to the tank is assumed to be completely and equally consumed by all fish over multiple exposure days. In some cases, kinetic studies use the diet adjusted initial slope from the chemical uptake curve and assumed feeding rate to estimate chemical AEs without consideration for elimination (e.g., Gobas et al. 1993a; Bruggeman et al. 1981). In other cases, non-linear statistical fits to a bioaccumulation model are performed in conjunction with independently measured whole body elimination rates, diet chemical concentration and assumed fish feeding rates to estimate AE (Fisk et al. 1998; Gobas et al. 1999). While the latter method adjusts for mean elimination that occurs during the dietary exposure period, these longer-term experiments are more susceptible to error propagation related to individual differences in chemical elimination, feeding rate and growth.

Nichols et al. (2001) reported near complete absorption (94.5 to 99.8%) of ^{14}C-PCB52 by rainbow trout using a mass balance approach after trout were fed a meal of contaminated fathead minnows, not unlike the high (~100%) AEs observed for lower chlorinated PCBs in the benthic invertebrate group from this study. Burreau et al. (1997) estimated AEs ranging from 35 to 90% using a mass balance approach for northern pike fed a meal of contaminated trout dosed with PCBs, polychlorinated naphthalenes and polybrominated diphenenyl ethers. Tanabe et al (1982) used the mass balance of chemical in food relative to egested feces determined over the first week of exposure of carp to PCB spiked fish pellets and determined AEs ranging from 67 to 93% across different PCB congeners. In contrast, several kinetic-based studies have reported PCB AEs in fish ranging from 24% to 75% (Fisk et al. 1998; Gobas et al. 1993ab; Bruggeman et al. 1981; 1984; Sijm

et al. 1992; Brown et al. 2002; Buckman et al. 2004). The one exception where relatively high PCB AEs (i.e., > 75%) were reported by Dabrowska et al. (1999) using kinetic calculation methods where yellow perch and rainbow trout were estimated to have AEs ranging from 74 to 95% and 79 to 99%, respectively. In this study, a mass balance approach was used, however, AEs were found to vary strongly by diet type, which produced AE estimates consistent with measurements determined in fish using mass balance and kinetic based approaches.

Nichols et al. (2001) and Burreau et al. (1997) suggested that the mechanism of incorporating chemicals into the food matrix could influence measurements of chemical AE. In the study by Nichols et al. (2001), the diet item (fathead minnows), which was dosed by exposure to ^{14}C-PCB52 in water to simulate normal bioaccumulation process by fish. Food fish was fed to rainbow trout housed in clean water conditions. Burreau et al. (1997) extracted lipids from rainbow trout, spiked the lipids with contaminants and injected the spiked lipids into live food fish, which were subsequently fed to pike. The latter investigation argued that use of rainbow trout oil as a medium for contaminant uptake would not alter the digestibility and absorption of lipids from ingested food. Both the above studies used mass balance approaches and reported high PCB AE values. In this study, the differences in PCB AEs between the two natural diets were as great as the differences observed between the natural and artificial diets. Specifically, the AEs between fish fed forage fish and pellet food were not significantly different from one another. Thus, it does not appear that artifacts related to spiking food versus those resulting from natural incorporation of PCBs into living diet items via bioaccumulation strongly influence measurements of PCB AEs.

Other studies have tested the influence of lipid content on PCB and organochlorine AEs (e.g., Drouillard and Norstrom 2003; Gobas et al. 1993a; Dabrowska et al. 1999). Sharifi et al. (1997) observed no difference in the AE for p,p-DDE for gold fish that were fed diets that varied in lipid contents from 2.9 to 10.9%. Dabrowska et al. (1999) fed yellow perch and rainbow trout low fat (5.7%) and high fat (14.1%) diets and did not observe consistent differences in PCB 153 AEs between the high and low lipid diets. Gobas et al. (1993a) fed gold fish and guppies low (<0.2%), medium (6.3%) and high fat (13.5%) diets spiked with a set of organochlorine compounds. For low K_{OW} compounds, the above study found no significant differences in AE values across the diets, however, AEs were significantly higher for highly hydrophobic compounds in the low fat diet compared to the high fat group. In the latter case, the differences reported between AEs across diet treatments were less than 10% (Gobas et al. 1993a).

In this study, the low lipid pellet had a lipid content similar to that of the benthic invertebrates, however, the mean difference in AEs measured between these diet treatments was 36.0±1.3%. These differences exceeded the differences in chemical AEs measured across low and high fat pellet treatments (9.6±0.1%), which exhibited a much larger gradient in lipid content. Feeding rate differences were also noted across the treatment groups with natural diets producing lower feeding rates compared to pellet diets, however, feeding rates were not correlated to PCB AEs. Gobas et al. (1993a) interpreted the higher chemical AEs they observed for goldfish in their low fat diet group to be related to the higher digestability of this diet. This was confirmed in their study by measuring fecal output in addition to feeding rate in experimental fish. Unfortunately, feces were not quantitatively collected in this study, which prevented diet digestion efficiency calculations across the diet treatments.

Although ornamental pond fish are intensively selected for culture conditions that involve artificial feeds, naturalized carp feed primarily on benthic invertebrates and presumably have a digestive physiology optimized for this food source. Therefore, differences in diet digestion efficiency cannot be ruled out as a contributing factor to the observed differences in PCB AEs resulting from the different diet treatment types.

Significant decreasing trends in PCB AEs with increasing PCB hydrophobicity were noted for each diet. The observed trends are consistent with the hydrophobicity effect on chemical AEs reported in other studies (Gobas et al. 1988; Fisk et al. 1998; Tanabe et al. 1982; Drouillard and Norstrom 2000; Buckman et al. 2004). However, the decline in AE with hydrophobicity has not been universally reported across all studies (Gobas et al. 1993a; Burreau et al. 1997). In some cases, the relationship may have been obscured by either mixing of chemical classes, including some compounds that undergo metabolic biotransformation, or by not including sufficient numbers of chemicals to provide statistical power for such inferences.

Several food web bioaccumulation models for organochlorine compounds include algorithms to estimate chemical AEs that decrease with increasing chemical K_{OW} (Arnot and Gobas 2004; Thomann 1989). Gobas et al. (1988) formulated a predictive algorithm for chemical AEs based on a two phases resistance model and fitted the data to empirical measurements reported by Bruggeman and others. The algorithm was subsequently updated and applied in a widely used food web bioaccumulation model by Arnot and Gobas (2004). Figure 2.2 provides the algorithm (AE = $1/ (2.0 + 3.0 \times 10^{-7} \times K_{OW;}$ dashed line) recommended by Arnot and Gobas (2004) contrasted against measured AE values determined in this study. With the exception of the low K_{OW} chemicals (log K_{OW} values <

6.25) in the medium and high fat diet treatments, the recommended algorithm of Arnot and Gobas (2004) underestimated PCB AE for the majority of congeners in the different diets used in this study. Similar observations regarding the accuracy of the predictive performance of this algorithm were observed by Burreau et al. (1997). In addition, the algorithm predicts a much stronger K_{OW} dependence of chemical AE than observed in the diet treatments used in this study, with the exception of the forage fish fed group where AE was higher, but a similar K_{OW} dependence was observed.

Non-linear and linear regressions were performed to fit the data from each diet treatment to the two-phase resistance and linear models (Table 2). For all diet treatments, the linear model explained more of the observed variation than the two-phase model. However, both models failed to explain a majority of the data variability (R^2 values for the linear model varied from 0.14 to 0.44) even when confined to a single diet type. The reduced fit of the two-phase resistance model relative to the linear model is likely due to the restricted K_{OW} range, particularly at the low K_{OW} boundary, over which PCB AE values were determined. Gobas et al. (1988) derived their original model based on a data set that included chemicals with log K_{OW} values as low as 4.0, whereas the minimum K_{OW} for compounds measured in this study (PCB 28 and 31) was 5.67. Extending the K_{OW} range of test chemicals would be helpful to validate the apparent asymptotic relationship of AE at the lower K_{OW} range. The data are also inconsistent with the parabolic relationship between AE and K_{OW} reported by Fisk et al (1998). The Fisk et al. (1998) study showed increases in AE between log K_{OW} values of 5 to 6.5 and decreases with further increases in chemical hydrophobocity. When linear regressions were restricted to compounds with log K_{OW} ranging from 5.67 to 6.5, three groups (forage fish, medium and high fat pellet group) exhibited significant ($p<0.05$)

negative relationships between chemical AE and K_{OW}, whereas the benthic invertebrate and low fat groups demonstrated weak negative, but insignificant ($p>0.05$) relationships. Given the robustness of the simple regression equation and the fact that the lowest PCB log K_{OW} value is 5.0 (Hawker and Connell 1988), it is recommended that the linear model be applied when predicting the K_{OW} dependence of AE for PCB bioaccumulation models.

2.5 Conclusion

Sensitivity studies on persistent organic contaminant food web bioaccumulation models have identified the chemical AE as one of the toxicokinetic parameters that strongly impacts model output and has a high degree of measurement uncertainty (Iannuzzi et al. 1996; Ciavatta et al. 2009). Although the dependence of AE on chemical hydrophobicity is widely recognized and incorporated into many HOC bioaccumulation models, few models consider the impact of different diet types and/or how changes in diet composition through time affects the magnitude of this key toxicokinetic parameter. This study demonstrates that diet properties can contribute to an equal amount of variation in chemical AEs as chemical hydrophobicity. The maximum difference between AE values across PCB congeners that varied three orders of magnitude in K_{OW} ranged from 28% in the medium fat pellet diet to 56% in the forage fish diet. However, for a given PCB congener, the differences in AE values across the diets ranged from 28% (PCB 118) to as high as 66% (PCB 128). Additional research to understand variation in chemical AEs, particularly across natural diet types of critical indicator species used in food web models, should be performed.

Table 2.1. Summary of mean (±S.E.) of the composition and PCB concentration of five diet treatments in this study.

	BI	FF	LF	MF	HF
Moisture content (% wet wt.)	8.98±1.53	80.90±1.84	5.31±0.27	3.24±0.44	2.890±0.17
Lipid content (% dry wt.)	4.1±0.091	14.3±2.12	6.74±0.68	18.4±4.11	24.0±4.17
Total PCB (ng/g dry wt.)	696.7±69.0	682.6±80.6	640.0±60.2	591.62±90.4	553.8±62.3
PCB31	12.52±0.33	10.01±0.67	24.70±3.29	23.02±2.06	25.89±3.12
PCB42	5.92±0.22	6.03±0.43	9.85±3.63	8.70±1.18	8.71±1.11
PCB44	13.36±0.52	11.89±0.92	16.55±4.53	14.35±2.23	14.28±1.70
PCB49	12.80±2.93	15.11±2.11	12.14±0.59	10.47±0.02	12.25±1.45
PCB52	17.14±3.06	19.36±4.10	24.36±2.65	21.39±1.62	23.68±2.28
PCB56/60	8.84±0.81	7.58±0.83	10.48±3.97	8.99±0.98	7.52±0.93
PCB64	8.62±1.15	8.81±0.47	13.14±5.45	11.68±1.25	9.71±0.58
PCB66/95	34.76±3.98	34.40±2.96	41.56±8.72	33.57±2.3	36.56±2.65
PCB70/76	7.69±1.54	13.70±2.17	21.72±2.73	19.82±2.01	20.93±1.83
PCB74	17.07±2.38	20.51±5.03	19.89±2.86	65.33±2.05	31.14±2.12
PCB87	7.33±10.03	6.61±0.14	16.46±1.15	11.06±0.63	13.77±1.49
PCB97	10.20±1.89	6.94±2.91	9.52±1.58	8.22±1.10	8.46±0.81
PCB99	21.14±2.73	11.88±0.90	10.04±1.61	8.90±0.62	9.38±1.11
PCB101	24.64±3.46	28.63±3.77	30.35±1.31	25.87±1.74	25.55±2.33
PCB105	5.38±0.44	7.57±0.82	10.41±0.72	8.68±0.73	7.60±0.88
PCB110	16.08±0.52	21.80±0.81	27.40±1.74	22.51±1.84	21.06±1.83
PCB118	16.53±3.33	17.10±2.01	20.42±1.23	17.48±1.06	18.71±1.31
PCB128	3.65±1.55	5.45±0.19	6.01±0.59	5.18±0.89	3.95±1.09

PCB138	59.20±8.56	55.98±6.22	41.48±3.94	35.22±1.74	34.34±0.73
PCB141	11.21±1.67	12.65±3.01	10.86±1.44	9.30±0.85	9.03±0.98
PCB146	11.22±1.51	12.53±1.00	5.95±1.24	5.11±0.95	4.78±0.84
PCB149	47.74±6.38	46.74±7.49	35.72±2.14	30.23±2.76	29.25±1.65
PCB151	19.78±0.49	16.09±0.91	13.68±1.36	11.78±1.07	10.07±2.31
PCB153	79.49±15.81	65.23±10.04	36.52±3.86	31.49±1.33	30.47±1.75
PCB158	3.81±0.23	4.40±0.18	3.93±0.71	3.08±0.32	3.10±0.72
PCB170/190	25.02±4.48	20.67±2.93	14.91±0.71	12.90±0.90	12.04±1.65
PCB171/156	8.89±0.33	12.48±0.47	10.79±0.44	8.97±0.80	8.65±1.07
PCB172	5.06±1.20	4.26±0.82	2.81±0.22	2.33±0.02	2.32±0.02
PCB174	12.03±1.35	17.69±2.44	13.72±1.22	11.40±0.62	10.79±1.20
PCB177	9.46±0.69	11.53±1.92	7.60±1.45	6.43±0.99	5.87±1.33
PCB178	4.98±0.53	5.40±0.54	4.84±0.84	3.84±0.41	3.74±0.81
PCB179	4.07±0.27	6.16±0.17	6.22±0.64	5.22±0.42	4.91±0.98
PCB180	54.02±8.98	45.86±12.31	29.51±2.51	25.04±2.56	23.18±2.51
PCB183	13.81±2.27	12.30±4.05	8.15±1.25	6.73±0.76	6.30±1.39
PCB187	19.89±2.71	29.83±1.42	16.41±1.99	13.87±1.09	12.46±0.72
PCB194	18.55±3.69	9.88±0.98	7.99±1.01	6.33±0.19	5.91±1.29
PCB195	4.39±0.97	4.01±0.98	3.47±0.44	2.77±0.14	2.63±0.54
PCB200	2.96±1.11	2.61±2.24	3.65±0.99	2.91±0.62	2.89±0.77
PCB201	7.08±1.09	9.93±4.67	7.26±1.17	6.09±0.66	5.47±0.79
PCB203	13.97±2.18	11.37±2.21	8.05±1.09	6.81±0.58	6.20±0.78
PCB206	5.03±1.01	2.56±1.07	2.46±0.09	2.00±0.1	1.93±0.45

Table 2.2. Summary of the mean (±S.E.) body total PCB concentration, body weight, body lipid content and the food consumption of each experimental group.

Fish group	Total PCBs (ng/g wet wt.)	Body weight (g wet wt.)	Lipid in Fish (% wet wt.)	Consumed food (g dry feed wt./g fish wet wt.)
Control (0 h)	1.96±0.06	8.82±0.44	5.28±0.88	n/a
Control (54 h)	1.88±0.44	7.87±0.35	5.06±0.58	n/a
BI	37.38±3.02	10.97±0.81	3.99±1.02	0.055±0.0046
FF	25.98±2.1	11.67±1.44	5.11±0.72	0.045±0.0059
LF	38.20±4.63	9.49±0.53	5.52±1.86	0.10±0.0033
MF	34.08±2.7	9.73±0.71	6.07±1.18	0.12±0.0079
HF	26.22±2.38	10.75±0.59	5.28±1.83	0.10±0.010

Table 2.3. Summary of the AE-K_{OW} regression models for five feed groups in present study

Feed type	Regression equation	R^2
	$AE = 1/ (1.100 + 3.712 \times 10^{-9} \times Kow)$	0.116
BI	$AE = 1.47 - 0.088 \times \log Kow$	0.168
	$AE = 1/ (1.57 + 3.24 \times 10^{-8} \times Kow)$	0.291
FF	$AE = 1.72 - 0.18 \times \log Kow$	0.442
	$AE = 1/ (1.78 + 1.43 \times 10^{-8} \times Kow)$	0.136
LF	$AE = 1.201 - 0.102 \times \log Kow$	0.219
	$AE = 1/ (2.142 + 9.97 \times 10^{-9} \times Kow)$	0.110
MF	$AE = 0.88 - 0.066 \times \log Kow$	0.214
	$AE = 1/ (2.236 + 1.09 \times 10^{-8} \times Kow)$	0.073
HF	$AE = 0.85 - 0.063 \times \log Kow$	0.136
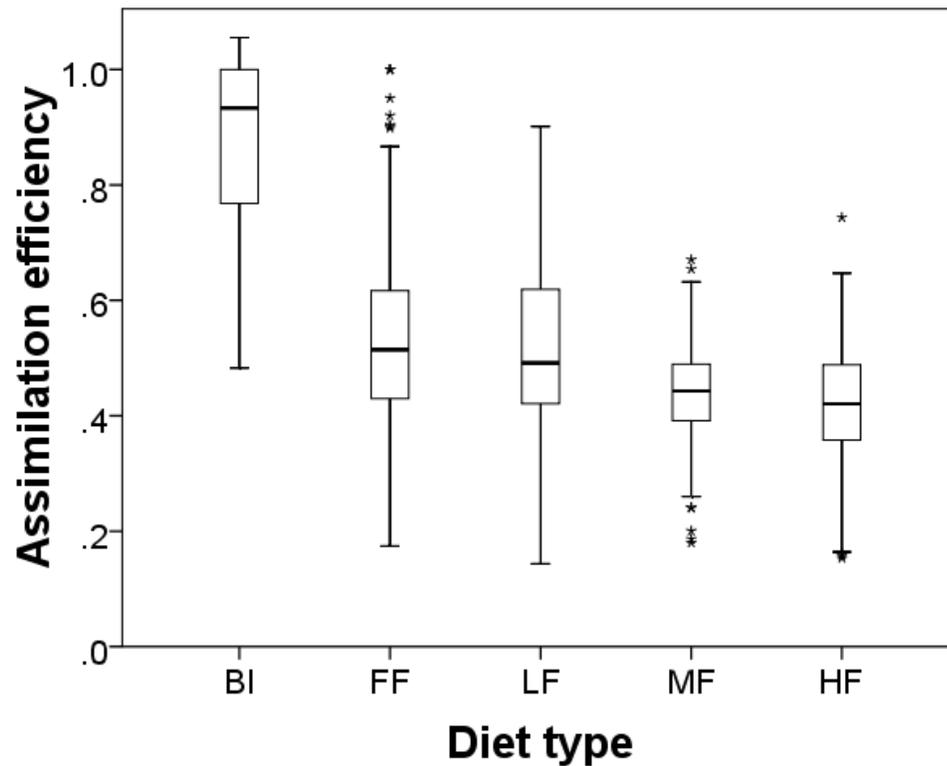
Figure 2.1. Box-whisker plot of the observed dietary assimilation efficiencies of PCB from

five diet treatment groups in this study. The upper and lower boundaries of whisker are the

maximum and minimum values, the upper and low boundaries of the box are the upper and

lower quartiles of the AE values. The line in the box represents the median AE value of the

group. The asterisk (*) stands for the outliers.

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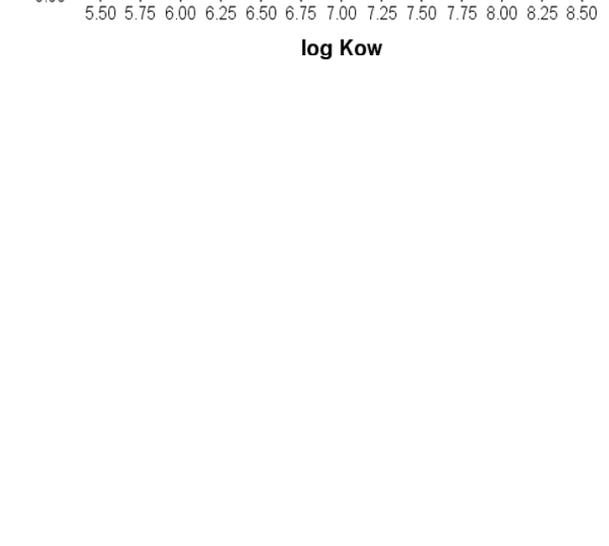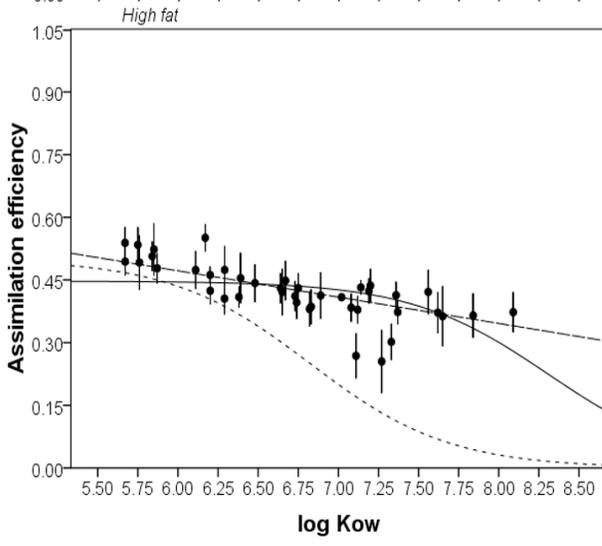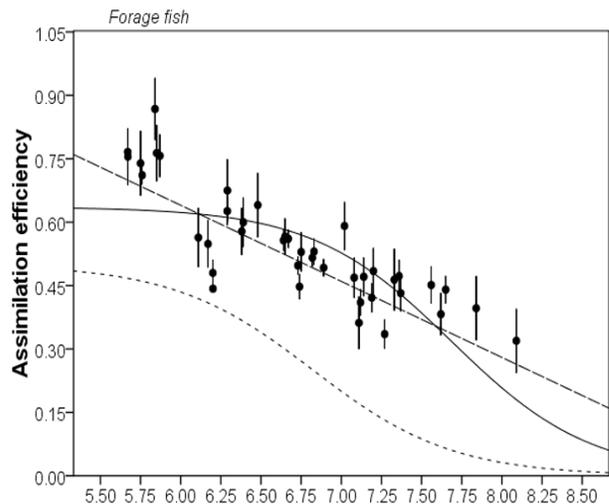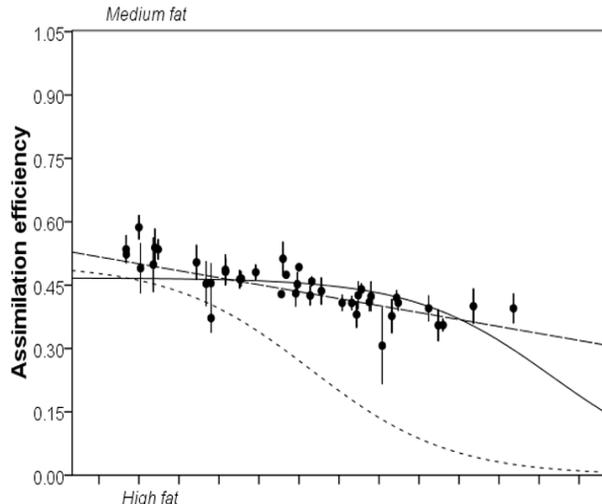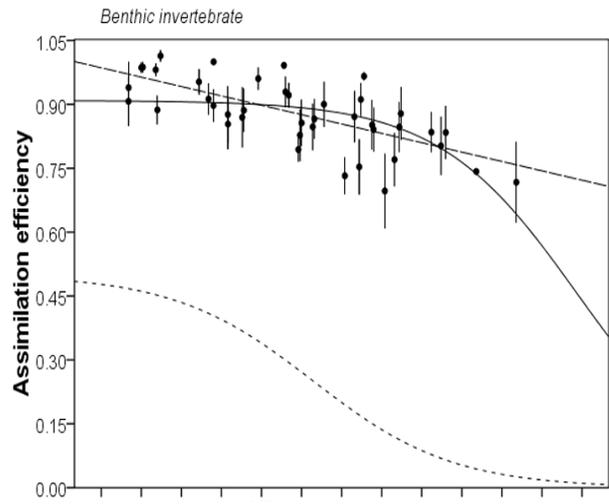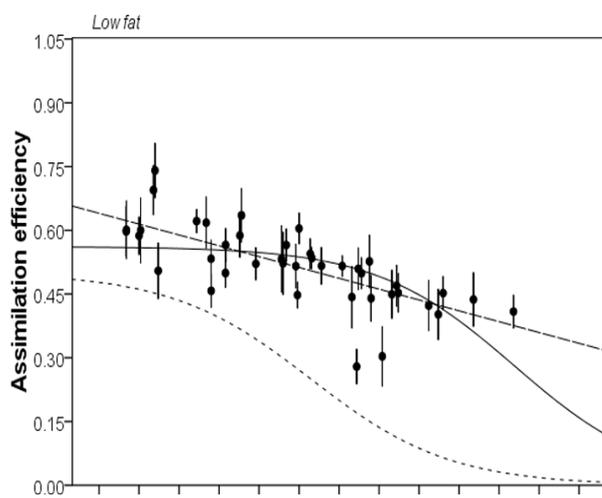
Figure 2.2. The mean (dot, ●) and SE (bar, |) of measured assimilation efficiencies of every

PCB congeners from each diet treatment group. The lines illustrate the regression relation

between chemical assimilation efficiency and its log K_{OW} value. (Solid lines represent the

non-linear regression models; dashed lines represent the linear regression models and the

dotted lines represent the function: $AE = 1/ (2.0 + 3.0 \times 10^{-7} \times K_{OW})$, suggested by Arnot and

Gobas (2004), which was used in the food web bioaccumulation model).

2.6 References

Arnot JA, Gobas FAPC. 2004. A food web bioaccumulation model for organic chemicals in

    aquatic ecosystems. *Environ Toxicol Chem* 23: 2343–2355.

Arnot JA, Gobas FAPC. 2006. A review of bioconcentration factor (BCF) and

    bioaccumulation factor (BAF) assessments for organic chemicals in aquatic

    organisms. *Environ Rev* 14:257–297.

Arnot JA, Mackay D. 2008. Policies for Chemical Hazard and Risk Priority Setting: Can

    Persistence, Bioaccumulation, Toxicity, and Quantity Information Be Combined?

    Environ Sci Technol 42: 4648-4654.

Barber MC. 2008. Dietary uptake models used for modeling the bioaccumulation of organic

    contaminants in fish. *Environ Toxicol Chem* 27: 755–777.

Brown SB, Fisk AT, Brwon M, Villella M, Muir DCG, Evans, RE, Lockhart WL, Metner

    DA, Cooley HM. 2002. Dietary accumulation and biochemical responses of juvenile

    rainbow trout (*Oncorhynchus mykiss*) to 3,3′,4,4′,5-pentachlorobiphenyl (*PCB* 126)

    Aquat Toxicol 59: 139-152.

Bruggeman WA, Martron LBJM, Kooiman D, Hutzinger O. 1981. Accumulation and

    Elimination Kinetics of Di-, Tri- and Tetra Chlorobiphenyls by Goldfish After

    Dietary and Aqueous Exposure. *Chemosphere* 10: 811-832.

Bruggeman WA, Opperhuizen A, Wijbenga A, Hutzinger O. 1984. Bioaccumulation of

    super-lipophilic chemicals in fish. *Toxicol Enivorn Chem* 7: 173-189.

Buckman AH, Brown SB, Hoekstra PF, Solomon KR, Fisk AT. 2004. Toxicokinetics of

    three polychlorinated biphenyl technical mixtures in rainbow trout (Oncorhynchus

    mykiss). *Environ Toxicol Chem* 23: 1725−1736.

Burreau S, Axelman J, Broman D, Jakobsson E. 1997. Dietary uptake in pike (*Esox lucius*) of some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers administered in natural diet. *Environ Toxicol Chem* 16: 2508–2513.

Ciavatta S, Lovato T, Ratto M, Pastres R. 2009. Global uncertainty and sensitivity analysis of a food web bioaccumulation model. *Environ Toxicol Chem* 28: 718–732.

Clark KE, Gobas FAPC, Mackay D. 1990. Model of organic chemical uptake and clearance by fish from food and water. *Environ Sci Technol* 24:1203–1213.

Clark KE, Mackay D. 1991. Dietary uptake and biomagnifications of four chlorinated hydrocarbons by guppies. *Environ Toxicol Chem* 10:1205–1217.

Connolly JP, Pedersen CJ. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environ Sci Technol* 22:99–103.

Corkum LD, Ciborowski JJH, Lazar R. 1997. The distribution and contaminant burdens of adults of the burrowing mayfly, Hexagenia, in Lake Erie. *J Great Lakes Res* 23:383–390

Dabrowska H, Fisher SW, Dabrowska K, Staubus AE. 1999. Dietary uptake efficiency of 2,2_,4,4_,5,5_-hexachlorobiphenyl in yellow perch and rainbow trout: Role of dietary and body lipids. *Environ Toxicol Chem* 18:938–945.

Daley, JM, Leadley,TA, Drouillard,KG. 2009. Bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch eggs during incubation. *Chemosphere* in press.

Doi AM, Lou Z, Holmes E, Li C-LJ, Venugopal CS, James MO, Kleinow KM. 2000. Effect of micelle fatty acid composition and 3,4,3_,4_-tetrachlorobiphenyl (TCB) exposure

on intestinal [14C]TCB bioavailability and biotransformation in channel catfish *in situ* preparations. *Toxicol Sci* 55:85–96.

Drouillard KG, Ciborowski JJH, Lazar R, Haffner GD. 1996. Estimation of the uptake of organochlorides by the Mayfly Hexagenia limbata (Ephemeroptera: Ephemeridae). *J Great Lakes Res* 22:26–35.

Drouillard KG, Norstrom RJ. 2000. Dietary absorption efficiencies and toxicokinetics of polychlorinated biphenyls in ring doves following exposure to Aroclor mixtures. *Environ Toxicol Chem* 19:2707–2714.

Drouillard KG, Norstrom RJ. 2003. The influence of diet properties and feeding rates on PCB toxicokinetics in the ring dove. *Arch Environ Contam Toxicol* 44:99–106.

Drouillard KG, Hagen H. Haffner GD. 2004. Evaluation of chloroform/methanol and dichloromethane/hexane extractable lipids as surrogate measures of sample partition capacity for organochlorines in fish tissues. *Chemosphere* 55: 395-400.

Drouillard KG, Paterson G, Haffner GD. 2009. A Combined Food Web Toxicokinetic and Species Bioenergetic Model for Predicting Seasonal PCB Elimination by Yellow Perch (Perca flavescens). *Environ Sci Technol* In press.

Feldman RS, Titus JE. 2001. Polychlorinated biphenyl accumulation differs among pumpkinseed sunfish during experimental field exposure: the role of invertebrate prey. *Aquat Toxicol* 51: 389-404.

Giesy JP, Kannan K. 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): Implications for risk assessment. *Crit Rev Toxicol* 28: 511-569.

Gobas FAPC, Muir DCG, Mackay D. 1988. Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.

Gobas FAPC, Clark KE, Shu WY, Mackay D. 1989 Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and elimination into the feces. *Environ Toxicol Chem* 8:231–245.

Gobas FAPC, McCorquodale JR, Haffner GD. 1993a. Intestinal absorption and biomagnification of organochlorines. *Environ Toxicol Chem* 12:567–576.

Gobas FAPC, Zhang X, Wells R. 1993b. Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals *Environ Sci Technol* 27: 2855-2863.

Gobas FAPC, Z'Graggen M, Zhang X. 1995. Time response of the Lake Ontario ecosystem to virtual elimination of PCBs. *Environ Sci Technol* 29: 2038-46

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133–141.

Harrison HL, Loucks OL, Mitchell JW, Parkhurs DF, Tracy CR, Watts DG, Yannacon VJ. 1970. Systems studies of DDT transport. *Science* 170: 503-508.

Hawker DW, Connell DW. 1985. Relationships between partition coefficient, uptake rate constant, clearance rate constant, and time to equilibrium for bioaccumulation. *Chemosphere* 14: 1205–1219.

Heath AG. 1995. *Water pollution and fish physiology* CRC Press, Boca Raton, FL, USA

Hendriks AJ, van der Linde A, Cornelissen G, Sijm DTHM. 2001. The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to

octanol-water partition ratio and species weight. *Environ Toxicol Chem* 20: 1399-1420.

Iannuzzi TJ, Harrington NW, Shear NM, Curry CL, Carlson-Lynch H, Henning MH, Su SH, Rabbe DE. 1996. Distributions of key exposure factors controlling the uptake of xenobiotic chemicals in an estuarine food web. *Environ Toxicol Chem* 15: 1979–1992.

Lazar R, Edwards RC, Metcalfe CD, Metcalfe T, Gobas FAPC, Haffner GD. 1992. A simple, novel method for the quantitative analysis of coplanar (non-ortho substituted) polychlorinated biphenyls in environmental samples. *Chemosphere* 25: 493-504.

Liem AKD, Furst P, Rappe C. 2000. Exposure of populations to dioxins and related compounds. *Food Addit Contam: Part A.* 17: 241-259.

Nichols JW, Fitzsimmons PN, Whiteman FW, Kuehl DW, Butterworth BC, Jenson CT. 2001. Dietary uptake kinetics of 2,2_,5,5_-tetrachlorobiphenyl in rainbow trout. *Drug Metab Dispos* 29:1013–1022.

Qiao P, Gobas FAPC, Farrell AP. 2000. Relative contributions of aqueous and dietary uptake of hydrophobic chemicals to the body burden in juvenile rainbow trout. *Arch Environ Contam Toxicol* 39:369–377.

Randall DJ, Brauner CJ. 1990. Toxicant uptake across fish gill. *Proceedings,* The seventeenth annual aquatic toxicity workshop. Vancouver, BC.

Saloranta TM, Næ K, Barton DN, Armitage JM, Cousins, IT, Haario H. 2008. Modeling the effects and uncertainties of contaminated sediment remediation scenarios in a Norwegian fjord by Markov chain Monte Carlo simulation. *Environ Sci Technol* 42: 200-206

Sharifi M, Connell DW, Gabric A. 1997. Influence of dietary fat on the intestinal absorption of lipophilic compounds in goldfish (*Carassius auratus*). *Ecotoxicol Environ Saf* 38: 316–321.

Sijm DTHM, Seinen W, Opperhuizen A. 1992. Life-cycle biomagnification study in fish. *Environ Sci Technol* 26: 2162-2174.

Tanabe S, Maruyama K, Tatsukawa R. 1982. Absorption efficiency and biological half-lives of individual chlorobiphenyls in carp (*Cyprinus carpio*) orally exposed to Kanechlor products. *Agric Biol Chem* 46:891–898.

Thomann RV. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ Sci Technol* 23: 699-707.

Thomann RV, Connolly JP, Parkerton TF. 1992. A equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ Toxicol Chem* 11: 615-629.

CHAPTER 3 THE IMPORTANCE OF FECAL ELIMINATION TO THE WHOLE BODY DEPURATION OF POLYCHLORINATED BIPHENYLS (PCBs) IN JAPANESE KOI *(CYPRINUS CARPIO)*

3.1 Introduction

Bioaccumulation is a function of chemical uptake and elimination to and from an organism. Toxicokinetic parameters required to model chemical bioaccumulation in fish include chemical uptake rate coefficients from water and food and the elimination rate coefficient. Among these processes, the elimination rate coefficient is the most important parameter for initial model calibration because the magnitude of this term is used to estimate the time required for an animal to achieve steady state with its environment and/or diet (Barron et al. 1994; Gobas and Morrison 2000). Most empirical studies that quantify chemical and species specific elimination rate coefficients characterize the whole body elimination rate coefficient (k_{tot}) under constant and species optimized laboratory conditions (e.g., Fisk et al. 1998; Buckman et al. 2004; Andersson et al. 2001). However, for a given animal model, the whole body elimination rate coefficient is not a constant, but rather is variable and dependent on a number of factors including the animal's age (Sijm et al. 1992), body size (Hendriks et al. 2001; Paterson et al. 2007a), whole body lipid content (de Freitas and Norstrom 1974), growth rate (DeBruyn and Gobas 2006), sex and reproductive status (Russell et al. 1999; Johnston et al. 2002), diet (Gobas et al. 1999) and seasonal/temperature related metabolic rate change (Paterson et al. 2007b) of the animal.

Further complicating matters is the fact that k_{tot} does not reflect a single process but represents cumulative loss of chemical through all possible elimination routes. Major

elimination mechanisms for hydrophobic organic contaminants typically include diffusive

loss via exchange across respiratory surfaces (k_2) or between the animal and its feces (k_{ex}),

maternal deposition of contaminant to reproductive tissues (k_{egg}) and/or metabolic

biotransformation (k_{met}). In order to improve bioaccumulation model performance and to

better account for variation in k_{tot} values over an animal's life history, it is necessary to

partition empirically measured whole body elimination rate coefficients into individual

elimination mechanisms and determine how each of these processes respond to different

types of environmental and physiological forcing functions.

To date, there have been few attempts to empirically determine the relative

contribution of different elimination mechanisms to whole body losses of hydrophobic

organic contaminants by fish. Of the mechanisms indicated above, metabolic

biotransformation and maternal depuration have received some attention with the purpose of

quantifying these loss mechanisms relative to diffusion based processes (Russell et al. 1999;

Buckman et al. 2006, 2007; Andersson et al. 2001). With respect to gill and fecal elimination,

inferences regarding the relative roles these processes play to whole body elimination have

largely been deduced from modeling exercises (Gobas et al.1989; Qiao et al. 2000; Gobas et

al. 1993; Drouillard et al. 2009). Losses of organic chemicals that experience negligible

biotranformation via respiratory surfaces to water is considered to be inversely related to

chemical K_{OW} and predicted to dominate whole body elimination for low to moderately

hydrophobic compounds. Gobas et al (1989) argued that for fish, fecal and gill elimination

of hydrophobic organic chemicals are of approximately equal importance for chemicals with

a log K_{OW} value of 6.5, whereupon fecal elimination dominates with further increases in

chemical hydrophobicity. Gobas et al (1993; 2008) further interpreted the transition from

respiration dominated chemical elimination to fecal dominated chemical elimination to be a primary factor which is responsible for delineating the hydrophobicity cutoff where chemical biomagnification occurs and also to control the K_{OW} dependence of chemical biomagnification.

Empirical studies on PCB fecal elimination in birds (Drouillard and Norstrom 2003) and rats (Rozman et al. 1983) demonstrated that fecal elimination is an important elimination route for non-biotransformed congeners in terrestrial vertebrates. However, for air respiring animals the potential losses of PCBs to air are several orders of magnitude lower compared to gill respiring animals because the volume of air respired per day and the capacity of air to partition PCBs is much lower than that of water (Kelly et al. 2007). To date, there have been no experiments conducted to directly measure the fecal elimination rates of PCBs by fish. In the present study, Japanese koi (*Cyprinus carpio*) were used in laboratory depuration trials to determine both whole body elimination rate coefficients (k_{tot}) and fecal elimination rate coefficients (k_{ex}) for a series of PCB congeners. The study was also replicated at three dosing levels to verify first order kinetics and diffusion limited toxicokinetics of PCB elimination. Specifically, the objective of this study was to test the hypothesis that PCB elimination to feces becomes a dominant elimination mechanism for highly hydrophobic PCBs and that the magnitude of k_{ex} approaches the magnitude of k_{tot} for compounds whose log K_{OW} exceeds a value of 6.5.

3.2 Material and Method

3.2.1 Experiment design

Seventy five koi (*Carassius auratus*) averaging 72.1±2.7 g (mean ± standard error) were obtained from a local aquaculture facility (Leadley Environmental Inc., Essex, ON, Canada). Replicate fish (n=5/tank) were randomly assigned to fifteen, 60 L aquaria maintained at 16°C under flow through conditions. The tanks were further separated into three groups (5 tanks/treatment group) that received different PCB doses (low, medium or high). Three fish per tank in each dosing group were dosed with PCBs and tagged using an external floy tag for identification. Treatment fish were dosed by intraperitoneal (IP) injection with a 1:1:1 mixture of Aroclor PCBs (Aroclor 1248/1254/1260) dissolved in safflower oil. The nominal dose for fish was established at 200 ng/g wet wt, 1000 ng/g wt and 2000 ng/g wet wt sum PCBs for the low, medium and high dose groups, respectively. The remaining two fish in each tank were sham dosed with clean safflower oil to serve as internal controls. After dosing, fish were acclimated for 30 days prior to beginning the depuration study in order to feed train them to a routine feeding schedule and to facilitate more consistent fecal collection.

During the acclimation and experimental periods, fish were fed commercial koi pellets (Martin Mills, Inc. Elmira, ON, Canada) at a daily ration of 1.5% body weight per day according to a structured feeding regime. The feeding cycle consisted of 5 days of feeding activity and a 2-day fasting cycle. During the feeding cycle, fish were given access to half their daily ration in the morning for a 3-hour period. After this period, excess uneaten food was removed from the tanks and the fish were fasted for 9 hours. The second half of the daily ration was provided for 3 hours in the evening and, after collecting excess food from the tanks, the fish were again fasted 9 hours until the following morning.

In order to determine whole body elimination rate coefficients (k_{tot}), fish were sacrificed (3 treatment and 2 controls from each of the three dosing groups) on days 0 (defined as 30 days after IP injection), 35, 70, 105 and 140 of the study. The PCB fecal elimination coefficients (k_{ex}) were calculated from measurements of the fecal egestion rate and the feces/carcass distribution coefficient (K_{EX}). During the feces collection periods, the dosed fish were cycled between depuration aquaria and a matching set of fecal collection aquaria that consisted of 60 L static aquaria under constant aeration and maintained at equivalent temperatures as the depuration tanks. The treatment and control fish were provided access to food twice per day according to the normal schedule in the depuration aquaria. During the two daily 9-hour fasting periods, the dosed fish were moved to the fecal collection tanks and allowed to evacuate their gut contents. The fecal collection tanks were allowed to accumulate feces over the 5 days period of fecal collections. Following the 5 days feeding period, fish were fasted for 2 days to allow removal of feces from the feces collection tank. The total mass of feces produced during this 5 days period was determined by filtering water through a 0.5 mm sieve to collect larger particles and then filtering the sieved water through 2 μm glass fiber filters using a Millipore® filtration system. Due to the limited quantities of feces and low concentrations of PCBs in fecal samples, feces from a given tank collected during 35-day intervals were pooled into a single sample. Fecal egestion rates were expressed on a dry weight feces per g wet body weight basis using the original total biomass of fish in each dosing group.

This study was performed under ethics approval from the University of Windsor's Animal Care Committee.

3.2.2 Sample processing and chemical analysis

Following sacrifice, the body weight, length, sex, gonad and liver mass from each fish was measured. Carcass samples were homogenized using a solvent-rinsed stainless steel Waring blender and the homogenate stored in the hexane-rinsed aluminum tins at $-20^{\circ}C$ until chemical analysis. Fecal samples from fecal collection tanks were stored frozen until chemical analysis.

Methods for PCB extraction followed the micro-extraction procedure described by Daley et al. (2009) and florisil clean-up described by Lazar et al (1992). Samples were spiked with 250 ng each of tribromobenzene (TBB) and PCB 30 for use as internal recovery standards. Lipid contents of carcass samples were determined by removing 10% of the hexane:dichloromethane (VWR, St. Catherines, ON, Canada) sample extracts for gravimetric lipid determination (Drouillard et al. 2004). The cleaned-up extracts were condensed to 1 ml by rotary evaporation, sealed in 2 ml gas chromatograph (GC) vials, and stored at $4^{\circ}C$ until GC analysis. Method blanks and an in-house reference homogenate (Detroit River carp) were co-extracted for every batch of six samples analyzed. All samples were analyzed using an Agilent Model 6890 gas chromatograph with a ^{63}Ni micro electron capture detector (GC-μECD) and an Agilent 7683 autosampler. Forty five PCB congeners were identified by retention time and quantified using the external standard response for equivalent peaks in a standard mixture (Lazar et al. 1992).

Blanks and reference tissues, quantified during each batch of sample extractions, were in compliance with the normal quality assurance procedures instituted by the organic analytical laboratory. Sample recoveries for the two internal standards averaged $66\pm 5.44\%$ and $85.7\pm4.87\%$ for TBB and PCB30, respectively. Chemical concentrations were not corrected for recovery.

3.2.3 Parameter estimation

The growth rate (d^{-1}) of each fish was calculated based on the wet weight at the dosing time and at sacrifice time according to:

$$k_g = (\ln W_t - \ln W_o)/t \qquad (1)$$

Where k_g (d^{-1}) is the daily growth rate of the fish, W_t and W_o are the fish weights at sacrifice and the time of dosing, and t is time. The concentration in each fish at sacrifice was control and growth corrected prior to determining whole body elimination rate coefficients. Control correction was performed by subtracting the mean concentration in control fish from the same dosing group at the time of sacrifice from each individual treatment fish. Growth correction was performed according to:

$$C_{cor} = C_t \times (1 + k_g \cdot t) \qquad (2)$$

Where C_{cor} (ng/g wet wt) is the growth corrected concentration and C_t (ng/g wet wt) is the control corrected carcass PCB concentration. In cases where negative growth occurred (weight loss), the C_t value was divided by the $(1 + k_g \cdot t)$ term in Eq. 2. The whole body elimination rate coefficient (k_{tot}) for individual PCB congeners was determined as the slope of the least-squares regression between the natural logarithm of C_{cor} over time for all fish sacrificed within a given dosing group.

The PCB fecal elimination rate, k_{ex} (d^{-1}) was calculated as per Drouillard and Norstrom (2003) according to the equations:

$$k_{ex} = G_{ex} \times K_{EX} \qquad (3)$$

$$K_{EX} = C_{ex}/C_{t'} \qquad (4)$$

Where G_{ex} is the fecal egestion rate (g dry feces/ g fish wet wt. d^{-1}) and K_{EX} is the

PCB feces/carcass concentration ratio, the ratio of chemical concentration in feces, C_{EX},

(ng/g dry wt) to the average of measured chemical concentration in fish sampled over the

fecal collection interval, $C_{t'}$, (ng /g fish wet wt.), which is the $((C_t + C_{t+35})/2)$

In order to test for metabolic biotransformation, individual PCB congeners were

separated into labile and recalcitrant groups based on structure activity relationships

developed for PCB biotransformation. For PCBs, the structure activity rules for

biotransformation vary across taxonomic lines. While more complicated classification

schemes exist for mammals (Boon et al. 1989, 1992,1994; Norstrom 1988), labile PCBs

across many taxonomic groups appear to be those that have vicinal hydrogen substituents at

the meta and para sites on one or both phenyl rings of the molecule (Drouillard and Norstrom

2003; Leney et al. 2006; Buckman et al. 2007). Labile PCBs were, therefore, categorized as

those congeners having vincinal hydrogen substituents at meta-, para- positions on at least

one phenyl ring, while recalcitrant congeners were chlorine hindered at these locations (i.e.

exhibiting chlorine substitutents at 4,4'-; 4, 3',5'-; 3,5,4'-; or 3,5,3',5'- positions). Of the

chemicals quantified in the current study, PCBs 42, 44, 49, 52, 64, 87, 97, 101, 110, 141, 149,

151, 174, 179, 187 were categorized as labile, and PCBs 74, 99, 105, 118, 128, 138,153,

158,170/190, 171/156, 172, 177, 180, 183, 194, 195, 201, 206 were categorized as

recalcitrant. Recalcitrant PCBs are assumed to be lost primarily through diffusion pathways,

while labile PCBs are assumed to be lost by a combination of diffusion and metabolic

biotransformation (Fisk et al. 1998, Buckman et al. 2006).

3.2.4 Data analysis

The differences in body weight, body lipid level and fecal egestion rate between dosing groups were tested by ANOVA and by post hoc comparisons using Tukey's honestly significant difference test. Differences in lipid and PCB concentration between treatment fish and control fish within a dosing group were tested by student's t-test. Within treatment (dosing) group differences in K_{EX} or k_{ex} values across time were tested by ANCOVA using time as the covariate. Congener specific PCB k_{tot} and k_{ex} values were compared across dosage groups using ANCOVA after adjusting for chemical K_{OW} as a covariate. Whole body PCB elimination coefficient rates k_{tot} were compared between labile and recalcitrant congener groups using ANCOVA after adjusting for chemical K_{OW} as covariate. Normality plots were performed prior to statistical testing. Data failing normality were log transformed and re-tested for normality prior to performing parametric statistical tests. All statistical tests were performed using SPSS ver. 16 for Windows (Chicago, IL, USA) with a criterion for significance of $p < 0.05$ used in all cases. Log K_{OW} values reported for all congeners were from Hawker and Connell (1988).

3.3 Results

Mean water temperatures from the flow through and static fecal collection aquaria were 16 ± 1 and $15.4\pm1.3^{o}C$, respectively. There were no trends in water temperature with time nor differences between flow through (depuration) and static (fecal collection) aquaria ($p>0.05$, t-test). All fish fully recovered within 12 hours after IP dosing of PCBs and there was no mortality experienced over the study period. The average body lipid contents of fish at sacrifice are listed in Table 3.1. The body lipid level varied from 2.8 to 4.8%. There was no significant difference in the lipid level among the dosage groups ($p>0.25$, ANOVA), and

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there were no significant differences in lipid levels between treatment fish and control fish (p>0.05, t-test).

Body weights are also provided in Table 3.1 and, similar to the lipids, there were no significant differences in body weights across the dosing treatments (p>0.62, ANOVA). Growth rates were determined for individual fish to provide growth corrected carcass concentrations as described by Eq. 1 and 2. Growth rates for individual fish were highly variable (-0.005 to 0.003 /d) but exhibited no consistent differences across the dosage groups. Most fish demonstrated weight loss during the acclimation period as they adjusted to the imposed feeding schedule. Following the acclimation period, most fish exhibited net growth, while a minority of fish exhibited a small degree of weight loss.

Total and congener specific PCB concentrations in dosed and control fish are provided in Table S3.1. PCB concentrations in the control groups showed an increasing trend over the study duration except for the medium dose groups, where the highest PCB concentration in the control group occurred on day 0. However, compared with the chemical concentration in the treatment fish, control fish had relatively low PCB concentrations. For the low dose group, total PCB concentration in treatments were 20 times higher than the control group at the beginning of the study, but this difference decreased to 5 times higher than controls by the end of the study. Across individual congeners in this group, treatment fish contained between 2 to 7 times higher concentrations than their respective controls on day 140.

3.3.1 Whole body elimination of PCBs

Out of 45 measured PCB congeners, 42 showed significant elimination during the study duration (p<0.05, ANOVA). Elimination trends for representative PCB congeners in

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each dosing group are provided in Figure 3.1. The elimination rate constants for individual

PCB congeners and across dosing treatments varied within the range from 0.001 to 0.017 d^{-1}

(Table S3.2). There was no significant difference in the whole body elimination rate, k_{tot},

between dosing treatments (p=0.679, ANCOVA). For all three dosing levels, log k_{tot} was

significantly correlated with log K_{OW} (p<0.001, ANOVA).

Figure 3.2 summarizes whole body PCB elimination as a function of chemical K_{OW}

with PCB congeners separated into labile and recalcitrant persistence groups according to the

structure activity relationships described earlier. Although there was overlap in individual

congener elimination rates between the two persistence groups, labile categorized PCBs

exhibited significantly greater k_{tot} values (p<0.0001, ANCOVA) compared to persistent

categorized congeners after adjusting for chemical K_{OW} as a covariate. Dose effects were also

separately examined between the two persistence categories to examine for differences in k_{tot}

across the different initial dose regimes. For the labile PCBs, k_{tot} did not significantly change

as a function of initial dose (p>0.7, ANCOVA) after adjusting for chemical K_{OW} as the

covariate. For the labile congeners, the linear regression relationship between log k_{tot} and log

K_{OW} was:

$$\log k_{tot} = -0.127 \log K_{OW} - 1.275 \quad (p=0.022, r^2 = 0.116) \tag{5}$$

A similar relationship was observed for recalcitrant PCBs (p>0.3; ANCOVA). For the

recalcitrant PCB congeners the linear regression relationship was:

$$\log k_{tot} = -0.192 \log K_{OW} - 1.079 \quad (p=0.022, r^2 = 0.097) \tag{6}$$

3.3.2 Fecal elimination of PCBs

Feeding and fecal egestion rates determined across all sampling periods are summarized in Table 3.1. The fecal egestion rate, G_{ex}, was relatively constant over the study duration and there was no significant difference between dose treatments ($p > 0.5$, ANOVA).

All 45 PCB congeners detected in carcass samples were detected in feces. Chemical K_{EX} values were determined for each group according to Eq. 4 (Table S3.2). The chemical K_{EX} value varied from 0.03 to 0.8 across 44 PCB congeners (a valid K_{EX} value was not obtained for PCB 74 due to an interference in the fecal matrix). Within a given dosing treatment there was relatively high variation in measured K_{EX} values, but no significant trends of K_{EX} values were noted over time ($p = 0.152$, ANCOVA). There were also no significant differences in K_{EX} between dosing treatments ($p = 0.365$, ANCOVA). Similar to k_{tot}, K_{EX} was found to be significantly ($p < 0.001$; ANOVA) related to chemical hydrophobicity. The combined linear relationship between log K_{EX} and congener log K_{OW} for the different dosing treatments is given by:

$$\log K_{EX} = -0.038 \log K_{OW} + 0.447 \ (p < 0.001, \ r^2 = 0.093) \qquad (7)$$

Figure 3.3 presents the relationship between mean K_{EX} determined across the different time points and chemical K_{OW} along with the linear regression fit described by Eq. 7.

PCB fecal elimination rate, k_{ex}, was calculated based on the measured K_{EX} and G_{ex} according to Eq. 3. PCB fecal elimination rate ranged from 0.00013 to 0.00049 d^{-1} (Table S3.2). Similar to K_{EX}, the fecal elimination rate didn't differ significantly between treatments ($p = 0.144$, ANCOVA) and in all treatments, there was no significant change in k_{ex} over the depuration period ($p = 0.14$, ANCOVA). Also, similar to K_{EX}, the fecal elimination rate constant correlated with chemical K_{OW}. The fitted linear regression between log k_{ex} and chemical K_{OW} is given by:

$$\log k_{ex} = -0.112 \log K_{OW} - 2.867 \ (p<0.001, \ r^2 = 0.125) \qquad (8)$$

Figure 3.4 presents the k_{ex} data as a function of chemical log K_{OW} and regression fit provided by Eq. 8. Figure 3.4 also provides the fitted regression lines determined for whole body elimination rates (k_{tot}) for labile and recalcitrant categorized PCBs (Eq. 5 and 6, respectively). In all cases, the k_{ex} values were considerably lower than measured k_{tot} values. Comparing the differences between fitted regression predictions provided by Eq. 5, 6 and 8, the k_{tot} for recalcitrant PCBs were approximately 20 to 25 times higher compared to the relationship generated for k_{ex}. Similarly, for the labile categorized PCBs, the k_{tot} values were 25 to 28 times higher than k_{ex}.

3.4 Discussion

Whole body k_{tot} values measured in this study varied from <0.001 to 0.017 d^{-1} for PCBs spanning a log K_{OW} range of 5.67 to 7.80. Tanabe et al (1982) reported PCB half lives in common carp that, when converted to k_{tot} values, ranged from <0.003 d^{-1} to 0.40 d^{-1}. In the above case, k_{tot} values were not growth corrected and the carp had demonstrated considerable growth, from 28g at the beginning of the experiment to 83g by the end. Bruggeman et al (1981) reported half-lives of goldfish (4.5 to 5.2 cm in length) for tri- and tetrachlorobiphenyls that were smaller than individuals used in the present study. The k_{tot} values for PCB 31 and 52 determined from Bruggeman et al (1981), converted from half-lives, were 0.021 and 0.015 d^{-1}, respectively. In this study, PCB28/31 had k_{tot} values across the dosage groups that ranged from 0.009 to 0.016 d^{-1} and PCB 52 from 0.0079 to 0.014d^{-1}. Given the differences in size, growth and temperature conditions of the different studies,

these different estimates of whole body PCB elimination in goldfish and koi appear comparable with one another.

Whole body PCB elimination rates determined from this study are also bracketed by data presented for other fish species. The k_{tot} values from this study were consistent with values reported for similar sized yellow perch (60g) depurated at their optimal growth conditions, but slower than reported for smaller (10g) yellow perch (Paterson et al. 2007a,b). Similarly, the range of k_{tot} values determined in this study in koi can be shown to be bracketed between the very slow PCB elimination determined for 900 g rainbow trout reported by Oliver and Niimi (1983), approached PCB elimination determined in juvenile (85g) rainbow trout (Buckman et al. 2007), and was slower compared to PCB losses experienced by small (5-10g initial weight) juvenile trout (Fisk et al. 1998).

PCB k_{tot} values were observed to decrease with increasing chemical hydrophobicity as has been reported elsewhere (e.g., Bruggeman et al. 1981; Tanabe et al. 1982; Oliver and Niimi 1983; Fisk et al. 1998; Buckman et al. 2004). However, there was a high degree of variation in the data across congeners and dose treatments. A number of congeners had marginally significant k_{tot} estimates, with values approaching 0.0010 to 0.0015 d^{-1}, that appeared to be independent of K_{OW}. Given the above elimination rates and 140 day depuration period, fish would have only lost between 13 and 18% of their initial body burden, which is approaching the resolution of the analytical methods used in this study. Growth and control correction artifacts may have also had greater impacts on k_{tot} estimates for the slowest eliminated congeners. Both longer depuration times and higher numbers of replicate fish sampled at each time point would have likely improved k_{tot} estimates for these compounds.

The whole body PCB elimination rate coefficients measured in this study were consistent with first-order kinetics and k_{tot} was independent of the dosing level and also observed to be varied logarithmically with time. Barron et al (1990) suggested that dosing level be varied by at least an order of magnitude to test for Michaelis Menton kinetics. In this study, sum PCB concentrations varied by a factor of 7 across the dosing groups. While strong evidence exists for selective PCB biotransformation by mammals, birds and amphibians (Matthews and Dedrick 1984; Norstrom 1988; Safe 1994; Hansen 1999; Leney et al. 2006), structure activity relationships for PCB biotransformation by fish have been less well defined. Most fish bioaccumulation models ignore biotransformation as a relevant elimination pathway (e.g., Gobas et al. 2002; Morrison et al. 2002; Arnot and Gobas 2006). However, a number of empirical studies provide some support for the observation that different species of fish are capable of biotransforming certain PCBs (Doi et al. 2006; Buckman et al. 2007, 2006; Li et al. 2003; Merhrtens and Laturnus 1999). The most common method for deducing biotransformation activity involves the detection of metabolites in feces, blood or animal tissues (Li et al. 2003; Buckman et al. 2006). However, the presence of metabolic products does not quantify the relative importance of biotransformation compared to other elimination processes. A second common approach is to use recalcitrant PCBs as reference compounds in conjunction with structure activity relationships (SAR) to deduce which congeners are likely to be undergoing biotransformation (Norstrom 1988; Fisk et al. 1998; Fisk et al. 2000; Buckman et al. 2007). This method assumes that the elimination of recalcitrant designated congeners occurs primarily by diffusion and that biotransformation rates of labile PCBs can be deduced based on the difference between labile and recalcitrant whole body elimination rates (Fisk et al. 1998).

This study demonstrated that labile categorized PCBs exhibited higher whole body elimination compared to recalcitrant categorized congeners. Comparing the separate regression equations (Eq. 5 and 6) generated for the two persistence groups, labile categorized PCBs exhibited between 1.62 to 1.88 times higher k_{tot} values compared to recalcitrant ones over the log K_{OW} range of 6.25 to 7.25 (i.e., where the K_{OW} values of both groups exhibited overlap). For American kestrels, difference in plasma clearance constants for labile and recalcitrant PCBs was 3.54 times greater after taking chemical hydrophobicity into consideration (Drouillard et al. 2001). Green frogs exhibited 1.5 to 4.9 times and leopard frogs exhibited 2.0 to 3.3 times higher elimination rates for labile PCB compared to recalcitrant ones depending on the life stage and after adjusting for chemical hydrophobicity (Leney et al. 2006). Thus, the biotransformation capacity of Japanese koi for labile PCBs appears to be lower than amphibians and birds but somewhat higher than that observed for rainbow trout. In the Buckman et al. (2006) study on rainbow trout, temperature was shown to exhibit an interactive effect with PCB biotransformation rates. Because the present study was conducted at only a single temperature, the full metabolic capability of Japanese koi could not be evaluated and thus some caution in comparing the metabolic capability across species is required.

The main objective of this study was to evaluate the relative role of fecal elimination to whole body PCB elimination by koi. Whole body elimination rates for recalcitrant PCBs were observed to be from 5 to 20 times higher than k_{ex} values (Figure 3.4) and accounted for an average of 9.1±0.9% of the whole body elimination. For labile congeners, k_{ex} explained an average of only 4.9±0.6% of whole body elimination. For recalcitrant congeners, elimination is assumed to occur primarily by diffusive loss mechanisms either to fecal

egestion or gill ventilation. Thus, for the recalcitrant congeners, approximately 91% of the whole body elimination is attributed to gill losses compared to the 9% quantified losses measured in feces. Assuming comparable diffusive losses for labile compounds, approximately 52% of whole body losses would be attributed to diffusion across the gills, 5% losses to feces and 43% loses related to biotransformation.

Errors in k_{ex} estimates from this study may have resulted from underestimates of the fecal egestion rate (G_{ex}), which was measured to be in the range of 8 to 10% of the feeding rate (Table 3.1). This suggests a higher food digestion efficiency than is commonly assumed for fish in bioaccumulation models (Arnot and Gobas 2006). Gobas et al (1988) estimated a fecal egestion rate of 33% for guppies and goldfish when provisioned a nominal feeding rate of 2% body weight per day. Other studies have reported diet digestion efficiencies in fish ranging from 60 to 75%, which corresponds to fecal egestion rates on the order of 25 to 40% of feeding rate (Fisk et al. 1998; Gobas et al. 1993ab; Bruggeman et al. 1981; 1984; Sijm et al. 1992).

The need to collect sufficient quantities of feces for chemical analysis in this study necessitated pooling feces from multiple fish and across multiple days. It is possible that fecal losses could have occurred due to the method of collection or as a result of consumption of feces by the fish held in the static aquaria during the fasting cycles. Fecal ingestion is not uncommon in goldfish, although the likelihood of losing large quantities of feces by this route in fish fed to satiation daily seems unlikely. To test for such effects, the fecal egestion rate (G_{ex}) was replaced with a value 3 times higher than the measured G_{ex}. In this case, the calculated fecal excretion rate was still less than 30% of the whole body elimination rate determined for most PCB congeners, including highly hydrophobic congeners such as PCB

194. Thus, underestimates in G_{ex} would not likely alter the overall conclusion that gill elimination of recalcitrant PCBs dominates the elimination of these compounds.

Gobas et al. (1989) used a bioenergetic/toxicokinetic model to predict gill and fecal elimination rate coefficient and predicted that there would be a transition from gill elimination dominated to fecal elimination dominated processes at a log K_{OW} of approximately 6.5. However, Drouillard et al.'s (2009) bioenergetic/toxicokinetics model calibrated to predict seasonal PCB losses by yellow perch, indicated that gill elimination dominated for a majority of PCB congeners.

Unfortunately, direct measurement of gill elimination rates of hydrophobic chemicals remains technically challenging. Dvorchik and Maren (1972) injected dogfish sharks with ^{14}C DDT and collected samples of expired water but were unable to detect any elimination by this route. Fitzsimmons et al (2001) developed a method using continuous column extraction of expired water, coupled with high resolution mass spectrometry to measure gill elimination in adult rainbow trout (average 950g) of four PCBs with log K_{OW} values ranging from 5.8 to 8.2. The gill elimination rate coefficients measured in this study varied from 0.00005 to 0.0003 d^{-1}. To the authors' knowledge, no other studies have been performed where both the fecal and whole body PCB elimination rates by fish were performed. In ring doves, the fecal elimination of PCB 28 was not found to be different from the whole body elimination rate (Drouillard and Norstrom 2003), indicating a greater role of fecal elimination of recalcitrant PCBs by birds. The magnitude of the water/biota partition coefficient for PCBs and volume of water flux through fish gills, however, are much higher than PCB air/biota partition coefficients and air flow into lungs. Thus, for air-respiring animals, the potential losses of PCBs across respiratory surfaces are expected to be orders of

magnitude lower compared to gill-respiring animals (Kelly et al. 2007). Kelly et al (2007) hypothesized that the low capacity of terrestrial animals to eliminate hydrophobic chemicals across their lungs results in much higher biomagnification potentials of lung breathing animals compared to aquatic species.

In summary, this study quantified both whole body and fecal elimination of individual PCB congeners in Japanese koi. Whole body and fecal elimination of PCBs was observed to be K_{OW} dependent and decreased with increasing chemical hydrophobicity. Although PCB elimination was dose independent, labile categorized PCBs demonstrated between 1.62 and 1.88 times higher elimination than recalcitrant categorized congeners, providing some support for selective metabolic biotransformation of PCBs having open *meta-*, *para-* positions on one of the phenyl rings in the molecule. For recalcitrant categorized PCBs, fecal elimination rates explained only 9.1% of the whole body elimination, suggesting that gill elimination dominates the losses of these compounds. For labile categorized PCBs, gill elimination and metabolic biotransformation appear to play approximately equal roles to whole body elimination (52% vs. 43%), with fecal losses accounting for only 5%.

Table 3.1. Mean (±S.E.) of body weight (g), body lipid level (% wet wt.), and fecal egestion

rate (g dry feces/g wet body wt. day) of each treatment over every sampling period.

		A(low dose)		B(med. dose)		C(high dose)	
		Treatment	Con.	Treatment	Con.	Treatment	Con.
Body weight (g)	0	76.0(±8.1)	n/a	62.0(±8.1)	n/a	80.2(±3.9)	n/a
	35	72.1(±4.2)	n/a	98.4(±11.7)	n/a	66.8(±7.3)	n/a
	70	58.9(±8.8)	n/a	83.2(±5.3)	n/a	62.2(±2.5)	n/a
	105	71.7(±6.7)	n/a	89.0(±5.1)	n/a	52.2(±6.6)	n/a
	140	63.5(±6.6)	n/a	69.1(±11.1)	n/a	86.3(±4.4)	n/a
Lipid (% wet wt.)	0	3.2(±0.1)	3.3	4.1(±0.3)	3.8	3.2(±0.2)	3.9
	35	3.2(±0.1)	3.6	3.8(±0.4)	3.2	3.7(±0.4)	3.3
	70	3.0(±0.4)	3.3	3.9(±0.7)	3.5	4.8(±0.4)	3.7
	105	3.6(±0.3)	3.2	4.4(±0.5)	3.5	3.7(±0.4)	3.8
	150	4.4(±0.6)	3.5	4.4(±0.6)	3.2	4.0(±0.6)	3.2
Fecal egestion rate (10^{-3}g dry feces/g wet wt. day)	0-35	1.3(±0.1)	n/a	1.3(±0.1)	n/a	1.3(±0.1)	n/a
	35-70	1.5(±0.1)	n/a	1.4(±0.1)	n/a	1.4(±0.1)	n/a
	70-105	1.3(±0.0)	n/a	1.3(±0.1)	n/a	1.4(±0.2)	n/a
	105-140	1.5	n/a	1.4	n/a	1.6	n/a

Table S3.1. Congener specific concentrations (ng /g wet weight) of both treatment and

control fish of each treatment at every sampling date.

		Low dose		Med. dose		High dose	
		Treatment	Control	Treatment	Control	Treatment	Control
Day 0	PCB31/28	1.78±0.187	0.087	31.262±0.624	0.904	45.315±2.890	0.490
	PCB52	2.77±0.084	0.135	27.462±0.752	0.794	53.248±0.502	0.561
	PCB49	2.95±0.190	0.144	17.909±0.886	0.518	37.154±2.863	0.397
	PCB44	1.036±0.063	0.051	11.515±0.940	0.333	22.874±0.492	0.252
	PCB42	0.489±0.053	0.024	3.767±0.045	0.109	6.062±0.328	0.032
	PCB64	0.591±0.126	0.049	4.925±0.905	0.142	10.349±1.554	0.115
	PCB74	0.738±0.009	0.066	3.775±0.426	0.109	6.279±0.325	0.054
	PCB70/76	5.435±0.623	0.181	18.257±0.858	0.491	36.325±1.313	0.382
	PCB66/95	5.741±0.456	0.203	25.010±0.427	0.500	45.346±1.846	0.477
	PCB60/56	2.293±0.277	0.106	9.087±0.419	0.263	17.386±1.104	0.197
	PCB101	3.216±0.160	0.109	14.443±0.551	0.307	23.325±1.562	0.217
	PCB99	2.074±0.099	0.120	7.573±0.853	0.194	20.132±1.930	0.213
	PCB97	1.651±0.244	0.108	3.013±0.051	0.087	6.026±0.468	0.061
	PCB87	5.425±0.278	0.125	26.512±0.814	0.767	51.856±1.831	0.506
	PCB110	1.211±0.434	0.391	24.039±1.122	0.695	53.893±0.995	0.507
	PCB151	6.017±0.040	0.244	30.672±0.180	0.887	60.647±0.997	0.606
	PCB149	5.761±0.322	0.272	25.629±1.780	0.741	50.096±2.823	0.509
	PCB118	7.559±0.511	0.329	37.659±1.181	1.089	77.680±5.643	0.739
	PCB153	16.401±0.582	0.789	31.046±1.767	0.898	56.649±2.432	0.535
	PCB105	1.879±0.034	0.102	11.327±0.556	0.328	22.237±0.710	0.216
	PCB141	2.255±0.211	0.140	9.477±0.401	0.274	18.086±0.951	0.194
	PCB179	3.426±0.201	0.107	15.226±0.128	0.440	27.859±2.005	0.283
	PCB138	16.641±1.073	0.883	57.509±8.495	1.663	85.246±1.846	0.866
	PCB158	1.700±0.088	0.119	7.804±0.598	0.226	14.855±0.915	0.131
	PCB187	5.358±0.207	0.242	17.438±0.801	0.504	22.043±1.706	0.224
	PCB183	1.449±0.0922	0.091	5.986±0.473	0.173	13.441±1.092	0.136
	PCB128	1.505±0.103	0.064	6.090±0.276	0.176	11.386±0.258	0.106
	PCB174	4.713±0.169	0.130	19.223±2.424	0.556	30.383±1.938	0.248

	PCB177	3.463±0.034	0.269	17.209±0.604	0.498	28.927±2.312	0.264
	PCB171/156	3.112±0.280	0.352	11.747±0.485	0.340	28.446±1.614	0.229
	PCB200	1.227±0.066	0.090	3.932±0.279	0.114	11.494±0.690	0.107
	PCB172	0.841±0.015	0.081	3.240±0.138	0.094	6.230±0.028	0.051
	PCB180	5.442±0.248	0.246	26.647±2.048	0.771	51.397±0.832	0.482
	PCB170/190	2.600±0.083	0.107	12.682±0.854	0.367	22.961±0.730	0.183
	PCB201	1.444±0.0299	0.171	6.886±0.416	0.199	14.362±0.651	0.126
	PCB203	1.566±0.052	0.077	7.858±0.615	0.227	15.533±0.545	0.118
	PCB195	0.825±0.037	0.140	4.170±0.195	0.121	7.684±0.278	0.038
	PCB194	1.459±0.043	0.041	6.554±0.272	0.190	13.734±0.525	0.109
	PCB206	0.824±0.013	0.040	1.589±0.073	0.046	3.111±0.037	0.022
	Total PCB	**141.18±1.52**	**7.025**	**601.70±11.08**	**17.135**	**1113.49±10.49**	**10.983**
Day 35	PCB31/28	0.709±0.076	0.155	17.128±0.663	0.320	27.094±2.005	0.334
	PCB52	0.744±0.119	0.138	14.363±2.027	0.268	23.121±1.447	0.321
	PCB49	1.362±0.268	0.090	10.727±0.763	0.200	12.006±0.853	0.192
	PCB44	0.516±0.113	0.103	7.100±0.760	0.132	7.921±0.797	0.097
	PCB42	0.335±0.076	0.098	3.406±0.406	0.064	4.267±0.389	0.048
	PCB64	0.430±0.111	0.083	3.457±0.296	0.064	4.500±0.229	0.062
	PCB74	0.222±0.017	0.097	1.603±0.099	0.030	1.876±0.0348	0.020
	PCB70/76	4.443±0.447	0.193	17.903±1.023	0.334	34.991±1.649	0.361
	PCB66/95	4.110±0.222	0.118	21.645±0.525	0.338	40.650±1.885	0.428
	PCB60/56	0.862±0.060	0.097	7.1578±0.470	0.134	13.680±0.439	0.119
	PCB101	2.743±0.434	0.101	12.347±0.193	0.175	19.131±0.521	0.207
	PCB99	1.440±0.165	0.106	6.213±0.456	0.103	17.670±0.721	0.167
	PCB97	0.808±0.221	0.063	3.400±0.665	0.063	5.536±0.378	0.059
	PCB87	4.614±0.189	0.357	20.672±1.163	0.386	40.997±2.068	0.457
	PCB110	9.9164±0.431	0.968	19.144±0.899	0.357	37.494±1.811	0.301
	PCB151	3.886±0.078	0.101	18.560±1.484	0.346	34.611±1.659	0.355
	PCB149	2.635±0.171	0.104	17.241±1.468	0.322	26.729±2.264	0.328
	PCB118	2.519±0.205	0.095	17.663±1.525	0.330	50.698±1.053	0.313
	PCB153	13.431±1.703	0.940	31.713±2.287	0.592	48.468±3.408	0.677
	PCB105	1.724±0.183	0.234	9.601±1.002	0.179	17.634±0.738	0.183
	PCB141	1.444±0.442	0.212	7.101±0.361	0.132	12.553±1.637	0.201

	PCB179	2.538±0.088	0.297	12.920±1.102	0.241	20.649±2.133	0.231
	PCB138	15.234±1.196	.880	53.749±6.565	1.003	72.455±7.252	1.001
	PCB158	1.438±0.186	0.311	5.851±0.354	0.109	14.884±1.801	0.139
	PCB187	2.732±0.355	0.112	13.915±1.665	0.260	18.927±2.008	0.193
	PCB183	0.880±0.224	0.068	5.684±0.527	0.106	8.587±0.634	0.118
	PCB128	1.345±0.130	0.104	6.539±0.833	0.122	9.103±0.373	0.166
	PCB174	4.221±0.133	0.367	16.281±2.118	0.304	24.109±1.301	0.346
	PCB177	3.269±0.161	0.353	16.491±0.435	0.308	26.711±1.123	0.328
	PCB171/156	2.172±0.066	0.268	9.005±1.136	0.168	24.304±3.087	0.290
	PCB200	1.307±0.0623	0.111	3.585±0.083	0.067	11.202±0.467	0.120
	PCB172	0.585±0.088	0.145	2.485±0.123	0.046	4.199±0.278	0.057
	PCB180	4.191±0.370	0.225	23.331±1.842	0.435	46.242±0.967	0.741
	PCB170/190	2.224±0.285	0.272	11.910±1.029	0.222	19.304±2.295	0.209
	PCB201	0.931±0.019	0.172	6.462±0.618	0.121	13.585±0.648	0.208
	PCB203	1.574±0.220	0.192	7.341±0.591	0.137	16.313±0.652	0.211
	PCB195	0.783±0.070	0.091	3.908±0.150	0.073	7.681±0.169	0.103
	PCB194	1.197±0.001	0.093	6.245±0.650	0.117	12.029±0.163	0.173
	PCB206	0.797±0.033	0.092	1.592±0.126	0.030	2.758±0.100	0.054
	Total PCB	**107.13±3.04**	**8.606**	**471.68±4.65**	**8.738**	**829.82±1.86**	**9.918**
Day 70	PCB31/28	0.529±0.172	0.82	6.146±0.594	0.114	12.157±0.535	0.232
	PCB52	0.463±0.101	0.065	5.042±0.644	0.151	11.386±0.188	0.177
	PCB49	0.638±0.068	0.052	5.446±0.253	0.163	11.237±1.186	0.284
	PCB44	0.250±0.032	0.034	4.682±0.466	0.104	4.832±0.305	0.092
	PCB42	0.349±0.079	0.034	1.790±0.085	0.043	3.552±0.119	0.088
	PCB64	0.278±0.0115	0.017	1.272±0.017	0.038	3.002±0.219	0.077
	PCB74	0.261±0.014	0.016	1.206±0.082	0.036	1.444±0.033	0.058
	PCB70/76	4.664±0.395	0.518	16.851±1.027	0.504	34.085±0.534	0.600
	PCB66/95	3.266±0.529	0.303	18.245±0.796	0.529	39.762±1.141	0.728
	PCB60/56	0.973±0.106	0.090	6.779±0.421	0.203	11.815±1.328	0.245
	PCB101	2.348±0.269	0.149	9.649±0.315	0.288	17.575±1.295	0.185
	PCB99	1.568±0.090	0.110	5.566±0.630	0.166	16.038±0.650	0.190
	PCB97	0.596±0.024	0.088	2.683±0.370	0.080	5.962±0.320	0.124
	PCB87	3.499±0.255	0.242	14.170±0.336	0.423	25.974±0.571	0.295
	PCB110	7.121±0.444	0.796	17.410±0.641	0.520	32.682±1.144	0.603

	PCB151	2.108±0.190	0.266	8.572±0.471	0.256	17.263±0.771	0.229
	PCB149	1.485±0.061	0.135	11.886±0.590	0.355	18.338±0.822	0.150
	PCB118	1.514±0.164	0.118	9.065±1.052	0.271	35.703±2.532	0.480
	PCB153	10.038±1.514	0.902	28.986±1.529	0.866	51.192±4.773	0.676
	PCB105	1.639±0.055	0.120	8.849±0.637	0.264	14.698±1.266	0.280
	PCB141	1.094±0.290	0.177	6.667±0.247	0.199	11.733±1.292	0.124
	PCB179	1.521±0.064	0.139	7.986±0.666	0.239	16.008±1.160	0.205
	PCB138	17.094±1.213	1.71	53.588±6.117	1.602	75.245±3.507	1.634
	PCB158	1.132±0.231	0.091	7.529±0.453	0.225	10.115±1.187	0.163
	PCB187	2.651±0.170	0.239	9.973±0.454	0.298	13.728±2.178	0.242
	PCB183	0.681±0.173	0.067	3.840±0.577	0.115	9.729±0.198	0.195
	PCB128	1.298±0.173	0.117	5.430±0.761	0.162	9.046±0.912	0.202
	PCB174	3.975±0.676	0.359	14.315±1.400	0.428	19.410±0.868	0.270
	PCB177	2.642±0.417	0.238	11.101±0.669	0.332	20.953±2.496	0.199
	PCB171/156	2.345±0.245	0.219	10.313±0.108	0.308	20.028±1.007	0.482
	PCB200	1.359±0.098	0.163	3.269±0.041	0.098	9.065±0.435	0.143
	PCB172	0.424±0.095	0.041	2.084±0.360	0.062	2.462±0.183	0.087
	PCB180	3.965±0.658	0.368	22.397±0.910	0.669	42.615±1.533	0.892
	PCB170/190	1.603±0.164	0.147	11.355±0.261	0.339	17.739±1.964	0.438
	PCB201	0.788±0.030	0.057	6.236±0.350	0.186	11.720±0.075	0.123
	PCB203	1.682±0.051	0.184	8.121±0.500	0.243	16.428±0.623	0.263
	PCB195	0.619±0.098	0.041	3.472±0.187	0.104	6.779±0.127	0.629
	PCB194	1.111±0.020	0.109	6.920±0.191	0.207	10.895±0.229	0.388
	PCB206	0.784±0.039	0.087	1.740±0.0627	0.052	2.846±0.069	0.254
	Total PCB	**91.02±3.20**	**9.428**	**381.44±6.46**	**11.242**	**692.54±12.54**	**12.726**
Day 105	PCB31/28	0.564±0.025	0.138	4.728±0.525	0.155	6.956±0.624	0.406
	PCB52	0.327±0.028	0.062	9.897±0.870	0.324	27.717±3.051	0.919
	PCB49	0.655±0.145	0.135	3.594±0.156	0.118	7.209±0.099	0.313
	PCB44	0.297±0.034	0.067	4.199±0.024	0.138	4.834±0.450	0.143
	PCB42	0.183±0.011	0.025	0.853±0.090	0.028	3.052±0.960	0.090
	PCB64	0.204±0.021	0.029	1.574±0.173	0.052	1.765±0.447	0.102
	PCB74	0.174±0.041	0.029	1.042±0.021	0.034	1.068±0.023	0.202
	PCB70/76	3.823±0.522	0.885	16.981±1.194	0.557	31.552±0.182	0.913
	PCB66/95	3.277±0.525	0.598	17.598±1.053	0.577	37.034±0.894	1.005

	PCB					
	PCB60/56	1.008±0.185	0.162	5.251±0.835	0.172	7.829±0.736	0.361
	PCB101	2.091±0.216	0.260	8.262±1.612	0.316	16.490±0.692	0.307
	PCB99	1.500±0.112	0.276	4.753±1.070	0.186	13.538±0.803	0.136
	PCB97	0.718±0.081	0.147	2.790±0.261	0.091	5.609±0.347	0.146
	PCB87	2.780±0.445	0.591	6.280±0.378	0.206	12.906±0.591	0.362
	PCB110	5.314±0.598	1.004	17.677±2.640	0.579	27.427±0.488	0.891
	PCB151	1.564±0.242	0.399	7.952±1.044	0.261	12.741±1.084	0.597
	PCB149	1.415±0.215	0.170	9.890±0.674	0.324	13.552±0.735	0.251
	PCB118	1.187±0.149	0.127	6.040±0.447	0.198	28.549±2.718	0.544
	PCB153	6.426±1.117	1.206	26.125±1.717	0.856	45.479±1.102	1.444
	PCB105	1.457±0.264	0.208	7.592±0.763	0.249	11.946±0.809	0.553
	PCB141	1.459±0.243	0.248	6.762±0.306	0.222	10.297±1.581	0.434
	PCB179	1.390±0.144	0.235	4.202±0.257	0.138	11.027±1.098	0.316
	PCB138	15.400±4.09	2.593	57.580±2.556	1.887	70.192±0.775	2.175
	PCB158	1.251±0.200	0.249	5.597±0.283	0.183	10.907±0.829	0.422
	PCB187	2.291±0.629	0.477	8.548±0.620	0.280	15.206±0.399	0.600
	PCB183	0.920±0.0840	0.186	4.939±0.470	0.162	8.709±0.172	0.457
	PCB128	1.087±0.215	0.197	5.618±1.025	0.184	9.063±0.757	0.268
	PCB174	2.216±0.592	0.323	9.552±0.558	0.313	17.701±0.592	0.623
	PCB177	2.401±0.241	0.358	7.994±0.928	0.262	19.978±0.372	0.401
	PCB171/156	1.716±0.123	0.428	10.124±0.435	0.332	18.385±0.756	0.503
	PCB200	1.365±0.045	0.561	3.588±0.179	0.118	11.847±0.068	0.350
	PCB172	0.404±0.092	0.177	1.491±0.206	0.049	2.517±0.356	0.104
	PCB180	3.567±0.730	0.581	22.218±2.858	0.728	38.449±0.507	1.307
	PCB170/190	1.681±0.084	0.221	10.368±0.872	0.240	17.911±0.301	0.349
	PCB201	0.828±0.055	0.188	6.057±0.694	0.109	10.248±0.110	0.243
	PCB203	1.292±0.097	0.207	7.254±0.808	0.238	11.904±0.824	0.552
	PCB195	0.443±0.069	0.075	2.801±0.329	0.092	5.008±0.134	0.448
	PCB194	1.039±0.060	0.168	6.042±0.696	0.198	9.900±0.093	0.393
	PCB206	0.772±0.045	0.187	1.674±0.054	0.055	2.691±0.170	0.180
	Total PCB	**74.40±6.72**	**14.177**	**347.78±14.06**	**11.211**	**615.67±10.46**	**19.810**
Day 140	PCB31/28	0.474±0.194	0.070	3.181±0.100	0.121	4.987±0.497	0.169
	PCB52	0.404±0.088	0.059	5.395±0.910	0.204	23.511±2.388	0.456
	PCB49	0.497±0.094	0.074	1.988±0.286	0.075	4.767±1.400	0.633

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PCB44	0.355±0.042	0.070	3.137±0.171	0.119	4.103±0.495	0.414
PCB42	0.248±0.020	0.052	0.565±0.018	0.021	2.414±0.506	0.097
PCB64	0.190±0.038	0.082	1.317±0.086	0.050	1.419±0.182	0.094
PCB74	0.433±0.290	0.064	5.379±4.54	0.204	9.413±0.017	0.076
PCB70/76	3.971±0.368	0.739	12.451±1.267	0.638	31.725±0.379	0.811
PCB66/95	2.482±0.045	0.539	13.113±1.519	0.646	39.129±1.447	1.012
PCB60/56	0.647±0.111	0.155	3.870±0.906	0.147	6.430±0.278	0.579
PCB101	1.895±0.233	0.322	7.116±0.706	0.346	15.640±0.997	0.336
PCB99	1.231±0.092	0.223	4.506±0.364	0.149	10.604±0.705	0.196
PCB97	0.532±0.074	0.190	2.968±0.442	0.162	6.676±0.239	0.286
PCB87	1.318±0.087	0.224	3.473±0.314	0.122	8.685±0.820	0.542
PCB110	3.605±0.192	0.612	20.549±0.148	0.769	22.870±0.790	0.938
PCB151	1.089±0.023	0.185	6.502±0.738	0.226	11.699±1.035	0.426
PCB149	0.908±0.007	0.154	8.016±0.605	0.324	10.807±0.580	0.302
PCB118	1.186±0.245	0.201	2.501±0.198	0.105	14.014±1.515	0.291
PCB153	6.509±0.436	1.105	16.918±1.094	0.631	24.995±1.274	0.997
PCB105	1.453±0.160	0.247	7.147±0.358	0.221	11.453±1.240	0.320
PCB141	0.795±0.118	0.135	5.674±0.537	0.265	8.274±0.517	0.131
PCB179	1.052±0.105	0.179	3.179±0.358	0.150	8.559±0.149	0.139
PCB138	15.400±6.739	2.655	69.966±11.685	2.652	56.237±5.539	1.569
PCB158	1.061±0.286	0.180	5.813±0.574	0.200	9.635±0.738	0.469
PCB187	1.58±0.143	0.239	4.636±0.218	0.136	13.993±1.569	0.290
PCB183	0.819±0.030	0.129	4.280±0.285	0.152	9.386±0.449	0.362
PCB128	0.743±0.184	0.116	5.295±0.839	0.221	9.016±0.850	0.352
PCB174	2.133±0.752	0.382	8.119±0.556	0.288	16.285±1.022	0.454
PCB177	2.221±0.204	0.397	7.926±1.328	0.310	16.744±3.427	0.567
PCB171/156	1.461±0.294	0.228	8.144±0.118	0.299	17.153±2.267	0.579
PCB200	1.118±0.077	0.150	3.086±0.151	0.147	10.382±0.982	0.290
PCB172	0.210±0.037	0.026	1.010±0.088	0.028	2.310±0.130	0.054
PCB180	3.314±0.295	0.543	18.391±1.571	0.597	34.664±1.060	0.967
PCB170/190	1.206±0.097	0.185	9.401±1.301	0.356	14.614±2.000	0.608
PCB201	0.705±0.022	0.140	5.294±0.155	0.291	9.621±1.004	0.468
PCB203	1.263±0.059	0.234	7.445±0.855	0.182	11.638±0.673	0.225
PCB195	0.521±0.043	0.098	2.544±0.299	0.090	5.414±0.356	0.181
PCB194	0.721±0.054	0.142	5.414±0.173	0.105	8.684±0.385	0.202

PCB206	0.840±0.077	0.163	1.706±0.072	0.165	2.733±0.138	0.376
Total PCB	**68.90±1.81**	**11.688**	**319.27±8.89**	**11.914**	**523.32±12.25**	**17.258**

Table S3.2. The measured mean (±S.E.) value of log k_{tot}, K_{EX}, and log k_{ex} from three treatments for each PCB congeners.

	Low dose			Med. dose			High dose		
	log k_{tot}	K_{EX}	log k_{ex}	log k_{tot}	K_{EX}	log k_{ex}	log k_{tot}	K_{EX}	log k_{ex}
PCB31/28	-2.05	0.20±0.04	-3.62±0.06	-1.77	0.19±0.03	-3.60±0.05	-1.77	0.16±0.02	-3.66±0.04
PCB42	-2.30	0.20±0.03	-3.61±0.04	-1.82	0.12±0.03	-3.83±0.10	-2.15	0.20±0.02	-3.57±0.04
PCB44	-2.10	0.22±0.02	-3.57±0.04	-2.05	0.21±0.03	-3.54±0.05	-1.96	0.21±0.03	-3.53±0.05
PCB49	-1.92	0.24±0.02	-3.51±0.03	-1.80	0.21±0.03	-3.53±0.05	-1.85	0.20±0.02	-3.55±0.04
PCB52	-1.85	0.28±0.03	-3.45±0.03	-2.00	0.26±0.03	-3.46±0.07	-2.10	0.24±0.04	-3.50±0.05
PCB60/56	-2.15	0.21±0.02	-3.59±0.04	-2.00	0.23±0.02	-3.50±0.06	-2.15	0.21±0.03	-3.54±0.05
PCB64	-2.10	0.23±0.01	-3.54±0.03	-2.00	0.22±0.02	-3.50±0.03	-1.85	0.22±0.02	-3.50±0.03
PCB66/95	-2.30	0.31±0.03	-3.41±0.04	-2.40	0.27±0.02	-3.42±0.06	-2.40	0.25±0.03	-3.47±0.05
PCB70/76	-2.70	0.29±0.02	-3.45±0.01	-2.70	0.27±0.03	-3.44±0.05	-3.00	0.26±0.02	-3.44±0.03
PCB74	-2.15	na	na	-2.40	na	na	-1.92	na	na
PCB87	-2.00	0.44±0.13	-3.31±0.14	-1.82	0.18±0.03	-3.60±0.05	-1.85	0.20±0.02	-3.55±0.05
PCB97	-2.15	0.17±0.02	-3.69±0.04	-2.15	0.19±0.02	-3.57±0.04	-2.15	0.22±0.02	-3.51±0.03
PCB99	-2.70	0.20±0.02	-3.60±0.05	-2.40	0.17±0.01	-3.61±0.03	-2.22	0.19±0.02	-3.59±0.04
PCB101	-2.40	0.27±0.02	-3.47±0.04	-2.30	0.26±0.02	-3.43±0.02	-2.52	0.22±0.02	-3.52±0.05
PCB105	-2.70	0.18±0.03	-3.71±0.10	-2.52	0.19±0.02	-3.58±0.05	-2.30	0.19±0.04	-3.65±0.08
PCB110	-2.05	0.20±0.02	-3.61±0.03	-2.22	0.19±0.02	-3.58±0.05	-2.22	0.19±0.02	-3.58±0.03
PCB118	-1.89	0.19±0.03	-3.64±0.07	-1.72	0.14±0.02	-3.71±0.05	-1.96	0.16±0.02	-3.67±0.06
PCB128	-2.30	0.15±0.03	-3.74±0.07	-2.40	0.18±0.02	-3.62±0.06	-2.40	0.21±0.02	-3.53±0.04
PCB138	-3.00	0.20±0.02	-3.62±0.06	-3.00	0.23±0.03	-3.52±0.07	-2.70	0.22±0.03	-3.52±0.05
PCB141	-2.22	0.29±0.02	-3.43±0.01	-2.52	0.23±0.02	-3.49±0.03	-2.30	0.21±0.02	-3.53±0.04
PCB149	-1.92	0.25±0.02	-3.49±0.03	-2.10	0.25±0.04	-3.49±0.08	-1.96	0.17±0.01	-3.61±0.03
PCB151	-1.92	0.23±0.02	-3.54±0.04	-1.96	0.24±0.03	-3.47±0.05	-1.92	0.23±0.02	-3.48±0.04
PCB153	-2.15	0.16±0.02	-3.71±0.05	-2.40	0.16±0.01	-3.66±0.04	-2.30	0.18±0.01	-3.60±0.03
PCB158	-2.40	0.16±0.02	-3.71±0.06	-2.70	0.14±0.02	-3.73±0.06	-2.52	0.16±0.01	-3.65±0.05
PCB170/190	-2.30	0.18±0.02	-3.65±0.05	-2.70	0.14±0.02	-3.77±0.09	-2.52	0.13±0.02	-3.76±0.05
PCB171/156	-2.30	0.19±0.02	-3.64±0.05	-2.70	0.18±0.02	-3.60±0.03	-2.40	0.16±0.02	-3.67±0.06
PCB172	-2.05	0.16±0.01	-3.68±0.03	-2.10	0.16±0.03	-3.69±0.09	-2.15	0.13±0.02	-3.77±0.06
PCB174	-2.15	0.18±0.01	-3.65±0.04	-2.22	0.14±0.02	-3.72±0.05	-2.40	0.16±0.02	-3.67±0.06

PCB177	-2.52 0.14±0.04 -3.74±0.07	-2.15 0.24±0.04 -3.49±0.05	-2.40 0.16±0.02 -3.68±0.07
PCB179	-2.05 0.15±0.01 -3.72±0.03	-1.92 0.12±0.02 -3.77±0.06	-2.05 0.12±0.02 -3.80±0.07
PCB180	-2.52 0.17±0.03 -3.71±0.07	-2.70 0.09±0.02 -3.95±0.08	-2.52 0.10±0.02 -3.89±0.06
PCB183	-2.52 0.16±0.04 -3.75±0.06	-2.70 0.14±0.02 -3.73±0.07	-2.70 0.14±0.03 -3.77±0.08
PCB187	-2.10 0.17±0.03 -3.71±0.07	-2.05 0.14±0.03 -3.74±0.07	-2.52 0.19±0.03 -3.61±0.06
PCB194	-2.40 0.20±0.03 -3.65±0.09	-2.70 0.20±0.03 -3.57±0.06	-2.52 0.19±0.03 -3.61±0.08
PCB195	-2.40 0.18±0.03 -3.67±0.04	-2.40 0.11±0.02 -3.87±0.07	-2.52 0.10±0.01 -3.88±0.06
PCB200	-3.00* 0.19±0.01 -3.61±0.04	-3.00* 0.16±0.02 -3.66±0.05	-3.00* 0.13±0.02 -3.78±0.07
PCB201	-2.40 0.15±0.03 -3.77±0.09	-2.70 0.26±0.05 -3.49±0.08	-2.52 0.11±0.02 -3.86±0.06
PCB203	-2.70* 0.24±0.05 -3.58±0.09	-3.00* 0.20±0.03 -3.59±0.08	-2.52* 0.14±0.03 -3.77±0.09
PCB206	-3.00* 0.19±0.02 -3.70±0.08	-3.00* 0.08±0.01 -3.95±0.06	-3.00* 0.12±0.02 -3.84±0.10

* indicates no significant elimination over the depuration period, p>0.05.
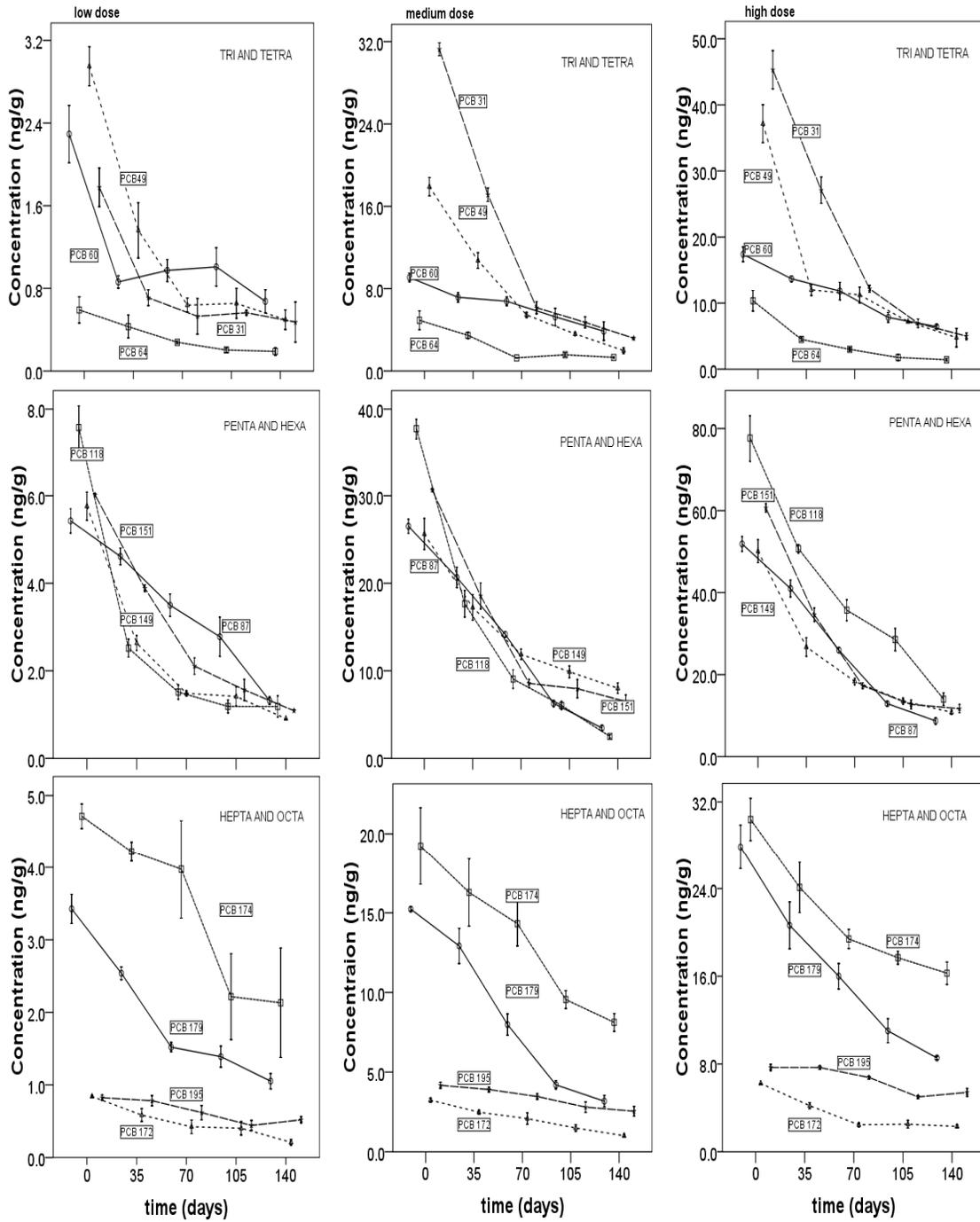
Figure 3.1. Elimination of selected PCBs from Japanese koi as a function of depuration time.
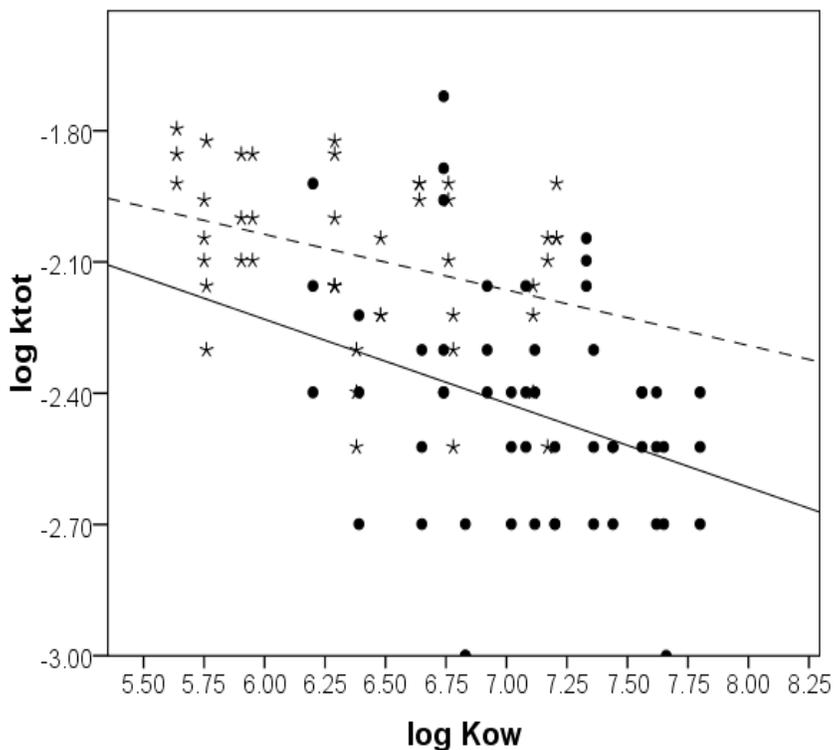
Figure 3.2. The relation between log k_{tot} and log K_{OW}. Solid dot and asterisk represent the estimated log k_{tot} value recalcitrant and liable PCB congeners, respectively. Solid line and dashed line represents the regression relationship between log k_{tot} and log K_{OW} of recalcitrant congeners (log k_{tot} = -0.192 log K_{OW}-1.079) and labile congeners (log k_{tot} = -0.127 log K_{OW}-1.275).
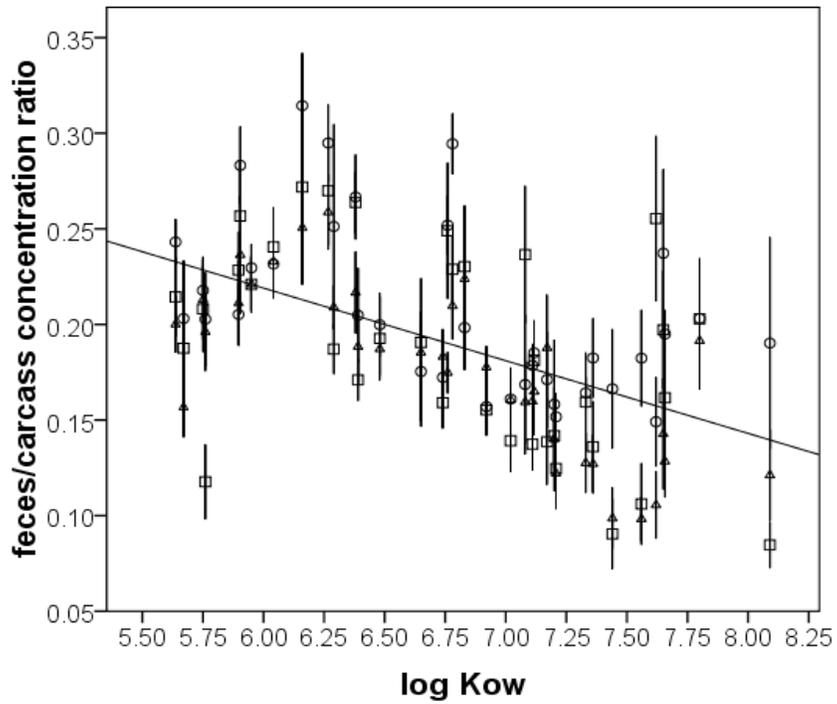
Figure 3.3. The mean (○ stands for low dose group,□ stands for medium dose group and △

stands for high dose group) and SE (bar,|) of chemical feces/organism concentration ratio,

K_{EX}, of every PCB congener from each dosing level group.
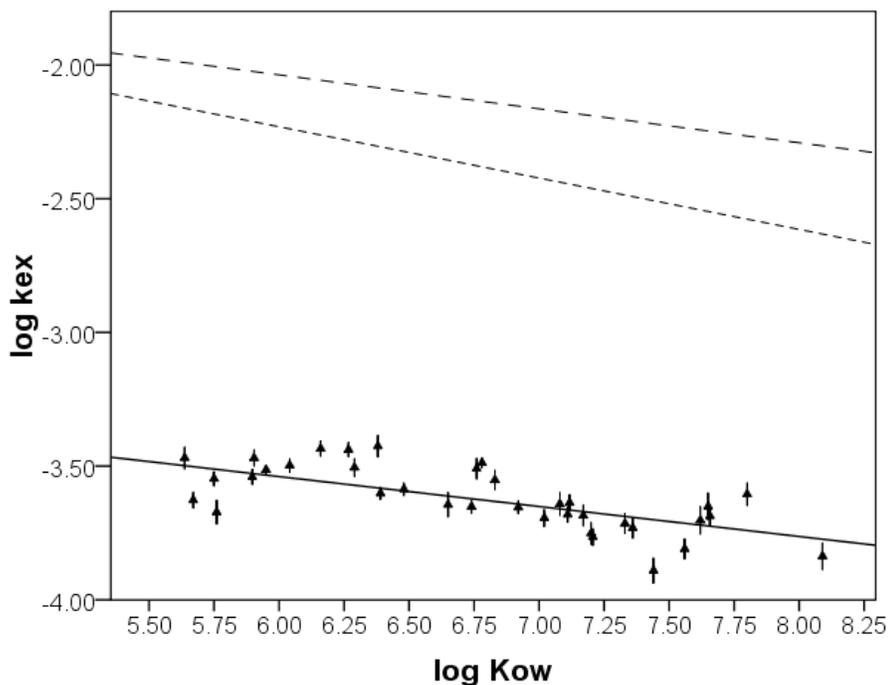
Figure 3.4. The mean and S.E. of chemical fecal elimination rate, k_{ex}, of every PCB congener from each dosing level group. The lines illustrate the regression relation between chemical k_{ex} and its log K_{OW} value. The solid line is the regression equation derived from least squares regression between these two variables and the dashed lines are the log k_{tot}/log K_{OW} regression lines (The upper dashed line is of labile congeners and the lower dashed line is of recalcitrant congeners).

3.5 References

Arnot JA, Gobas FAPC. 2006. A review of bioconcentration factor (BCF) and
  bioaccumulation factor (BAF) assessments for organic chemicals in aquatic
  organisms. *Environ Rev* 14:257–297.

Andersson PL, Berg AH, Bjerselius R, Norrgren L, Olsen H, Olsson PE, Orn S and Tysklind
  M. 2001. Bioaccumulation of selected PCBs in zebrafish, three-spined stickleback,
  and arctic char after three different routes of exposure. *Arch Environ Contam Toxicol*
  40: 519-530.

Boon JP, Eijgenraam, Everaarts JM, and Duinker JC. 1989. A structure activity relationship
  (SAR) approach towards metabolism of PCBs in marine animals from different
  trophic levels. *Mar Environ Res* 27: 159-176.

Barron MG. 1990. Bioconcentration. *Environ Sci Technol* 24: 1612-1618.

Barron MG, Yurk JJ, and Crothers DB. 1994. Assessment of potential cancer risk from
  consumption of PCBs bioaccumulated in fish and shellfish. *Environ Health Perspet*
  102: 562-567.

Boon JP, van Arnhem E, Hansen S, Kannan N, Petrick G, Schulz D, Duinker JC, Reijnders
  PJH, and Goksoyr A. 1992. In: Walker CH, Livingstone DR (Eds) Persistent
  Pollutants in Marine Ecosystems. Pergamon Press. Oxford, 119-159.

Boon JP, Oostingh I, van der Meer J, and Hillebrand MTJ. 1994. Amodel for the
  bioaccumulation of chlorobiphenyl congeners in marine mammals. *Eur J Pharmacol*
  270: 237-251.

Bruggeman WA, Martron LBJM, Kooiman D, and Hutzinger O. 1981. Accumulation and elimination kinetics of di-, tri-, and tetrachloro biphenyls by goldfish after dietary and aqueous exposure. *Chemosphere* 10:831-832

Buckman AH, Brow SB, Hoekstra PF, Solomon KR, and Fisk AT. 2004. Toxicokinetics of three polychlorinated biphenyls technical mixtures in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 23: 1725-1736.

Buckman AH, Wong CS, Chow EA, Brown SB, Solomon KR and Fisk AT. 2006. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. *Aquat toxicol* 78:176-185.

Buckman AH, Brown SB, Small J, Solomon KR and Fisk AT. 2007. Role of temperature and enzyme induction in the biotransformation of polychlorinated biphenyls and bioformation of hydroxylated polychlorinated biphenyls by rainbow trout (*Oncorhynchus mykiss*). *Environ Sci Technol* 41: 3856-3863.

Daley, JM, Leadley,TA, Drouillard,KG. 2009. Bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch eggs during incubation. *Chemosphere* in press.

DeBruyn AMH and Gobase FAPC. 2006. A Bioenergetic Biomagnification Model for the Animal Kingdom. *Environ Sci Technol* 40: 1581-1587.

de Freitas AS and Norstrom RJ. 1974. Turnover and netabolism of polychlorinated biphenyls in relation to their chemical structure and movement of lipids in the pigeon. *Can J Physiol Pharmacol* 52: 1080-1094.

Dvorchik BH and Maren TH.1972. The fate of p,p'-DDT in the dogfish (*Squalus acanthias*). *Comp Biochem Physiol A Comp Physiol* 42: 205-211

Doi AM, Lou Z, Holmes E, Venugopal CS, Nyagode B, James MO and Kleinow KM. 2006. Intestinal bioavailability and biotransformation of 3,3',4,4'-tetrachlorobiphenyl (CB 77) in in situ preparations of channel catfish following dietary induction of CYP1A. *Aquat Toxicol* 20: 33-42.

Drouillard KG, Fernie K, Smits JE, Bortolotti, Bird DM, and Norstrom RJ. 2001. Bioaccumulation and toxicokinetics of 42 PCB congeners in American kestrels (*Falco sparverius*). *Environ Toxicol Chem* 20: 2514-2522.

Drouillard KG, Norstrom RJ. 2003. The influence of diet properties and feeding rates on PCB toxicokinetics in the ring dove. *Arch Environ Contam Toxicol* 44:99–106.

Drouillard KG, Hagen H. Haffner GD. 2004. Evaluation of chloroform/methanol and dichloromethane/hexane extractable lipids as surrogate measures of sample partition capacity for organochlorines in fish tissues. *Chemosphere* 55: 395-400.

Drouillard KG, Paterson G, Haffner GD. 2009. A combined food web toxicokinetic and species bioenergetic model for prediction seasonal PCB elimination by yellow perch (*Perca flavescens*). *Environ Sci Technol* In press.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ Toxicol Chem* 17:951–961.

Fisk AT, Tomy GT, Cymbalisty CD, and Muir DCG. 2000. Dietary accumulation and quantitative structure-activity relationship for depuration and biotransformation of short (C_{10}), medium (C_{14}) and Long (C_{18}) carbon-chain polychlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 19: 1508-1516.

Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC and Nichols JW. 2001.
Branchial elimination of syperhydrophobic organic compounds by rainbow trout
(*Oncorhynchus nykiss*). *Aquat Toxicol* 55:22-34.

Gobas FAPC, Muir DCG, Mackay D. 1988. Dynamics of dietary bioaccumulation and fecal
elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.

Gobas FAPC, Clark KE, Shu WY, Mackay D. 1989 Bioconcentration of polybrominated
benzenes and biphenyls and related superhydrophobic chemicals in fish: role of
bioavailability and elimination into the feces. *Environ Toxicol Chem* 8:231–245.

Gobas FAPC, McCorquodale JR, Haffner GD. 1993a. Intestinal absorption and
biomagnification of organochlorines. *Environ Toxicol Chem* 12:567–576.

Gobas FAPC, Zhang X, Wells R. 1993b. Gastrointestinal magnification: The mechanism of
biomagnification and food chain accumulation of organic chemicals *Environ Sci
Technol* 27: 2855-2863.

Gobas FAPC, Z'Graggen M, Zhang X. 1995. Time response of the Lake Ontario ecosystem
to virtual elimination of PCBs. *Environ Sci Technol* 29: 2038-46

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. 1999. Mechanism of
biomagnification in fish under laboratory and field conditions. *Environ Sci Technol*
33:133–141.

Gobas FAPC, Morrison HA 2000. Bioconcentration and bioaccumulation in the aquatic
environment. *In* Boethling R, Mackay D (eds) Handbook for environmental
properties. CRC Press, Boca Raton, FL.

Hansen LG. 1999. The Ortho Side of PCBs. Kluwer Academic Publisher, Boston, MA, USA.
260p.

Hawker DW, Connell DW. 1985. Relationships between partition coefficient, uptake rate

    constant, clearance rate constant, and time to equilibrium for bioaccumulation.

    Chemosphere 14: 1205–1219.

Hendriks AJ, van der Linde A, Cornelissen G, Sijm DTHM. 2001. The power of size. 1. Rate

    constants and equilibrium ratios for accumulation of organic substances related to

    octanol-water partition ratio and species weight. *Environ Toxicol Chem* 20: 1399-

    1420.

Johnston TA, Fisk AT, Whittle DM and Muir DCG. 2002. Variation in Organochlorine

    bioaccumulation by a predatory fish: Gender, geography and data analysis methods.

    Environ Sci Technol 36: 4238-4244.

Kelly BC, Ikonomou MG, Blair JD, Morin AE and Gobas FAPC. 2007. Food web specific

    biomagnification of persistent organic pollutants. *Science* 317: 236-239.

Lazar R, Edwards RC, Metcalfe CD, Metcalfe T, Gobas FAPC, Haffner GD. 1992. A simple,

    novel method for the quantitative analysis of coplanar (non-ortho substituted)

    polychlorinated biphenyls in environmental samples. *Chemosphere* 25: 493-504.

Leney JL, Drouillard KG and Haffner GD. 2006. Dose metamorphosis increase the

    susceptibility of frogs to highly hydrophobic contaminants? *Environ Sci Technol* 40:

    1491-1496.

Li H, Drouillard KG, Bennett ER, Haffner GD and Letcher RJ. 2003. Plasma- associated

    halogenated phenolic contaminants in benthic and pelagic fish species from the

    Detroit River. *Environ Sci Technol* 37: 832-839.

Matthews HB and Derick RL. 1984. Pharmacokinetics of PCBs. *Annu Rev Pharmacol*

    Toxicol 24: 85-103.

Mehrtens G and Laturnus F. 1999. Mixed function oxidase dependent biotransformation of
    polychlorinated biphenyls by different species of fish from the North Sea.
    Chemosphere 38: 2995-3002.

Morrison HA, Whittle DM, and Haffner GD. 2002. A comparison of the transport and fate of
    polychlorinated biphenyl congeners in three Great Lakes food webs. *Environ Toxicol
    Chem* 21: 680-692.

Norstrom RJ. 1988. Bioaccumulation of polychlorinated biphenyls in Canadian Wildlife. In
    Crine JP (Ed). Hazards, Decontaminations and Replacement of PCBs. Plenum
    Publishing, New York, NY, USA. 85-100.

Oliver BG and Niimi AJ. 1983. Biological half-lives of polychlorinated biphenyl (PCB)
    congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*) *Can J Fish
    Aquat Sci* 40: 1388-1394.

Paterson, G, Drouillard KG, and Haffner GD. 2007(a). PCB elimination by yellow perch
    (*Perca flavescens*) during an annual temperature cycle. *Environ Sci Technol* 41: 824-
    829.

Paterson G, Drouillard KG, Leadley TA, and Haffner GD. 2007(b). Long-term
    polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca
    flavescens*) *Can J Fish Aquat Sci* 64: 1222-1233.

Qiao P, Gobas FAPC, Farrell AP. 2000. Relative contributions of aqueous and dietary uptake
    of hydrophobic chemicals to the body burden in juvenile rainbow trout. *Arch Environ
    Contam Toxicol* 39:369–377.

Rozman K, Ballhorn L and Rozman T. 1983. Enhanced intestinal excretion of

    hexachlorobenzens in rats by intraluminal injection of hexadecane. *J Appl Toxicol* 3:

    48-50.

Russell RW, Gobas FAPC, and Haffner GD. 1999. Maternal transfer and in ovo exposure of

    organochlorines in oviparous organisms: a model and field verification. *Environ Sci*

    Technol 33: 416-420.

Safe, S. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and

    toxic responses, and implications of risk assessment. *Crit Rev in Toxicol* 24, 87-149.

Servos MR, Muir CG, Webster GRB 1992. Bioavailability of polychlorinated dibenzo-p-

    dioxin in lake enclosures. *Can J Fish Aquat Sci* 49: 435-742.

Sijm DTHM and Van der Linde A. 1995. Size-dependent bioconcentration kinetics of

    hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric

    relationships. *Environ Sci Technol* 29: 2769-2777.

Tanabe S, Maruyama K, Tatsukawa R. 1982. Absorption efficiency and biological half-lives

    of individual chlorobiphenyls in carp (*Cyprinus carpio*) orally exposed to Kanechlor

    products. *Agric Biol Chem* 46:891–898.

CHAPTER 4 GENERAL DISCUSSION

The purpose of this thesis was to provide enhanced calibration of a generalized fish
bioaccumulation model applicable to persistent, hydrophobic organic compounds such as
polychlorinated biphenyls. The generalized fish bioaccumulation is commonly formulated as
follows:

$$\frac{dC_f}{dt} = k_1 \cdot C_{wat} + k_{feed} \cdot C_{food} - C_f \cdot (k_2 + k_{ex} + k_{met}) \tag{1}$$

Where C_f (ng/g fish), C_{wat} (ng/mL water), and C_{food} (ng/g food) are the wet weight
chemical concentration in fish, freely dissolved concentration in the water and the
concentration in the food, respectively. The rate coefficients denoted by k_1 (mL/ g fish · d)
and k_{feed} (g feed/ g fish · d) are the rate coefficients for uptake via the gills and from the diet,
respectively. The rate coefficients denoted by k_2 (d^{-1}), k_{ex} (d^{-1}), and k_{met} (d^{-1}) are the rate
coefficients for elimination through gills, fecal egestion and metabolic biotransformation.

It has been long recognized, however, that the constants (k_1, k_2, k_{feed}, k_{ex}, k_{met}) are, in
fact, variable for fish and are often scaled to animal metabolic rate, feeding rate and diet
digestion efficiencies. *A priori* prediction of toxicokinetic parameters described above has
been performed by coupling bioenergetic sub-models with more detailed toxicokinetic sub-
models. Using these approaches the toxicokinetic parameters can be individually defined
according to:

$$k_1 = G_v \cdot E_w \tag{2}$$

$$k_2 = G_v \cdot E_w \cdot \frac{1}{K_{BW}} \tag{3}$$

$$k_{feed} = G_{feed} \cdot AE \qquad (4)$$

$$k_{ex} = G_{ex} \cdot AE \cdot \frac{1}{K_{BEX}} \qquad (5)$$

Where G_v, G_{feed}, G_{ex} refer to gill ventilation rates (mL/g fish·d), animal feeding rate (g feed/g fish·d) and fecal egestion efficiencies (g feces/g fish·d). The terms E_w and AE refer to diffusive transfer efficiency terms of chemical across the respiratory surface and digestive tract. The terms K_{BW} and K_{BEX} are the biota/water and biota/feces partition coefficients. At present, the term k_{met} remains an empirical expression, although, in theory, this parameter can obey Michaelis Menton type kinetics if enzyme induction is involved in the biotransformation process.

Combining the above equations yields a more generalized bioenergetics-based bioaccumulation model:

$$\frac{dC_f}{dt} = G_v \cdot E_w \cdot C_{wat} + G_{feed} \cdot AE \cdot C_{food} - C_f \cdot (G_v \cdot E_w \cdot \frac{1}{K_{BW}} + G_{ex} \cdot AE \cdot \frac{1}{K_{BEX}} + k_{met}) \quad (6)$$

In the generalized expression of Eq. 6, the terms G_v, G_{feed} and G_{ex} are bioenergetics parameters that are considered to be variable in time or across environmental conditions and are scaled to animal metabolic rate. These parameters are predicted separately using a dedicated species optimized bioenergetic sub-model (Drouillard et al. 2009). The terms E_w, AE, K_{BW}, K_{BEX} and k_{met} are fundamental toxicokinetic parameters and are typically assumed to be constant for a given chemical and bioindicator species (Arnot and Gobas 2004; Thomann 1992; Sijm et al. 1992).

While a number of studies have reported empirical measurements of uptake and/or whole body elimination rate constants for hydrophobic chemicals such as PCBs in fish (Bruggeman et al. 1984; Paterson et al. 2007; Sijm et al. 1992), fewer studies have provided

detailed examination of fundamental toxicokinetic parameters described in Eq. 6 and how these parameters respond to different dietary or environmental conditions. Several studies have quantified the magnitude of dietary AEs in fish and established relationships between dietary AE and chemical hydrophobicity (Gobas et al. 1988; Burreau et al. 1997; Fisk et al. 1998). Few studies, however, have established the constancy of AE as a function of different diet types (Gobas et al. 1993; Clark et al. 1990; Dabrowska et al. 1999) and no studies were identified that quantified dietary PCB AEs in fish fed different types of naturally contaminated diets (i.e. diets that were contaminated in the field through natural bioaccumulation pathways).

Similarly, the relative importance of different elimination routes, k_2, k_{ex} and k_{met}, to PCB whole body elimination by fish remains poorly characterized. Most studies have focused on measurement of the whole body elimination rate constant ($k_{tot} = k_2 + k_{ex} + k_{met}$; Fisk et al. 1998; Buckman et al. 2004; Andersson et al. 2001) but have not quantified individual elimination routes and how these vary under different dosing, environmental or feeding conditions. Although some modelling studies have attempted to estimate the magnitude of k_2 and k_{ex} for fish (Gobas et al.1989; Qiao et al. 2000; Gobas et al. 1993; Drouillard et al. 2009), these studies have generally done so by assuming $k_{met} = 0$. A limited number of studies have attempted to characterize the magnitude of k_{met} relative to k_{tot} using a reference compound approach (Fisk et al. 2000; Buckman et al. 2004; Buckman et al. 2006). To date, there has been little to no empirical support to validate model predictions on the magnitude of fecal or gill elimination and to determine which of these diffusive loss mechanisms dominate PCB whole body losses from fish.

This thesis focused on characterizing a set of toxicokinetic parameters (dietary AE, k_{ex} and k_{met}) for a series of PCB congeners that range in hydrophobicity in a model fish species, Japanese koi (*Cyprinus carpio*). It is important to better understand these toxicokinetic parameters as they are critical parameters that have been incorporated in more advanced individual based and food web bioaccumulation models (Arnot and Gobas 2006). The emphasis in this thesis was placed on testing whether or not the above toxicokinetic parameters remain constant under different experimental conditions, as is commonly assumed when parameterizing fish bioaccumulation models to real world conditions. For example, Chapter 2 tested the consistency of AE across different experimental diet treatments. Chapter 3 tested the consistency of k_{tot}, k_{ex} and k_{met} over different dose treatments. Specific hypotheses and results for each chapter are summarized below, and this chapter also discusses the general implications of this thesis, and suggested areas of future research.

4.1 Chapter 2

Chapter 2 of this thesis focused on dietary AE and characterized the influence of food properties on this uptake parameter. Dietary uptake of PCBs is considered to be the most important exposure route (Gobas et al. 1989; Connolly and Pedersen 1988; Heath 1995; Baber 2008) and model sensitivity studies indicate that small errors in the estimated magnitude of AE can translate into large changes in model output (Iannuzzi et al. 1996; Ciavatta et al. 2009). Chapter 2 measured PCB AEs in Japanese koi fed two naturally contaminated diets (mayflies and ground up fish) and three artificial fish pellet diets spiked with PCB mixtures. The different diets also varied in their lipid contents. The specific hypotheses tested by Chapter 2 were:

2.1) Dietary assimilation efficiency of individual PCB congeners is controlled by chemical hydrophobicity

2.2) The relationship between dietary AE and chemical hydrophobicity is best fitted using a two-phase resistance model, as recommended by Gobas et al (1989):

$$AE = (AK_{OW} + B)^{-1}$$

2.3) Dietary assimilation efficiency of individual PCB congeners is independent of dietary lipid content.

2.4) Dietary assimilation efficiency of individual PCB congeners is similar between natural foods collected from contaminated sites and PCB-spiked artificial pellet fish diets.

Based on the results of Chapter 2, Hypothesis 2.1 was accepted. As shown in Figure 2.2, there was a highly significant relationship ($p<0.001$; ANOVA) between chemical K_{OW} and dietary AE for all experimental diets. However, the more specific hypothesis regarding the relationship between PCB AE and chemical hydrophobicity provided by hypothesis 2.2 was rejected. For each dietary treatment, a simple linear regression between dietary AE and chemical K_{OW} explained more variation among PCB AEs compared to the recommended two phase resistance model. The relatively poor performance of the two phase resistance model maybe related to the small K_{OW} range of congeners tested (log K_{OW} from 5.5 to 7.8). Inclusion of less hydrophobic, poorly biotransformed chemicals (i.e., log K_{OW} values approaching 4 or less) is necessary to evaluate inflection points in the AE versus K_{OW} relationship especially for the lower K_{OW} compounds. Gobas et al (1989) developed the two phase resistance model using a series of organochlorine compounds that exhibited log K_{OW} values ranging from 3 to 8. For PCBs, the minimum log K_{OW} is 4.46 (PCB 1; Hawker and Connell 1988) and there is some evidence that indicates fish can biotransform

monobiphenyls (Hutzinger et al. 1972). Environmentally relevant PCB congeners have a more restricted K_{OW} range compared to the full suite of possible compounds and commence at a log K_{OW} of approximately 5.25 (PCB 17; Hawker and Connell 1988). Thus, when restricting the predictive AE algorithm to environmental PCBs, a linear regression equation appears to provide a better predictive relationship than the two phase resistance model suggested by Gobas et al (1989). It should be noted, however, that both the linear and two phase resistance models provided overall poor descriptions of the total variation in the data. The linear regression models only described 14 to 44% of the variation in the data for a given dietary treatment. Clearly, other factors are influencing the within diet congener specific dietary AEs apart from chemical hydrophobicity and these factors need to be explored further.

Hypothesis 2.3 was accepted as there was no significant relationship between PCB AE and dietary lipid content. It is clear from the data of Chapter 2 that other factors had a strong influence on modulating the between diet PCB AEs. Fish fed benthic invertebrates had significantly higher PCB AEs across the individual PCB congeners compared to the other groups ($p<0.001$), while there were no significant differences among the other groups ($p>0.135$, Tukey's HSD). The low lipid diet and benthic invertebrate diet had similar fat contents but exhibited an average of 36% difference in their respective PCB AEs, while the difference in AEs between high lipid diet (22%) and low lipid diet (6%) was only 9%.

Hypothesis 2.4 was also accepted as there were no systematic differences in dietary AEs determined in fish fed one of the naturally contaminated diets compared to fish fed artificially prepared diets. The AEs between fish fed ground up forage fish and pellet food were not significantly different from one another ($p>0.135$, Tukey's HSD). However, there was a significant difference between PCB AEs of fish fed the benthic invertebrate diet versus

the artificial diet groups ($p<0.001$, Tukey's HSD), as well as those fed the forage fish diet ($p<0.001$, Tukey's HSD). Past investigators have argued that diets prepared by sorbing PCBs onto the food matrix may result in lower bioavailbility compared to diets that have incorporated PCBs into lipids by normal bioaccumulative processes (Nichols et al. 2001; Burreau et al. 1997). The above investigators criticized the wide spread use of artificial pellet diets and practice of adding PCBs to such diets by spiking the food when conducting experiments designed to quantify dietary AEs for fish. Based on the equivalent AEs between fish fed naturally contaminated forage fish compared to spiked pellet formulations, such criticism would appear unwarranted.

However, the observation that the two natural diets (benthic invertebrates and ground up fish) exhibited significant differences from one another was an unexpected result and indicated that dietary AEs can vary widely among different types of natural diets. This is the first study to demonstrate differences in PCB AEs in fish fed different naturally contaminated diets. At present, it is not known why Japenese koi were able to assimilate PCBs so efficiently from the mayfly diet. Common carp and related species in this genus normally consume a high proportion of benthic invertebrates in their wild diet (Scott, W.B. and E.J. Crossman) and it is possible that these species are adapted to maximize nutrient absorption from such diet types. However, more research would be required to quantify differences in PCBs and relate such differences to diet digestability of proximate components in order to better understand such processes.

4.2 Chapter 3

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Chapter 3 of this thesis investigated the roles of fecal elimination and metabolic biotransformation to whole body elimination of individual PCB congeners in Japanese koi. The study used a depuration experimental design and was further replicated at three dosing levels to verify first order kinetics. The following specific hypotheses were tested as part of the Chapter 3 study design:

3.1) Whole body PCB elimination by fish is a first order kinetic process and PCB elimination rate coefficients are independent of chemical concentration in animal tissues

3.2) Whole body PCB elimination rate coefficients are dependent on chemical hydrophobicity.

3.3) Metabolic biotransformation of PCBs does not occur or occurs to a negligible extent as determined by a reference compound approach and structure activity relationships described for other vertebrates.

3.4) Fecal PCB elimination rate coefficients exhibit a lower magnitude than whole body elimination rate coefficients for congeners having log K_{OW} values < 6.5.

3.5) Fecal elimination PCB rate coefficients approach the magnitude of whole body elimination rate coefficients for congeners having log K_{OW} values > 6.5.

Hypothesis 3.1 was accepted: The whole body PCB elimination rate coefficient, k_{tot}, was consistent with first order kinetics since it was independent of the dosing level ($p=0.679$, ANCOVA) and also observed to be log linear over time (Figure 3.1).

Hypothesis 3.2 was also accepted. As shown in Figure 3.2, there was a decreasing trend of whole body PCB elimination rate coefficients, k_{tot}, with increasing chemical K_{OW}. This relation was found to be significant in all three dosing levels ($p<0.001$, ANOVA).

Hypothesis 3.3 was rejected. PCBs categorized as labile, i.e. those with vicinal hydrogen substitutions at *meta* or *para* sites on at least one of the phenyl rings in the PCB molecule, had significantly (p<0.0001, ANCOVA) higher k_{tot} values compared to PCB congeners categorized as recalcitrant reference compounds. The above observation occurred for all three dosing levels. Labile categorized PCBs were eliminated between 1.62 and 1.88 fold faster compared to recalcitrant PCBs after considering for differences in congener specific hydrophobicity. These results reaffirm conclusions from other studies (Buckman et al. 2006; Buckman et al. 2007; Li et al. 2003; Mehrtens and Laturnus 1999; Dio et al. 2006) that fish possess some capability to biotransform PCBs according to similar structure activity relationships as described for amphibians (Leney et al. 2006) and birds (Drouillard et al. 2001). The results also indicate that k_{met} needs to be considered when evaluating the relative roles of gill and fecal elimination to whole body elimination of PCBs in fish.

Hypothesis 3.4 and Hypothesis 3.5 were rejected: As shown in Figure 3.4, whole body elimination rate coefficients were 5 to 20 fold higher than the fecal elimination rate coefficients for PCBs across the range of hydrophobicity tested. For recalcitrant categorized PCBs, fecal elimination accounted for an average of 9.1% of the whole body elimination, indicating that approximately 91% of the whole body elimination was attributed to losses through the gills. For labile congeners, fecal elimination accounted for an average of 5% of whole body elimination, while approximately 52% and 43% of whole body elimination were attributed to losses through gill and biotransformation, respectively.

Chapter 3 was the first empirical study to quantify the relative importance of different elimination routes: gill diffusion, fecal depuration and metabolic biotransformation to whole body PCB losses in fish. The results were not consistent with previous modeling efforts,

which concluded that fecal elimination dominates whole body PCB losses for highly

hydrophobic congeners (low K_{OW} > 6.5; Gobas et al. 1989). Even for the most hydrophobic

PCB congeners (e.g. PCB 194), gill elimination dominated total elimination under the

experimental conditions of this study. Gill and fecal elimination are both subject to changes

in gill ventilation and feeding rates in response to environmental temperature and animal

metabolic rates. Recent modeling studies suggest that gill transfer efficiencies may change

as a function of temperature under conditions of high gill ventilation rates (Drouillard et al.

2009). Similarly, metabolic biotransformation of PCBs by fish appears to be influenced by

water temperature (Buckman et al. 2007). The effect of temperature on the importance of

different elimination routes was not tested in Chapter 3. However, given the conclusion that

gill and metabolic biotransformation (labile categorized PCBs) appear to dominate PCB

elimination by carp, however, further experiments to understand the temperature dependence

of these processes are warranted.

4.3 Implications and future research

The research included in this thesis has several important implications for our

understanding and current practices of quantitative modeling of organic contaminant

bioaccumulation in fish. Chapter 2 reaffirmed that PCB AEs decrease with increasing

chemical hydrophobicity and such observations were made for all diet types tested. Chapter

2 also demonstrated that the mechanism of incorporating chemical into the food matrix

(spiking food vs. natural incorporation of PCBs into living diet items) did not influence

measurements of chemical AE. However, it was revealed that different types of diet can

contribute to a high degree of variation of chemical AEs. These across diet differences in

PCB AEs were not related to the neutral lipid content of the diet, but may be related to other factors such as diet and energy digestability of proximate components such as proteins and lipids. Other factors such as the effect of digesta residence times and how this varies across diet types cannot be ruled out. While most fish bioaccumulation models account for the effect of chemical hydrophobicity on chemical AE, none of the common bioaccumulation models utilized today consider how diet type affects the magnitude of chemical AE. In Chapter 2, the amount of variation in PCB AEs associated with different diet types was as large as that contributed by congener hydrophobicity. Model sensitivity studies indicate that variation in AE has a major influence on model outputs (Iannuzzi et al. 1996; Ciavatta et al. 2009). Most fish species undergo ontogenetic diet shifts over their life history and also exhibit considerable omnivory over seasonal cycles. Thus, there is a need to determine how differences in PCB AEs across diets that bound the natural range of diet items ingested by a given bioindicator species influence fish exposures to persistent organic contaminants.

In Chapter 3, indirect evidence of PCB biotransformation was produced using a reference compound approach that separately categorized PCB congeners into labile and recalcitrant groups based on structure activity relationships developed for PCB biotransformation in vertebrates. Labile categorized PCBs, which are considered to be degraded by CYP2B type activity (Drouillard and Norstrom 2003; Leney et al. 2006; Buckman et al. 2007), exhibited between 1.62 to 1.88 fold faster elimination compared to recalcitrant congeners. However, elimination of labile PCBs was not dependent on dose level and among other vertebrates, CYP2B-like enzymes do not appear to be readily inducible by persistent organic pollutants in the same manner as CYP1A-type enzymes, which are induced in response to planar aromatic hydrocarbons (Ronis and Walker 1989; Buckman et al. 2006).

Because Chapter 3 did not validate the production of PCB metabolites, the reference compound approach that was used can only be an indirect measure of PCB biotransformation by fish. Other studies have quantified hydroxyl-PCB metabolites in fish blood (Li et al. 2003; Buckman et al. 2006). Further research to quantify production rates of PCB metabolites in conjunction with whole body elimination of parent PCBs would be required to better characterize the magnitude of k_{met}.

A major assumption related to the reference compound approach used in Chapter 3 is that persistent categorized PCBs undergo little or negligible metabolic biotransformation. For these congeners, the difference between whole body and fecal elimination rate was attributed to gill elimination. It is possible that the gill elimination contribution could be overestimated if metabolic biotransformation of these compounds occurred especially given that whole body elimination rates exceeded fecal losses for all recalcitrant compounds studied. Further research to directly quantify gill elimination of PCBs from Japanese koi would be required in order to verify such an assumption. Quantifying the gill elimination of hydrophobic chemicals remains technologically challenging, although use of metabolism chambers in conjunction with solid phase extraction of water that has passed through the gills, as described by Fitzsimmons et al. (2001), represents one possibility for future research consideration. Further studies are needed in both qualifying and quantifying PCB biotransformation in fish. Comparisons should be made between different species under similar environmental conditions to investigate the difference in biotranformation capacity across species as well as to characterize temperature-dependent biotransformation effects. These types of studies could potentially improve fish bioaccumulation model predictions, particularly as they relate to congener-specific predictions.

Finally, the role of fecal egestion was explored by evaluating the magnitude of k_{eg} estimated by determining the animal/feces PCB distribution (K_{BEX}) ratio and fecal egestion rate (G_{ex}). In practice, the empirically-measured K_{BEX}, which is equal to $1/K_{EX}$ (Chapter 3), incorporates both a partition coefficient and the fecal transfer efficiency term (AE; Eq. 5). Additional measurements to determine the actual feces/carcass partition coefficient using vial equilibration techniques as described by Gobas et al (1999) and/or Drouillard et al (2001) would be required to independently determine the equilibrium K_{BEX} value. The difference between the equilibrium K_{BEX} and empirically-measured K_{BEX} determined in Chapter 3 could then be used to establish the fecal transfer efficiency term (AE). This type of study would be useful to evaluate the common assumption that the dietary AE (Chapter 2) is equivalent to the fecal transfer efficiency term. Similarly, as observed in Chapter 2, different diet types would be expected to produce different fecal transfer efficiencies that ultimately influence the magnitude of k_{ex}. Chapter 3 represents the first study to directly quantify PCB k_{ex} in fish and compare it to the magnitude of k_{tot}. Future studies should focus on evaluating the variability of k_{ex} as it relates to different diet properties and relating such variation to dietary assimilation efficiencies. These studies could be useful to provide additional insights into factors that control the maximum biomagnification potential in fish (Drouillard 2008).

4.4 References

Andersson PL, Berg AH, Bjerselius R, Norrgren L, Olsen H, Olsson PE, Orn S and Tysklind M. 2001. Bioaccumulation of selected PCBs in zebrafish, three-spined stickleback, and arctic char after three different routes of exposure. *Arch Environ Contam Toxicol* 40: 519-530.

Arnot JA, Gobas FAPC. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ Toxicol Chem* 23: 2343–2355.

Barber MC. 2008. Dietary uptake models used for modeling the bioaccumulation of organic contaminants in fish. *Environ Toxicol Chem* 27: 755–777.

Bruggeman WA, Opperhuizen A, Wijbenga A, Hutzinger O. 1984. Bioaccumulation of super-lipophilic chemicals in fish. *Toxicol Enivorn Chem* 7: 173-189.

Buckman AH, Brow SB, Hoekstra PF, Solomon KR, and Fisk AT. 2004. Toxicokinetics of three polychlorinated biphenyls technical mixtures in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 23: 1725-1736.

Buckman AH, Wong CS, Chow EA, Brown SB, Solomon KR and Fisk AT. 2006. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. *Aquat toxicol* 78:176-185.

Buckman AH, Brown SB, Small J, Solomon KR and Fisk AT. 2007. Role of temperature and enzyme induction in the biotransformation of polychlorinated biphenyls and bioformation of hydroxylated polychlorinated biphenyls by rainbow trout (*Oncorhynchus mykiss*). *Environ Sci Technol* 41: 3856-3863.

Burreau S, Axelman J, Broman D, Jakobsson E. 1997. Dietary uptake in pike (*Esox lucius*) of

    some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated

    diphenyl ethers administered in natural diet. *Environ Toxicol Chem* 16: 2508–2513.

Ciavatta S, Lovato T, Ratto M, Pastres R. 2009. Global uncertainty and sensitivity analysis of

    a food web bioaccumulation model. *Environ Toxicol Chem* 28: 718–732.

Connolly JP, Pedersen CJ. 1988. A thermodynamic-based evaluation of organic chemical

    accumulation in aquatic organisms. *Environ Sci Technol* 22:99–103.

Doi AM, Lou Z, Holmes E, Venugopal CS, Nyagode B, James MO and Kleinow KM. 2006.

    Intestinal bioavailability and biotransformation of 3,3',4,4'-tetrachlorobiphenyl (CB

    77) in in situ preparations of channel catfish following dietary induction of CYP1A.

    Aquat Toxicol 20: 33-42.

Drouillard KG, Norstrom RJ. 2003. The influence of diet properties and feeding rates on

    PCB toxicokinetics in the ring dove. *Arch Environ Contam Toxicol* 44:99–106.

Drouillard KG, Fernie K, Smits JE, Bortolotti, Bird DM, and Norstrom RJ. 2001.

    Bioaccumulation and toxicokinetics of 42 PCB congeners in American kestrels

    (*Falco sparverius*). *Environ Toxicol Chem* 20: 2514-2522.

Drouillard KG. 2008. Biomagnification. In: Jorgensen SE, Fath BD (Eds). Ecotoxicology

    Vol. 1 of Encyclopaedia of Ecology, vol. 5. Elsevier, Oxford, pp, 441-448.

Drouillard KG, Paterson G, Haffner GD. 2009. A combined food web toxicokinetic and

    species bioenergetic model for prediction seasonal PCB elimination by yellow perch

    (*Perca flavescens*). *Environ Sci Technol* In press.

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG. 1998. Dietary accumulation and

    depuration of hydrophobic organochlorines: Bioaccumulation parameters and their

relationship with the octanol/water partition coefficient. *Environ Toxicol Chem* 17:951–961.

Fisk AT, Tomy GT, Cymbalisty CD, and Muir DCG. 2000. Dietary accumulation and quantitative structure-activity relationship for depuration and biotransformation of short (C_{10}), medium (C_{14}) and Long (C_{18}) carbon-chain polychlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 19: 1508-1516.

Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC and Nichols JW. 2001. Branchial elimination of syperhydrophobic organic compounds by rainbow trout (*Oncorhynchus nykiss*). *Aquat Toxicol* 55:22-34.

Gobas FAPC, Muir DCG, Mackay D. 1988. Dynamics of dietary bioaccumulation and fecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.

Gobas FAPC, McCorquodale JR, Haffner GD. 1993. Intestinal absorption and biomagnification of organochlorines. *Environ Toxicol Chem* 12:567–576.

Gobas FAPC, Clark KE, Shu WY, Mackay D. 1989 Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and elimination into the feces. *Environ Toxicol Chem* 8:231–245.

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133–141.

Hawker, D. W. and Connell, D. W. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ Sci Technol* 22: 382-387.

Heath AG. 1995. *Water pollution and fish physiology* CRC Press, Boca Raton, FL, USA

Hutzinger O, Nash DM, Safe S, DeFreitas ASW, Norstrom RJ, Wildish DJ, and Zitko V.

    1972. Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons,

    Rats and Brook Trout. *Science* 178: 312-314.

Iannuzzi TJ, Harrington NW, Shear NM, Curry CL, Carlson-Lynch H, Henning MH, Su SH,

    Rabbe DE. 1996. Distributions of key exposure factors controlling the uptake of

    xenobiotic chemicals in an estuarine food web. *Environ Toxicol Chem* 15: 1979–1992.

Leney JL, Drouillard KG and Haffner GD. 2006. Dose metamorphosis increase the

    susceptibility of frogs to highly hydrophobic contaminants? *Environ Sci Technol* 40:

    1491-1496.

Li H, Drouillard KG, Bennett ER, Haffner GD and Letcher RJ. 2003. Plasma- associated

    halogenated phenolic contaminants in benthic and pelagic fish species from the

    Detroit River. *Environ Sci Technol* 37: 832-839.

Mehrtens G and Laturnus F. 1999. Mixed function oxidase dependent biotransformation of

    polychlorinated biphenyls by different species of fish from the North Sea.

    Chemosphere 38: 2995-3002.

Nichols JW, Fitzsimmons PN, Whiteman FW, Kuehl DW, Butterworth BC, Jenson CT. 2001.

    Dietary uptake kinetics of 2,2_,5,5_-tetrachlorobiphenyl in rainbow trout. *Drug*

    Metab Dispos 29:1013–1022.

Paterson, G, Drouillard KG, and Haffner GD. 2007. PCB elimination by yellow perch (*Perca*

    flavescens) during an annual temperature cycle. *Environ Sci Technol* 41: 824-829.

Qiao P, Gobas FAPC, Farrell AP. 2000. Relative contributions of aqueous and dietary uptake

    of hydrophobic chemicals to the body burden in juvenile rainbow trout. *Arch Environ*

    Contam Toxicol 39:369–377.

Ronis MJ and Walker CH 1989. The microsomal monooxygenases of birds. *Rev Biochem Toxicl* 10: 301-384.

Scott, W.B. and E.J. Crossman; Freshwater Fishes of Canada, Galt House Publications, Oakville, ON, Canada, 966 pp

Sijm DTHM, Seinen W, Opperhuizen A. 1992. Life-cycle biomagnification study in fish. *Environ Sci Technol* 26: 2162-2174.

Thomann RV, Connolly JP, Parkerton TF. 1992. A equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ Toxicol Chem* 11: 615-629.

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