Factors regulating the exposure dynamics of polychlorinated biphenyls in two fish species, Oncorhynchus mykiss and Pimephales notatus.

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FACTORS REGULATING THE EXPOSURE DYNAMICS OF POLYCHLORINATED BIPHENYLS IN TWO FISH SPECIES, ONCORHYNCHUS MYKISS AND PIMEPHALES NOTATUS.

by

Susan Ann Coristine

A thesis submitted to the Faculty of Graduate Studies and Research through the Department of Biological Sciences in Partial Fulfillment of the requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

1995

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This work is dedicated to all my boys, Ron, Brennen, Cameron, and Aaron, for their patience and understanding, without which this goal would have been unattainable.
Abstract

The aim of this research was to quantify the relative importance of biological and chemical properties in regulating the exposure dynamics of organic chemicals in aquatic ecosystems. This was achieved by 1) calibrating the bluntnose minnow (*P. notatus*) as a biomonitor, 2) examining the influence of chemical properties such as hydrophobicity and substitution patterns on chemical elimination rates and 3) examining the kinetics of the more toxic non-ortho PCBs. Elimination rate constants ($k_2$) for bluntnose minnow ranged from 0.00987 to 0.00116 (ug/kg/day) for compounds with octanol-water partition coefficients varying from 5.69 to 7.80. It was further determined that substitution patterns in PCBs, as well as $K_{ow}$, were important factors regulating chemical kinetics in rainbow trout. Multivariate analysis of covariance revealed a highly significant interaction between chlorine substitution pattern and time ($p<0.001$), suggesting that substitution pattern influences elimination kinetics. A significant relationship ($p<0.001$) was also observed between elimination rate constants and octanol-water partition coefficients (log $K_{ow}$). The non-ortho (coplanar) congeners, IUPAC # 81 and # 77 had the highest elimination rate constants of 0.0062 and 0.0090 (ug/kg/day) respectively, significantly faster than other tetrachlorobiphenyls present. The $k_2$ values for rainbow trout (*Oncorhynchus mykiss*) ranged from 0.0030 to 0.0090 (ug/kg/day) for congeners with log
$K_{ow}$ values varying from 5.85 to 7.42. A closer examination of the kinetics of the toxic coplanar congeners in bluntnose minnow revealed a range in uptake rate constants ($k_1$) of 8.2, 5.3, and 3.02 (ug/kg/day) for congeners #77, #126, and #169 respectively. Elimination rate constants ($k_2$) and bioconcentration factors for congeners #77, #126, and #169 were 8.2, 5.3, and 3.02 (ug/kg/day), and 2500, 1220, and 960 respectively.
ACKNOWLEDGEMENTS

I would like to express my appreciation to Rodica Lazar for her patience and analytical talents, to all the various G.L.I.E.R summer students who were on the other (deep) end of the seine net, and most of all to my mentor and friend Douglas Haffner. Thank you Doug for your guidance, your love for discovery and your unwavering support.
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CHAPTER ONE

Introduction

To understand the development of ecotoxicology as a science it is important to recognize the history behind the chemical assault on the environment. The legacy of chemical use and dependency has saddled future generations with the costs of clean up as well as failing health and a stress on the life supporting characteristics of the planet.

This human dependency on synthetic convenience is presently displayed in a myriad of concerns such as ozone depletion over both poles of the earth. Mounting evidence couples this overall thinning with increased occurrences in skin cancers. Acid rain is another example that shows the general lack of responsibility industry had toward its role in maintaining harmony in the surrounding ecosystem (Person 1989). Sulfur dioxide emissions coupled with water vapour in the air create acid deposition resulting in acid shock and subsequent death of many northern lakes incapable of buffering the acid inputs.

DDT, once thought of as a miracle compound, decreasing the incidence of malaria and increasing crop productivity is now seen as one cause in the decrease of whole populations of birds, due to eggshell thinning and teratogenic effects in offspring (Person 1989).
DDT is just one example of the many organochlorines that have been synthesized in the past 50 years. A book entitled ‘Silent Spring’ (1962) written by Rachael Carson publicized the toxicity of DDT and other pesticides. Although challenged by industry and government, it was this book that enlighten the public to the potential and very real dangers that organochlorines can inflict on wildlife and ultimately human life. These molecules are synthesized along a carbon backbone with chlorine atoms substituted onto the carbon atoms in place of hydrogens. These synthetic chemicals tend to persist in the environment and create a variety of problems when biological systems are exposed to them.

An organochlorine group of considerable environmental importance is the polychlorinated biphenyl (PCB) family. PCBs were first commercially synthesized in the 1920’s and were later mass produced in the 1940’s for over 20 years. De Voogt (1987) estimated that over 1.5 million metric tonnes of PCB were produced, and only a third of this production has entered the environment (Tanabe et al. 1987). The PCB molecule consists of two conjugated phenyl rings with 1 to 10 chlorine atoms substituted around these biphenyl rings. Due to the array of substitution patterns which may result, there are 209 possible isomers in the PCB family ranging from a biphenyl to a hexachlorobiphenyl. The physical properties of these compounds allowed for their diverse application. Low flammability and high thermal stability allowed their use in heat transfer fluids and lubricants. The high electrical resistance and favourable dielectric
constants benefitted their use in capacitors and transformers. Resistance to acids, oxidation, alkalis, and hydrolysis rendered them useful for plasticizers and adhesives (Poland et al. 1982). However, these same chemicals with their physical properties that industry favoured, stability and low water solubility, resulted in a variety of unfavourable responses from the environment.

In the late 1960's, environmental effects were beginning to become prevalent. Declines in bird populations, particularly those situated around the Great Lakes region, became apparent. Birds are especially sensitive to toxic chemicals due to their high basal metabolic rate and low body fat (Carson 1962). Those birds which were colonial, fish eating, and/or scavengers showed significant declines in population size, including the Peregrine Falcon (Hickey et al. 1969), the American Bald Eagle (Broley 1958), and the double-crested cormorant (Gilbertson et al. 1991). It is interesting to note that PCB production and use in open systems was ceased in 1972 (Frank et al. 1993), approximately twelve years after PCBs were first implicated in the occupation of toxic effects in Great Lakes wildlife.

PCBs elicit numerous toxicological responses both in vitro and in natural populations. The 209 PCB congeners have slightly different physical properties due to the number and placement of substituted chlorine atoms. The more chlorine atoms substituted on the biphenyl molecule, the more hydrophobic the molecule becomes (Gobas 1987). Each of these 209 congeners has been designated a numerical name according to the International Union of Pure and
Applied Chemistry (IUPAC) standard notation (Ballschmiter and Zell 1980). PCBs with the same number of chlorine atoms are given a common numerical prefix, such as ‘tetra-chlorinated biphenyl’ for molecules substituted with four chlorines. The mono to tetra chlorinated PCBs have been shown to be more highly metabolized, unlike the penta to deca PCBs which resist metabolism and persist in biological systems (Sundstrom et al. 1976, Safe et al. 1985, Poland and Knutson 1982).

A commonly used measure of a chemical’s hydrophobicity is the octanol water partition coefficient ($K_{ow}$). For PCBs the $K_{ow}$ increases with the number of chlorines on a molecule, and it is measured at equilibrium as the

$$K_{ow} = \frac{[\text{concentration of chemical in octanol}]}{[\text{concentration of chemical in water}]}$$

where octanol is used as a surrogate of lipid. Gobas et al. (1987) observed $K_{ow}$ to be an effective predictor of bioconcentration potential for those compounds with log $K_{ow}$’s up to 6. After this point a breakdown in the relationship occurs. Within the literature, there also appears to be a vague relationship between the $K_{ow}$ of the compound and the observed toxicity. Higher $K_{ow}$ compounds tend to accumulate in target tissues (those tissues which have a high affinity for chemical, i.e lipids, receptor sites) and are also associated with greater toxicity in organisms (Tanabe et al. 1987).
Substitution patterns are another chemical property highly associated with toxicity. A very strong relationship between toxicity and substitution pattern has been observed in both *in vivo* and *in vitro* studies (Safe et al. 1985, Tanabe et al. 1987, Kubiak et al. 1989). There are three substitution patterns on the biphenyl rings, ortho, meta, and para. Substitution of more than one chlorine atom in the ortho position creates a large sterically hindered molecule (Figure 1.1). The greater the number of ortho substitutions, the more contorted and bulky the molecule becomes. Substitution into the meta and para positions only, produces a more flattened, streamlined molecule, after referred to as ‘coplanar’. The coplanar structure results in different chemical behaviours in the environment (Koslowski 1994), in toxicological endpoints among different organisms (Safe 1991), and even in different target sites within an organism.

There are three main areas of toxicological concern associated with the PCB family: neurotoxicity, reproductive and teratogenic toxicity, and activation of the cytochrome P450 mono-oxygenase and monoclonal antibody systems. These are not mutually exclusive effects, for the homeostatic balance within organisms may be disrupted by the incapacitation of any one of its systems as a result of the presence of many different congeners at the same time. It is not possible to correlate any particular $K_{ow}$ with these effects. However, based on the literature there appears to be a distinction between the effects that ortho-substituted congeners generate versus effects of non-ortho substituted (coplanar) congeners.
Figure 1.1

Ortho, meta, and para substitution positions.
Similar neurotoxic responses have been observed among many different species, including *Homo sapiens*. During the 1970’s considerable research was focussed on cytochrome P450 monooxygenase systems and how certain PCB congeners induced this system (Poland et al. 1982, Safe 1987). Further interest was developed on the effects PCBs caused in humans after two unfortunate incidents of contamination in cooking oil (Yusho and Yushang). Both incidents revealed that contaminants were transferred from the mother through the placenta to the fetus, causing deleterious effects in the infant at birth. Shorter gestation periods were observed and were accompanied by reduced birth weights as well as behavioural and learning disfunctions in the newborns of contaminated mothers (Seegal et al. 1987). Tilson et al. (1990), in a cross species (rat, dog, rhesus monkey, mouse) comparison of PCBs and their effects on the developing nervous system, observed similar shortened gestation periods, lighter birth weights and decreased litter size. Depending on the dose of Aroclor 1254 (a commonly used commercial mixture of PCBs) that was given to the mother, young belonging to highly dosed mothers exhibited spinning syndrome (continual circular motion in one direction). Common to all prenatally exposed young, behavioural learning tests showed decreases in active avoidance as well as decreases in judgement and reasoning. Seegal et al. (1989) observed a dose dependant relationship with increasing PCB concentration resulting in decreased cellular dopamine levels and norepinephrin concentrations over time. A series of Aroclor 1254 dosing experiments
conducted on the pig-tailed macque (*Macaca nemestrina*) revealed that nearly 100 percent of the PCB congeners in the brain were specifically 2,4,4’ trichlorobiphenyl (IUPAC # 28), 2,2’,5,5’-tetrachlorobiphenyl (IUPAC # 52) and 2,2’,4,4’ tetrachlorobiphenyl (IUPAC # 47). These were shown to be concentrated in the substantia nigra region of the brain which is also the dopamine centre of the brain (Dahlsom *et al.* 1964). These congeners were synergistic in their ability to decrease concentrations of dopamine. Dopamine balance is important to the normal development of a newborn and an imbalance is associated with spinning syndrome (Dankova *et al.* 1978).

Ecologically, any stress affecting the birth rate of a population can have profound effects on its success. Therefore congeners which induce teratogenic effects or stress the reproductive process of populations, can regulate population size. Coupled with the compounded changes in community dynamics, PCB exposure can result in drastic decreases in whole populations (Morarity 1990). Reproductive and teratogenic effects have been observed in a variety of bird populations resident to the Great Lakes region. Congenital bill deformities such as cross bills have been observed in the common tern (*Sterna hirundo*) (Gochfeld 1975), herring gull (*Larus argentatus*), and double-crested cormorant (*Phalacrocorax auritus*) (Kubiac *et al.* 1989). Such toxicological stress reduces the bird’s ability to obtain sufficient food to reproduce let alone acquire sufficient energy for body maintenance. Lengthened incubation periods, infant mortality and nest abandonment were observed in Forster’s
Terns (Kubiak et al. 1989). Caspian terns, in a heavily contaminated area of Saginaw bay, experienced reduced hatching success and no survival past fledgling (Tillit et al. 1991). Eggshell breakage, assumably associated with egg shell thinning, was observed in double-crested cormorants (Phalacrocorax auritus) (Gilbertson et al. 1991). Analysis of bird tissue and eggs revealed unusually high concentrations of coplanar PCBs. Unusually high female to male ratios possibly resulting from embryo feminization and/or excessive male mortality has been observed in herring gulls (Gilbertson et al. 1991). In mink (Mustela vison) the presence of coplanar and di-ortho substitution groups resulted in embryotoxicity and reduced neonate weights. The impairments observed were a proposed result of the partial estrogenic actions of these chemicals causing imbalances in progesterone concentrations as well as progesterone receptor imbalances (Patnode and Curtis 1994). Mink are considered to be appropriate mammalian models for reproductive toxicity, due to their status as high-trophic-level consumers in both aquatic and terrestrial systems.

The third category of effects observed in organisms exposed to PCBs are those associated with the induction of the Cytochrome P-450 mono-oxygenase system. This system is thought to play a key role in xenobiotic and endobiotic metabolic processing of fatty-acids, steroids, drugs, carcinogens, mutagens etc. (Poland and Knutson 1982). The induction process begins with the binding of a chemical to the Ah receptor site in the cytoplasm of the cell. The activated
receptor complex moves into the nucleus and binds to a recognition site on the DNA causing transcription for specific genes and subsequent translation of corresponding proteins resulting in a variety of biochemical responses (Dufresne 1987). This is a multi-isozymic system in which the different isozymes - 'vern the type of substrate that will be accepted and hence the alternate metabolic routes producing different end pathological consequences (Gelboin 1994). The end result produces metabolites which are detoxified by conjugation and excreted, or metabolites which are mutagenic or carcinogenic alkylating critical cellular macro-molecular targets such as DNA (Safe 1992). Two classes of mixed function oxidase inducers most commonly associated with PCBs are the Phenolbarbitol (PB) type and the 3-Methylcholanthrene (MC) type. Each of these is subsequently related to several monooxygenase dependant enzyme activities. Phenolbarbitol tends to induce the CYP2B1 (P-450b) and the CYP2B2 (P-450e) while 3-Methylcholanthrene induces the CYP1A1 (P-450c, EROD) and CYP1A2 (P-450d, AHH), and both induce the CYP2A1 (P-450a) (Safe 1992). Aroclor mixtures have been shown to induce all five of these systems (Safe 1992).

The P,450 aryl hydrocarbon hydroxylase (AHH) cytosolic receptor site is located primarily in the liver. It is highly specific to the planar halogenated aromatic hydrocarbons (HAH). The most toxic HAHs are the 2,3,7,8,-tetrachloro-p-dibenzo-dioxin (2,3,7,8-TCDD) and its dibenzo-furan counterpart (2,3,4,7,8-TCDF) (Poland and Knutson 1982) (Fig.1.2). The 2,3,7,8-TCDD
Figure 1.2

Comparison of the structure of the coplanar PCB molecules to that of 2,3,7,8-Tetrachloro-p-dibenzo dioxin (TCDD).
#77

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

#126

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

#169

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

\[\text{2,3,7,8-TCDD}\]

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]
isomer is known to be the most potent inducer of the MC type mixed function oxidase system (Poland and Knutson 1982, Safe 1987). A group of PCBs which are structurally similar to this compound are the non-ortho or coplanar congeners. Because they are relatively flat, they have a similar stereochemistry to TCDD and TCDF (Figure 1.2).

A commonly used measure of dioxin like toxicity of PCBs is the Toxic Equivalent Factor (TEF). The TEF is a measure of relative potencies of chemicals to induce the AHH or EROD systems as based on in vitro bioassays using rat and mouse cell lines (H4IIIE and Hepa clone 4) (Safe 1987). Since 2,3,7,8-TCDD is the most potent inducer, it is used as the reference standard against which all other environmental contaminants are compared. Smith et al. (1990) tested a variety of environmental matrices (eg. fish, bird eggs) for levels of AHH and EROD induction. They observed the following orders of potency, TCDD > PCB 126 > PCB 169 > PCB 77 for both the AHH and EROD assays. Actual TEF values vary depending on the time of response, duration of treatment, and strain or species of animal used. Safe (1991) determined TEF values for most of the coplanar PCBs using the rat hepatoma H-4-II E cell line. He observed a similar order of toxicity TCDD > 126 > 169 > 77. These TEFs are employed in Toxic Equivalence (TEQ) assessments. TEQs are determined by multiplying the TEF of a chemical by its concentration in the environmental matrix and then summed to produce a total TEQ.

The structure-activity relationship among PCBs and their affinity for the
AHH cytosol receptor, show a positive correlation with their ability to produce a variety of toxic responses. This suggests that the capacity of a congener to elicit a toxic response from AHH system indicates the potential for that chemical to produce the definitive toxic response (Poland and Knutson 1982). Effects associated with these congeners include mass wasting syndrome, thymic atrophy, subcutaneous edema, immune suppression, and hormonal alterations (Tanabe et al. 1987). An approximate 20 percent decrease in thymus size was observed in rats, pigs, rabbits, monkeys and mice when exposed to Aroclor mixtures (Poland and Knutson 1982, Safe 1987). Damage to the hepatic area has been documented for a large number of species exposed to coplanar PCBs. In rats, the liver lobes increased in size, accompanied by lipid accumulation, development of large multinucleated cells, and pigment deposition (Poland and Knutson 1982, Muirk et al. 1991).

Exposure Dynamics

In today’s industrial world, there is an ever growing increase in the number and variety of foreign chemical compounds that are being introduced into the environment, which makes it difficult to predict the toxic effects. The route taken through the environment, and the uptake into the organism’s tissues along with elimination rates are important parameters of toxicological exposure. This emphasizes the importance of understanding the exposure dynamics of chemicals and the role this understanding will play in predicting environmental
impact.

Chemicals such as PCBs are commonly released into the air and water compartments. Their transport in the air compartment is governed by their vapour pressures which range from $10^{-4}$ to $10^{-11}$, classifying these chemicals as semi-volatile organic compounds (SOCs) (Bidleman 1988). Depending on the air temperature, PCBs occur in aerosol (gaseous) or particulate form. The net flux of PCBs between the atmosphere, and earth's surface and water occur by rain and snow scavenging of both aerosol and particle PCBs, dry-particle deposition, and exchange of vapour across the air-water interface (governed by Henry's law constants). Approximately 90% of the atmospheric PCB burden exists in the vapour phase (Junge 1980). It has been suggested that input of PCBs by dry and wet deposition is a major source of contamination to the Great Lakes watershed (Eisenriech 1981). Atmospheric transport and deposition accounts for possibly 60-90% of total inputs to these Great Lakes with high surface areas such as Lake Michigan and Lake Superior (64% of total basin area is comprised of the surface area) (Muir et al. 1988).

PCBs existing in the particulate phase occur in the form of Aitken nuclei (produced by gas-to-particle conversion), and coagulated Aitken nuclei. These complexes are much lighter than the PCB aerosols therefore they do not undergo rapid gravitational settling, and are only slowly removed by wet and dry deposition (Bidleman 1988). This is an important source of PCB contamination for far reaching areas due to the high atmospheric retention time.
which allows these particles to be transported great distances. PCBs in particular are manufactured in heavily industrialized centres yet have been shown to occur in such far reaching sites as the Arctic, Antarctica, and Northwest Territories (Addison et al. 1986, Calamarl et al. 1991). Dispersal of PCBs from industrialized centres is a result of the intrinsic nature of the weather patterns. Volatilized PCBs are translocated up and northward by air fluxes originating in the temperate and tropical regions. Depending on the Henry’s law constants of the PCBs and the ambient air temperature, wet and dry deposition will result in the contamination of sites unfamiliar to PCB production. PCBs have been discovered in foodwebs indigenous to these nonindustrialized regions such as the Arctic and its marine food chain. Measurable quantities of PCBs were found in arctic cod (Boreogadus saida), ringed seals (Phoca hispida) and polar bears (Urus maritimus) (Addison 1986, Muir et al. 1988).

Paracelsus stated that all substances have the potential to be toxic if they occurred in large enough doses. Therefore the dose makes the poison (Molarity 1987). The toxicity originally associated with PCBs was most often measured as acute toxicity, which required that unusually high doses be administered to the test organism. Those organisms in the environment which exhibit the more common chronic effects of PCBs, as discussed earlier, would have to be in contact with relatively high PCB concentrations such as point source locations to be similarly exposed. The greater the PCB dose, the greater
potential for the expression of acute and/or chronic effects. In other words, the
greater the exposure (the uptake and residence time) to PCBs the more severely
an organism is affected. Toxicity then is not just a function of active toxicity
at a target site within the organism, but must also take into account the
delivery (exposure) of chemical to the target site. This is dependant on the
amount of chemical the organism is being exposed to. Toxicity is therefore a
function of
Toxicity = activity (potency) x exposure.

Exposure dynamics refers to both transport and fate of a chemical when
it is discharged into the environment as well as the uptake and elimination of
the chemical by organisms. The environment itself may be compartmentalized
into air, water, and soil/sediments. Based on the laws of thermodynamics in
an ideal system, chemicals released into the environment will equilibrate among
the compartments. The concentration of chemicals in each compartment vary
based on Henry’s law constants, $K_{ow}$, and other properties which are related to
the physical chemistry of the molecules as well as the capacity of the compartment to hold the chemical. Once PCBs are released into the
environment, their partitioning among the compartments can be predicted by
their $K_{ow}$ (Connolly 1988). PCBs in the aqueous phase will partition to phases
with higher capacity such as particulate organic carbon in the water column,
particulate carbon in the sediment/soil phase, the atmosphere, and organisms.
The higher the $K_{ow}$, the stronger the tendency for the chemical to partition out of the water phase to the lipid phase of organisms. Therefore the original relative concentrations of PCB congeners in commercial mixtures are highly modified as congeners partition among the various phases of the environment. For example, an enrichment of the higher $K_{ow}$ congeners occurred in the tissue samples of birds in Saginaw bay when compared to both the original Aroclor profiles, and sediment profiles (Tillit et al. 1991).

A general decrease in the environmental concentrations of Total PCBs has occurred since their peak levels were observed in the mid 1970’s. In Great Lakes fish PCB levels decreased rapidly from the 1970’s to the late 1980’s, at which point levels have remained somewhat constant (Borgmann et al. 1991). This trend has also been observed in soil/sediment cores, bird populations and other environmental matrices in the Great Lakes region (Swartz et al. 1991, Hebert et al. 1994, Addison et al. 1986). These same trends have been observed in other countries such as Spain, Britain, and Sweden and etc. (Port et al. 1994, Ormerod et al. 1992, Jarnverg et al. 1993).

Based on the second law of thermodynamics, chemicals in any matrix will have a tendency to passively disperse from an area of high concentration to one of lower concentration. The assumption of passive dispersal is the backbone upon which the modelling of exposure dynamics of PCBs is based. There have been two approaches developed to predict the movement of chemicals through the environment and into and out of organisms. The
equilibrium approach describes the mechanism of the movement of chemicals based on transport parameters and capacities of environmental phases to hold the chemical. The kinetic approach quantifies the rate of the chemical exchange between phases.

Fugacity is the tendency of a chemical to partition out of a compartment or phase. The fugacity equilibrium approach is based on the thermodynamic assumption that at equilibrium, all compartments, soil/sediment, air, water, and biota, will have equal fugacities. This does not mean however, that phases will have the same concentrations, but rather the same tendency of the chemical to leave any one of the compartments. This would predict that the lipid phase of all organisms would have the same fugacity capacity, and that all organisms should have similar contaminant concentrations on a per unit lipid basis regardless of the trophic level occupied by that species. Numerous food chain contaminant studies have shown this predicted similarity in lipid concentration to be the exception rather than the rule. For a chemical to partition into any phase, it would be determined by the fugacity capacity of that particular phase and the chemical’s concentration. Fugacity is measured as;

\[ f = \frac{C}{Z} \]  

(1)

Where \( C \) is the chemical concentration and \( Z \) is the fugacity capacity of a particular phase. The fugacity of a chemical in water is then
\[ f_w = \frac{C_w}{Z_w} \]  

(2)

The fugacity capacity of water \((Z_w)\) is equal to the inverse of the Henry's Law constant \(1/H\), and therefore equation (2) may be written as

\[ f_w = C_w \cdot H \]  

(3)

The fugacity capacity of a chemical in an organism is based on the assumptions that lipid is the storage site of the chemical and that the fugacity capacity of lipid is the same as the surrogate octanol \((K_{ow})\). Therefore the fugacity of a chemical in an organism is;

\[ f_A = \frac{C_A}{Z_A} \]  

(4)

where \(f_a\) is the fugacity of the chemical in the organism, \(C_A\) is the concentration of the chemical in the organism and \(Z_A\) is the fugacity capacity of the organism. As \(K_{ow}\) is assumed to represent the ratio of the concentration between water and the lipid phase, then the ratio of the fugacity capacities of the two phases is such that;

\[ K_{ow} = \frac{Z_A}{Z_w} \]  

(5)
Rearranging this results in $Z_A = K_{ow} \cdot Z_w$, but $Z_w = 1/\text{H}$, so substituting these back into equation (4) results in the fugacity capacity in the animal:

$$ f_A = \frac{C_A \cdot H}{K_{ow}} \quad (6) $$

and the ratio of $f_A$ to $f_w$ can be determined as

$$ \frac{f_A}{f_w} = \frac{[C_A/C_w]}{K_{ow}} \quad (7) $$

in which $f_A$ and $f_w$ are the fugacity of the chemical in the fish and water respectively, $C_A$ and $C_w$ are the concentrations of the chemical in the fish and water respectively, and $K_{ow}$ is the octanol water partition coefficient for the chemical of interest. The ratio $C_A/C_w$ is the lipid based bioconcentration factor (BCF) for the fish and is directly proportional to the $K_{ow}$.

It is the assumption with this fugacity approach, that the organism is in equilibrium with its surroundings which would mean that the ratio of $f_A/f_w = C_A/C_w = k_1/k_2$ (ratio of uptake and elimination) should equal 1. A ratio larger than 1 may be an indicator of food chain transfer, since it is apparent that the fish is continuing to accumulate chemical even after reaching equifugacity (Gobas et al. 1987). This process of chemical enrichment with increasing trophic level is termed biomagnification. As lipids of the prey food are digested
in the gastro-intestinal tract of the predator, the volume of lipid decreases while the amount of contaminant within the lipid remains the same, thus the chemical concentration increases. This results in an increased fugacity of the chemical in the digested lipids. Since the fugacity gradient runs from high to low, contaminants in the digested food will pass into the predator increasing its contaminant burden (Gobas et al. 1987, Connolly et al. 1988).

The bioaccumulation factor (BCF) is the fish/water partition coefficient and is expressed thermodynamically as

\[
\frac{V_F * Z_F * df_F}{dt} = \frac{V_L * Z_L * df_L}{dt} = D_F * (f_w - f_l)
\]  

(8)

In which \(V_F, V_L, Z_F, Z_L, f_F,\) and \(f_w\) are the volume of the fish and lipids, fugacity capacity of the fish and lipids and fugacity of the chemical in fish and water respectively. \(D_F\) is a transport parameter between the fish and water. This differential equation describes the exchange of chemicals between fish and water such that with time there is a change in the fugacity based on the volume of the fish, the fugacity capacity of the fish and the fugacity of the chemical in the fish. This is also the case for the lipid phase of the fish if the assumptions of equifugacity hold true. Thus the change in chemical fugacity is dependant on the volume of the lipid phase, the fugacity capacity of the lipid phase and the chemical’s fugacity in the lipid of the fish. Integration of equation (8) with a constant chemical fugacity in water \(f_w\) and an initial fugacity in the fish \(f_l\)
of zero yields,

\[ f_F = f_w \cdot (1 - \exp(-D_F \cdot t/V_F \cdot Z_F)). \]  \hspace{1cm} (9)

Substituting \( C_F/Z_F \) in for \( f_F \) and \( C_w/Z_w \) in for \( f_w \) gives,

\[ C_F = C_w \cdot Z_F \cdot (1 - \exp(-D_F \cdot t)). \] \hspace{1cm} (10)

\[ \frac{Z_w}{V_F \cdot Z_F} \]

The fugacity capacity of the fish \( (Z_F) \) may also be stated as \( D_F/(V_F \cdot Z_F) \) which is equal to the rate of uptake of a chemical \( (k_1) \). The fugacity capacity of the water \( (Z_w) \) likewise may be stated as \( D_F/(V_F \cdot Z_w) \) which is equal to the elimination rate \( (k_2) \) of the chemical from the organism. Therefore the BCF ratio \( Z_F/Z_w \) may be restated as \( k_1/k_2 \) (Gobas 1987). The one compartment first order kinetic equation equivalent to equation (10) is

\[ C_F = C_w \cdot k_1 \cdot (1 - \exp(-k_2 \cdot t)). \] \hspace{1cm} (11)

\[ \frac{k_2}{k_2} \]

From equation (11), it is apparent that the rate at which the organism eliminates the chemical from its body \( (k_2) \) is not only an integral part of the BCF, but is an important parameter in determining the overall chemical concentration in the organism. This is particularly true with aquatic organisms.
such as fish which may experience periodic pulses of chemicals (spring run off, contaminant purges from industry, etc.) from their environment. During the period following a pulse when the chemical concentration in the water is at very low concentrations, the $k_2$ will determine how quickly the fish will re-equilibrate with their surroundings. Thus the $k_2$ is invaluable in determining how closely an organism is tracking its chemical environment. As noticed in equations (8), (9), and (10), $k_2 (D_F/V_F*Z_F)$ is proportional to the size of the organism, thus after a pulse of chemical into the environment large organisms will be slower to return to equilibrium than small organisms (Leblanc 1995).

The elimination rate also has important implications in the long debated arguments of the relative importance of bioconcentration versus biomagnification. Morarity (1992) dismissed biomagnification as an insignificant route of chemical uptake in comparison to absorption through gills. Observations of biomagnification were refuted due to nonequilibrium conditions or differences in capacity among organisms with varying lipid levels. Hebert (1992) analyzed contaminant levels in three species of forage fish, the spottail shiner (*Notropis hudsonius*), the brook silversides (*Labidesthes sicculus*), and the bluntnose minnow (*Pimephales notatus*). Different lipid concentration levels of contaminants (in particular hexachlorobenzene, pentachlorobenzene and octachlorostyrene) were observed among the three species at the same sample sites. This was counter to the fugacity assumption that all organisms should have the same lipid normalized concentrations if they were in equifugacity.
within their environment. What is more curious is that the three species occupied the same trophic level. Gut content analyses and evaluation of morphological design of the fishes’ feeding structures suggested these fish had specialized feeding habits at different regions within the same water column. Hebert (1992) theorized that bioaccumulation was the explanation for the observed chemical differences. Although all species had equal exposure through bioconcentration, they had additional exposure based on the food which they were consuming. However, the concentration within a fish is also dependant upon $k_2$. For Hebert’s (1991) conclusion to be correct, these species would require similar elimination rates.

The elimination rate determines the ability of an organism to clear a chemical, and also have implications on toxicity. As was mentioned earlier in the introduction, there appears to be a relationship between $K_{ow}$, $k_2$, and toxicity. Higher $K_{ow}$ compounds have been observed to have both lower $k_2$ values and greater toxicity than low $K_{ow}$ chemicals (Connell et al. 1988). Research into the toxic effects that PCBs elicit in organisms has not yet considered if the $K_{ow}$ is the only factor which regulates $k_2$. No research has addressed if substitution patterns that are related to toxic activity can also be related to $k_2$. The important point to make is whether or not the $k_2$ is affected by the substitution pattern of the congener and can the $k_2$ in turn affect end toxicity observed?

Utilizing three separate studies, this thesis attempts to examine the
general dynamics of PCBs and more specifically coplanar PCBs in two different fish species. It aims to determine those factors which influence the elimination rate constant of PCBs i.e is \( k_2 \) species driven or chemically driven?

1) In Chapter 2 I calibrate \( k_2 \) values for a range of organochlorine chemicals including 12 PCBs by means of a depuration study with *Pimphales notatus*. Null hypothesis: There will be no differences in \( k_2 \) values among the congeners tested.

2) Chapter 3 examines the role that chemical structure plays in the elimination rate of a range of PCBs with varying substitution patterns. Null hypothesis: There are no differences between the elimination rate constants among mono-ortho, di-ortho and non-ortho substitution patterns.

3) Chapter 4 examines the exposure dynamics of coplanar PCBs in the forage fish *Pimphales. notatus*. Null hypothesis: There are no differences in the \( k_1 \), \( k_2 \) and bioconcentration of coplanar congeners in comparison with other substitution patterns.
CHAPTER TWO

Calibration of the Elimination of 12 PCBs from *Pimephales notatus*.

Introduction

The aquatic environment is so dynamic that measurements of the contaminant levels at any one time often do not truly indicate the actual chemical bioavailability nor the exposure organisms are subjected to. Hence, it becomes a priority to calibrate the organisms themselves to truly quantify both their exposure and availability of chemical in the environment. Calibrated organisms can be termed biomonitors, and the application of biomonitors is founded on the premise that organisms attain chemical equilibrium with their surroundings in a predictable time frame. Until recently, biomonitors were assumed to be in equilibrium or (equifugacity) with the surrounding water and thus analysis of the fish would have given an accurate level of bioavailable chemicals in the water body (Kauss and Hamdy 1985).

The fugacity approach to monitoring is based on the assumption that given enough time all compartments of the environment will be in equilibrium. This being the case, the laws of thermodynamics predict the nature of the partitioning of chemicals amongst these equilibrated phases (McKay 1987). The advantage to the fugacity approach lies in its ability to predict in which
direction chemicals will flow, in order to attain an equilibrium state. Fugacity expresses chemical transport not as the movement of chemicals from high potential to low potential but rather the transport of a chemical from a high fugacity (escaping tendency) to a low fugacity. This is best explained by Gibb’s maximization of entropy hypothesis; it is chemical nature to reside in an environment which allows for maximum thermodynamic and entropic stability, i.e., the lowest Gibbs free energy state. This is observed as the partitioning of a chemical between two phases so that its chemical potential or activity (not necessarily concentration) in each phase is equal. Lewis (1901) restated these observations using partial pressures rather than chemical potential. Since partial pressure refers more accurately to a gas, the fugacity term was applied to characterize equilibrium partitioning of mass for all phases. This describes the drive of a chemical to move from high fugacity to low fugacity. The fugacity capacity (in each phase) is unique for each chemical and is determined from such properties as vapour pressure, aqueous water solubility and $K_{ow}$. A compartment with a low fugacity capacity does not hold chemical well while conversely a high fugacity capacity phase accumulates chemicals readily (i.e. water versus lipid). The fugacity model employs four common parameters:

- $Z$ which is the fugacity capacity of some phase (mol/m$^3$ Pa),
- $f$ which is the fugacity of some chemical (Pa),
- $V$ which is the volume of a phase (m$^3$) and,
- $D$ which is a transport parameter for a chemical between two phases.
\[(\text{mol}/\text{Pa} \cdot \text{h})\text{(Mackay 1985)}\].

The direction of equilibrium in a system may be determined once all the required fugacities are known.

A one compartment model derived by fugacity for fish is described by Gobas and Mackay (1985) and is written as

\[
V_F * Z_F * df_f/dt = D_F * (f_w - f_f) + D_A * f_A * D_E * f_E * D_R * f_f \tag{1}
\]

where \( V, f \) and \( Z \) are the target volume, the chemical’s fugacity, and the chemical’s fugacity capacity in a phase respectively.

\( D_F \) is the overall transport parameter \((\text{mol}/\text{m}^3 \cdot \text{Pa})\) for chemical movement between the fish and water through the gills.

\( D_A \) is the transport parameter for chemical uptake from food into the fish across the gastro-intestinal (GI)-tract.

\( D_E \) describes chemical transfer from the GI-tract to feces.

\( D_R \) is the transport parameter for metabolism of chemical in the fish.

The disadvantage of this approach is that to predict water concentration from organism concentration one must assure the two are in chemical equilibrium unless the transport coefficients are known.

Complimentary to the fugacity model is the kinetic approach which is based on first order rate constants rather than transport parameters. The two concepts are centered on the thermodynamic behaviour of chemicals. Kinetic models quantify the movements of the chemicals through the different phases.
and rate coefficients are more readily derived from experimental evidence. The one compartment, first order, linear model can be expressed using kinetic constants as

\[
dC_F/dt = k_1 * C_W - k_2 * C_F + k_A * C_A - k_e * C_F - k_r * C_F,
\]

(2)

Where \( C_F \) is the change in chemical in an organism and \( k_1, k_2, k_A, k_e, \) and \( k_r \) are the rate constants for uptake via gills, elimination via gills, uptake via food, elimination via faeces, and metabolism, respectively (Clark, et al. 1990). \( C_W, C_A \) and \( C_F \) refer to the concentration of the chemical in water, the chemical in food, and the amount of chemical in the fish (mol/m³) respectively. Equation (2) describes the chemical concentration within an organism at any given time, and attempts to quantify the relationship between an organism and the chemicals in its environment. The rate at which an organism uptakes and eliminates the chemicals determines how quickly the organism can reach equilibrium. A desirable biomonitor closely tracks its environment, and therefore preferably has rapid uptake and elimination rates (Gobas et al. 1990). Passive partitioning of chemical from water across the gills is thought to be the dominant means of chemical uptake (Rubinstein et al. 1984). However, for very hydrophobic or high log \( K_{ow} \) compounds such as the Polychlorinated Biphenyls (PCBs), diet is a major source of uptake (Niimi and Dookhram 1989). Uptake of chemicals by means of water and consumption of food is termed Bioaccumulation.
Differences have been observed in lipid normalized concentrations of three forage fish species: the brooksilversides (*Labidesthes sicculus*), the spottail shiner (*Notropis hudsonius*), and the bluntnose minnow (*Pimephales notatus*). Bioaccumulation was hypothesized to be the reason for differences apparent in this single trophic level (Hebert 1992). A better understanding of the rates of chemical elimination in these fish species, however, is essential to support this postulate of bioaccumulation.

As a fish equilibrates with its environment by taking up contaminants, it must also eliminate these chemicals in order to maintain a dynamic equilibrium. By placing a contaminated fish into uncontaminated water and feeding it uncontaminated food, it is possible to calculate the chemical elimination rates. By using equation (2) and assuming the $C_w = 0$, the food is uncontaminated ($C_a = 0$), and there is no change in the lipid content of the organism, the resulting equation is:

$$\frac{dC_F}{dt} = -k_2C_F - k_eC_F - k_RC_F$$  \hspace{1cm} (3)

which after integration yields

$$C_F = C_{F,t=0} \cdot \exp(-(k_2 + k_e + k_R)t).$$  \hspace{1cm} (4)

Logarithmically transforming both sides of equation (4) produces:
\[ \ln C_F = \ln CF_o - (k_2 + k_E + k_R) \cdot t \] (5)

Equation (5) yields a straight line in which the total depuration rate constant \( k_F \) (the sum of \( k_2 \), \( k_E \), and \( k_R \)) is the slope of the line created when graphing \( \ln \) concentration against time (Gobas et al. 1990). However, this is dependant on the assumptions that the water concentrations remain constant, the fish undergo no growth or change in lipid levels, and uptake and elimination rates are constant (Gobas 1990).

The elimination rates determine how quickly the fish equilibrates with its environment, such that the biological half-life of a chemical in an organism can be estimated. Half-lives are expressed as:

\[ T_{1/2} = \frac{0.693}{k_2 + k_E + k_R} \] (6)

and represent the time that it takes for the contaminant within an organism to reach half its initial concentration while the fish depurates in uncontaminated water (Gobas et al. 1990).

An important environmental monitoring parameter is the time to reach 95% of steady state (equilibrium) and this is expressed as

\[ t_{0.95} = \ln 0.05/(k_2 + k_E + k_R) = \frac{3}{(k_2 + k_E + k_R)} \] (7)

The aquatic organism to be calibrated in this study is the forage fish *Pimephales notatus*, commonly referred to as the bluntnose minnow. This
minnow is characteristically a bottom feeder in the water column, feeding on oligochaetes, algae, invertebrates, and other detritus mixed in with bottom sediment (Scott and Crossman 1982, and T. Leadley University of Windsor gut analysis, personal communication 1993, University of Windsor). A naturally contaminated population of bluntnose minnows originally from the Trenton Channel, an offshoot from the Detroit River, were collected. Subsequently, these fish were moved to uncontaminated water, and elimination rates for the chemicals which the fish contained were calculated and compared with the chemicals' respective Log $K_{ow}$ values. Theoretically, it is expected that as the log $K_{ow}$ of the chemicals increases, the chemicals' tend to stay in the non-aqueous phase, this being a more stable state. This is observed through previous data, as a decreasing elimination rate with increasing log $K_{ow}$. This study quantifies the elimination rate coefficients ($k_1$), for polychlorinated biphenyls ranging in log $K_{ow}$, and tests the hypothesis that increasing $K_{ow}$ results in proportionally decreasing rates of elimination.
Materials and Methods

Fish Collection

The fish *Pimephales notatus* (blunt nose minnow) used in this study were collected from two different sites of close proximity in the Trenton Channel, which is known for its high polychlorinated biphenyl concentrations (Furlong et al. 1988), (Fig. 2.1). A blunt nose population was collected from waters off the shore of Horse Island using a 10 m wide, 1 m deep, 6 mm mesh bagsience net.

On September 15, 1989, these fish were caught in approximately 1 m depth of noticeably clouded water. This site was odorous, dense with macrophytes, and the water flow-through rate was minimal. The water's surface was covered by an oily residue. Undesirable species were sorted out, and the blunt nose minnows were retained by a floating enclosure in the water, until all seining was completed. The fish in the retainer were then moved into large, opaque plastic bags filled with one part water and two parts pure oxygen. This practice decreased the vision of the fish in the hope of decreasing oxygen demand caused by higher respiration and metabolism rates, induced by stress. The bags were then placed in large coolers to prevent damage by sloshing. This detailed sampling procedure was used to minimize mortality, allowing large numbers of fish to be brought into the lab with minimal stress levels. Identical sampling procedures were employed during the collection of the second population located offshore of Celeron island. This site was located in a flow.
Figure 2.1
Sample site along the Detroit R. and entrance to L. Erie. Sample sites Celeron and Horse IIs. are marked by triangles.
through, dynamic area of the Trenton Channel. It consisted of a sandy bottom, with a slightly cooler water temperature. This site had low macrophyte and algal biomass and appeared to be physically cleaner than Horse Island.

**Lab Conditions**

The bags containing the fish were then acclimated in holding tanks for one hour. Acclimation brought the water, containing the fish populations, to the same temperature as the water in the tanks. When this was achieved the fish were released into separate tanks. After overcoming the initial stress period, the fish populations were pooled and moved into eight identical ten-gallon aquaria, located in incubation chambers. The temperature within the incubator was 60 °F and the fluorescent lights were on a 12h day:12h night schedule. Identical aquaclear 200 carbon filters were mounted on each aquarium (Fig. 2.2). On September 17, 1989, a bacteria bloom resulted in a massive die off of the collected fish. The surviving fish were removed from the holding tank and were replaced into three of the above dechlorinated aquaria.

**Experimental Procedures**

Sampling began on the day of collection denoted as day 0, and subsequently on days 3, 7, 22, 63, 78. For each sample, three replicates (one from each tank) of three grams of fish (3-5 fish) were taken. These were then placed in hexane rinsed foil and frozen for later extraction and gas
Figure 2.2
Experimental setup: 3, 30 gallon aquaria placed inside an environmental chamber. Each tank has an aquaclear 200 carbon filter to remove depurated chemical from the water.
Aquaclear 200,
charcoal filters

10 gallon

10 gallon

10 gallon
chromatograph-electron capture analysis (Lazar et al. 1991). The chemical concentrations for the chlorinated benzenes, octachlorostyrene and polychlorinated biphenyls were lipid normalized, ln transformed, and subsequently plotted versus time. Simple linear regressions were used to calculate the rates of elimination. The resulting elimination rates were then graphed against their corresponding chemical Log $K_{ow}$ values in order to observe any relationship between $K_{ow}$ and $k_2$. 
Results

A suite of fifteen different chemical compounds were analyzed in each sample taken during the 78 day study (Table 2.1). The ln lipid normalized concentrations were plotted against time (days) and regression coefficients were calculated for each chemical analyzed (Figs. 2.4 - 2.17). A regression was performed on the percent lipid against the time length of the experiment. The mean (± S.E., n = 3) lipid increased from 3.0 % ± 0.1 in the initial samples to 5.1 % ± 0.3 in the samples analyzed on day 78 (Fig. 2.3 b). PP'-DDT, the parent isomer of the pesticide DDT, had a k₂ of 0.0062 and a corresponding half life of 110 days. The PCB congeners 28, 153, 138, and 182 had the highest elimination rate constants with k₂ of 0.0086, 0.0007, 0.0085, and 0.0081, (ug/kg/day) respectively, and half lives of 81, 428, 82, and 86 days respectively. The PCB congeners which required the longest time to depurate were PCBs 52, 99, 66, 110, and 194, with elimination rate constants of 0.0048, 0.0066, 0.0068, 0.0068, and 0.0068 (ug/day), respectively, and half lives of 144, 105, and 102, respectively.

The elimination rate coefficients were compared with Kₐw to determine the predictive power of Kₐw with respect to chemical elimination. The slope of the resulting regression was 0.004 with an R² value of 0.122 (Fig. 2.18).
Table 2.1

Total elimination constants with corresponding R^2 values, half life values (days) and time to 95% steady state (days) for each chemical.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $K_{cw}$</th>
<th>$k_2$</th>
<th>$R^2$</th>
<th>$T_{1/2}$</th>
<th>$T_{95}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP'-DDT**</td>
<td>5.7</td>
<td>0.0063</td>
<td>0.81</td>
<td>110</td>
<td>475</td>
</tr>
<tr>
<td>PCB 28**</td>
<td>5.8</td>
<td>0.0086</td>
<td>0.97</td>
<td>81</td>
<td>350</td>
</tr>
<tr>
<td>PCB52</td>
<td>6.1</td>
<td>0.0048</td>
<td>0.84</td>
<td>144</td>
<td>625</td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>6.1</td>
<td>0.0012</td>
<td>0.02</td>
<td>597</td>
<td>2586</td>
</tr>
<tr>
<td>PCB 66*</td>
<td>6.6</td>
<td>0.0066</td>
<td>0.88</td>
<td>105</td>
<td>453</td>
</tr>
<tr>
<td>Octachlorostyrene**</td>
<td>6.4</td>
<td>0.0099</td>
<td>0.97</td>
<td>70</td>
<td>304</td>
</tr>
<tr>
<td>PCB 101**</td>
<td>6.4</td>
<td>0.0071</td>
<td>0.90</td>
<td>98</td>
<td>424</td>
</tr>
<tr>
<td>PCB 110*</td>
<td>6.5</td>
<td>0.0068</td>
<td>0.71</td>
<td>102</td>
<td>440</td>
</tr>
<tr>
<td>PCB 87**</td>
<td>6.5</td>
<td>0.0072</td>
<td>0.91</td>
<td>96</td>
<td>418</td>
</tr>
<tr>
<td>PCB 118**</td>
<td>6.7</td>
<td>0.0075</td>
<td>0.89</td>
<td>93</td>
<td>402</td>
</tr>
<tr>
<td>PCB 138*</td>
<td>6.8</td>
<td>0.0085</td>
<td>0.86</td>
<td>82</td>
<td>357</td>
</tr>
<tr>
<td>PCB 153*</td>
<td>6.9</td>
<td>0.00070</td>
<td>0.84</td>
<td>99</td>
<td>428</td>
</tr>
<tr>
<td>PCB 182*</td>
<td>7.2</td>
<td>0.0081</td>
<td>0.83</td>
<td>86</td>
<td>366</td>
</tr>
<tr>
<td>PCB 180</td>
<td>7.4</td>
<td>0.0070</td>
<td>0.77</td>
<td>98</td>
<td>426</td>
</tr>
<tr>
<td>PCB 194*</td>
<td>7.8</td>
<td>0.0077</td>
<td>0.81</td>
<td>102</td>
<td>442</td>
</tr>
</tbody>
</table>

P<0.05 = **, P<0.1= *.
Figure 2.3

a) The change in the mean weight (g) of the fish through time (days). (High/low bars represent ± standard error).

b) The change in the mean percent lipid of the fish through time (days). (High/low bars represent ± standard error).
a) $m = -0.008$
$R^2 = 0.20$

b) $m = 0.03$
$R^2 = 0.82$
Figure 2.4
Mean Ln transformed lipid normalized concentration levels of PP'-DDE in *P. notatus* through time (error bars may be hidden in the symbol).
Concentration = -0.0063 (+/- 0.003) x + 3.2.

Figure 2.5
Mean Ln transformed lipid normalized concentration levels of PCB 28 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0086 (+/- 0.004) x + 1.4.
\[ \text{Ln Lipid Norm. Concentrations (ug/Kg)} \]

- **pp'-DDE**
  - \[ k_2 = -0.0063 \]
  - \( R \text{ squared} = 0.86 \)

- **PCB 28**
  - \[ k_2 = -0.0086 \]
  - \( R \text{ squared} = 0.97 \)
Figure 2.6
Mean Ln transformed lipid normalized concentration levels of Octachlorostyrene in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = $-0.0099 \, (+/- 0.003) \times + 0.81$.

Figure 2.7
Mean Ln transformed lipid normalized concentration levels of PCB 52 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = $-0.0048 \, (+/- 0.003) \times + 2.7$. 
Figure 2.8
Mean Ln transformed lipid normalized concentration levels of PCB 101 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0071 (+/-0.003) x + 3.2.

Figure 2.9
Mean Ln transformed lipid normalized concentration levels of PCB 110 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0068 (+/-0.005) x + 2.6.
The diagrams show the natural logarithm of lipid normalized concentrations (μg/Kg) over time (Days) for two different PCBs:

**PCB 101**
- k2 = -0.0071
- R squared = 0.90

**PCB 110**
- k2 = -0.0068
- R squared = 0.71
Figure 2.10
Mean Ln transformed lipid normalized concentration levels of PCB 87 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0072 (+/-0.003) x + 2.2.

Figure 2.11
Mean Ln transformed lipid normalized concentration levels of PCB 66 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0066 (+/-0.004) x + 3.1.
Figure 2.12
Mean Ln transformed lipid normalized concentration levels of PCB 118 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0075 (+/- 0.004) x + 2.9.

Figure 2.13
Mean Ln transformed lipid normalized concentration levels of PCB 138 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0085 (+/-0.005) x + 3.7.
For PCB 118:

- \( k_2 = -0.0075 \)
- \( R \text{ squared} = 0.88 \)

For PCB 138:

- \( k_2 = -0.0084 \)
- \( R \text{ squared} = 0.87 \)
Figure 2.14
Mean Ln transformed lipid normalized concentration levels of PCB 180 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.007 (+/-0.005) x + 3.3.

Figure 2.15
Mean Ln transformed lipid normalized concentration levels of PCB 182 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0081 (+/-0.005) x + 2.8.
$k_2 = -0.0074$
$R\ squared = 0.77$

$k_2 = -0.0081$
$R\ squared = 0.83$
Figure 2.16
Mean Ln transformed lipid normalized concentration levels of PCB 153 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0007 (+/-0.004) x + 3.7.

Figure 2.17
Mean Ln transformed lipid normalized concentration levels of PCB 194 in *P. notatus* through time. (error bars may be hidden in the symbol).
Concentration = -0.0077 (+/-0.004) x + 1.4.
Figure 2.18
Log inverse of elimination rate constant plotted over a range of $K_{ow}$. The $k_2$ here represents a total elimination constant.
Discussion

A common trend observed in the data were the larger standard errors for samples taken during the first thirty days of the elimination experiment. The mixing of the two populations due to the fish die off can account for the individual variability observed. Mentioned earlier was the fact that the two collection sites varied greatly in their environmental characteristics. The waters around Horse Island were observed to be stagnant and non-flowing, while the sediment appeared extremely fine and silty. This could be an accumulation site for waterborne chemicals as they tend to adhere to suspended fine particulate matter such as silts. Some researchers (Weber et al. 1983, and Capel 1990) have shown that chemicals have a higher affinity for smaller sediment particle sizes due to a higher fraction of organic carbon into which the chemicals may partition. Celeron Island, a sandy, more dynamic site due to stronger currents, may not have the same affinity for chemical accumulation, leaving fish populations characteristic to this site with lower total body burdens. Sand, having lower organic carbon content for chemicals to adhere to would seem to support this observation. Compounding variables such as size and age differences between the two populations may have also resulted in a larger variability in concentrations than would be expected from samples taken from a homogeneous population. There may also be differences in fish contaminant body burdens due to sex. The fish were collected after breeding season during which females could have depurated into egg lipids, reducing the level of

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contaminants in female fish. These factors are common problems associated with calculating elimination rate constants from natural populations.

The elimination half-life calculated for the trichloro-PCB substitution group was 81 days, higher than those published for goldfish of 15-36 days (Bruggeman 1981). However the size of the goldfish were not available to allow for comparison with the minnows in this experiment. The tetrachlorobiphenyl substitution group in this study (half life of 144 days) was much higher than published values of 51-87 days for goldfish and 21-53 days in the guppy (Niimi 1987). The specific congeners in the guppy and goldfish experiments were not listed. Congener 52 is known to preferentially accumulate in the brain of mammals (Seegal et al. 1988). It is possible that 52 is retained in the fish taking longer to eliminate than its $K_{ow}$ would predict.

The heptachloro- and octachloro- biphenyl groups gave similar half life values (98-102 days) as those observed in guppies (100 days). In comparison to other available data, it becomes clear that the elimination rate constant is size specific and species specific.

Although chemical uptake and elimination are considered to be passive processes, the complex interactions between the organism and the chemicals render the organism’s biology a potentially important factor. The organism’s physiology, i.e gill ventilation rate, gut absorption-desorption efficiency, growth rate, etc. all have an impact on the estimated uptake and elimination coefficients of chemicals. A smaller fish has a higher metabolism and therefore,
more rapid uptake and elimination rates than a large adult fish (Connolly et al. 1987, Gobas 1987). This has been observed in Niimi’s review of biological half lives of chemicals in fish, 1987. Hexachlorobenzene among many other chemicals has a half life ranging from 173 to 224 days in trout (which has a substantially larger biomass) versus a half-life of 12 days in guppy.

The experimental design may also influence the elimination rate constants reported. For example the depuration rates of octachlorobiphenyls and heptachlorobiphenyls in this study conformed to published values while the hexachlorobiphenyls disagreed. Some of the variability in the data may be a result of dosing techniques and dosing concentrations. Heptachloro- and octachlorobiphenyls have typically log $K_{ow}$ of 7+. Gobas (1990) observed that compounds with such high $K_{ow}$ ’s have restricted absorption efficiencies hence differences in dosing techniques and initial concentrations can affect the elimination rate constants because the first order model might not be equally approximate for all congeners.

Another important factor which could cause elimination coefficient $k_2$ to vary is the possibility of congener metabolism. This is particularly true for specific substitution patterns which leave two adjacent meta and para or ortho and meta unsubstituted carbon atoms such as congeners 153 and 118 (Tanabe et al. 1987, Safe et al. 1985). In this study, the elimination rate coefficients represent total elimination including elimination via gills, gut and metabolism. Desorption from the gut is thought to be a slow process and metabolism is
assumed to be small based on studies conducted with other similar fish species (Lutz 1987). Very little information is known regarding the ability of *P. notatus* to metabolize contaminants. There was, however, no evidence of metabolism observed for the chemicals used in this study.

Noticeable growth occurred over the course of the study. The change in the size of an organism will affect the physiological parameters that govern the rates of uptake and elimination, such that the larger the organism, the lower its ability to depurate the chemical, particularly for higher $K_{ow}$ compounds (Connolly 1987). In kinetic experiments it is assumed that the fugacity capacity of the organism remains constant over time (Gobas 1990). In this depuration study, the percent lipid of the fish did increase slightly over time, however observing the standard errors, this increase was considered to be minimal (1 % increase).

**Summary**

Elimination rate coefficients were calculated for octachlorostyrene, PP'-DDT, trans-nonachlor, and twelve PCB congeners in an elimination study using *Pimephales notatus*. Corresponding half lives and T95% values were estimated based on the $k_2$ coefficients obtained in the study. No significant relationship was observed between $k_2$ values and corresponding log $K_{ow}$ values.
CHAPTER THREE

A Comparison of Elimination Rates for Mono-ortho, Di-ortho, and Non-ortho Substituted Tetra- to Hexachloro-Biphenyls in Rainbow Trout (*Oncorhynchus Mykiss*).

Introduction

Environmental contamination related to polychlorinated biphenyls (PCBs) has been of concern since the 1970’s. PCBs are ubiquitous in the environment and have been detected far from original sources (Hooper 1990, Tanabe 1987, Safe 1991). The persistence and biomagnification potential of PCBs is evident in many predator species including birds, seals, fish and bears (Muir 1988, Kubiak 1983, Koslowski 1993). PCBs are a priority to understand in terms of their exposure dynamics and their effects which include embryotoxicity, teratogenicity, genotoxicity, and induction of the cytochrome P450 isoenzyme systems in selected organisms (Hooper 1990, Poland 1982, Safe 1991, Bolla 1994, Hontela 1992). PCB toxicity has been demonstrated in both laboratory assays and the wild. Kubiak *et al.* (1989) concluded that over 90% of the toxicity observed in Forester terns was a result of exposure to coplanar PCBs.

There are ten substitution sites on the PCB molecule that can produce 209 possible isomers. However, of these isomers, approximately 103 are prevalent in the environment (Tanabe *et al.* 1987). Congeners which have
chlorines substituted at adjacent ortho and meta positions are known to be more readily biodegraded (Hutzinger et al. 1975, Mathew et al. 1975). Congeners with substitutions in the meta and para positions with less than two substitutions in the ortho positions, exhibit a coplanar conformation that is similar to the highly toxic 2,3,7,8-tetra-chlorodibenzo-p-dioxin (2,3,7,8-TCDD)(Safe 1990, Tanabe et al. 1987, Safe et al. 1987). Of particular concern are the non-ortho substituted (coplanar) congeners with IUPAC numbers 77 (3,3',4,4'-tetrachlorobiphenyl), 126 (3,3',4,4',5,-pentachlorobiphenyl), and 169 (3,3',4,4',5,5'-hexachlorobiphenyl), which have toxic potencies within 1-3 orders of magnitude of TCDD (Safe 1990). More recently Harris et al. (1994) showed that the coplanar PCB congener 81 (3,4,4',5-tetrachlorobiphenyl) was toxic to fish.

In reference to their toxicological hazard, coplanar PCBs are compared with 2,3,7,8-TCDD which is known to be the most potent inducer of both the aryl hydrocarbon hydroxylase (AHH) and ethoxy-resorufin-O-deethylase (EROD) enzyme systems (Tanabe et al. 1987, Safe et al. 1987, Safe 1985). The planar structure of the non-ortho substituted PCB molecules, results in an induction potential similar to 2,3,7,8-TCDD (Tanabe et al. 1987, Safe 1991), and the relative toxicity is related to the binding affinities of the PCBs to the Ah receptor. This TCDD like toxicity of PCB congeners is represented by conversion factors (Keqs) which have been established for many congeners. Of all the PCBs, the coplanar congeners have the highest
affinities such that $126 > 169 > 77$ (Haspellier et al. in press 1995, Safe 1991). Mono-ortho substituted congeners, in particular PCBs 105, 118, and 156 also exhibit similar affinities for the Ah receptor site (Smith et al. 1990, Safe 1985), yet are believed not to be as strong inducers as the non-ortho congeners.

It is estimated that an excess of $2.0 \times 10^9$ kg of PCBs have been produced worldwide (Hutzinger 1974). Although levels of total PCB have declined since their ban in use and production, it has been difficult to determine and predict the exposure dynamics and effects of total PCBs in the environment. Much of this problem relates to the assumption that total PCB measurements accurately reflect the environmental hazard of all congeners.

The predominant method for assessing environmental toxicity of PCBs is the Toxic Equivalents (TEQ) approach. TEQs predict toxicity as an additive function of the relative toxicity of specific congeners. The relative toxic potencies (Toxic equivalence factor, TEF) of the coplanar congeners are known for the aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylase enzyme induction assays in mouse and rat hepatoma cell lines. The toxicity of each congener determined from these assays is then multiplied by their respective concentrations in the environmental matrix to produce a TEF. These values are subsequently summed to produce the total toxicity attributed to PCBs for the sample. Recent research in Lake
Michigan salmonids suggest there is a close correlation between total PCBs and measured TEQs (Williams et al. 1992).

TEQs are dependant on chemical concentrations being indicative of the chemical kinetics, i.e., exposure. Unfortunately, there has been very little published research available on the kinetics of coplanar PCBs (Tanabe et al. 1987, Niimi et al. 1983, Sericano et al. 1992). Niimi and Oliver (1983), questioned whether the dynamics of coplanar PCBs were simply related to the water partition coefficient ($K_{ow}$) or if the substitution pattern also played an important role in regulating chemical exposure dynamics. Nimii and Oliver (ibid) speculated that substitution patterns might also determine the exposure dynamics of the more active coplanar congeners.

Elimination studies, to date, have not been designed to test the relative importance of structure versus that of hydrophobicity. An elimination study using *Oncorhynchus mykiss* was designed to quantify the elimination rate constants of selected di-ortho, mono-ortho, and non-ortho PCB congeners within a narrow range of hydrophobicity. The study was implemented to provide a detailed examination of the role of chlorine substitution patterns and water solubility in regulating the elimination rate constants for PCBs in fish populations. Of particular interest to this study is whether non-ortho, mono-ortho, and di-ortho PCBs of similar $K_{ow}$, have the same half-life. A complication which may arise in accomplishing this is the possibility of the congeners’ differential metabolism in the trout. Previous
studies have shown that fish do not readily metabolize and excrete PCBs with four or more chlorines (Hutzinger 1972). Even in birds and mammals, metabolism of highly chlorinated PCBs is extremely slow. The main mechanism of PCB metabolism in vertebrates is by monooxygenase-catalyzed binding of singlet oxygen between adjacent carbon atoms on biphenyl rings, which yields a transient arene oxide that is degraded to a more polar hydroxylated PCB (Mathews 1984). Therefore, in order to be metabolized by this mechanism, PCB congeners must have at least one area with an adjacent pair of unsubstituted carbons at either the **meta-para** or **ortho-meta** positions. According to some studies (Sundstrom 1976, Tanabe 1988, Wolf 1983), congeners with an adjacent, unsubstituted pair of **meta-para** carbons are metabolized more rapidly than those congeners with unsubstituted **ortho-meta** positions. These studies indicate that organisms may not be able to efficiently metabolize and eliminate PCB congeners chlorinated at adjacent **meta** and **para** positions. Among tetrachloro- and pentachlorobiphenyls, this group includes the non-**ortho** PCB congeners and also some mono-**ortho** congeners.

A one compartment model, based on first-order linear rate kinetics (Gobas 1990 and Barron 1990), is used in this study. Theoretically, the change in chemical concentrations in fish can be quantified as the net difference between chemical uptake and elimination.

\[
\frac{dC_f}{dt} = k_1 C_w - k_2 C_F
\]  

(1)
where $C_F$ and $C_W$ are the concentrations in the fish and water respectively, and $k_1$ and $k_2$ are the uptake and elimination rate constants. To measure the elimination rate constant ($k_2$), spiked trout were placed in clean water and allowed to depurate chemical for 266 days (assuming a $C_W=0$) such that:

$$\frac{dC_F}{dt} = -k_2 C_F$$  \hspace{1cm} (2)

which when integrated yields,

$$C_F = C_{F_0} \cdot e^{(k_2 \cdot t)}$$  \hspace{1cm} (3)

or

$$k_2 = \frac{\ln C_{F_0} - \ln C_F}{t}$$  \hspace{1cm} (4)

where $C_{F_0}$ is the concentration at time $t=0$. The half-life of the chemical is inversely proportional to the $k_2$ and is determined as:

$$T_{1/2} = \frac{\ln 2}{k_2} = 0.693 \frac{k_2}{k_2}$$  \hspace{1cm} (5)

In biomonitoring programs and environmental assessment, half-lives are invaluable as they discern how quickly organisms respond to chemical fluxes in their environment. Of particular interest to this study is whether or not non-ortho and di-ortho PCBs of similar $K_{ow}$s have the same half-life. If not, the use of total PCB chemical concentrations does not adequately represent the exposure dynamics of the different congeners.
Materials and Methods

a) Fish

To measure the rates of chemical elimination, 120 rainbow trout initially weighing approximately 45 g, were obtained from the Linwood Acres hatchery near Peterborough, Ontario. Sixty fish were anesthetized with MS-222(Sigma) and intraperitoneally injected with corn oil spiked with 13 PCB congeners dissolved in Dimethyl Sulfoxide (DMSO). The PCB congeners present in the spike were obtained from Ultra Scientific (Rhode Island) with a purity of 99%. The congeners selected for the spike represented various substitution patterns over a narrow range in $k_{ow}$ (Table 3.1). Sixty control fish were injected intraperitoneally with corn oil containing DMSO. The ratio of DMSO to corn oil for both experimental and control injections was 10:90 (v/v), and all fish were injected with total volumes equivalent to 1 mL/kg.

The sixty spiked trout were adipose fin clipped for identification and placed in three holding tanks with the 60 control trout. The three 1000 L holding tanks were connected to the same partial recirculation water system (Fig. 3.1). The water was continually recirculated through a settling tank, fibre filters, and UV sterilization. To maintain sufficient oxygen levels and optimum water conditions, Otanabee River water was sand filtered and added to the water system at the rate of approximately 2 L/min. Optimum water temperatures (10-15 °C) were maintained with either Minocool chilling
Table 3.1
Congeners and their concentration present in the corn oil spike solution injected into the experimental trout. Their concentrations in the food (trout chow) are also listed beside the spike concentrations.

<table>
<thead>
<tr>
<th>IUPAC</th>
<th>Substitution</th>
<th>Pattern</th>
<th>$K_{ow}$</th>
<th>Spike (mg/L)</th>
<th>Food (ug/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>2,2',5,5'</td>
<td>di-ortho</td>
<td>5.85</td>
<td>2.0</td>
<td>6.50</td>
</tr>
<tr>
<td>47</td>
<td>2,2',4,4'</td>
<td>di-ortho</td>
<td>5.85</td>
<td>2.0</td>
<td>4.80</td>
</tr>
<tr>
<td>66</td>
<td>2,3',4,4'</td>
<td>mono-ortho</td>
<td>6.20</td>
<td>2.0</td>
<td>23.70</td>
</tr>
<tr>
<td>81</td>
<td>3,4,4',5</td>
<td>non-ortho</td>
<td>6.36</td>
<td>2.0</td>
<td>NA</td>
</tr>
<tr>
<td>77</td>
<td>3,3',4,4'</td>
<td>non-ortho</td>
<td>6.36</td>
<td>2.0</td>
<td>3.80</td>
</tr>
<tr>
<td>101</td>
<td>2,2',4,5,5'</td>
<td>di-ortho</td>
<td>6.38</td>
<td>2.36</td>
<td>9.70</td>
</tr>
<tr>
<td>105</td>
<td>2,3,3',4,4'</td>
<td>mono-ortho</td>
<td>6.65</td>
<td>2.25</td>
<td>1.80</td>
</tr>
<tr>
<td>118</td>
<td>2,3,3',4,4',5</td>
<td>mono-ortho</td>
<td>6.74</td>
<td>2.25</td>
<td>6.80</td>
</tr>
<tr>
<td>119</td>
<td>2,3',4,4',6</td>
<td>mono-ortho</td>
<td>6.58</td>
<td>2.25</td>
<td>NA</td>
</tr>
<tr>
<td>126</td>
<td>3,3',4,4',5,5'</td>
<td>non-ortho</td>
<td>6.89</td>
<td>2.25</td>
<td>1.50</td>
</tr>
<tr>
<td>153</td>
<td>2,2',4,4',5,5'</td>
<td>di-ortho</td>
<td>6.92</td>
<td>2.48</td>
<td>7.50</td>
</tr>
<tr>
<td>156</td>
<td>2,3,3',4,4',5</td>
<td>mono-ortho</td>
<td>7.18</td>
<td>2.50</td>
<td>NA</td>
</tr>
<tr>
<td>169</td>
<td>3,3',4,4',5,5'</td>
<td>non-ortho</td>
<td>7.42</td>
<td>2.48</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Figure 3.1
Experimental setup: The 120 trout were randomly placed in one of three 1000 L tanks. A continual recirculating filtration system conditioned the water and removed any depurated chemical. Cooling systems maintained cold water conditions favourable to Rainbow trout.
units or immersible heating elements, depending on the season.

Control trout were used to act as indicators of any chemical recirculation which might occur within the tanks. To minimize the possibility of chemical contamination during the experiment, the recirculation units were also equipped with an activated carbon filter. The trout were kept on a low maintenance diet (1% of biomass) of commercial Trout Chow (Martin Feed Mills, Guelph, Ont.) to minimize variations which might be introduced by growth. One week post-intraperitoneal injection, sampling was initiated and continued over eight months to the close of the experiment. On each of days 0, 21, 49, 111, 179, and 266, ten spiked and ten control trout were sacrificed weighed and frozen for later analysis.

b) Analysis of PCBs

Each trout sample was cut into pieces and homogenized using an Osterizer blender. A 5 g tissue subsample was then taken and prepared for analysis of PCBs as describe in detail by Lazar et. al 1993. Briefly, the tissue subsample was ground in anhydrous sodium sulphate and was subsequently extracted by a "cold column" technique into a 1:1 mixture of Hexane:Dichloromethane (DCM). Sample extracts were cleaned up by adsorption chromatography with Florisil. Florisil was activated in a muffle furnace and was then placed in an oven to keep dry. Chromatography columns were washed and rinsed with three separate solvents, acetone, petroleum ether, and hexane. The columns were packed with 30 mL of the
activated Florisil and topped with 10 mL anhydrous sodium sulphate. The initial extract was placed on the activated Florisil packed column. The Florisil column was eluted with 50 mL of Hexane to collect di-ortho and mono-ortho PCB congeners (Fraction I). The column was then eluted with 50 mL of 85% Hexane: 15% DCM to collect the non-ortho substituted congeners (Fraction II). The Trout Chow pellets were crushed with pestal and mortar and ground with anhydrous sodium sulphate. Extraction of the PCBs from the trout food followed the same procedure as above. A blank was run with every 6 tissue samples to ensure quality control. The percent lipid was determined by pipeting 2.5 mL of the initial tissue extract into a preweighed aluminum dish, evaporating hexane off and drying the dish in a 105°C oven for 20 minutes. The cooled dish was reweighed and percent lipid was calculated.

Fraction I was rotary-evaporated to 10 mL and Fraction II was rotary-evaporated to 5 mL. These samples were analyzed on a Hewlett Packard model 5890 gas chromatograph (GC) with an electron capture detector. The GC carrier gas was helium at a flow of 30 cm/sec (@ 100°C) and the temperature program was 100°C to 270°C @ 3°C/min. The sample was injected in a splitless mode.

A major concern in coplanar analysis is the coelution of specific congeners in capillary columns (Duinker 1991). The above extraction technique for coplanar congeners has been shown to provide high and
reliable recoveries in a variety of biological matrices and has also been shown to completely separate congener 77 from 101 (Lazar et al. 1992, Duinker 1991).

The PCB wet weight concentrations of the samples were determined by the measured sample peak area relative to a prepared standard for the 13 PCB congeners injected. A range of unspiked PCB concentrations were also measured relative to the Canadian Wildlife Survey (CWS) standard.

c) Elimination Rates

Congener wet weight concentrations were In normalized and elimination rate constants and half lives of the specific congeners were obtained from linear regressions (concentrations vs time) using equations (4) and (5). The elimination rates measured are a function of both chemical clearance and growth dilution. To correct for growth, multivariate multiple linear regressions were employed to remove the error associated with the growth of the fish while preserving the variability among the congener concentrations. The independent variables used for the analysis were time (days) and lipid (%). The main objective of this study was to compare the relative elimination rates of non-ortho, mono-ortho and di-ortho tetra to hexachlorobiphenyls and to determine if these rates were influenced by the chlorine substitution pattern, the log $K_{ow}$ or both. To accomplish this, a 3 x 4 factorial multivariate repeated-measures multiple analysis of covariance

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(MANCOVA) was employed using the GLM procedure of the SAS statistical package. Each PCB congener was classified as belonging to

a) one of three structural configurations (non-ortho, mono-ortho, di-ortho), and,

b) one of a group of PCBs with log $K_{ow} < 6.3$ (PCBs 47, 52, 66, 81), < 6.7 (PCBs 77, 101, 105), < 6.9 (PCBs 118, 119, 126), or < 7.4 (PCBs 153, 156, 169).

Structural configuration of a chemical was concluded to be important in determining elimination rates if the analysis indicated that there was a significant structure x time interaction. Hydrophobicity was judged to be important in determining elimination rates if there was a significant $K_{ow}$ x time interaction.
Results

Despite the maintenance diet in the eight month experiment, the fish grew in size from an average weight of 45 g to 168.3 g (Figures 3.2-3.3). To account for dilution of chemical by growth a multivariate multiple regression was performed to determine if there was a significant change in concentration with time. These regressions removed the error associated with growth by preserving the variability among the congener concentrations within a specific fish based on the individual weight of the fish. This statistical method allowed the elimination rate constants to be calculated by measuring the change in variance of the concentrations over the duration of the experiment. To quantify if there were significant differences in elimination rates among the congeners and between the chlorine substitution groups, a MANCOVA was performed.

An important assumption often overlooked in elimination studies pertains to the possible change in fugacity capacity of the organism over the duration of the study. In this experiment, the amount of lipid in the trout increased with weight (Fig. 3.4). Since the percent lipid in the fish (approximately 8%) did not change markedly over the experiment there was no significant change in the capacity of the trout to retain these lipophilic compounds in the tissue. The change in lipid normalized concentrations although higher, are mirror images of the wet weight concentrations.
Figure 3.2
The change in the mean weight (g) of the experimental trout through time (days).

Figure 3.3
The change in the mean weight (g) of the control trout through time (days).
Figure 3.4
The change in the mean lipid (g) of the trout through time (days).

Figure 3.5
The change in the fugacity capacity of the trout presented as the regression of the change in lipid (g) against the change in weight (g).
observed over time, thus accentuating that the fugacity capacity of the trout remained relatively constant (Figures 3.6-3.9).

The concentration of PCB analyte in the trout food are summarized in Figure 3.10. The concentrations in food of total PCB analytes (74.6 ug/kg), were well below that observed in the inoculated fish (376.3 ug/kg). All trout specimens utilized in this study had been raised on this nutritional mixture, thus it is assumed that both control and experimental trout have been equally exposed and are in equilibrium with any PCB congeners present in their food supply. If contamination were occurring via ingestion then an increase in concentration of the 13 PCB analytes in the control fish would reflect this over the duration of the study. The concentrations in food of congeners 153, and 101 (8 ug/kg and 10 ug/kg respectively) although elevated, were below that observed in the inoculated fish (32 ug/kg and 31 ug/kg respectively). Similarly, congener 66 was observed to occur in relatively high concentrations in the food (23 ug/kg). However, there was no significant (P > 0.01, MANCOVA) uptake of the 13 polychlorinated biphenyls in the control fish with the exception of congener 101 (Table 3.2). Congener 77 was observed to significantly eliminate from the control fish (P > 0.05). Congener 138, a common congener in Aroclor mixtures and ubiquitous in the environment was chosen as an internal control for this study. There was no apparent contamination of congener 138 as the slopes of both the experimental and control fish did not significantly differ (0.0026
Figure 3.6

Comparison of mean Ln lipid normalized concentrations (ug/kg) with mean Ln wet weight concentrations (ug/kg) over time (days). The dotted line represents the lipid normalized concentrations and the solid line represents the wet weight concentrations. (some standard error bars may be hidden in the symbols).

a) Comparison of congener 52 lipid normalized to wet weight concentrations.

b) Comparison of congener 47 lipid normalized to wet weight concentrations.

c) Comparison of congener 66 lipid normalized to wet weight concentrations.

d) Comparison of congener 81 lipid normalized to wet weight concentrations.
Figure 3.7

Comparison of mean Ln lipid normalized concentrations (ug/kg) with mean Ln wet weight concentrations (ug/kg) over time (days). The dotted line represents the lipid normalized concentrations and the solid line represents the wet weight concentrations. (some standard error bars may be hidden in the symbols).

a) Comparison of congener 77 lipid normalized to wet weight concentrations.

b) Comparison of congener 101 lipid normalized to wet weight concentrations.

c) Comparison of congener 119 lipid normalized to wet weight concentrations.

d) Comparison of congener 105 lipid normalized to wet weight concentrations.
Figure 3.8

Comparison of mean Ln lipid normalized concentrations (ug/kg) with mean Ln wet weight concentrations (ug/kg) over time (days). The dotted line represents the lipid normalized concentrations and the solid line represents the wet weight concentrations. (some standard error bars may be hidden in the symbols).

a) Comparison of congener 118 lipid normalized to wet weight concentrations.

b) Comparison of congener 126 lipid normalized to wet weight concentrations.

c) Comparison of congener 153 lipid normalized to wet weight concentrations.

d) Comparison of congener 156 lipid normalized to wet weight concentrations.
Figure 3.9

Comparison of mean Ln lipid normalized concentrations (ug/kg) with mean Ln wet weight concentrations (ug/kg) over time (days). The dotted line represents the lipid normalized concentrations and the solid line represents the wet weight concentrations. (some standard error bars may be hidden in the symbols).

a) Comparison of congener 169 lipid normalized to wet weight concentrations.

b) Comparison of congener 138 lipid normalized to wet weight concentrations.
Figure 3.10

The mean wet weight concentration (ug/kg) of contaminants in the trout food (Trout Chow) fed to the trout before and during the experiment.
PCB congeners present in Trout Chow.
Table 3.2.
Kinetics observed in the control trout.
Significance * p<0.05, ** p<0.01 is determined by MANCOVA.

<table>
<thead>
<tr>
<th>IUPAC No.</th>
<th>Kow</th>
<th>$k_1$</th>
<th>S.E.</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>5.84</td>
<td>0.34</td>
<td>± 0.021</td>
<td>0.093</td>
</tr>
<tr>
<td>47</td>
<td>5.85</td>
<td>0.052</td>
<td>± 0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>66</td>
<td>6.20</td>
<td>0.074</td>
<td>± 0.02</td>
<td>0.31</td>
</tr>
<tr>
<td>81</td>
<td>6.36</td>
<td>0.001</td>
<td>± 0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>77 **</td>
<td>6.36</td>
<td>-0.012</td>
<td>± 0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>101 **</td>
<td>6.38</td>
<td>0.007</td>
<td>± 0.025</td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>6.58</td>
<td>0.044</td>
<td>± 0.023</td>
<td>0.101</td>
</tr>
<tr>
<td>105</td>
<td>6.65</td>
<td>0.08</td>
<td>± 0.043</td>
<td>0.08</td>
</tr>
<tr>
<td>118</td>
<td>6.74</td>
<td>0.018</td>
<td>± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>126</td>
<td>6.89</td>
<td>-0.021</td>
<td>± 0.039</td>
<td>0.077</td>
</tr>
<tr>
<td>153</td>
<td>6.92</td>
<td>0.034</td>
<td>± 0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>156</td>
<td>7.18</td>
<td>0.025</td>
<td>± 0.047</td>
<td>0.055</td>
</tr>
<tr>
<td>169</td>
<td>7.42</td>
<td>0.01</td>
<td>± 0.04</td>
<td>0.045</td>
</tr>
<tr>
<td>87 *</td>
<td>6.29</td>
<td>0.04</td>
<td>± 0.023</td>
<td>0.17</td>
</tr>
<tr>
<td>110 *</td>
<td>6.48</td>
<td>0.024</td>
<td>± 0.015</td>
<td>0.21</td>
</tr>
<tr>
<td>138</td>
<td>6.83</td>
<td>0.53</td>
<td>± 0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>129 **</td>
<td>6.73</td>
<td>0.02</td>
<td>± 0.009</td>
<td>0.29</td>
</tr>
<tr>
<td>180</td>
<td>7.36</td>
<td>0.04</td>
<td>± 0.04</td>
<td>0.077</td>
</tr>
</tbody>
</table>
and 0.0020 for the experimental and control fish respectively) (Fig 3.11 and 3.12).

The elimination rate constants for the di-ortho congeners (52, 47, 101, and 153) calculated from wet weight concentrations and corrected for growth dilution ranged from 0.0039 ug/kg/d to 0.0050 ug/kg/d (Table 3.3, Figure 3.13). The $R^2$ values ranged from 0.74 to 0.89 ($P<0.05$), which shows that significant decline in chemical concentration had occurred during the study. The slowest elimination rate observed was for congener 101 with a $K_{ow}$ of 6.38. A possible explanation of this slow rate of elimination might be related to the substantial uptake (Table 3.3) of this chemical in the control trout, suggesting a source of contamination of PCB 101 within the experimental system.

Mono-ortho congeners (66, 105, 118, and 156) showed a range in elimination rate constants of 0.0042 ug/kg/d to 0.0056 ug/kg/d (Figure 3.14). The range in $R^2$ was 0.59 to 0.74, once again showing a significant decline of chemical concentration with time. The slowest elimination rate was for congener 66, which has a $K_{ow}$ of 6.20. Congener 156 with a $K_{ow}$ of 7.18 has the highest elimination rate constant (0.0056 ug/kg/d).

The non-ortho substituted PCBs (81, 77, 126, and 169) had a range in elimination rate constants of 0.0057 ug/kg/d to 0.009 ug/kg/d (Figures 3.15). The range in $R^2$ was 0.49 to 0.68. Congener 169 with a $K_{ow}$ of 7.42 had the lowest elimination rate constant. The highest elimination rate
Figure 3.11
Mean Ln wet weight concentrations of PCB 138 through time in *Oncorhynchus mykiss*. The solid line represents the concentration in the experimental trout and the dotted line represents the concentrations in the control trout. (some standard error bars may be hidden in the symbols).

Figure 3.12
Mean Ln wet weight concentrations of PCB 180 through time in *Oncorhynchus mykiss*. The solid line represents the concentration in the experimental trout and the dotted line represents the concentrations in the control trout. (some standard error bars may be hidden in the symbols).
Table 3.3

Growth corrected elimination rate constants determined through multiple linear regressions are presented with corresponding standard errors, $R^2$ values and calculated half lives (days). All $k_2$ values are actually total elimination values and are significant to $* P<0.0001$ (MANCOVA).

<table>
<thead>
<tr>
<th>IUPAC Pattern</th>
<th>Substitution</th>
<th>$k_{ow}$</th>
<th>$k_2$</th>
<th>S.E.</th>
<th>$R^2$</th>
<th>$T_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>52*</td>
<td>2,2',5,5'</td>
<td>5.85</td>
<td>0.0050</td>
<td>±0.0003</td>
<td>0.76</td>
<td>139</td>
</tr>
<tr>
<td>47*</td>
<td>2,2',4,4'</td>
<td>5.85</td>
<td>0.0050</td>
<td>±0.0003</td>
<td>0.89</td>
<td>139</td>
</tr>
<tr>
<td>66*</td>
<td>2,3',4,4'</td>
<td>6.20</td>
<td>0.0042</td>
<td>±0.0004</td>
<td>0.71</td>
<td>165</td>
</tr>
<tr>
<td>81*</td>
<td>3,4,4',5</td>
<td>6.36</td>
<td>0.0062</td>
<td>±0.0010</td>
<td>0.49</td>
<td>112</td>
</tr>
<tr>
<td>77*</td>
<td>3,3',4,4'</td>
<td>6.36</td>
<td>0.0090</td>
<td>±0.0010</td>
<td>0.68</td>
<td>77</td>
</tr>
<tr>
<td>101*</td>
<td>2,2',4,4',5</td>
<td>6.38</td>
<td>0.0030</td>
<td>±0.0003</td>
<td>0.71</td>
<td>231</td>
</tr>
<tr>
<td>119*</td>
<td>2,3',4,4',5</td>
<td>6.58</td>
<td>0.0053</td>
<td>±0.0005</td>
<td>0.75</td>
<td>131</td>
</tr>
<tr>
<td>105*</td>
<td>2,3,3',4,4'</td>
<td>6.65</td>
<td>0.0053</td>
<td>±0.0005</td>
<td>0.74</td>
<td>131</td>
</tr>
<tr>
<td>118*</td>
<td>2,3,4,4',5</td>
<td>6.74</td>
<td>0.0049</td>
<td>±0.0006</td>
<td>0.62</td>
<td>141</td>
</tr>
<tr>
<td>126*</td>
<td>3,3',4,4',5</td>
<td>6.89</td>
<td>0.005</td>
<td>±0.0006</td>
<td>0.65</td>
<td>122</td>
</tr>
<tr>
<td>153*</td>
<td>2,2',4,4',5,5</td>
<td>6.92</td>
<td>0.0039</td>
<td>±0.0003</td>
<td>0.74</td>
<td>178</td>
</tr>
<tr>
<td>156*</td>
<td>2,3,3',4,4',5</td>
<td>7.18</td>
<td>0.0056</td>
<td>±0.0007</td>
<td>0.59</td>
<td>124</td>
</tr>
<tr>
<td>169*</td>
<td>3,3',4,4',5,5</td>
<td>7.42</td>
<td>0.0057</td>
<td>±0.0007</td>
<td>0.60</td>
<td>122</td>
</tr>
</tbody>
</table>
Figure 3.13

Mean Ln wet weight concentrations of the di-ortho substituted PCBs through time in *Oncorhynchus mykiss*. The solid line represents the concentration in the experimental trout and the dotted line represents the concentrations in the control trout. (some standard error bars may be hidden in the symbols). a) 47, b) 52, c) 101, and d) 153.
Figure 3.14

Mean ln wet weight concentrations of mono-ortho PCBs through time in *Oncorhynchus mykiss*. The solid line represents the concentration in the experimental trout and the dotted line represents the concentrations in the control trout. (some standard error bars may be hidden in the symbols).

a) 66,  b) 105,  c) 118,  d) 119, and  e) 156.
Figure 3.15

Mean Ln wet weight concentrations of non-ortho PCBs through time in *Oncorhynchus mykiss*. The solid line represents the concentration in the experimental trout and the dotted line represents the concentrations in the control trout. (some standard error bars may be hidden in the symbols). a) 81, b) 77, c) 126, and d) 169.
constant of the non-ortho congeners was for # 77, with a $K_{ow}$ of 6.36. The rate constants for congeners 126 and 169 were not significantly different. Congeners 81 and 77 were eliminating more rapidly than would be predicted by their $K_{ow}$ values.

The growth compensated kinetics observed in the elimination study along with corrected half lives of the congeners are summarized in Table 3.3. The non-ortho 77 and 81 had higher elimination rate constants than either the di-ortho or mono-ortho congeners with similar $K_{ow}$ values. This supports the observation that chlorine substitution patterns are relatively important in regulating the elimination of PCBs in rainbow trout. The chemical concentrations in the control fish remained constant over the duration of the experiment for all coplanar congeners, except 77 which declined significantly (Table 3.2). Congener 77 is thought to be one of the most degradable congeners of the coplanar group, and metabolism is a possible explanation for the significant decline of 77 in control and experimental trout.

No significant trend occurred when $1/k_2$ values were plotted against time

$$(m = -0.024, R^2 = 0.01)$$ (Figure 3.16). However, when individual substitution patterns were regressed with $K_{ow}$ the di-ortho substitution group had a positive slope of 0.13 $R^2 = 0.35$, the mono-ortho group had a negative slope of -0.11 $R^2 = 0.69$, and the non-ortho group had a positive relationship of $m = 0.12$ with $R^2 = 0.45$ (Figures 3.17-3.18). To determine if
Figure 3.16
Correlation between log \(1/k_2\) values of all congeners tested with log \(K_{ow}\).

Figure 3.17
Correlation between log \(1/k_2\) values of only di-ortho substitution patterns with log \(K_{ow}\).
\[ \text{Log } \left( \frac{1}{k_2} \right) \]

Log Kow

\[ m = -0.024 \]
\[ r^2 = 0.01 \]

\[ \text{Log } \left( \frac{1}{k_2} \right) \]

Log Kow

\[ m = 0.13 \]
\[ r^2 = 0.35 \]
Figure 3.18
Correlation between log $1/k_2$ values of only mono-ortho substitution patterns with log $K_{ow}$

Figure 3.19
Correlation between log $1/k_2$ values of only non-ortho substitution patterns with log $K_{ow}$
\[ \log \left( \frac{1}{k_2} \right) \]

**Mono-ortho**

\[
m = -0.11 \\
r^2 = 0.69
\]

**Non-ortho**

\[
m = 0.12 \\
r^2 = 0.45
\]
Table 3.4

MANCOVA results using the growth corrected kinetics.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.f.</th>
<th>Wilk's Lambda</th>
<th>F value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure x Time</td>
<td>2</td>
<td>0.667</td>
<td>10.98</td>
<td>0.0001</td>
</tr>
<tr>
<td>$K_{ow}$ x Time</td>
<td>3</td>
<td>0.357</td>
<td>25.83</td>
<td>0.0001</td>
</tr>
<tr>
<td>Structure x Time x $K_{ow}$</td>
<td>6</td>
<td>0.181</td>
<td>28.68</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
the substitution pattern significantly affected the $k_2$, each congener was classified according to its $K_{ow}$ and substitution pattern. A MANCOVA was performed on the ranked congeners for statistical rigour. The substitution pattern was found to significantly affect the elimination rate $k_2$ ($P < 0.0001$) (Table 3.4). A statistical relationship was observed between the $k_2$ of the spiked PCBs and their $K_{ow}$ values also significant ($P < 0.001$).
Discussion

In this study, the percent lipid remained constant over time. Ideally, elimination rate constants $k_2$, should be derived from an experimental design such that the organism being used does not grow over the duration of the experiment. The change in body mass of the organism may allow the chemical to be diluted by growth as opposed to being eliminated from the body. Most published elimination studies do not address the possible change in capacity of the experimental organism as it grows. As chemicals partition to the lipid phase of an organism, it is important to measure the change in the ability of an organism to hold the chemical (i.e. fugacity capacity) by measuring the change in percent lipid of the organism.

The elimination rate constants summarized in Table 3.3 are somewhat higher than values reported by Niimi and Oliver (1983) for rainbow trout (Table 3.5). For instance, half-lives for congeners 77 and 153 in the earlier study were 107 and $>1000$ days respectively. The cause of this difference is not known, but might be a result of the differences in dosing method (injection vs feeding), which might result in different uptake and depuration kinetics. Differences could also reflect the shorter depuration period for the Niimi and Oliver study. Tanabe et al. (1987), reported elimination rate constants that were an order of magnitude higher for PCBs in *Perna viridis* (Green-lipped mussels) than rates reported here. Elimination rate constants
Table 3.5
Comparison of elimination half life values obtained from other sources in the literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>weight/sample</th>
<th>Congener</th>
<th>Expt. length</th>
<th>T_{1/2}life</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. mykiss</td>
<td>600g</td>
<td></td>
<td>105d</td>
<td>650 #</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>77</td>
<td></td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66</td>
<td></td>
<td>890</td>
</tr>
<tr>
<td></td>
<td></td>
<td>119</td>
<td></td>
<td>+1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>153</td>
<td></td>
<td>+1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>156</td>
<td></td>
<td>+1000</td>
</tr>
<tr>
<td>P. viridis</td>
<td>5-6 g</td>
<td>20 indiv</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52</td>
<td>32d</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66</td>
<td></td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101</td>
<td></td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>118</td>
<td></td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105</td>
<td></td>
<td>6.4</td>
</tr>
<tr>
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<td></td>
<td>153</td>
<td></td>
<td>8.8</td>
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<td>138</td>
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<td></td>
<td></td>
<td>156</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>P. viridis</td>
<td>5-6 g</td>
<td>77</td>
<td>32d</td>
<td>9.3 @</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>169</td>
<td></td>
<td>85</td>
</tr>
<tr>
<td>C. virginica</td>
<td></td>
<td>77</td>
<td></td>
<td>87 &amp;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126</td>
<td></td>
<td>108</td>
</tr>
<tr>
<td>P. hoyi</td>
<td>6-10 mg</td>
<td>153</td>
<td>6h</td>
<td>888</td>
</tr>
<tr>
<td>M. relictta</td>
<td>43 mg</td>
<td>153</td>
<td></td>
<td>+1000</td>
</tr>
</tbody>
</table>

O. mykiss from Niimi et al. 1983. #
P. viridis from Tanabe et al. 1987. *
&
P. viridis from Kannan et al. 1989. @
are often species specific. Sericano (1992) reported $k_2$ values in *Crassostrea virginica* (American oyster), that were similar to values presented here (eg. 0.0079 ug/kg/day and 0.0064 ug/kg/day for congeners 77 and 126 respectively).

Sericano et al. (1992) and Kannan et al. (1986) have suggested that the coplanar congeners 169 and in particular 126 were trophically enriched in comparison to other PCB congeners. Koslowski (1993), however, observed in a food chain study on the Western Basin of Lake Erie, that coplanars 77, 126 and 169 were not trophically enriched and indeed suggested that trophic dilution was occurring relative to other PCB congeners of similar $K_{ow}$. In this experiment, the coplanar congeners 77 and 81 eliminated more rapidly than the di-ortho and mono-ortho substitution patterns, which were present in the spike mixture. Congeners 126 and 169 were eliminated somewhat more rapidly than their homologues in the spike mixture. This may be a result of non significant statistical relationship observed between the non-ortho substitution group and the body lipid of the trout ($P<0.05$). Whereas most congeners belonging to the di-ortho and to a lesser extent mono-ortho substitution groups were significantly correlated to the percent lipid ($P>0.0001$), ($P>0.05$). Congeners, such as 52 and 47, may become bound up in the lipid, and retained for longer periods of time, retarding elimination rates compared with non-ortho congeners.

Niimi and Oliver (1983) discussed the selective elimination of PCB
congeners in rainbow trout, and observed that elimination was dependent on both water solubility and chlorine substitution patterns. A strong correlation was observed between elimination rate constants and chlorine substitution patterns. Our experiment observed a statistical relationship between the elimination rate constants and $K_{ow}$, unlike that observed in mussels and oysters (Scharenberg 1991, Norstrom 1988, Smith et al. 1990). However, only compounds with a limited range in $K_{ow}$ were used in this study. Connell and Hawker (1988), among other researchers predicted a breakdown in the relationship between $K_{ow}$ and $k_2$ values for superhydrophobic compounds above $K_{ow}$ of approximately 5.8-6. The range of $K_{ow}$ values used in this experiment (5.8-7.42) fall into this $K_{ow}$ range, and it is possible these results are further evidence of the lack of correlation between the kinetics of superhydrophobic chemicals and $K_{ow}$ in this specific range.

The literature has consistently shown that coplanar 77 is a more dynamic compound than would be predicted from its $K_{ow}$ of 6.36 (Scharenberg 1991, Hoffman 1987). PCB 77 had the highest elimination rate constant of all compounds in this depuration experiment followed by coplanar congener 81. PCB 81, a less commonly studied coplanar PCB, has the same $K_{ow}$ of 6.36. This experiment shows that the non-ortho congeners 81 and 77 have relatively short half-lives compared with other PCBs of similar or even lower $K_{ow}$ values. As there are a wide range of $K_{ow}$ values (5.62-6.67) reported for congener 77 [Tanabe et al. 1987, Safe 1991,
Poland and Knutson 1982), our data indicate that the lowest log $K_{ow}$ of 5.62 reported by Rapaport et al. 1984, may be the most accurate.

Individual PCB congeners have significantly different kinetics. Some of the coplanar congeners which are the most toxic (Hoff 1992, Bidleman 1988, Addison 1986, Ankley 1991, Colborn 1991, Golub 1991) are the most dynamic in this study. Congener 77, which exists in higher concentrations in the environment, (Muir 1988) is also more dynamic than 126 or 169.

TEF values determined by Safe (1994) rank coplanar toxicity as 126 > 169 > 77 with values of 0.1, 0.05, and 0.01 respectively. Further to this study, a more conservative TEF value of 0.0005 was established for congener 77 (Ahlborg et al. 1994, Ahlborg 1992). However of greater interest is the role that the $k_2$ may play in the toxicity of congeners 77 and 126. It is possible that congener 77 being more dynamic and more highly concentrated in the environment, out competes other congeners for the Ah receptor sites. Due to 77’s lower induction affinity, the organism experiences less toxicity than might be expected if the receptor site were occupied by congener 126. However, once 77 has been eliminated from the organism, receptor sites may then become occupied by the more toxic congener 126. This suggests that as the concentration ratio of 77/126 changes, the level of toxicity the organism is experiencing may also change. The TEQ approach to toxicity is based on the assumption that the toxicity
experienced is additive. This does not take into account the pharmacokinetics of the individual congeners. Jantz et al. (1991) showed that when a mixture of congener 77 and 2,3,7,8-TCDD was tested on an AHH induction assay with rainbow trout, the toxicity level produced was less than expected. Although TCDD is fairly dynamic itself, congener 77 may be reaching the receptor sites prior to TCDD. A study by Brown et al. (1994) confirmed that kinetics dominated in the competition of Ah receptor sites rather than the principal of equilibrium partitioning previously thought. They observed that the first ligand to be added to the assay occupied a disproportionate number of the receptor sites. The TEQ approach to toxicity which is based on the level of induction multiplied by the concentration in the environment, assumes that all congeners have the same exposure kinetics. However, based on observations in this study, different substitution groups have substantially different dynamics. Concentrations observed in the sample represent only a moment in time. Toxicity may be very different at later dates in time due to differences in individual congener kinetics. Therefore using total PCB measurement to determine toxicity may lead to inaccurate conclusions.
Summary

Thirteen PCBs with a range in substitution patterns (di-ortho, mono-ortho, and non-ortho), and log $K_{ow}$ (5.8 - 7.42) were intraperitoneally injected with corn oil into 60 rainbow trout (Oncorhynchus mykiss). The non-ortho PCBs specifically 77, and 81 eliminated most rapidly from the fish (0.006 to 0.009 ug/kg/d). The range of elimination rate constants for the mono-ortho PCBs was 0.004 to 0.006 ug/kg/d, and the range for the di-ortho PCBs was 0.003 to 0.005 ug/kg/d. A highly significant interaction between chlorine substitution pattern and time was observed ($P<0.001$). A highly significant relationship ($P<0.001$) was observed between elimination rate constants and octanol-water partition coefficients ($log K_{ow}$).
CHAPTER FOUR

The Bioconcentration of Coplanar Polychlorinated Biphenyls in *P. notatus*.

Introduction

PCBs released into the environment via water (i.e., breach of containment from landfills, dumps, and sediment remediation sites), may subsequently partition into any one of the following compartments: water, air, and soil/sediment (Connolly and Pederson 1988). These chemicals may be taken up into organisms inhabiting these compartments. Uptake is based on a chemical’s bioavailability or the availability of the organism to be exposed through inhalation or ingestion. The bioavailability of a chemical is different in each compartment, and is a function of the chemical properties such as molecular structure, degree of chlorination or substitution, solubility, hydrophobicity, etc. (Ying Shiu and Mackay 1986). These important properties are somewhat related to the chemical’s log $K_{ow}$ value. Because it describes a chemical’s relative tendency to be driven into the lipid phase, $K_{ow}$ can be used as a predictive indicator for the compartments in which the compounds may accumulate. In the aquatic compartment, a chemical with a log $K_{ow} = 3$ is likely to stay along in the water phase while a chemical with a Log $K_{ow} = 8$ will adhere to the sediment phase, and possibly be taken up by benthic organisms via the food chain (Connolly and Pederson, 1988, and Gobas, et al. 1989). In terms of uptake from water, Mackay (1982) predicted that
bioconcentration increases with increasing \( K_{ow} \). This has been observed most strongly for chemicals with a \( \log K_{ow} < 6.0 \).

If fish and water are considered as homogeneous compartments, then the bioconcentration process is described as

\[
\frac{dC_f}{dt} = k_1 C_w - k_2 C_f \quad (1)
\]

where \( C_f, C_w, k_1, \) and \( k_2 \) represent the fish and water chemical concentrations, and uptake and elimination rate constants respectively. Assuming \( C_w \) is constant, the integrated form of equation (1) is:

\[
C_f = \frac{k_1}{k_2} \cdot C_w (1 - e^{-kt}). \quad (2)
\]

Thus as \( t \) becomes larger, equilibrium conditions will prevail and,

\[
BCF = \frac{C_f}{C_w} = \frac{k_1}{k_2}. \quad (3)
\]

However the ratio \( k_1/k_2 \) describes the partitioning of a chemical between the aqueous environment and organism's lipid phase. This by definition is the \( K_{ow} \), and substitution into equation (3) yields

\[
BCF_L = K_{ow}. \quad (4)
\]

In the literature, bioconcentration factors (\( K_c \)) have been strongly correlated to the 1-octanol/water partition coefficient. Correlation coefficients approaching or exceeding 0.90 have been reported for many \( K_{ow} \) and BCF relationships such that the BCF may be predicted within reason based on the relationship \( K_c = 0.48 \cdot K_{ow} \) (Niimi and Kissoon 1989, Connell and Hawker 1988).

Kannan et al. (1989) observed the enrichment of the coplanar congeners \# 77, \# 126, and \# 169 in \textit{Perna viridis} (Green-lipped mussels). Comparison of
the concentrations of coplanar PCBs in commercial PCBs to their levels in total PCBs taken up by mussels, reveals the considerable bioconcentration of these coplanar congeners relative to other congeners. Based on their observations on aquatic forms, they suggest that these highly toxic PCBs are potentially bioaccumulative in biological systems. Similarly, Koslowksi (1993) observed an increase in total lipid PCB concentrations with increasing trophic level in a Lake Erie contaminant food chain and distribution study. Although biomagnification of the coplanar congeners appeared evident, unlike that observed by Kannan (1989), the coplanar congeners 77, 126 and 169 did not enrich relative to total PCBs. In fact the percent composition of coplanar congeners was observed to actually dilute among higher trophic levels. The occurrence of congener 77 was found to be ubiquitous in the food chain whereas congeners 126 and 169 appeared to be concentrated in the sediment, benthic organisms, and terrestrial organisms (bird eggs). This suggests that even within the non-ortho substitution group the exposure dynamics (bioconcentration potentials) are different and results of the study in chapter 3, predict that $k_2$ may play a role in this observation.

This study aims to quantify uptake and elimination rate constants of the coplanar PCBs, 77, 126, and 169 in the forage fish species *Pimephales notatus*. Bioconcentration factors will be calculated and compared to corresponding $K_{ow}$ values.
Materials and Methods

1) Sample sites and collection

A forage fish species *Pimephales notatus* (bluntnose minnow), was gathered from the Chanel Carte in Walpole Island. This site had previously tested low in PCB residues and had an abundance of bluntnose minnows larger than young of the year (Hebert and Haffner 1992). Intrinsic to the kinetic models employed is that the test organism does not grow over the course of the study. Typically minnows > 3.5 cm are past young of the year hence their growth rate declines (Scott and Cross 1973).

The collection procedures were such that a 1 m by 10 m bag seign net with a mesh size of 6.0 mm was employed. A sweep of an area entailed two people walking in the same direction at the same moderate pace. Once a stretch had been seigned (approx. 20 m) the two leads of the net were united and the net was gathered. The fish were scooped from the submerged bag using a 15 cm by 10 cm aquarium net into a 60 cm X 40 cm X 120 cm holding tank filled with the same water as the sample site. This holding tank floated in the water to allow for more rapid collection and also to acclimate the fish to captivity. Upon completion of collection the holding tank was lifted into a garbage can filled with water from the site and placed in Novaqua, a chemical stress reducer which was added to the water (10 mL) to prepare the fish for transport. Pure oxygen was bubbled through the tank until reaching the
University of Windsor. The containment chamber was lifted into the blue 500 gallon holding tanks on the 4th floor of the biology building where the fish were allowed to acclimate to the cool dechlorinated, oxygenated water for 1/2 hour. Following this period, the fish were released and were contained here until all remaining fish were captured and the dosing was to begin. Extensive procedures and care were taken in the collection and transportation of these fish since there was typically 20-30% mortality experienced by the end of the collection process.

2) **Spiking mixture**

Six compounds (PCBs 77, 101, 138, 126, 153, 169) were chosen from those kept in stock at the Great Lakes Institute. Of greatest interest were the non-ortho congeners 77, 126, and 169. Due to the relatively high elimination rates observed in their depuration from rainbow trout, I was curious as to whether the effect of substitution pattern would be reflected in the $k_1$ rate constant and the Bioconcentration factor relative to the other congeners in this study. The crystals for each chemical were weighed to the nearest 10th of a gram and dissolved in 10 mL of Hexane. Due to the small amounts of some of the chemicals available, two solutions were developed. Solution A consisted of five compounds with a concentration of 900 mg/L. Solution B contained the five remaining compounds and had a concentration of 360 mg/L. Once in the hexane, all standards were placed in a sonic bath for 1 hr to dissolve any
remaining crystals. To ensure that all congeners had similar initial concentrations at the start of the experiment solution A was diluted to 360 mg/L. Four mL of spike solution B and 4 mL of spike solution A were pipetted onto sterile glass wool, and placed in a fume hood where the hexane carrier was allowed to evaporate off over 36 hours. This produced a 1440 µg/L spike compound.

3) Experimental Design

Four 50 gallon tanks were placed in an environmental chamber on the fourth floor of the biology building and were filled with tap water. Two aquaclear 200 filters packed with activated carbon were placed on each of the aquaria and were allowed to run for 1 week in order to dechlorinate and aerate the water in preparation to receive the fish. Following this period, two Fluvial 403 filters were attached to the aquaria. They were modified such that one drew aerated water from the 1st tank and delivered it to the remaining three tanks. The second Fluvial removed waste water from the three tanks and delivered it back to the 1st tank (Figure 4.1). Six 12 inch bubble wands (Aquarium services outlet) were installed in the 1st tank and were subsequently connected to three maxum elite air pumps. On July 15 bluntnose minnows were placed into these aquaria. Approximately 600 fish in all were placed in three of the prepared aquaria such that each aquarium received roughly 300 fish from the mixture of species.
Figure 4.1

Experimental set up: four 50 gallon aquaria were placed in an environmental chamber maintained at approximately 19 °C. Two fluvial 403 filters circulated the water through the oxygen tank and back to the three experimental tanks.
Dosing began by placing the spiking column in Fuvial # 1. (Figure 4.2). The spiked glass wool was placed in the second chamber of a three chambered Fluvial filter column. The first chamber was filled with Ammonex (Mail Order Pet Shop, CA. USA). This was to remove nitrogenous wastes generated by such a large number of fish. The third chamber was filled with sterilized glass wool to remove any particulate matter which might still be in the water and also to ensure against any re-crystallized PCBs from reaching the experimental fish. Flow regulation of each tank was achieved by a series of valves located on the delivery tubes. The flow rate was approximately 1 L/3 min. Each of the 4 tanks was completely sealed with translucent plastic covers which were taped around the edges to prevent as much leakage of any volatilized PCBs as possible. Small retractable doors were made to allow feeding of the fish. Each tank was given 2 g of Tetramin fish food (mail order pet shop) once a day. The experiment was originally designed to run for three months, encompassing both an uptake period and a depuration period to allow estimations of both $k_1$ and $k_2$. However, on Aug. 3 a die off occurred causing the termination of the experiment.

Sampling was initiated on July 15 (day 0), and subsequently on days 3, 5, 11, 15, and 17. A minimum of 3 g of tissue was required for each sample. On average 5 fish were sacrificed to acquire approximately 3 g. On the determined sample days, three 3 g samples were taken, one from each of the three tanks. These samples were wrapped in hexane rinsed foil and frozen until
Figure 4.2
Design of both the spiking column and cleanup column.
extraction at the completion of the experiment. Each tank was measured for
temperature, dissolved O₂, conductivity and pH. A 1 L water sample was
removed from each tank and placed in a hexane rinsed solvent bottle for later
extraction.

4) **Extraction and Analysis**

The extractions were carried out 5 samples and one blank at a time. All
procedures were identical to those followed for the trout tissue analysis (see
Lazzar *et al.* 1992). To account for any drift in the GC analysis, samples were
randomly extracted and analyzed at the end of the experiment.
Results

Death occurred in the bluntnose population on day 10 of the dosing. During this period abnormal, circular swimming behaviour and decreased activity were observed in various minnows. This suggests a possible toxicological response to the presence of the coplanar PCBs (as this was not observed in Chapter 2). There were enough fish present however, to continue a shorter (9 day depuration) than originally planned (3 month). Sample concentrations in the water were measured for the three coplanar congeners 77, 126, 169 (Fig. 4.3). The maximum water concentration reached for each congener was approximately 1.002 ug/L. The available chemical was absorbed quickly in the fish by day 5 of the dosing. A resurgence in the level of chemical in the water occurred on days 10-12 following the large die-off. The reappearance of the chemical in the water may be a result of bacteria from decomposing fish as well as less total chemical from the surviving fish. Table 4.1 displays the measured physical parameters for each of the three experimental tanks. The tanks showed good consistency among the temperature, conductivity, pH and most importantly oxygen levels. This also supported the assumption that all the tanks had the same rate of turnover from the fluvial filters. On the last sample date, day 11, the oxygen levels dropped by 1 mg/L to 6 mg/L. Only the dosing period was monitored as this was the
Figure 4.3

The Ln water concentrations (ng/L) through day 0 to day 11 of the dosing study for coplanar congeners a) 77, b) 126, and c) 169.
Table 4.1

Environmental parameters measured during the dosing in each of the three tanks. Temperature is measured in °C.

<table>
<thead>
<tr>
<th>Date</th>
<th>tank</th>
<th>Temperature</th>
<th>pH</th>
<th>Conductivity (ppm)</th>
<th>Oxygen (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>18.6</td>
<td>7.26</td>
<td>0.33</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.6</td>
<td>7.22</td>
<td>0.33</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.5</td>
<td>7.22</td>
<td>0.33</td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>19.04</td>
<td>7.4</td>
<td>0.33</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19.0</td>
<td>7.4</td>
<td>0.35</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.9</td>
<td>7.4</td>
<td>0.33</td>
<td>8.2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>18.43</td>
<td>7.30</td>
<td>0.42</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.5</td>
<td>7.31</td>
<td>0.42</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.5</td>
<td>7.2</td>
<td>0.42</td>
<td>8.4</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>19.1</td>
<td>6.9</td>
<td>0.37</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19.1</td>
<td>6.9</td>
<td>0.33</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>19.2</td>
<td>6.9</td>
<td>0.34</td>
<td>7.0</td>
</tr>
</tbody>
</table>
most stressful time for the fish, and the depuration allowed for greater oxygen penetration because the tanks were no longer covered.

The average weight increased slightly from approximately 3.5 g to 5.1 g. This 0.084 (g/d) rate of increase in body weight had a $R^2 = 0.57$ (Fig. 4.4). The variability may be a result of the death of the smaller minnows at day 10 leaving only the larger fish for sampling near the end of the study. This was mirrored in a negligible increase in percent lipid of 0.024 with a non-significant correlation to time $R^2 = 0.16$.

Uptake over time for PCB congeners 77, 101, 126, 138, 169, 153, and 194 are presented in Figures 4.6-4.12. The di-ortho congener 153 with a $K_{ow}$ of 6.9 had the highest observed uptake rate constant ($k_1$) of 32.0 ug/kg/day followed by congener 194 (10 ug/kg/day). The coplanar congeners 77, 126, and 169 had the next highest $k_1$ of 8.0, 5.3, and 3.0 ug/kg/day respectively (Table 4.2). Congener 180 a prevalent PCB in the environment was measured as a chemical control. Uptake of PCB 180 would indicate an outside source of chemical other than the spiking column. No uptake of congener 180 was observed $m=0.0007$, $R^2 = 0.0002$ with the high variability expected of background contamination in variable sized minnows (Figure 4.2).

Elimination over time for these same spiked congeners are shown in Figures 4.6 to 4.12. The duration of the depuration was 5 days. The two coplanar congeners 77 and 126 were observed to have the highest elimination rate constants ($k_2$) of 0.537 ug/kg/day and 0.217 ug/kg/day respectively (Table
Figure 4.4

Change in the weight (g) of *Pimephales notatus* over the duration of the 19 day study. (Standard error bars may be hidden in the symbols).
$m = 0.084$
$r^2 = 0.57$
Figure 4.5

a) Dosing study, mean Ln wet weight concentrations of PCB 180 through time in *Pimephales notatus*.

b) Elimination study, mean Ln wet weight concentrations of PCB 180 through time in *P. notatus*. 
Figure 4.6

a) Dosing study, mean Ln wet weight concentrations of PCB 101 through time in *Pimephales notatus*.

b) Elimination study, mean Ln wet weight concentrations of PCB 101 through time in *P. notatus*. 
Figure 4.7

a) Dosing study, mean Ln wet weight concentrations of PCB 138 through time in *Pimephales notatus*.

b) Elimination study, mean Ln wet weight concentrations of PCB 138 through time in *P. notatus*.
Figure 4.8

a) Dosing study, mean Ln wet weight concentrations of PCB 153 through time in *Pimephales notatus*.

b) Elimination study, mean Ln wet weight concentrations of PCB 153 through time in *P. notatus*.
Figure 4.9

a) Dosing study, mean Ln wet weight concentrations of PCB 194 through time in *Pimephales notatus*.

b) Elimination study, mean Ln wet weight concentrations of PCB 194 through time in *P. notatus*.
Figure 4.10

a) Dosing study, mean Ln wet weight concentrations of PCB 77 through time in *Pimephales notatus.*
Concentration = 8.2 (+/-0.4) x + 0.22.

b) Elimination study, mean Ln wet weight concentrations of PCB 77 through time in *P. notatus.*
Concentration = -0.53 (+/-0.023) x + 8.6.
Figure 4.11

a) Dosing study, mean Ln wet weight concentrations of PCB 126 through time in *Pimephales notatus*.
Concentration = 5.3 (+/-0.41) x -0.19.

b) Elimination study, mean Ln wet weight concentrations of PCB 126 through time in *P. notatus*.
Concentration = -0.22 (+/-0.068) x + 3.7.
a) Dosing study, mean Ln wet weight concentrations of PCB 169 through time in *Pimephales notatus*.
Concentration = 3.02 (+/-0.20) x -1.1.

b) Elimination study, mean Ln wet weight concentrations of PCB 169 through time in *P. notatus*.
Concentration = -0.036 (+/-0.053) x + 1.3.
Table 4.2.
The estimated uptake and elimination rate coefficients of seven PCB congeners for *P. notatus*. The bioconcentration factors are presented for the dosed congeners only. * PCB 180 was not in the spike mixture and is used here as a an experimental control.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_{ow}$</th>
<th>$k_1$</th>
<th>$R^2$</th>
<th>$k_2$</th>
<th>$R^2$</th>
<th>logKoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 77</td>
<td>6.36</td>
<td>8.2*</td>
<td>0.95</td>
<td>0.53*</td>
<td>0.98</td>
<td>3.4</td>
</tr>
<tr>
<td>PCB 101</td>
<td>6.4</td>
<td>2.0</td>
<td>0.027</td>
<td>0.042</td>
<td>0.45</td>
<td>4.1</td>
</tr>
<tr>
<td>PCB 138</td>
<td>6.83</td>
<td>0.91</td>
<td>0.67</td>
<td>0.073</td>
<td>0.30</td>
<td>4.0</td>
</tr>
<tr>
<td>PCB 126</td>
<td>6.89</td>
<td>5.3*</td>
<td>0.94</td>
<td>0.22*</td>
<td>0.86</td>
<td>3.1</td>
</tr>
<tr>
<td>PCB 153</td>
<td>6.92</td>
<td>32.0</td>
<td>0.50</td>
<td>0.045</td>
<td>0.40</td>
<td>4.0</td>
</tr>
<tr>
<td>PCB 180*</td>
<td>7.36</td>
<td>0.00071</td>
<td>0.00027</td>
<td>0.12</td>
<td>0.63</td>
<td>n/a</td>
</tr>
<tr>
<td>PCB 169</td>
<td>7.42</td>
<td>3.02*</td>
<td>0.98</td>
<td>0.036*</td>
<td>0.23</td>
<td>3.0</td>
</tr>
<tr>
<td>PCB 194</td>
<td>7.8</td>
<td>10.0</td>
<td>0.51</td>
<td>0.007</td>
<td>0.00096</td>
<td>2.4</td>
</tr>
</tbody>
</table>

P<0.05 = *
4.2). The lowest elimination rate constant was observed for PCB 194 with a $k_2$ of 0.007 ug/kg/day.

The maximum measured bioconcentration factors (BCF) are listed in Table 4.2. Congener 77 reached its maximum BCF on day 5 while the more toxic coplanars 126 and 169 attained their highest BCF on day 11. However, congener 77 had the highest log BCF value of 3.4, from the coplanar group. The highest BCF obtained among the spiked PCBs was congener 101 (4.1).

A positive relationship was observed between $k_1$ and log $K_{ow}$ with a slope of 55 and a $R^2 = 0.61$ (Fig 4.13). A positive relationship was also observed between $1/k_2$ and log $K_{ow}$ slope = 70 and $R^2 = 0.61$, however this is heavily weighted by the behaviour of congener 194 (Fig 4.14). Excluding PCB 194 produces a correlation with a slope of +13 and less significant $R^2 = 0.22$. A negative relationship was observed between the log BCF values and $K_{ow}$ with slope = -2.0 and $R^2 = 0.68$ (Figure 4.15). Statistical analysis was not carried out in greater detail as a result of the limited number of sample dates and sample replicates.
Figure 4.13

The correlation observed between the $k_1$ values and the log $K_{ow}$ of the spiked congeners.

Figure 4.14

The correlation between $1/k_2$ values and log $K_{ow}$ for the spiked congeners.
Figure 4.15  
The correlation between log $K_c$ and log $K_{aw}$ for the spiked congeners.
m = -0.87
r^2 = 0.67
Discussion

The large mortality of fish experienced on days 10-11 are thought to be a result of the presence of unusually high concentrations of the coplanar congeners. The fish exhibited low activity and some displayed a circular swimming motion, both indicative of toxicological stress in fish (Opperhuizen 1988).

Bioconcentration factors ($K_c$) may be determined one of two ways; 1) the actual $C_r/C_w$ ratio may be calculated at equilibrium by measured concentrations in the fish and water or, 2) by the equation $K_c = k_1/k_2$ in which these uptake and elimination rate constants may be calculated by linear regressions of change in concentrations over time. Characteristically the second method is preferred as equilibrium conditions are not reached in most laboratory studies. The estimated $k_2$ values obtained from this data set were an order of magnitude higher than those observed in the literature and in a previous study for fish of similar size (Table 4.4). Longer elimination studies without toxic stress provide lower $k_2$ values (Hawker 1986, Nilmi 1989). This may be associated with the fact that elimination of these hydrophobic compounds is blood flow limited. Elimination may be more accurately thought of as elimination from two basic compartments, 1) the blood (and aqueous phases) directly, and 2) the lipid via the blood. Chemical elimination from muscle (which has high blood perfusion rates) was observed to be twice that of the lipid in a comparative study of PCB
Table 4.4.
Comparison of uptake, elimination, and $K_c$ values present in the literature for small fish (guppies, fathead minnows)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>$K_{ow}$</th>
<th>$k_1$ (/d)</th>
<th>$k_2$ (/d)</th>
<th>log$K_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCBs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52*</td>
<td>5.84</td>
<td>1220</td>
<td>0.015</td>
<td>5.76</td>
</tr>
<tr>
<td>153</td>
<td>6.92</td>
<td>794</td>
<td>0.004</td>
<td>6.16</td>
</tr>
<tr>
<td>194</td>
<td>7.8</td>
<td>151</td>
<td>0.007</td>
<td>6.24</td>
</tr>
<tr>
<td>52@</td>
<td>5.84</td>
<td>741</td>
<td>0.015</td>
<td>4.26</td>
</tr>
<tr>
<td>153</td>
<td>6.92</td>
<td>794</td>
<td>0.004</td>
<td>5.32</td>
</tr>
<tr>
<td>194</td>
<td>7.8</td>
<td>151</td>
<td>0.0071</td>
<td>4.35</td>
</tr>
<tr>
<td>2,3,7,8-TCDD**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.64</td>
<td>603</td>
<td>0.046</td>
<td>4.11</td>
</tr>
</tbody>
</table>

* Gobas et al. 1989
@ Bruggeman et al. 1984
** Opperhuizen et al. 1985
depuration from lipid versus muscle tissue in *Oncorhynchus mykiss* (Niimi 1983). Thus elimination slopes may be slightly biphasic such that depuration at the very early stages may reveal rapid elimination from the more aqueous compartments.

A great amount of variability for k₂ values occurs in the literature (Niimi 1987). This is partly a result of the presentation of the data as wet weight, lipid normalized, and body burden, making it difficult to compare values among studies. Elimination rate constants are species specific (Niimi 1987, Sericano et al. 1992, Kannan 1986). The k₂ value is actually a total elimination rate constant rather than an elimination via gills as the models assume. Thus k₂ = kₑ + kᵣ where kₑ and kᵣ are assumed to be negligible. The elimination via faeces and urine, and metabolism of PCBs are physical parameters specific to each species. However the kₑ has been observed to be insignificant (5% of dose) especially for the higher Kₐw compounds (Lutz 1987). The kᵣ presents a source of variation as the literature suggest that different species have different biotransformation capabilities. In general, lower chlorinated congeners are more readily metabolized as are those substitution groups which have unsubstituted adjacent meta and para carbons. Studies among rats, dogs, and monkeys have shown that metabolism rates may vary by orders of magnitude depending on the chemical (Lutz 1987). The lipid fraction of an organism accumulates most of the chemicals, changes in the lipid fraction may affect rate constants. Increase in lipid content may result in lower elimination of chemicals due to the
lower perfusion rate of blood through the lipids. Gobas and Mackay (1987) predicted an increase in storage lipid may change the ratio of target to non-target lipid thereby changing the rate of chemical flux to the target area (site of toxic action). Heuvel (1991), observed higher elimination kinetics of pentachlorophenol in low lipid fish versus slower elimination from high lipid fish, however the trend was insignificant due to high variability. This has toxic implications in that organisms with less body fat may experience higher toxicity than organisms with high body fat. Any mobilization of lipid in organisms with high body fat can produce greater toxicity due to the partitioning of chemicals. When plotted against $K_{ow}$, $1/k_2$ showed a positive relationship, but this was heavily waited by the behaviour of congener 194. Connell et al. (1988) and Gobas (1989) predicted and observed a positive correlation between $1/k_2$ and $K_{ow}$. However a breakdown occurs in this relationship around $K_{ow}$ of 5.8-6. Connell (IBID) and Gobas (IBID), hypothesized this was due to a breakdown in the relationship between lipid and the surrogate phase octanol, decreasing the predictive capability of $K_{ow}$ for superhydrophobic compounds.

The uptake rate constants were low when compared to the published values for guppy (Table 4.4). Gobas (1989) calculated a minimum uptake value of $10^{3.11}$ (Gayer 1985) for chemicals with a log $K_{ow}$ > 6.5. The coplanar congeners had strong significant $R^2$ values of 0.95, 0.94, and 0.98 for congeners 77, 126, and 169 respectively based on linear regressions. However non-linear regression may provide a better $R^2$ value for the remaining congeners.
The lipid dependence of the di-ortho and mono-ortho substitution patterns may resulting in delays of partitioning into the lipid fraction complicating the fit of the data to linear uptake kinetics.

The coplanar congeners had similar $k_1$ constants thus stressing the importance and dominance of elimination constants in predicting exposure dynamics. Although the chemical was pumped into the water before the fish, it is possible that such a large number of fish took up the chemical so completely, the uptake rates reflect the limitations of the pump to spike the water. A positive relationship was observed between $k_1$ and log $K_{ow}$ (Fig. 4.13). Published literature has shown that $k_1$ versus $K_{ow}$ results in a parabolic curve at a $K_{ow}$ value of approximately 5.5. This is thought to be a result of the increase in molecular size of the compounds which decreases diffusion coefficients. Thus compounds with a $K_{ow} > 6$ are controlled by diffusion through the aqueous phases of the fish (membrane permeation) (Gobas 1986, Connell 1988).

Although most published studies report bioconcentration factors calculated from the first-order equations and corrected for lipid, the $k_1$ and $k_2$ values observed in this study appeared unreliable for this purpose. BCFs were derived from the ratio of actual fish to water concentrations for each of the sample dates day 0 to day 11 (table 7). The advantage to utilizing equations is the ability to determine $K_c$ when the study is not in equilibrium. The duration of this experiment was too brief to allow steady state to be reached. This is
reinforced by the observation that for any one chemical, no $K_c$ was the same for any of the sample dates. The determined $K_c$ values for this study were an order of magnitude lower than those presented in the literature. The BCF is affected by many factors, such as species, age, size, metabolism and environmental factors such as temperature, salinity, dissolved oxygen and light (Gayer 1985). Congener 77 attained its maximum $K_c$ before the other coplanar congeners in the spike. This supports observations of congener 77's dynamic nature and the possibility it blocks out other toxic chemicals from the aryl hydrocarbon hydroxylase receptor sites. The rapidly attained, large value for $K_c$ serves to stress the deterministic quality of $k_2$ values in kinetics. The plotting of $K_c$ versus $K_{ow}$ is expected to yield a positive relationship with higher $K_{ow}$ compounds having higher BCFs. A negative correlation between $K_{ow}$ and $K_c$ ($m = -0.09$, $R^2 = 0.67$) was observed in this study. Other experimental results have shown that rather than a linear relationship existing between $K_{ow}$ and $K_c$ ($\log \text{BCF} = -0.23 + 0.76 \log P$) (Niimi 1989), a parabolic one appears to better fit the data for compounds with a $K_{ow}$ higher than 6 (Gobas 1989). Depending on the species this may be in part caused by the metabolism of the chemical. The elimination rate constant $k_2$ is actually a total elimination via the gills, faeces, and metabolism. The occurrence of metabolism or faecal elimination would result in lower $K_c$ values. The concentration of these superhydrophobic chemicals in the water phase may be over estimated since due to their low water solubility, they adsorb onto any form of organic matter.
in the water in turn reducing their actual bioavailability to be taken up into the fish. This could also cause a lower $K_c$ than would be expected.

The relationships between $k_1$, $1/k_2$, and $K_c$ with $\log K_{ow}$, observed in this study are unreliable due to the small range in $\log K_{ow}$ (6.36 to 7.8), thus all chemicals tested were above the crucial value of $K_{ow}$ 6. A more thorough study would include a larger number of substitution pattern replicates and varying $K_{ow}$ values.

An advancement in modelling accuracy occurs when the model more closely approximates the actual mechanism. This is the case with a two compartment and multicompartment model approach. Physiological pharmacokinetic modelling (PKB) is one step closer to representing the real kinetic movement of a chemical within an organism and includes as many compartments as the tester requires. Lutz (1994) developed a model containing blood, liver, gut lumen, muscle, skin, and fat compartments. Imperative to the PKB model are the anatomical data such as tissue size and organ volumes; equilibrium partitioning coefficients; clearance, blood flow and metabolism rates; and diffusion coefficients and membrane permeabilities. These parameters may be available in the literature for some species but many may have to be established before the model would predict effectively. These models appear time consuming and are highly species specific but with calibration could provide greater insight into the movement of chemicals within an organism.
Summary

Little is known about the exposure dynamics of the most toxic group of polychlorinated biphenyls, the non-ortho substituted, (coplanar) PCB congeners. In this study the relative uptake ($k_1$) rate constants of 8.2, 5.3, and 3.0 (ug/kg/day) and elimination ($k_2$) rate constants of 0.53, 0.22, and 0.036 (ug/kg/day) were estimated for the feral fish species, the bluntnose minnow (P. notatus). Correlations between $k_1$ and $1/k_2$ with log $K_{ow}$ resulted in a positive relationship with $R^2 = 0.61$ for both parameters and supports similar observations in the literature. Corresponding $K_c$ values were determined. A negative relationship was observed between log $K_c$ and log $K_{ow}$ ($m = -2.0$, $R^2 = 0.68$).
CHAPTER FIVE

General Conclusions

These studies have further resolved the relative importance of chemical and biological properties in regulating the exposure dynamics of PCBs in aquatic ecosystems. Elimination rate constants, measured over a limited range of $K_{ow}$ for the bluntnose minnow, varied poorly with $K_{ow}$. It was further observed that $K_{ow}$ alone does not regulate $k_2$ in rainbow trout, and that substitution pattern also plays an important role. An underlying relationship evidenced in this study was the strong correlation of the di-ortho and mono-ortho substitution groups with percent lipid, unlike the non-ortho congeners which did not exhibit a significant correlation to lipid content. This factor may affect the kinetics of PCBs as well as influence their differential toxicity. Uptake and elimination studies confirmed, however, that $K_{ow}$ can play an important role, but is not sufficient to predict the exposure dynamics of the more toxic coplanar PCB congeners such as 77, 126 and 169. The rapid kinetics observed in this research supports the conclusion of Koslowski et al. (1993) in that coplanar PCBs are not predicted to be trophically enriched as speculated by Tanabe (1987).

Environmental toxic assessment rests heavily upon the TEO approach. The assumption maintained is that the variety of PCB congeners behave similarly, thus exposure, measured as Total PCB concentration, is used in
hazard assessment (Williams 1992). This study has shown that PCB congeners can have very different exposures (kinetics). Hence TPCB concentrations may lead to erroneous conclusions.

Due to high mortality, elimination studies with feral fish (ie. P. notatus) are difficult to perform. Even if kept alive, it is possible that stress, leading to high metabolic activity might result in an overestimation of elimination rate coefficients.

These studies may be altered to favour an in situ design. The loss of control of such parameters as pH, dissolved oxygen, aqueous chemical concentrations and food consumption would be sacrificed for more accurate calibration of chemical kinetics in an unstressed, naturally occurring population. The use of feral fish populations indigenous to a particular region may act as better monitors of contaminant levels than the use of caged mussels or clams uncharacteristic to the site.
The various concentrations analyzed and used in the three studies with *P. notatus* and *O. mykiss* are presented in the following tables.

a) Wet weight concentrations for chemicals examined in *P. notatus* (study 1).

b) Lipid corrected concentrations for chemicals examined in *P. notatus* (study 1).

c) Wet weight concentrations for chemicals examined in *O. mykiss*, controls fish (study 2).

d) Wet weight concentrations for chemicals examined in *O. mykiss*, experimental fish (study 2).

e) Wet weight concentrations for chemicals examined in *P. notatus* (study 3).
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<td>83.48721</td>
</tr>
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