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Chemical exposure dynamics of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in green frog (Rana clamitans) and bullfrog (Rana catesbeiana) tadpoles

Kimberley McCormack
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CHEMICAL EXPOSURE DYNAMICS OF POLYCHLORINATED BIPHENYLS (PCBs) AND ORGANOCHLORINE PESTICIDES (OCs) IN GREEN FROG (*RANA CLAMITANS*) AND BULLFROG (*RANA CATESBEIANA*) TADPOLES

by

Kimberley McCormack

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CO-AUTHORSHIP STATEMENT

I hereby declare that this thesis incorporates material that is the result of joint research, as follows: The two chapters included in this thesis (Chapters 2 and 3) will be published as co-authored papers. G.D. Haffner and K.G. Drouillard contributed intellectually to the work in Chapters 2 and 3 by providing initial study ideas and consultation throughout the process.

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

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

ABSTRACT

Studies were conducted to determine the effects of different environments and temperatures on chemical exposure dynamics in tadpoles. In the first study, green frog tadpoles (*Rana clamitans*) were dosed with Performance Reference Compound polychlorinated biphenyls (PRC PCBs) and placed into agricultural, municipal and reference watersheds near Windsor, Ontario, Canada. PRC PCBs were eliminated significantly faster than in previous laboratory studies, with elimination rate coefficients between 0.200 and 1.295 day^{-1}. Tadpoles reached steady state within 23 days and tracked differences in chemical bioavailability among sites.

The elimination of PRC PCBs and DDT were evaluated in green frog and bullfrog (*Rana catesbeiana*) tadpoles at two temperatures (18 and 25°C). Temperature had no effects on elimination of PRCs or DDT. These results indicate that tadpoles are tolerant of moderate temperature changes in this range. Green frogs eliminated PRCs and DDT faster than bullfrogs (e.g. PRC 43 at 1.278 versus 0.421 day^{-1}).

DEDICATION

This thesis is dedicated to all students who truly desire and deserve the opportunity to do a Master's, but fail due to such things as grades.

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LIST OF ABBREVIATIONS

AF – Aquaculture Facility
ANCOVA – Analysis of covariance
ANOVA – Analysis of variance
BCF – Bioconcentration factor
BF – Bullfrog
BR – Belle River
BSAF – Biota-sediment accumulation factor
C – Chemical concentration
C_{org} – Chemical concentration in an organism
$C_{org(t)}$ – Chemical concentration in an organism at time *t*
$C_{org(t=0)}$ – Chemical concentration in an organism at the start of the elimination period
C_{ss} – Concentration at steady state
DCM – Dichloromethane
DDD – Dichloro-diphenyl-dichloroethane
DDE – Dichloro-diphenyl-dichloroethylene
DDT – Dichloro-diphenyl-trichloroethane
GF – Green frog
HCB – Hexachlorobenzene
OC – Organochlorine pesticide
PCB – Polychlorinated biphenyl
PFOS – Perfluorooctanyl sulfonate
PRC – Performance Reference Compound
k_1 – Net uptake rate coefficient
k_2 – Elimination rate coefficient
K_{ow} – Octanol-water partition coefficient
OCS - Octachlorostyrene
SE – Standard error of the mean
SPMD – Semi-permeable membrane device
t – Time
t_{95} – Time to achieve 95% steady state concentration
TC – Turkey Creek
α-HCH – α-hexachloro-cyclohexane
β-HCH – β-hexachloro-cyclohexane
γ-HCH – γ-hexachloro-cyclohexane
δ-HCH – δ-hexachloro-cyclohexane
Σ – Sum
1234-TCB – 1234-tetrachlorobenzene
1245-TCB – 1245-tetrachlorobenzene

CHAPTER 1 – GENERAL INTRODUCTION

1.1 – Amphibian Declines

Amphibian populations have been in decline worldwide since the 1970s (Barinaga, 1990). No one factor has been definitively identified as the cause of the declines. Instead, many different hypotheses have been raised, including UV radiation (Blaustein *et al.*, 1998), habitat loss (Riley *et al.*, 2005; Delis *et al.*, 1996), acidification of waters (Hart and Hoffman, 1989; Bridges *et al.*, 2002), nutrient inputs (Edwards *et al.*, 2006), climate change (Reading, 2007; Carey and Alexander, 2003), pathogenic infections (Lips *et al.*, 2006) and chemical exposure (Blaustein and Wake, 1990; Wake, 1998; Houlahan *et al.*, 2000).

1.2 – Amphibian Chemical Exposure

The environments which amphibians rely on can be contaminated with a number of different chemicals through effluent (e.g. sewage, waste, water) from industries or urban areas, aerial pesticide applications (Berrill *et al.*, 1994; 1995), agricultural runoff (Gaizick *et al.*, 2001) and atmospheric deposition. Absorption of contaminants by amphibians can potentially occur during all life stages (Sparling *et al.*, 2001) because they have permeable skin and gills, and eggs that are not protected by a hard shell (Bantle *et al.*, 1992). Exposure to contaminants in amphibians can occur in both the aquatic and terrestrial environments, since they are dependent on the aquatic environment as tadpoles and more dependent on the terrestrial environment as adults (Howe *et al.*, 1998). Chemical exposure also occurs through the diet from aquatic and terrestrial sources, given that they are herbivorous as tadpoles and eat insects and other invertebrates as adults (Howe *et al.*, 1998). As a result of these combined factors (their unique life cycle, shifts in trophic status and their physiology), amphibians are often exposed to environmental chemicals, and have been described as super-accumulators (Gillan *et al.*, 1998). Since they serve as prey for other animals, amphibians can contribute significantly to the accumulation of chemicals in other species from both the aquatic and terrestrial food webs (DeGarady and Halbrook, 2003).

1.3 – Amphibian Sensitivity to Contaminants

The sensitivity of amphibians to contaminants depends on their developmental stage (Greulich and Pflugmacher, 2003). These differences in chemical sensitivity were frequently ignored in past studies. The tadpole and metamorph phases of the amphibian life cycle are generally the most sensitive to contaminants (Hall and Swineford, 1980; Freda and McDonald, 1993; Kane *et al.*, 1993). For example, the LC_{50} (concentration at which 50% of tested animals perish) for common frog (*Rana temporaria*) tadpoles exposed to DDT was 10,000 ng/g, while that for adults it was 24,000 ng/g (Cooke, 1970; Harri *et al.*, 1979).

This sensitivity in the early life stages of amphibians is probably due to energy and development requirements. Lipids provide the energy for embryos to develop and are actively transferred to membranes in early development (Ferrari *et al.*, 2008), which can disrupt the lipid-bilayer function. Similarly, during metamorphosis amphibians cease feeding and rely entirely on the lipids stored in their tails for their energy requirements. This use of lipids during metamorphosis can result in a rapid loss of mass. As a consequence, moderate to highly hydrophobic chemicals found in the lipids can increase in relative activity, potentially increasing toxic stress associated with the chemicals (Leney *et al.*, 2006a). Because of life cycle differences in sensitivity, it is imperative to examine the responses of amphibians to chemicals during all of their life stages, but especially during the tadpole and metamorph stages since these are most sensitive to contaminant exposure.

Not many studies have compared amphibian sensitivity to contaminants as compared to other species. A study by Bridges *et al.* (2002) examined the sensitivity of tadpoles compared to fish using two amphibian species (southern leopard frogs and boreal toads) and three fish species (bluegill sunfish, fathead minnows and rainbow trout). Tadpoles were more sensitive to copper than fish. However, for chemicals like carbaryl and permethrin, fish were more sensitive than tadpoles. Rainbow trout were the most sensitive to carbaryl, PCP and permethrin, while boreal toads were the most sensitive to 4-nonylphenol and copper. Another study revealed that the early life stages of some amphibians (i.e. northern leopard frogs and American toads) are more sensitive to Aroclors 1016, 1242 and 1254 than early life stage fish species (i.e. redear sunfish and

channel catfish) (Harfenist *et al.*, 1989). Rainbow trout fry were the most sensitive to contaminants in this study, followed closely by northern leopard frogs.

Amphibians have been identified as good indicators of ecosystem health (Blaustein *et al.*, 2003). Despite this fact, their use as bioindicators, or biomonitors, has not been well developed. When choosing an animal to serve as an indicator of contamination, a few criteria need to be met. These criteria are: the species can survive in the presence of the contaminants; the species can easily absorb the contaminants to levels higher than ambient concentrations; the species is easily sampled in the area where it was exposed to contaminants; the use of the species allows for a number of different habitats, or one specific type of habitat, to be investigated; the species is common enough to allow for sampling without raising concerns about continued survival of populations; and individuals of the species are large enough in body size to measure residues or physiological changes as a result of contaminant exposure (modified from: Sparling *et al.*, 2000).

For the most part, the tadpole stage of the amphibian life cycle meets the above criteria. Tadpoles are easily deployed, collected and analyzed for contaminant burdens (one tadpole can easily provide enough tissue for a single replicate). A variety of chemicals can also be tracked by tadpoles, including PAHs (Leney *et al.*, 2006b and 2006c), PCBs and OCs (e.g. Russell *et al.*, 1997). Frogs also have a very limited capacity to metabolize chemicals (Leney *et al.*, 2006b), which is one of the criteria behind the selection and use of mussels as biomonitors (e.g. Brown, 1992; Ravera, 2001; Stegeman and Lech, 1991). Cages of tadpoles can also be deployed in a variety of environments where other biomonitors would not be useful. For example, tadpoles can be deployed into stagnant ponds with low oxygen levels, since they can respire at the air-water interface by gulping air. Tadpoles can also be placed into areas with low water flow, unlike mussels and some fish which require flowing water to respire (Hildreth, 1976; Belal, 2008).

1.4 – Chemical Toxicity and Bioaccumulation

When exposed, organisms undergo various levels of interaction with a chemical, which include: 1) contact with the chemical; 2) uptake into the organism; 3) metabolism; 4) storage at inert sites; 5) excretion; 6) transport to target sites; and 7) interaction with

target sites (modified from: Eto, 1974). These interactions are influenced by the biology of the exposed organism and the properties of the chemical (Eto, 1974), which will both also influence a chemical's relative toxicity. The toxicological hazards of a chemical are generally evaluated using three criteria: persistence in the environment, tendency to bioaccumulate in food chains and toxicity to organisms. These criteria are related to the structure of the chemical, specifically solubility. Solubility in water is usually expressed using the octanol-water partition coefficient, or K_{ow} (octanol is a non-polar substance used as a surrogate for lipids). A chemical's K_{ow} reflects the partitioning of that chemical between a mixture of octanol and water when the system is at equilibrium. The K_{ow} of the chemical is defined as:

$$K_{ow} = \frac{C_o}{C_w} \tag{1.1}$$

where C_o is the concentration of the chemical in octanol at equilibrium and C_w is the concentration of the chemical in water. Values of K_{ow} can range over several orders of magnitude and are often expressed on a log scale. Chemicals are considered moderately hydrophobic at a log K_{ow} between 5 and 6 and super-hydrophobic at a log K_{ow} greater than 6.

1.5 – Determining the Bioaccumulation of Chemicals

Thousands of new chemicals are produced each year, so it is impossible to test toxicity on a chemical-by chemical basis. Instead, the fate of the chemicals in the environment (i.e. which environmental compartments it will partition into: air, water, or sediments) and their effects on organisms need to be predicted. The properties of the chemical can be used to achieve both these goals through the development of chemical exposure dynamics models. However, because of differences in the rates of uptake, elimination and metabolism of chemicals among species, it is necessary to develop chemical exposure dynamics models for key species in order to develop an ecosystem understanding of a chemical's fate and effects.

One type of chemical exposure dynamics model is a first-order, one-compartment kinetic model (e.g. Barron *et al.*, 1990). These models are widely used in aquatic

toxicology studies, allowing for comparisons with other species. Few such studies, however, have been done in amphibians.

The following equation is the basic one-compartment model that predicts the change in concentration of a chemical in an aquatic organism over time when it is placed into a contaminated environment:

$$\frac{dC_{org}}{dt} = k_w C_w + k_f C_f - k_{eg} C_{org} - k_g C_{org} - k_{met} C_{org} - k_{diff} C_{org} - k_{rep} C_{org} \qquad (1.2)$$

where: C_{org} is the concentration within the organism,

k_w is the uptake rate coefficient (or constant) from water,

C_w is the concentration of the chemical in water,

k_f is the uptake rate coefficient from food,

C_f is the concentration of the chemical in food,

k_{eg} is the elimination rate coefficient of the organism via fecal egestion,

k_g is the elimination rate coefficient of the organism via growth,

k_{met} is the elimination rate coefficient of the organism via metabolism,

k_{diff} is the elimination rate coefficient of the organism via gills, and

k_{rep} is the elimination rate coefficient of the organism via reproduction.

In practice, the whole body elimination rate coefficient ($k_{eg} + k_g + k_{met} + k_{diff} + k_{rep} = k_2$) of a chemical from a contaminated organism into a clean environment, where C_w is essentially zero, can be estimated using:

$$\frac{dC_{org}}{dt} = -k_2 C_{org} \qquad (1.3)$$

(Barron *et al.*, 1990), where k_2 represents the whole body elimination rate coefficient. In order to calculate k_2, we must first modify equation 1.3 to:

$$C_{org(t)} = C_{org(t=0)} e^{-k2t} \qquad (1.4)$$

or

$$\ln C_{org(t)} = \ln C_{org(t=0)} - k_2 t \qquad (1.5)$$

where $C_{org(t=0)}$ is the concentration of the chemical in the organism at the beginning of the elimination experiment.

From equation 1.5, the total elimination rate coefficient, k_2, can be measured as follows:

$$k_2 = \frac{\ln C_{org(t=0)} - \ln C_{org}}{t} \tag{1.6}$$

Once k_2 is determined, the time to 95% steady state (where uptake equals elimination) can then be determined using the following equation:

$$t_{95} = \frac{3}{k_2} \tag{1.7}$$

When an organism reaches steady state with its environment, there are several methods of quantifying the relative accumulation potential of an organism in its environment. One is the biota-sediment accumulation factor (BSAFs), which is the ratio of a chemical in an organism to that in the surrounding sediment. In green frogs, BSAFs increase with log K_{ow} value in a linear fashion (Gillan *et al.*, 1998). Such methods assume that the organism and its environment are in either equilibrium or steady state, yet often there is little evidence to support such an assumption.

A more quantitative method of determining if an organism is at steady state with its environment is a toxicokinetic approach where the organism is calibrated using Performance Reference Compounds (PRCs). Performance Reference Compounds are analytically non-interfering organic compounds that are selected to match the physicochemical properties of chemicals of interest, and usually have moderate to high K_{ow}s (i.e. ≥ 5). Originally injected into Semi-Permeable Membrane Devices (SPMDs) prior to deployment in the field (Huckins *et al.*, 2002), PRCs have begun to be used in biomonitors (e.g. O'Rourke *et al.*, 2004; Raeside *et al.*, 2009). Performance reference compounds allow researchers to calibrate biomonitors with respect to *in situ* chemical uptake and elimination rates in order to evaluate whether or not steady state can be expected to be achieved in a given life stage or over the age range of animals being sampled.

In order to calibrate biomonitors, a few steps must be taken. After dosing the biomonitors with the PRCs, the elimination rate coefficients of the PRCs are determined. Then a model is used to estimate the exposure dynamics of a chemical over time using the elimination rate coefficient of the PRC closest to it in terms of log K_{ow} value. This model,

developed in Raeside *et al.* (2009), assumes that steady state is achieved for each congener, and that the concentration of the chemical in the environment is coefficient over the time frame of the study. The exposure dynamics of a chemical over time can be established as:

$$C_{org(t)} = C_{org(t=0)} + [C_{org(ss)} - C_{org(t=0)}] \cdot (1 - e^{-k_2 t}) \qquad (1.9)$$

Where $C_{org(ss)}$ is the concentration of the chemical in the organism at steady state (Raeside *et al.*, 2009). The predictions of this model can be compared to actual time series data, and the model evaluated with respect to accuracy and precision.

The initial groundwork for toxicokinetic modeling in amphibians was carried out by Huang and Karasov (2000) and Leney *et al.* (2006a,b,c) using frogs. In their studies on frogs dosed with polychlorinated biphenyls (PCBs) it was revealed that elimination kinetics were first order. Studies on toxicokinetics in frogs are few, and have been carried out on many different species (Ankley *et al.*, 2004; Honkanen and Kukkonen, 2006; Greulich and Pflugmacher, 2004; Fridman *et al.*, 2004). The chemicals studied include industrial solvents, pesticides and hormonal modulators (e.g. PCBs, PFOS, bisphenol-A, atrazine, cypermethrin and estradiol).

The distribution among the life stages studied in the literature is also fairly even. Leney *et al.* (2006b) showed, however, that uptake dynamics and chemical metabolism differed among life stages. There is therefore a need to establish life cycle models that develop a framework to understand and compare the sensitivity of amphibian species around the globe. Polychlorinated biphenyls provide an excellent suite of congeners to develop and test such models, since individual PCB congeners can be selected to compare with other chemicals of interest based on their log K_{ow} or susceptibility to metabolism due to their structure and chlorination patterns.

1.6 – The Effects of Temperature on Toxicokinetics

In aquatic cold-blooded animals like frogs, environmental temperature can change activity, growth rates (Rombough, 1996), the permeability of membranes and enzyme activity (Kennedy and Walsh, 1996). These factors will affect both chemical uptake and elimination processes. Higher temperatures are predicted to increase exposure dynamics as a result of higher metabolic rates (Carey and Alexander, 2003; Reading, 2007) and

higher growth rates (Beebee, 1995). A study on the effects of temperature on the accumulation of bisphenol A in tadpoles (*Rana temporaria*) found that at higher temperatures (19°C compared to 7°C) uptake and elimination rate coefficients were higher (Honkanen and Kukkonen, 2006). Tadpoles at 19°C reached steady state (where chemical uptake was equal to chemical elimination) in ~4 days, while those at 7°C did not reach steady state. Licht (1976) found that uptake of DDT was faster at 15°C than at 21°C in wood frog (*Rana sylvatica*) tadpoles. Alternately, elimination rate coefficients were approximately twice as large at 21°C.

Other studies have demonstrated that temperature increased the toxicity of chemicals during early frog development (Boone and Bridges, 1999; Broomhall, 2004). It was not investigated, however, whether these changes in toxicity were due to differences in tissue concentrations, sensitivities, or a combination of the two. Furthermore, temperature can negatively affect body condition and survival (Reading and Clark, 1995; Reading, 2007). The effects of temperature changes on the toxicokinetics of amphibians are therefore an important factor to consider when attempting to interpret biomonitoring results using native collected animals and transplanted organisms. Understanding how temperature changes chemical exposure dynamics is also integral to developing environmentally-relevant life cycle models.

1.7 – Life History of Green Frogs and Bullfrogs

Green frogs, *Rana clamitans*, were used in the following experiments because they are widespread, easily obtained in the study area and have been previously studied (e.g. Leney *et al.*, 2006a,b,c; Berrill *et al.*, 1998; Ralph and Petras, 1997; Ralph *et al.*, 1996; Boone *et al.*, 2001; Russell *et al.*, 1995, 1997). Green frog eggs are laid beginning in late May until early June and hatch after three to six days (Stebbins, 1951). Tadpoles, acting as herbivores, then grow over the course of the summer, overwinter and undergo metamorphosis usually by August the following summer (Stebbins, 1951). As adults, green frogs eat insects and other invertebrates, retaining a close association with the water, and also tend to return to a particular pond year after year (Stebbins, 1951). Therefore, green frogs in a particular area could be exposed to the same chemicals

generation after generation. These repeated exposures would greatly contribute to bioaccumulation.

Bullfrogs (*Rana catesbeiana*) were used in these studies for similar reasons as green frogs, although toxicokinetics in bullfrogs have not been well-studied. Bullfrogs lay eggs from mid-May until well into July which hatch after three to six days (Harding, 2000). Some bullfrog tadpoles will undergo metamorphosis in their second summer, but many will overwinter again and metamorphose during their third summer (Harding, 2000). Bullfrog tadpoles are both herbivorous and carnivorous. Bullfrog tadpoles are also larger than green frog tadpoles, so comparing chemical toxicokinetics between the two species will provide some indication of size effects on time to steady state for organic contaminants such as PCBs. As adults, bullfrogs also have a more diverse diet including fish, other tadpoles and frogs, snakes, turtles, young birds and small mammals (Harding, 2000). Therefore, bioaccumulation of chemicals in bullfrog adults may also differ from that in green frogs.

1.8 – Chemicals of Interest in This Thesis

1.8.1 – Polychlorinated Biphenyls

Polychlorinated biphenyls are a class of chemicals that consist of two joined phenyl rings with up to 10 possible attachment locations for chlorine atoms seen in Figure 1.1.

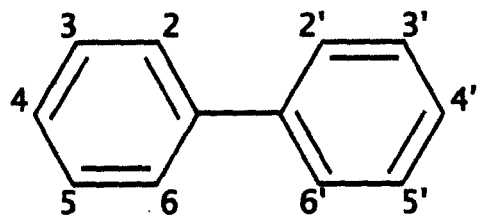


Figure 1.1. The biphenyl backbone of a polychlorinated biphenyl; each number represents a site where a chlorine atom can bind to the biphenyl backbone. If a chlorine atom is not present, that binding site is filled with a hydrogen atom.

There are 209 different combinations of how many chlorine atoms can be attached to the biphenyl backbone and where they can be located, and these combinations are called congeners. The number and position of chlorine atoms determine the chemical

properties and behaviour of a PCB congener and influence an animal's ability to metabolize a given PCB (e.g. Kannan *et al.*, 1995; Leney *et al.*, 2006b). All PCB congeners are considered to be hydrophobic and have log K_{ow} values between 4.09 and 8.18 (Hawker and Connell, 1988). Because of their hydrophobicity, PCBs partition easily into organic media, including lipids and organic rich sediments.

Polychlorinated biphenyls are of anthropogenic origin. In North America, eight mixtures of PCBs were created with varying percentages of chlorine by weight. These mixtures were used for industrial and electrical applications for over 50 years under the North American trade name Aroclor (Swackhamer, 1996). In the late 1970s, polychlorinated biphenyls were banned due to their toxicity, persistence in the environment (Tanabe, 1988; Vallack *et al.*, 1998) and ability to accumulate and biomagnify in food webs (Connolly and Pederson, 1988; Oliver and Niimi, 1988; Russell *et al.*, 1999). Polychlorinated biphenyls have also been documented in most environmental compartments (Tanabe, 1988) due to their ability to be transported long distances (MacDonald *et al.*, 2000). The effects of PCBs on organisms are therefore of great concern.

1.8.2 – Polychlorinated Biphenyls in Amphibians

It has been shown that frogs accumulate PCBs in the environment and have been referred to as super-accumulators of these and other hydrophobic compounds (Gillan *et al.*, 1998). A study on leopard frogs and green frogs in Ontario found PCB concentrations between 50 and 112 ng/g wet weight (Phaneuf *et al.*, 1995). Adult frogs in a wetland in the St. Lawrence River watershed were found to contain between 3800 to 9900 ng/g PCBs wet weight (Fadden, 1994). Little work on tadpole or metamorph environmental PCB burdens has been reported to date. This is a critical knowledge gap in current research, as tadpoles and metamorphs are the most sensitive stages of the amphibian life cycle to contaminants. Polychlorinated biphenyls have been linked to amphibian declines (Gillardin *et al.*, 2009; Reeder *et al.*, 2005; Karasov *et al.*, 2005), and information on *in situ* body burdens of PCBs in tadpoles could greatly increase our understanding of the factors behind amphibian declines.

1.8.3 – Amphibian Responses to Polychlorinated Biphenyls

Polychlorinated biphenyls are known to produce toxic effects in amphibians. For example, total PCBs in northern leopard frogs (*R. pipiens*) have been found at a concentration of 9900 ng/g in the St. Lawrence watershed (Fadden, 1994). Meanwhile, a concentration of total PCBs of 700 ng/g has been observed to induce hepatic ethoxyresorufin-*O*-deethylase (EROD) activity in 50% of tested *R. pipiens* (Huang *et al.*, 1999). This observation of EROD activity at such a low concentration is of concern, since EROD activity has been proposed as a biomarker of chemical toxicity (Huang *et al.*, 1999).

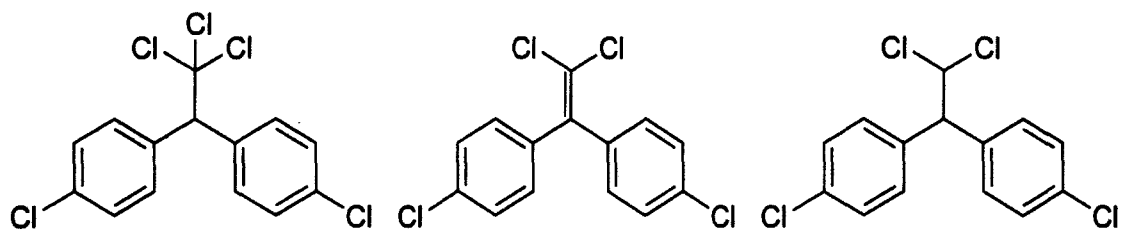
Concentrations of total PCBs in adult frogs have been observed at 3800 ng/g to 9900 ng/g in the St. Lawrence River watershed (Fadden, 1994). In red-legged frog eggs (*Rana aurora*), total PCBs have been observed at concentrations between 420 ng/g and 1080 ng/g (de Solla *et al.*, 2002). However, there have been no reports of PCB contamination in wild tadpoles. Adverse effects (i.e. mortality of 50% of test subjects) of Aroclor 1254 have been observed at concentrations between 1030 ng/g in *R. pipiens* tadpoles and 3740 ng/g in Fowler's toad tadpoles (*Bufo fowleri*) (Harfenist, 1989). Although these effects cannot be compared to concentrations observed in wild tadpoles, if the above concentrations observed in adults are indications of what can be expected to be observed in tadpoles, there is serious need for concern about the toxicity of PCBs to amphibians.

Compared to the early life stages of other animals, tadpoles are fairly sensitive to PCBs. Data outlined in Harfenist (1989) compares the LC_{50}s for three species of amphibians (northern leopard frogs, American toads and Fowler's toads) to four species of fish (rainbow trout, goldfish, redear sunfish and channel catfish) exposed to Aroclors 1242 and 1254. For both Aroclors 1242 and 1254, Fowler's toad tadpoles (*Bufo fowleri*) are the least sensitive. Leopard frog tadpoles (*R. pipiens*), on the other hand, are one of the most sensitive species, with only rainbow trout (*Oncorhynchus mykiss*) being more sensitive to Aroclors 1242 and 1254, and redear sunfish (*Lepomis microlophus*) having more sensitivity to Aroclor 1254.

1.8.4 – p,p'-Dichloro-diphenyl-trichloroethane (p,p'-DDT)

Organochlorine pesticides (OCs) were widely used up until the 1970s, when their use was banned or restricted. Dichloro-diphenyl-trichloroethane, or DDT, is the most well-known organochlorine pesticide. It was the first efficient pesticide developed (Stenerson, 2004) and was initially put into use during World War II as an agricultural insecticide to combat mosquitoes spreading malaria and typhus (Brooks, 1974). The use of DDT was banned in North America in the 1970s and 1980s due to its toxicity, persistence and ability to bioaccumulate. However, it is still prevalent in the environment (Russell *et al.*, 1997) and is still used in many countries worldwide for malaria control (Hoffman *et al.*, 2003).

The most commonly used form of DDT was *p,p'*-DDT, because it is more toxic than the *o,p'*-DDT conformation (Stenerson, 2004). Unless otherwise stated, all references to DDT and its metabolites will be regarding the *p,p'* conformation. Metabolism of DDT forms dichloro-diphenyl-dichloroethylene (DDE) and dichloro-diphenyl-dichloroethane (DDD). Both of these metabolites are as lipid soluble as their parent compound, but water solubility of DDD is an order of magnitude higher than DDE (Mackay *et al.*, 2006) and the metabolites are more toxic than DDT (Sparling *et al.*, 2000). The reported log K_{ow} of DDT is 6.5, while DDE has a log K_{ow} of 6.96 and DDD a log K_{ow} of 5.69 (Verschueren, 2001). The structures of DDT, DDE and DDD are illustrated below (Orme and Kegley, 2006):



DDT DDE DDD

Figure 1.2. The structures of dichloro-diphenyl-trichloroethane (*p,p'*-DDT) and its metabolites dichloro-diphenyl-dichloroethylene (DDE) and dichloro-diphenyl-dichloroethane (DDD).

1.8.5 – Residues of DDT in Amphibians

Few studies have examined residues of DDT and its metabolites in amphibians. Those that have reveal the need for great concern about the effects these compounds are having on wild populations and their food webs. A study in Point Pelee National Park, Ontario, Canada by Russell *et al.* (1995) found that spring peepers still contained DDT and its metabolites, even though spraying had ended 25 years before. In this study, Russell *et al.* found DDT in spring peepers at concentrations greater than 160 ng/g. Surveys done at several sites in southern Ontario reported concentrations of DDT in the tissues of green frogs (*Rana clamitans*) and northern leopard frogs (*Rana pipiens*) varied between 60 and 121 ng/g (Gillan *et al.*, 1998; Gilbertson *et al.*, 2003).

Compared to reptile species, amphibians have similar concentrations of DDT and its metabolites. According to a study in Iowa, USA by Punzo *et al.* (1979), male American toads had a mean DDE concentration of 19 ng/g compared to 2.8 ng/g in male northern water snakes and 2 ng/g in male midland painted turtles. Female northern leopard frogs, on the other hand, had 1.5 ng/g DDE compared to 4 ng/g in female red-sided garter snakes.

1.8.6 – Amphibian Responses to DDT

The characteristic effects of DDT on tadpoles are abnormal snouts and hyperactivity (Sparling *et al.*, 2000). Hyperactivity consists of a flight response, followed by uncoordinated spasms of jerking and tail twitching (Sparling *et al.*, 2000). Much of the groundwork for research on the responses induced by DDT on amphibians was done by Cooke in the 1970s and 1980s using frogs (e.g. Cooke, 1970, 1973 and 1981). Cooke found that responses to DDT differed between the different life stages of frogs. For example, mortality of common frog tadpoles (*Rana temporaria*) at the hind-limb-bud stage was higher than for older tadpoles; 70% at 120 hours compared to 30% (Cooke, 1970). When Cooke exposed tadpoles to a concentration of 20 ng/mL, they lost weight, were hyperactive, had spine curvature, deformed snouts and decreased alertness, (Cooke, 1970). Hyperactivity has also been caused by DDT in exposed wood frogs (*Rana sylvatica*) at 25 ng/mL (Schwen and Mannering, 1982; Licht, 1985) and in *Rana temporaria* tadpoles at 2000 ng/mL (Cooke, 1973). Immunotoxicity has also been

associated with DDT exposure in frogs. Albert *et al.* (2007) found that when northern leopard frog adults were exposed to DDT at 75 ng/g body weight, antibody production and secondary delayed-type hypersensitivity were significantly suppressed. Since concentrations up to 4000 ng/g body weight have been found in leopard frogs in the Colorado area (Finley and Pillmore, 1963), these effects are likely to be occurring in wild frog populations. It is difficult to compare amphibian sensitivity to DDT with that in other species because most of the toxicity studies that have been done on amphibian responses to DDT only examine the concentration in the exposure medium (e.g. ng/mL water) and do not determine the body concentration of DDT.

1.9 – Project Overview

This study aims to quantify chemical kinetics in amphibians. The toxicokinetics of PCBs and DDT will be examined along with the effects of temperature on the exposure dynamics of these chemicals during the tadpole and metamorph stages of green frogs and bullfrogs. Polychlorinated biphenyls are very useful in developing chemical exposure dynamics models, but no studies to date have quantified *in situ* PCB uptake dynamics in amphibian species. Performance Reference Compounds (PRCs) will be used to simultaneously track *in situ* uptake and elimination dynamics. This approach will also control for differences in metabolism that may be caused by the temperature treatments, toxicity, or environmental stress. Studies on the toxicokinetics of organochlorine pesticides have not been reported, which is a critical knowledge gap since OCs still exist in the environment, and are picked up by amphibians.

1.9.1 – Overall Objectives

This thesis contributes to a long term project whose objective is:

To develop a life cycle model of the green frog, *Rana clamitans*, and the bullfrog, *Rana catesbeiana*.

By examining the exposure dynamics of various chemicals during the different life stages of the green frog and bullfrog, we can develop life cycle models for these species and apply these models when considering other chemicals. These models will provide a framework to integrate toxicokinetic studies on amphibians using different

exposure models for an array of environmental chemicals. This thesis contributes to this overall objective by examining the following specific objectives.

The specific objectives of this thesis are:

1) To study chemical exposure dynamics in natural settings.

No study has examined *in situ* chemical exposure dynamics in amphibians. Environmental conditions are more complex than those in the laboratory and an understanding of chemical exposure dynamics under natural conditions is essential for the development of life cycle models for amphibians. Furthermore, bioaccumulation can be a slow process with respect to the time it takes for an organism to reach steady state. Furthermore, the timing of chemical exposures and achieved tissue concentrations in an animal can differ in laboratory-reared versus environmental populations. To avoid confounding factors often associated with the effects of different environments on chemical exposure dynamics, Performance Reference Compound polychlorinated biphenyls (PRC PCBs) will be used to calibrate tadpoles in terms of uptake and elimination of environmental chemicals.

2) To investigate the effects of temperature on chemical exposure dynamics in tadpoles.

As of yet, only two studies have measured the effects of temperature on chemical exposure dynamics in amphibians. One of the previous studies examined the chemical exposure dynamics of bisphenol A in *Rana temporaria* tadpoles at 7°C vs. 19°C (Honkanen and Kukkonen, 2006); the other (Licht, 1976) examined the exposure dynamics of DDT in wood frog (*Rana sylvatica*) tadpoles at 15°C vs. 21°C. There is evidence that global warming is connected to amphibian declines, and it is important to understand how smaller changes in temperature as predicted in global change models might interact with toxicant exposures. Therefore, the chemical exposure dynamics of both PCBs and DDT will be examined in two amphibian species (green frogs, *Rana clamitans*, and bullfrogs, *Rana catesbeiana*).

1.9.2 – Objectives of Chapter 2

Chapter 2 of this thesis will focus on the chemical exposure dynamics of PCBs and organochlorine pesticides at three different sites in the Essex County region of south-western Ontario, Canada. This study will use PRC PCBs as metabolic tracers to control for the effects of these different environments on the chemical exposure dynamics of these two classes of chemicals.

In Chapter 2, the hypotheses to be tested are:

2.1) The elimination rate coefficients of PRC PCBs are significantly different among sites.

2.2) The relationship between k_2 and log K_{ow} (as measured by the slope of the line) for PRC PCB and Aroclor congeners will differ from field and laboratory observations.

1.9.3 – Objectives of Chapter 3

The purpose of Chapter 3 is to examine the effects of different temperatures on the chemical exposure dynamics of DDT and PCBs during the tadpole and metamorph stages of the green frog and bullfrog. Usually, when an organism is dosed with a chemical and placed into clean water to depurate, the concentration of the chemical will decrease over time. However, it has been shown that reduced temperature decreases the kinetics of chemicals (Honkanen and Kukkonen, 2006). This chapter will examine the differences in the elimination rate coefficients of PRC PCBs and DDT at two temperatures. As well, interspecies differences in toxicokinetics between bullfrog and green frog tadpoles will be investigated. PRC PCBs will be used to control for differences in metabolism between the two temperature treatments.

The hypotheses tested in Chapter 3 will be as follows:

3.1) There are temperature effects on elimination rate coefficients of PRC PCBs and DDT.

3.2) There are no interspecific differences in elimination rate coefficients of PRC PCBs and DDT.

1.11 – References

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CHAPTER 2 – EVALUATION OF ENVIRONMENTAL EFFECTS ON CHEMICAL EXPOSURE DYNAMICS OF POLYCHLORINATED BIPHENYLS (PCBs) IN GREEN FROG TADPOLES (*RANA CLAMITANS*)

2.1 – Introduction

Amphibians have been in decline worldwide since the 1970s (Barinaga, 1990). Exposure to toxic chemicals is among the many hypotheses that have been raised to explain these declines. It is arguable, however, whether chemicals are a direct or indirect contributor to amphibian declines. Exposure to chemicals has been shown to interact with other factors contributing to the declines, such as UV-radiation and pathogen/toxicant interactions (Croteau *et al.*, 2008; Kiesecker, 2002). It is well established that amphibians accumulate chemicals present in the environment (e.g. Cooke, 1981, Russell *et al.*, 1995, 1997, Gillan *et al.* 1998). Therefore, to determine the potential for a chemical etiology to amphibian declines, a first step is to quantify chemical exposure dynamics in amphibians.

Toxicokinetic studies on amphibians have included species such as: leopard frogs (*Rana pipiens*), common frogs (*Rana temporaria*), yellow-bellied toads (*Bombina variegate*), moor frogs (*Rana arvalis*), Argentine toads (*Bufo arenarum*) and green frogs (*Rana clamitans*) (Ankley *et al.*, 2004; Honkanen and Kukkonen, 2006; Greulich and Pflugmacher, 2004; Fridman *et al.*, 2004). These studies, however, are not comparable to each other due to differences in exposure route, chemical choice and life stages studied. Therefore, there is a need for complete chemical exposure dynamics life cycle models for key species using standardized chemicals and exposure routes. Initial work on life cycle models was carried out by Leney *et al.* (2006a, 2006b, 2006c) who provided the essential framework for life cycle models using polychlorinated biphenyls (PCBs) in both the northern leopard frog (*R. pipiens*) and the green frog (*R. clamitans*). Polychlorinated biphenyls provide an excellent model system for developing chemical exposure dynamics life cycle models because PCB dynamics can be compared with other chemicals of interest based on their log K_{ow}, chemical structure and the degree of biotransformation they undergo in animals.

The studies by Leney *et al.* (2006a,b,c) were conducted under laboratory conditions, which are commonly viewed as ideal. To date, no toxicokinetic studies on amphibians have been carried out under natural conditions, and none have simultaneously

studied uptake and elimination processes. These are major issues in current research, since an understanding of how environmental conditions alter the toxicokinetics and bioaccumulation potential of chemicals are necessary for developing life cycle chemical exposure dynamics models.

This study is unique in that it examines the chemical exposure dynamics of PCBs and organochlorine pesticides (OCs) in green frog (*R. clamitans*) tadpoles under natural conditions. In order to control for environmental effects on toxicokinetics, Performance Reference Compound polychlorinated biphenyls (PRC PCBs) were used. Performance reference compound PCBs used in this study were a suite of five PCBs (International Union of Pure and Applied Chemistry [IUPAC] numbers 7, 23, 61, 109 and 173) that do not occur in the environment and have a range of solubilities (log K_{ow}s between 5.07 and 7.02; Hawker and Connell, 1988). Performance Reference Compounds are analytically non-interfering organic compounds that are selected to match the physicochemical properties of chemicals of interest, and usually have moderate to high K_{ow}s. Performance reference compounds are used to better understand the effects of different environments on the uptake kinetics of chemicals, have been developed for use with passive environmental samplers (Huckins *et al.*, 2002) and recently used in biota (Raeside *et al.*, 2009). Normally, elimination and uptake of environmental chemicals cannot be measured simultaneously, so only the overall change in concentrations of environmental chemicals can be determined. However, elimination rate coefficients (k_2) of PRCs can be used to estimate the elimination rate coefficients of environmental chemicals in order to determine the *in situ* exposure dynamics of environmental chemicals. The PRC approach has been used previously to measure *in situ* elimination rate coefficients of Semi-Permeable Membrane Devices (SPMDs) and mussel biomonitors (O'Rourke *et al.*, 2004; Raeside *et al.*, 2009) but not amphibian species.

2.2 – Materials and Methods

2.2.1 – PRC Dosing Procedures

Green frog tadpoles were collected in May 2006 from ponds at Leadley Environmental Co., which is a rural aquaculture facility in Essex County, near Windsor, Ontario, Canada. Tadpoles were sorted based on size and development stage, and any

tadpoles that had limb buds or emerged limbs were not used in this experiment. Two hundred and ten tadpoles were placed evenly into two indoor, aerated 50 L aquaria. One tank became the control tank and the other the dosed tank. Dosed tadpoles were fed PRC PCB-spiked flaked fish food and control tadpoles were fed clean fish food for seven days.

Performance reference compound PCBs included in the PRC PCB mixture were 2,4-dichlorobiphenyl (IUPAC #7); 2,3,5-trichlorobiphenyl (IUPAC #23); 2,3,4,5-tetrachlorobiphneyl (IUPAC #61); 2,3,3',4,6-hexachlorobiphenyl (IUPAC #109) and 2,2',3,3',4,5,6-heptachlorobiphenyl (IUPAC #173). The PRC PCB-spiked fish food was dosed in the following manner: 4 mL of 0.5 mg/mL total PRC solution was placed into 600 mL of hexane, and well mixed in a glass jar. One hundred grams of Nutrafin Basix Staple fish food (Montreal, QC, Canada) was added to the jar. This mixture was then placed into a fume hood and the hexane was allowed to evaporate. The food was mixed twice a day until the mixture was almost dry (~24 hours). The moist mixture was then poured out into a hexane-rinsed metal baking tray and allowed to dry completely in a fume hood (~24 hours). The dry flakes were then poured back into a glass jar and mixed again. Three subsamples totalling three grams were removed and placed into aluminum dishes for later chemical analysis. Performance Reference Compound PCB-dosed food was analyzed and concentrations of PRC PCBs in dosed food are summarized in Table 2.1. The control fish food was prepared in the same manner, except PRC PCBs were not added to the mixture. Control fish food did not contain any PRC PCBs.

After the 7-day dosing period, all tadpoles were fasted for 48 hours to ensure the tadpoles sampled on day 0 of the experiment did not contain PRC PCB-contaminated food in their intestines. Eighteen individuals were removed on day 0 (nine PRC PCB dosed and nine control) for analysis of initial PCB concentrations including PRC PCBs. The remaining tadpoles were then sorted based on size and divided evenly among the three sampling sites. Tadpoles were transported to the three sampling sites and placed into mesh cages (measuring 30 cm x 30 cm x 90 cm); one cage for the PRC dosed group and a second for the control group. Cages were approximately 70% immersed in water and were in contact with the sediments at each site. Six individuals (three controls and three PRC dosed) were sampled from each site at each time point of 0, 6, 22 and 34 days. Tadpoles were transported back to the laboratory in clean plastic containers with water

from the site and placed in a cooler with ice. Upon arrival at the laboratory, tadpoles were euthanized, their weights, total lengths, tail lengths and development stage were recorded. Tadpoles were then wrapped in hexane-rinsed tinfoil and placed in a –80°C freezer until analysis. Temperature loggers were also attached to each cage and set to take measurements every hour for the duration of the study.

All procedures were approved by the University of Windsor Animal Care Committee.

2.2.2 – Monitoring Sites

Figure 2.1 provides a map of the locations of the various monitoring sites in the Essex County region, Ontario, Canada. The specific location of the sites and brief descriptions are as follows.

(1) Turkey Creek (TC) is primarily a municipal drain for the city of Windsor. Cages were placed a few meters from shore in shallow, slow moving water and securely held in place with ropes. The cages were placed ~500m upstream of the junction with the Detroit River.

(2) Belle River (BR) is a small stream draining an intensive agricultural area. Cages were placed a few meters upstream of a foot bridge at 1704 County Road 46, South Woodslee, ON, Canada.

(3) The Aquaculture Facility (AF) is a farm with limited or no pesticide use, and as such provides a background reference site for the study. Cages were placed near the shoreline of a large, human-made pond at Leadley Environmental Inc., an aquaculture facility near Essex, ON, Canada. The Aquaculture Facility was also the site of tadpole collection.

This selection of sites provided an opportunity to test the toxicokinetics of amphibians in both urban and agricultural settings, and to determine if exposure to different suites of chemicals would alter uptake and elimination kinetics.

2.2.3 – Chemical Analysis

Green frog tadpoles were analyzed to determine whole-body and lipid concentrations of PCBs and organochlorine pesticides (OCs) according to Leney *et al.*

(2006a). Once thawed, tadpoles were weighed and homogenized. The tadpole homogenate was dried with 35 g of anhydrous sodium sulphate using a glass mortar and pestle, then wet packed in a 60 x 2.5 cm glass column with 50 mL dichloromethane (DCM):hexane (1:1 v/v). Columns were then spiked with 100 µL of an internal recovery standard solution, a mixture of 2 labelled PCBs ([C^{13}] PCB 52, [C^{13}] PCB 153 at 200 ng/mL) for recovery correction. Another 250 mL of 1:1 DCM:hexane was added, and the column was left to stand for 1 hour before being eluted. The extract was rotoevaporated down to about 1 mL and diluted to a volume of 10 mL with hexane. A 1 mL aliquot was removed and placed into an aluminum weigh boat and dried at 110°C for one hour for gravimetric lipid determination (Drouillard *et al.*, 2004). The remaining 9 mL of sample was rotoevaporated to 2 mL. The remaining sample was then added to a 35 x 1 cm glass column packed with 6 g of activated florisil and 1 cm of sodium sulphate. After being eluted from the column with 50 mL of hexane and 50 mL of DCM:hexane (15:85 v/v), the extract was rotoevaporated to less than 1 mL and brought up to 1 mL with isooctane. The sample was then transferred to a 2 mL gas chromatograph vial for storage at 4°C until instrumental analysis. A method blank and reference tissue homogenate (carp from the Detroit River) were run with each set of 6 samples.

Gas chromatographic analysis of PCBs and OCs was performed using a Hewlett-Packard 5890/5973 with a mass selective detector (MSD), a 7673 autosampler, and a 60 m x 0.250 mm x 0.25 µm DB-5 column (Chromatographic specialties, Brockville, ON, Canada). The carrier gas was He with a flow rate of 1 mL/min, injection port temperature was 250°C, and injection volume was 2 µL (splitless). The oven program was started at 90°C and remained at this temperature for 3 minutes, increased at 7°C/min to 150°C, then increased at 3°C/min to 280°C and remained at this temperature for 5.10 minutes, for a total run time of 60 minutes. The MSD was operated in selective ion mode (SIM) and the ions and time windows are described in O'Rourke *et al.* (2004).

Polychlorinated biphenyls, PRC PCBs and OCs were identified by retention time and quantification of individual compounds was based on the response factors from well characterized standard mixtures that were run with each set of 8 samples, including the reference homogenate and blank. Percent recoveries of the internal surrogate standards, C^{13} PCBs 52 and 153, were all above 70%, therefore no recovery corrections were made.

Mean percent recovery ± SE for C^{13} PCB 52 was 86.6 ± 2.0% and for C^{13} PCB 153 was 86.2 ± 2.3%.

2.2.4 – Data Analysis

For this study, a one compartment chemical exposure dynamics model was used (Barron *et al.*, 1990). The elimination of organic contaminants (polychlorinated biphenyls) is first-order (Huang and Karasov, 2000; Leney *et al.*, 2006b,c) and for elimination experiments, the elimination rate coefficient, k_2, is measured as:

$$k_2 = \frac{\ln C_{org(t=0)} - \ln C_{org(t)}}{t} \qquad (2.1)$$

Where $C_{org(t=0)}$ is the concentration of chemical in an organism at the start of the elimination period and $C_{org(t)}$ is the concentration of chemical in the organism at time *t*. Once k_2s are known for PRC PCBs, they are plotted against their corresponding log K_{ow} values. Elimination rate coefficients for environmental PCBs are estimated from the equation of the line derived from the k_2 versus log K_{ow} relationship.

Concentration at steady state (C_{ss}) for environmental PCBs is then calculated as:

$$C_{ss} = \frac{C_t}{1 - e^{-k2t}} \qquad (2.2)$$

where C_t is the concentration of a chemical after a specific uptake period (time *t*). The time to 95% steady state (t_{95}) can be determined:

$$t_{95} = \frac{3}{k_2} \qquad (2.3)$$

A model can be developed from the above relationship (Equation 2.2) to estimate the exposure dynamics of a chemical over time, assuming that steady state is achieved for each congener, and that the concentration of chemical in the environment is coefficient over the time frame of the study. This model is expressed as:

$$C_{org(t)} = C_{org(t=0)} + [C_{org(ss)} - C_{org(t=0)}] \cdot (1 - e^{-k_2 t}) \qquad (2.4)$$

where $C_{org(ss)}$ is the concentration of the environmentally accumulated concentration of the chemical in the organism at steady state (Raeside *et al.*, 2009). This model predicts steady state concentration, and the efficacy of the model can be verified by comparing predicted results with experimental observations.

Least Squares Regression (LSR) analysis was used to determine the elimination rate coefficients (k_2) of PRC PCBs. To determine whether PRC dosed versus control and site to site differences in elimination rate coefficients occurred, Analysis of Covariance (ANCOVA) was used. To investigate the relationship between PRC PCB k_2s and log K_{ow}, ANCOVA was used. Finally, to determine overall differences in tadpole length, PCB or OC concentrations, or percent lipid over time or among sites, Analysis of Variance (ANOVA) was carried out. All statistical tests were performed using Systat 8.0 (SPSS, Inc.). Results were considered significant at a value of $p<0.05$.

2.3 – Results

2.3.1 – Temperature

Temperature data over time are plotted in Figure 2.2. There was no significant difference between temperatures measured by the data loggers attached to the control and dose cages at each site. In May (days 0 and 6), water temperatures averaged 21 to 23°C and ranged between 14 and 36°C at all three sites. In June (days 22 and 34), water temperatures ranged widely from 16 to 38°C, and averaged between 20 and 25°C. Water temperature at the Belle River site was significantly lower (4°C) in June than at the Aquaculture Facility (ANOVA, $p=0.012$).

2.3.2 – Tadpole Size and Lipid Content

Total tadpole length was compared between control and PRC-dosed groups at each site and there were no significant differences in size over time (ANOVA, $p>0.05$). There were also no significant differences in total length of tadpoles among sites over time (ANOVA, $p>0.05$). Tadpole growth over time is plotted in Figure 2.3. Total length of tadpoles decreased over time, at rates between -0.005 and -0.047 cm/day. Total length was significantly correlated with developmental stage at all three sites, decreasing with development stage (ANOVA, $p=0.0001$).

An ANOVA was carried out to evaluate percent lipid over time among control and PRC-dosed tadpoles at the three sites. There were no significant differences in percent lipid between control and PRC-dose groups at each site or among sites ($p>0.05$). Percent lipid did not decrease significantly over time (ANOVA, $p>0.05$). Lipids were

variable between individuals and all tadpole concentrations were lipid normalized in order to account for inter-individual variability in capacity to accumulate chemicals.

2.3.3 – PRC PCB Elimination

Control tadpoles did not contain any PRC PCBs at any time point, so there was no evidence of recycling of PRC PCBs within the cages at any site. Although PRC PCB 7 was eliminated rapidly at all sites, a significant elimination rate coefficient was not measured at any of the sites due to a lack of time points earlier than day 6. For PRC PCBs 23 and 61, elimination rate coefficients were significant at all three sites (LSR, $p<0.05$). Significant elimination rate coefficients for PRC PCBs 109 and 173 were also observed at the Aquaculture Facility and Turkey Creek sites (LSR, $p<0.05$), but not at Belle River (LSR, $p>0.05$). This lack of estimating chemical elimination rate coefficients at Belle River was because of a flooding event after day 22 that caused the loss of all tadpoles. Until day 22, PRC PCBs 109 and 173 were eliminated at rates similar to the other sites (ANCOVA, $p>0.05$). Elimination dynamics are summarized in Figure 2.4. The elimination rate coefficients, coefficients of variation (R^2) and p-values for all five PRC PCBs are presented in Table 2.2.

Elimination rate coefficients for individual PRC PCBs did not differ among sites (ANCOVA, $p>0.05$). Therefore, metabolic activities of tadpoles were concluded to be similar among the three sites despite the temperature differences observed among the sites. Any difference observed in chemical uptake dynamics among sites was therefore directly related to bioavailable concentrations of chemicals in the local environment. When elimination rate coefficients of PRC PCBs were regressed against their corresponding log K_{ow}s, a significant relationship was observed (ANCOVA, $p=0.01$, see Figure 2.5). The slope of k_2 versus log K_{ow} was -0.487.

2.3.4 – PCB and OC Accumulation Dynamics

Analysis of Variance revealed that there were no significant differences in Σ PCBs between control and PRC-dosed groups at each site. Therefore, PCB data for control and dose groups were pooled within each site in order to evaluate site to site differences in PCB accumulation dynamics. Sum PCB concentrations in tadpoles over time are

summarized in Table 2.3. There was a significant difference in Σ PCBs between sites from day 6 onward (day 6: p=0.0001; day 22: p=0.008; day 34: p=0.006 respectively). This difference was mostly driven by Turkey Creek, where tadpoles had average Σ PCB concentrations after day 6 of 22,577 ± 4282 to 55,205 ± 15,228 ng/g compared to Belle River (between 1496 ± 510 and 2154 ± 204 ng/g) and the Aquaculture Facility (from 1144 ± 174 to 3799 ± 1596 ng/g). Sum PCB concentrations were significantly higher in tadpoles at Belle River compared to the Aquaculture Facility site by day 22 (2154 ± 304 compared to 1144 ± 174 ng/g respectively, p=0.025). Tadpoles at Turkey Creek had higher Σ PCB concentrations (between 22,577 ± 4282 and 55,205 ± 15,228 ng/g) compared to the Aquaculture Facility (between 1144 ± 174 and 3799 ± 1596 ng/g) on days 6, 22 and 34 (p=0.001, 0.005 and 0.006 respectively). Sum PCB concentrations were also higher in tadpoles at Turkey Creek than at Belle River on day 6 (22,577 ± 4282 compared to 1496 ± 510 ng/g respectively; p=0.002) (see Table 2.3). When specific PCB congeners were examined, it was observed that for most PCB congeners, Turkey Creek contained higher concentrations on day 34 (e.g. 3525 ng/g compared to 162 ng/g for PCB 87 at the Aquaculture Facility; 7243 ng/g versus 89 ng/g of PCB 138 at Belle River).

Extrapolating from the k_2 versus log K_{ow} relationship developed using the PRC PCB elimination rate coefficients (Figure 2.5), time to steady state for environmental PCBs was calculated based on Equation 2.3. It was calculated that steady state was reached for all environmental PCB congeners within 23 days.

Equation 2.5 was used to predict environmental PCB concentrations over time by setting $C_{org(ss)}$ as the measured concentration on day 34 at each site (except for Belle River where concentrations at day 22 were used) and using the PRC-extrapolated congener specific k_2 values (see Section 2.2.4 – Data Analysis for methods). Apart from day 34 concentration data which were used to parameterize the model, the model was effective at hindcasting PCB concentrations at earlier time points (see Figure 2.6). The accuracy of the model increased with increasing K_{ow} of the congeners being modeled. The trend lines generated did not differ significantly from those for the observed accumulation trend data (ANOVA, $p>0.05$). When the PCB concentrations predicted by the model were plotted against the observed concentrations, it was confirmed that the model was highly effective;

when compared to the slope of a 1:1 relationship, there was no significant difference at any of the sites (ANCOVA, $p>0.800$; see Figure 2.6).

There were no significant differences in OC concentrations among sites over time (ANCOVA, $p>0.403$). Nine of the twenty one OCs analyzed for were not observed at any of the three sites [1245-tetrachlorobenzene (1245-TCB), 1234-TCB, α-hexachloro-cyclohexane (α-HCH), γ-HCH, δ-HCH, octachlorostyrene (OCS), *cis*-nonachlor, photomirex and dieldrin]. The concentrations of OCs over time were modeled using Equation 2.5 in the same way as for the PCBs above. It was observed that the model was also effective at predicting OC concentrations over time (see Figure 2.7). None of the trend lines generated by the model were significantly different from those for the actual data (ANOVA, $p>0.05$). Also, when the OC concentrations predicted by the model were plotted against the observed concentrations, it was discovered that the relationship developed did not differ significantly from a 1:1 relationship at any of the sites (see Figure 2.7; ANCOVA, $p>0.687$).

2.4 – Discussion

2.4.1 – PRC PCB Elimination

All PRC PCBs were eliminated at significant rates, although it was not possible to calculate elimination rate coefficients for PRC 7, and PRCs 109 and 173 at the Belle River site. Elimination rate coefficients of PRC PCB congeners were not significantly different among sites. The similarity of PRC elimination rate coefficients among sites indicated that there were no metabolic differences in tadpoles among sites despite significant temperature differences. Without the use of the PRC PCBs, differences in concentrations of PCBs and OCs among sites would not be strongly associated with differences in bioavailable concentrations of these chemicals.

2.4.2 – PCB Elimination Rate Coefficients In Situ *Compared to Laboratory Estimates*

In situ tadpoles exhibited rapid kinetics of chemicals, reaching steady state within 23 days even for PCBs with high log K_{ow}s. This rapid time to steady state is much faster than predicted by past experiments in the laboratory (Leney *et al.*, 2006b). Elimination

rate coefficients of PRC PCBs in this study were 7 to 9 times faster than those observed in the laboratory by Leney *et al.* (2006b).

All PRC PCBs belonged to metabolic group 4, which have hydrogens in both the *meta-para* and *ortho-meta* positions (Kannan *et al.*, 1995). Polychlorinated biphenyls that fall into this group are metabolized by both the phenobarbital (PB)-type and 3-methylcholanthrene (3-MC)-type enzymes. These are cytochrome P450 (CYP) enzymes that make up part of the mixed function oxydase (MFO) system that metabolizes xenobiotic chemicals. When the k_2 versus log K_{ow} relationship for PCBs from metabolic group 4 from Leney *et al.* was compared to that for PRC PCBs in this study, it was observed that the relationship between k_2 and log K_{ow} was still significantly greater in this study.

These different elimination rate coefficients have important implications, in that although laboratory studies are commonly considered "ideal" situations, *in situ* toxicokinetics can be much more rapid as a result of increased stress or feeding dynamics. Due to their rapid toxicokinetic abilities, tadpoles can track high frequency environmental pulses of chemicals, and rapidly come to steady state in these environments.

2.4.3 – Comparison of Tadpole Toxicokinetics with Other Species

Compared to other monitors of contamination, tadpoles may be superior for use in short term monitoring studies. Tadpole elimination rate coefficients of PRC PCBs were between 3.5 and 10 times faster than those observed in mussels in the field (Raeside *et al.*, 2009). Tadpoles also eliminated PRC PCBs between 17 and 130 times faster than semi-permeable membrane devices (SPMDs, Booij *et al.*, 2006). Since tadpoles reach steady state so rapidly in the environment and tend not to metabolize chemicals as compared with other species (e.g. birds and fish; Leney *et al.*, 2006b), tadpoles would provide a more accurate snapshot of *in situ* chemical contamination than the other commonly used monitors.

There are certain limits to using tadpoles as biomonitors, however. Many species of frogs metamorphose quickly; tadpoles are also only around at certain times of year, most commonly in the spring and early summer. This short time period during which tadpoles are available severely limits the scope of biomonitoring projects that can use

them. Another possible drawback to using tadpoles as biomonitors is their relative sensitivity to some contaminants. If a biomonitoring project is carried out on one of the contaminants that tadpoles are sensitive to, then the tadpoles could perish before the desired sampling takes place. There is also limited knowledge of the ability of tadpoles and metamorphs to metabolize chemicals. More research on toxicokinetics and chemical metabolism in tadpoles is needed in order to fully explore the usefulness of tadpoles as biomonitors.

2.4.4 – Calibration of Tadpoles

The successful predictions of the model (Equation 2.5) indicates that using PRC PCB congeners is an effective method of calibrating tadpoles as biomonitors *in situ* where uptake and elimination are occurring simultaneously. Quantitative biomonitors such as tadpoles can provide valuable insight into in situ dose-effect studies which are essential for both hazard and risk assessment.

2.5 – Conclusions

Elimination rate coefficients of PRC PCBs were not significantly different among sites, so metabolic activities in tadpoles were the same from site to site. This lack of difference among metabolic activities in tadpoles indicates that chemical concentrations in the tadpoles reflected environmental contamination. Tadpoles display fast kinetic rates for eliminating and taking up organic chemicals, reaching steady state rapidly for both PCBs and OCs. These observations give strong confidence that the chemical burdens tadpoles possess in the wild are an accurate reflection of *in situ* conditions. Tadpoles also have limited potential to metabolize environmental chemicals. This limited metabolic potential allows for accurate estimation of time to steady state and the concentration achieved at steady state. These factors (rapidly reaching steady state and not metabolizing chemicals) elucidate the possible mechanisms behind chemical exposure as a factor in amphibian declines.

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Table 2.1 Performance reference compound PCB congener concentrations (mean ± SE, ng/g) in dosed food. Log K_{ow} values are from Hawker and Connell (1988).

PRC PCB Congener	log K_{ow}	Concentration
7	5.07	3226 ± 326
23	5.57	1974 ± 193
61	6.04	1966 ± 178
109	6.48	3242 ± 295
173	7.02	1889 ± 160
Total		**12297 ± 1132**

Table 2.2 Elimination rate coefficients (k_2, mean ± SE, day^{-1}), number of samples (n), coefficients of variation (R^2) and significance values (p-value) for PRC PCBs at all three sites.

PRC PCB Congener (log K_{ow}) Study Site	k_2	R^2	p-value
PRC 7 (5.07)			
Aquaculture Facility	0.259 ± 0.285 (15)	0.060	0.380
Belle River	0.251 ± 0.331 (13)	0.049	0.449
Turkey Creek	0.259 ± 0.285 (15)	0.060	0.380
PRC 23 (5.57)			
Aquaculture Facility	0.967 ± 0.090 (15)	0.898	0.0001
Belle River	0.967 ± 0.129 (13)	0.836	0.0001
Turkey Creek	0.790 ± 0.134 (15)	0.728	0.0001
PRC 61 (6.04)			
Aquaculture Facility	0.617 ± 0.281 (15)	0.271	0.047
Belle River	0.165 ± 0.080 (18)	0.211	0.045
Turkey Creek	0.111 ± 0.041 (21)	0.279	0.014
PRC 109 (6.48)			
Aquaculture Facility	0.432 ± 0.043 (18)	0.864	0.0001
Belle River	0.196 ± 0.083 (18)	0.259	0.031
Turkey Creek	0.289 ± 0.043 (18)	0.701	0.0001
PRC 173 (7.02)			
Aquaculture Facility	0.216 ± 0.026 (22)	0.777	0.0001
Belle River	0.028 ± 0.037 (18)	0.034	0.464
Turkey Creek	0.146 ± 0.028 (21)	0.587	0.0001

Table 2.3 Sum PCB concentrations (mean ± SE, ng/g lipid) and number of samples (n) at each study site on each sampling day. Areas of the chart where a value of Not Applicable (N/A) has been entered indicate that no samples were able to be collected at this time point. This occurred due to flooding events.

Day Site	Control	Dose	Control + Dose
Day 0			
All Sites	953 ± 118 (9)	1753 ± 563 (9)	1376 ± 311 (18)
Day 6			
Aquaculture Facility	1717 ± 722 (3)	830 ± 301 (3)	1274 ± 403 (6)
Belle River	2105 ± 877 (3)	1430 ± 503 (3)	1496 ± 510 (6)
Turkey Creek	19458 ± 1996 (3)	25696 ± 8829 (3)	22577 ± 4282 (6)
Day 22			
Aquaculture Facility	1120 ± 301 (3)	1168 ± 243 (3)	1144 ± 174 (6)
Belle River	N/A	2154 ± 204 (3)	2154 ± 204 (3)
Turkey Creek	80305 ± 20743 (3)	30105 ± 9954 (3)	55205 ± 15228 (6)
Day 34			
Aquaculture Facility	2571 ± 189 (3)	5026 ± 2761 (3)	3799 ± 1596 (6)
Belle River	N/A	N/A	N/A
Turkey Creek	35742 ± 12275 (3)	55904 ± 21298 (3)	45823 ± 11882 (6)

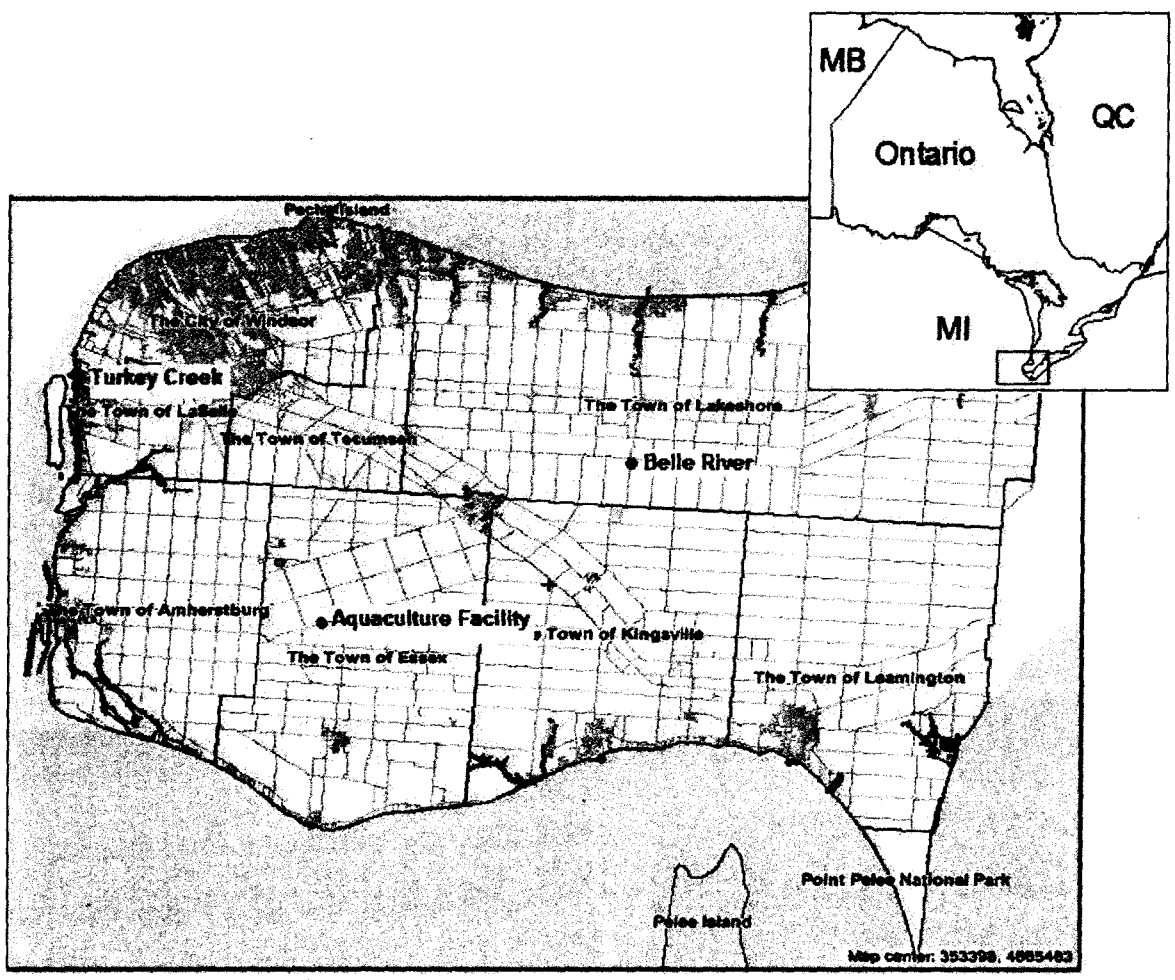


Figure 2.1 Map of the three field sites where tadpoles were deployed in cages in the Essex County region of south-western Ontario, Canada. Turkey Creek is in a municipal watershed, Belle River is in an agricultural watershed and the Aquaculture Facility is the reference site. Adapted from: http://www.townofessex.on.ca/gis.htm.

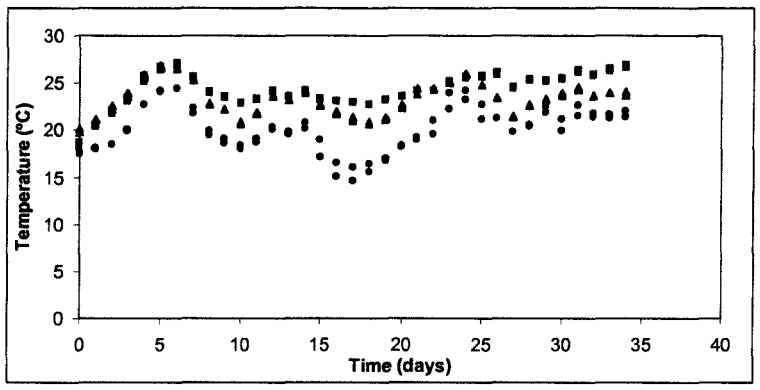
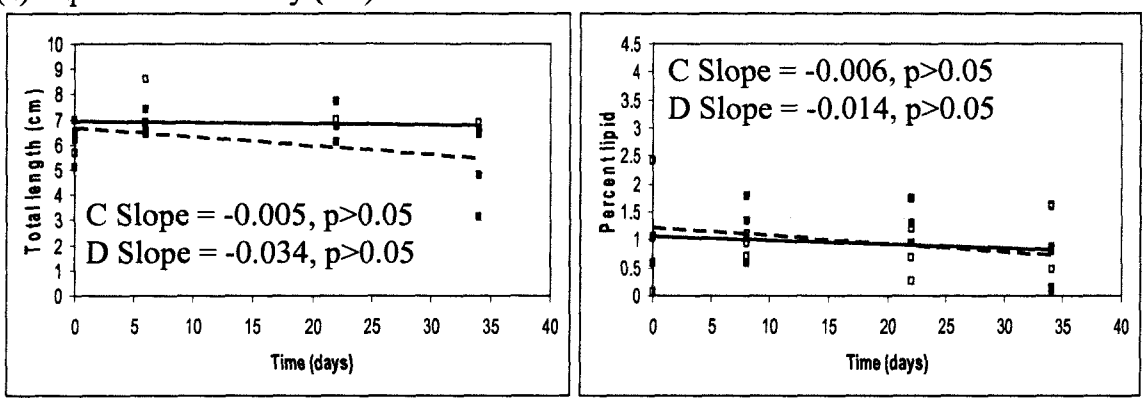
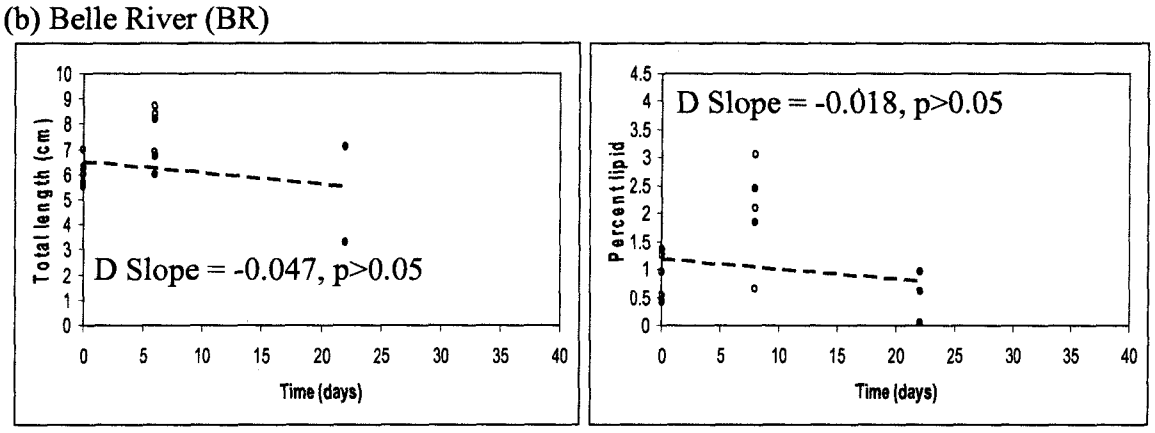


Figure 2.2 Temperature (°C) of water at all three sampling sites over time (Aquaculture Facility: ■; Belle River: ●; Turkey Creek: ▲).

(a) Aquaculture Facility (AF)



(b) Belle River (BR)



(c) Turkey Creek (TC)

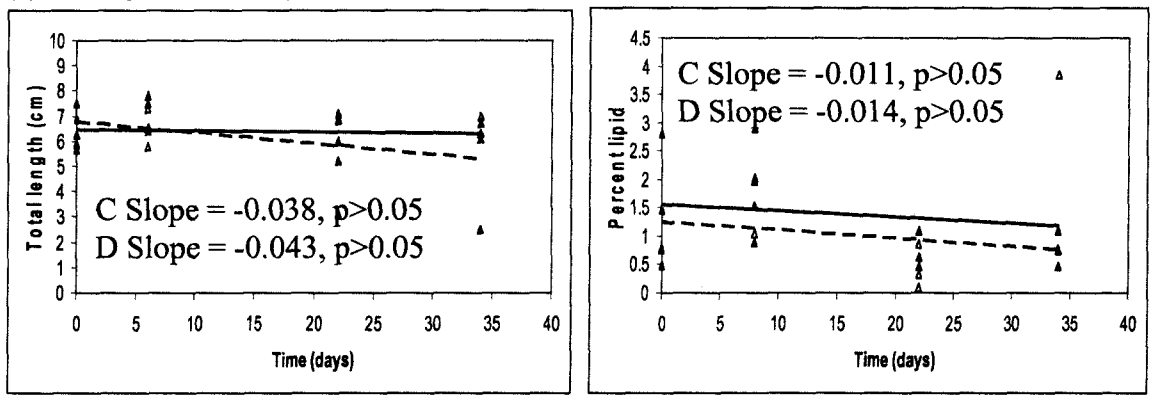


Figure 2.3 Total length (cm) and percent lipid of control (C) and PRC PCB dosed (D) tadpoles at the three study sites over time (AF: Control □ and ——; Dose ■ and – – –; BR: Control ○ and ——; Dose ● and – – –; TC: Control Δ and ——; Dose ▲ and – – –).

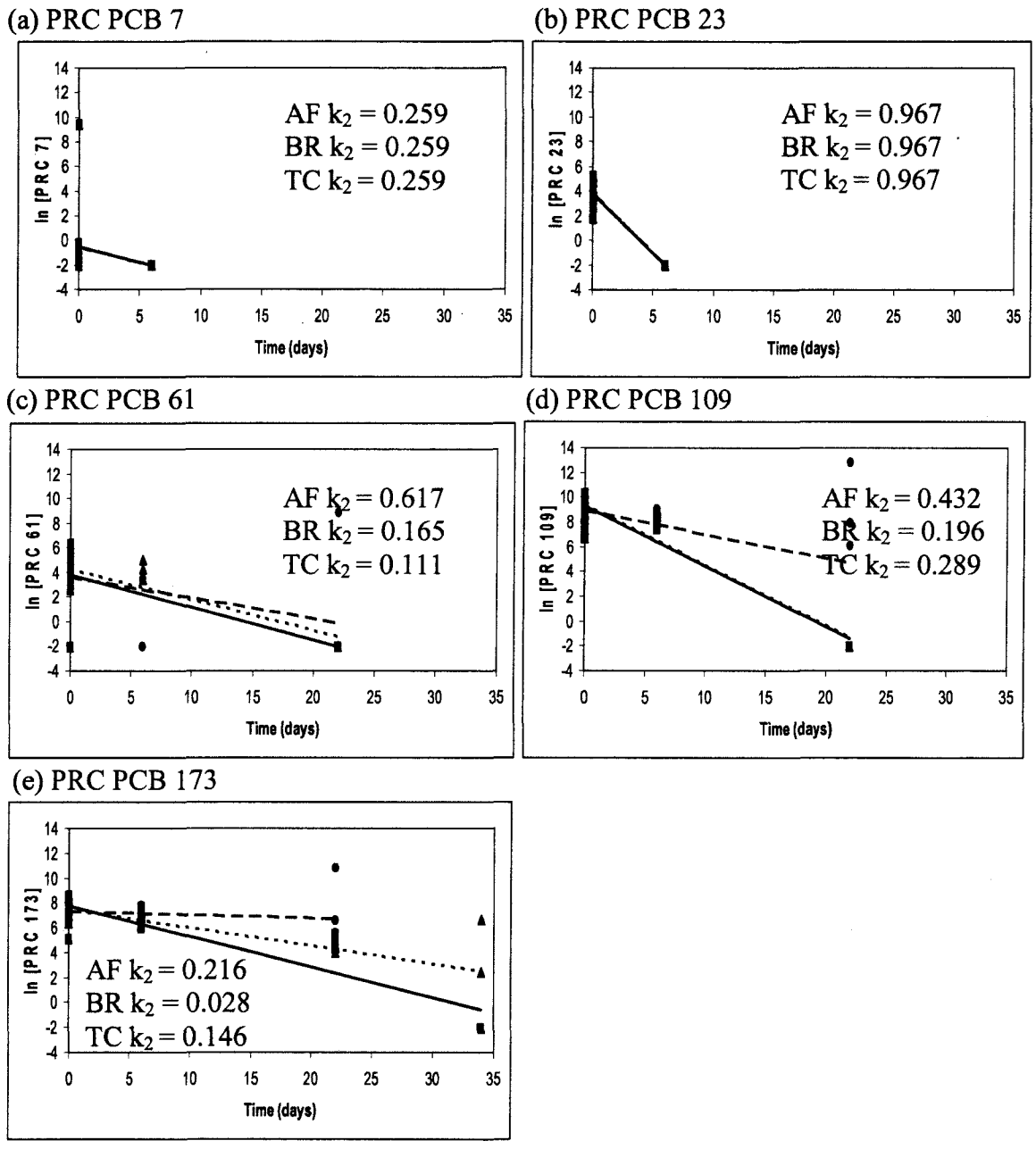


Figure 2.4 Elimination of PRC PCB congeners over time, plotted as the ln concentration (ng/g lipid), at all three sampling sites (Aquaculture Facility, AF: ■ and ——; Belle River, BR: ● and – – –; and Turkey Creek, TC: ▲and ·····).

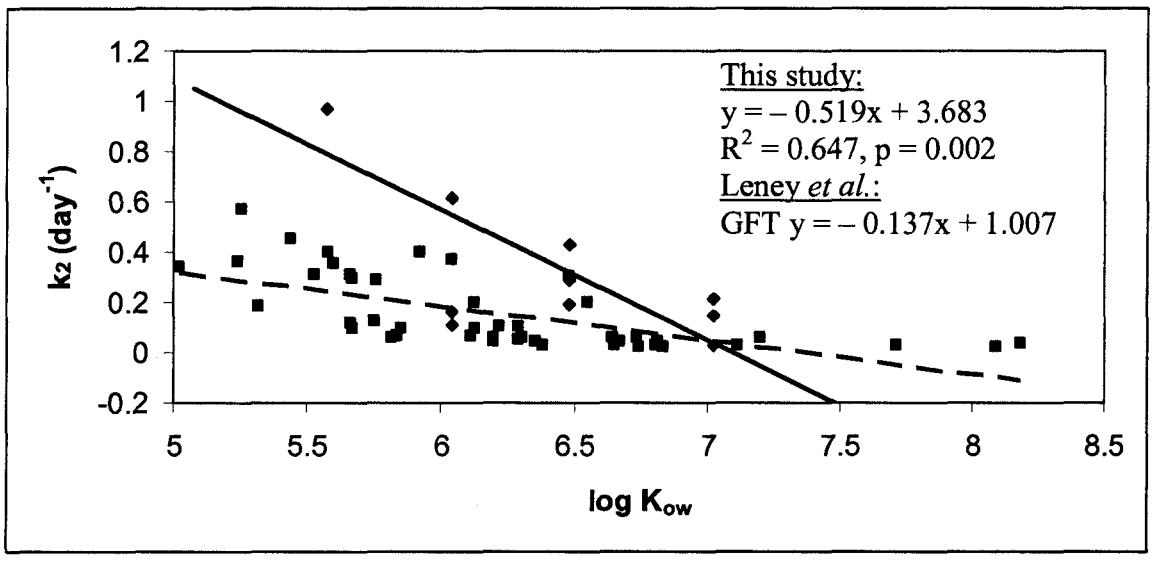
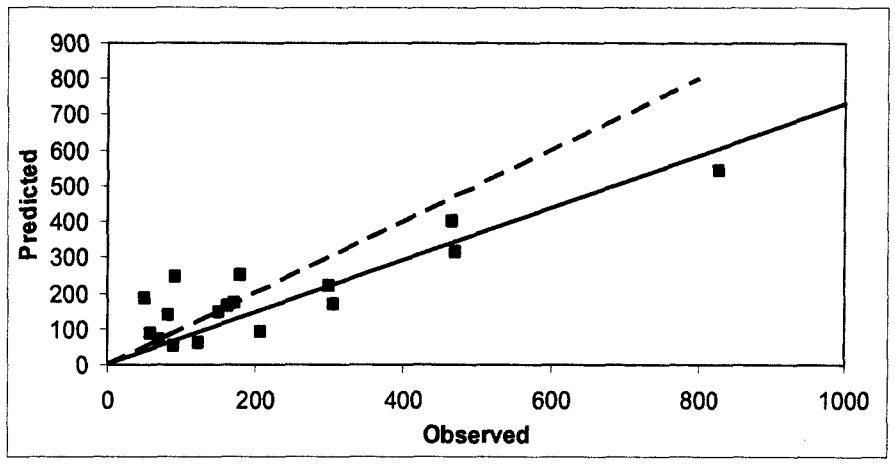
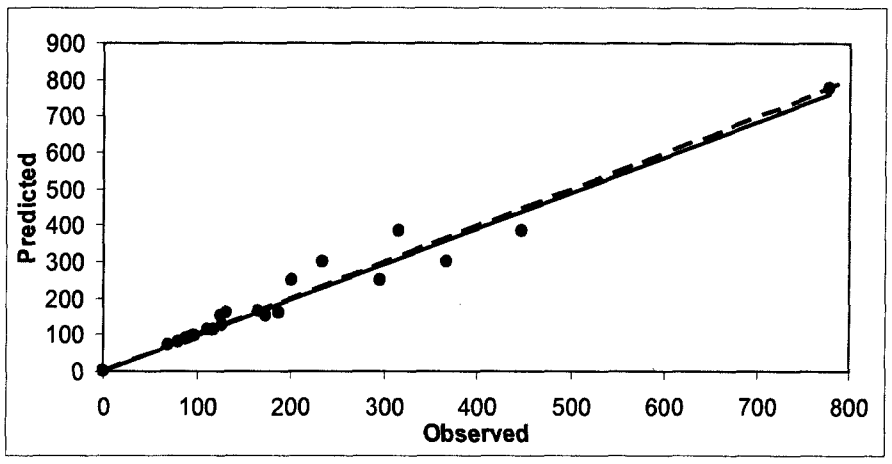


Figure 2.5 Plot of elimination rate coefficients, k_2, from this study (♦ and ——) and the Leney *et al.* (2006b) study (green frog tadpole, GFT: ■ and ---) versus log K_{ow}.

(a) Aquaculture Facility



(b) Belle River



(c) Turkey Creek

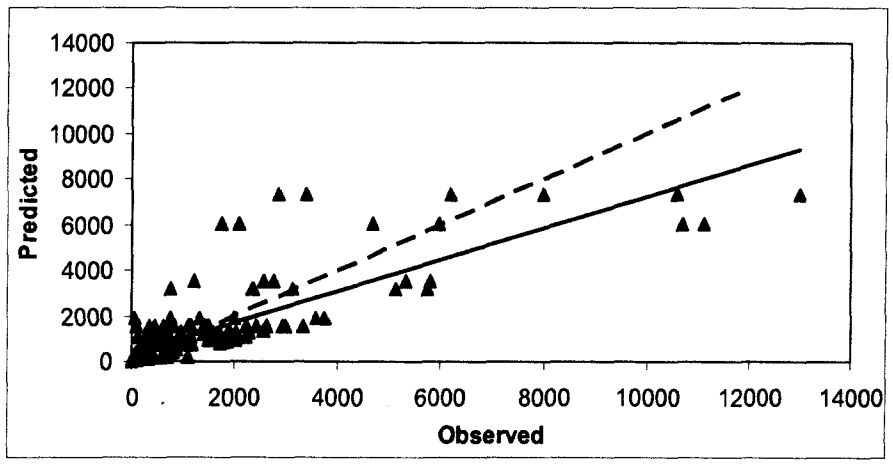
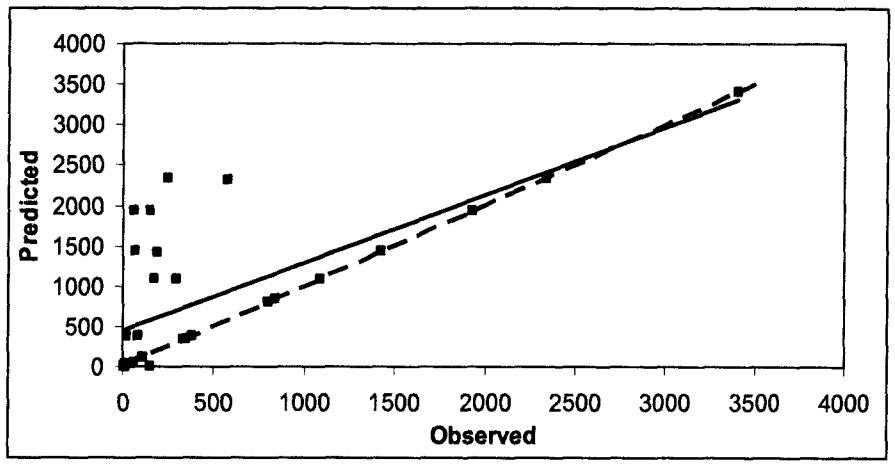
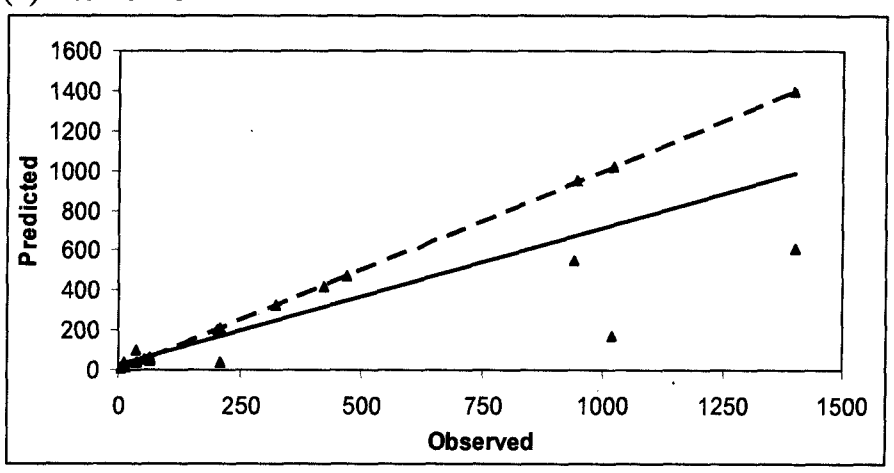


Figure 2.6 Comparison between observed PCB concentrations (ng/g lipid) and those predicted by the model (Equation 2.5). The solid line is the line of best fit for the relationship between predicted and observed values. The dashed line (– – –) represents a 1:1 relationship between predicted and observed values.

(a) Aquaculture Facility



(b) Belle River



(c) Turkey Creek

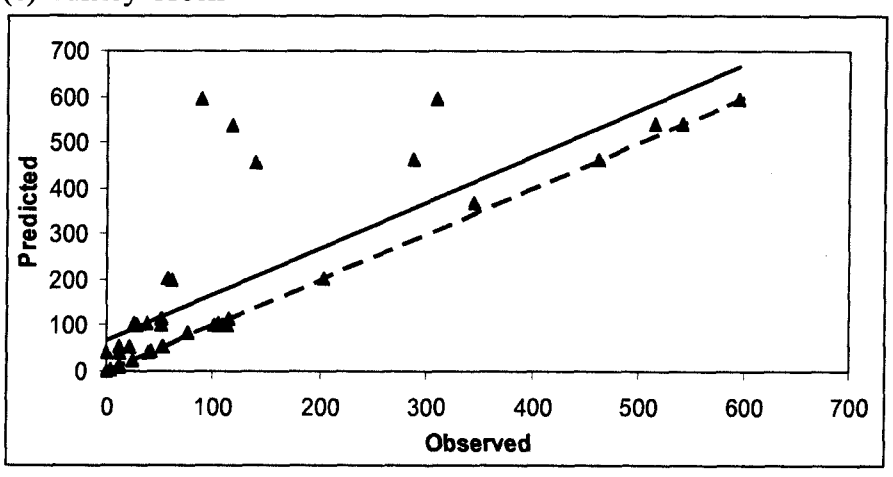


Figure 2.7 Comparison between observed OC concentrations (ng/g lipid) and those predicted by the model (Equation 2.5). The solid line is the line of best fit for the relationship between predicted and observed values. The dashed line (– – –) represents a 1:1 relationship between predicted and observed values.

CHAPTER 3 – TEMPERATURE EFFECTS ON ELIMINATION RATES OF *p,p'*-DDT IN GREEN FROG (*RANA CLAMITANS*) AND BULLFROG (*RANA CATESBEIANA*) TADPOLES

3.1 – Introduction

Many hypotheses have been raised in an attempt to explain amphibian declines, which have been occurring since the 1970s (Barinaga, 1990). One of the hypotheses suggested is exposure to toxic chemicals. Not only are amphibians exposed to chemicals in both the aquatic and terrestrial environments (Howe *et al.*, 1998), but each of their life cycle stages has different sensitivities to the same chemical exposure (Greulich and Pflugmacher, 2003). The most sensitive stages are the tadpole and the metamorph (Hall and Swineford, 1980; Freda and McDonald, 1993; Kane *et al.*, 1993). This sensitivity may be in part because during the process of metamorphosis, where tadpoles can rapidly lose body mass and lipids, tadpoles experience an increase in the toxic potential of contaminants contained in their bodies (Leney *et al.*, 2006a).

In order to understand the fate and effects of chemicals, we must understand how a chemical moves through various phases in the environment (e.g. water, air, sediments) and what factors regulate chemical dose to organisms. To do the latter, we must develop chemical exposure models for key representatives of ecosystems. Amphibians play an important role in ecosystems, acting as both herbivores and insectivores and serving as prey items for many higher order organisms (Harding, 2004). The exposure dynamics of chemicals in amphibians varies throughout their life cycle (Leney *et al.*, 2006b); therefore it is imperative to understand the toxicokinetics of chemicals throughout all life stages. Current research on toxicokinetics in frogs is fairly well distributed among life stages and chemicals studied (e.g. Ankley *et al.*, 2004; Honkanen and Kukkonen, 2006; Greulich and Pflugmacher, 2004; Fridman *et al.*, 2004). Only one group of researchers, however, has begun to develop life cycle models for amphibians: Leney *et al.* (2006a,b,c) on the green frog (*Rana clamitans*) and the leopard frog (*Rana pipiens*).

The most commonly used chemical exposure dynamics model is the one-compartment model (Barron *et al.*, 1990). Studies on frogs have shown that elimination of organic contaminants (polychlorinated biphenyls) is first-order (Huang and Karasov, 2000; Leney *et al.*, 2006a,b,c). It is therefore predicted that pesticides will undergo first-

order elimination in frogs. The overall elimination rate coefficient, k_2, can be calculated using the equation:

$$k_2 = \frac{\ln C_{org(t=0)} - \ln C_{org(t)}}{t} \quad (3.1)$$

(Barron *et al.*, 1990), where $C_{org(t=0)}$ is the chemical concentration within the animal at time zero and $C_{org(t)}$ is the chemical concentration within the animal at the end of the elimination period.

In aquatic cold-blooded animals like frogs, environmental temperature regulates activity and growth rates (Rombough, 1996) as well as the permeability of membranes and enzyme activity (Kennedy and Walsh, 1996). These factors will have impacts on both chemical accumulation and elimination processes. Only two studies on the effects of temperature on chemical exposure dynamics in amphibians have been reported. Honkanen and Kukkonen (2006) observed that at higher temperatures uptake and elimination rates of bisphenol A increased in common frog (*Rana temporaria*) tadpoles. Licht (1976) found that uptake of *p,p'*-dichloro-diphenyl-trichloroethane (hereafter referred to as DDT) was faster at 15°C than at 21°C in wood frog (*Rana sylvatica*) tadpoles. Alternately, elimination rate coefficients were approximately twice as large at 21°C. Few studies have examined exposure dynamics of DDT in amphibians despite its persistence in the environment, toxicity and ability to accumulate to high levels in amphibians (Russell *et al.*, 1995, 1997, Gillan *et al.*, 1998). This is a major issue since DDT and its metabolites are still present in the environment, and since DDT is still used in many countries for malaria control.

This study examined the chemical exposure dynamics of DDT in pre-metamorphic green frogs (*Rana clamitans*) and bullfrogs (*Rana catesbeiana*) at two temperatures (18 and 25°C). Performance reference compound polychlorinated biphenyls (PRC PCBs) were used in this study as an internal reference to quantify the effects of different temperatures on the exposure dynamics of DDT. Performance Reference Compounds are analytically non-interfering organic compounds that are selected to match the physicochemical properties of chemicals of interest, and usually have moderate to high K_{ow}s. It is predicted that elimination of PRC PCBs and DDT will increase with

temperature and that elimination of PRC PCBs and DDT will be similar between green frog and bullfrog tadpoles.

3.2 – Materials and Methods

3.2.1 – Dosing Procedures

Performance reference compound PCBs used in this study are a suite of ten PCBs (International Union of Pure and Applied Chemistry [IUPAC] numbers 23, 43, 61, 68, 109, 129, 168, 173, 198, 205) that were not used in the original Aroclor mixtures and thus do not exist in the environment. The PRC PCB control food was made in the following manner: 20 mL of 1 mg/mL total PRC solution was placed into 600 mL of hexane and mixed well in a glass jar. To that, 100 g of Nutrafin Basix Staple fish food (Montreal, QC, Canada) was added. For the DDT-dosed food, PRCs were added as in the PRC PCB-dosed food, and 5 mL of 0.1 mg/mL DDT was added to the 100 g of Nutrafin Basix food and 600 mL hexane mixture. For both the control and DDT-dosed food, the food and hexane mixtures were mixed thoroughly with hexane-rinsed glass rods, and placed in a fume hood to allow the hexane to evaporate. The solutions were mixed periodically until dry (~24 hours). Three subsamples of each type of flake food were removed for chemical analysis. Concentrations of PRC PCBs and DDT in food are summarized in Table 3.1. The PRC PCBs and *p,p'*-DDT and were obtained from AccuStandard, Inc. (New Haven, CT, USA).

Green frog tadpoles were collected in October 2007 from ponds at Leadley Environmental, a rural aquaculture facility in Essex County, near Windsor, Ontario. Bullfrog tadpoles were obtained from Ward's Natural Science (Rochester, NY). Bullfrog and green frog tadpoles were selected for use in the study based on size and development stage; it was ensured that tadpoles did not have limb buds or emerged limbs. Tadpoles of both species were divided into two groups, a PRC-control group and a PRC + DDT treated group, and placed into two dosing tanks with approximately 1050 L of water, with 1500 L reservoirs attached on a flow-through system. Control tadpoles were fed PRC PCB-dosed food for 7 days, while the PRC + DDT exposed tadpoles were fed food dosed with both PRC PCBs and DDT for 7 days. After this initial dosing period, tadpoles were fasted for 48 hours; 3 individuals of each species were then removed and euthanized from

each treatment group. The remaining tadpoles were divided evenly between two tanks, one at 25°C and the other kept at 18°C. Each tank was divided into four sections, two sections for green frogs (PRC-control and PRC + DDT treated) and two for bullfrogs (PRC-control and PRC + DDT treated; see Figure 3.1). Tadpoles were then fed a mixture of Nutrafin Basix fish food, boiled romaine lettuce and Aquarian® Algae chips (Aquarium Pharmaceuticals, Inc., Chalfont, PA) every three days for the remainder of the study. At each time point (days 0, 8, 22 and 39), three individuals of each species at each temperature and from each treatment group were sampled and euthanized (for a total of 12 individuals per species per sampling day). Then tadpoles were individually wrapped in hexane-rinsed foil and frozen at -80°C until they could be analyzed for contaminants.

All procedures were approved by the University of Windsor Animal Care Committee.

3.2.2 – Chemical Analysis

Sampled frogs were analyzed to determine whole-body and lipid-normalized concentrations of PRC PCBs, *p,p'*-DDT and its metabolites according to the methods of Leney *et al.* (2006a). Once thawed, tadpoles were weighed and homogenized. The tadpole homogenate was dried with 35 g of anhydrous sodium sulphate using a glass mortar and pestle, then wet packed in a 60 x 2.5 cm glass column with 50 mL dichloromethane (DCM):hexane (1:1 v/v). Columns were then spiked with 100 µL of an internal recovery standard solution; a mixture of 2 labelled PCBs ([C^{13}] PCB 52, [C^{13}] PCB 153 at 200 ng/mL) for recovery correction. Another 250 mL of 1:1 DCM:hexane was added, and the column was left to stand for 1 hr before being eluted. The extract was rotoevaporated down to about 1 mL and diluted to a volume of 10 mL with hexane. A 1 mL aliquot was removed and placed into an aluminum weigh boat and dried at 110°C for one hour for gravimetric lipid determination (Drouillard *et al.*, 2004). The remaining 9 mL of sample was rotoevaporated to 2 mL. The remaining sample was then added to a 35 x 1 cm glass column packed with 6 g of activated florisil and 1 cm of sodium sulphate. After being eluted from the column with 50 mL of hexane and 50 mL of DCM:hexane (15:85 v/v), the extract was rotoevaporated to less than 1 mL and brought up to 1 mL with isooctane. The sample was then transferred to a 2 mL gas chromatograph vial for storage at 4°C until

instrumental analysis. A method blank and reference tissue homogenate (carp from the Detroit River) were run with each set of 6 samples.

Gas chromatographic analysis of PCBs and OCs was performed using a Hewlett-Packard 5890/5973 with a mass selective detector, a 7673 autosampler and a 60 m x 0.250 mm x 0.25 μm DB-5 column (Chromatographic specialties, Brockville, ON, Canada). The carrier gas was He with a flow rate of 1 mL/min, injection port temperature was 250°C and injection volume was 2 μL (splitless). The oven program was started at 90°C and remained at this temperature for 3 minutes, increased at 7°C/min to 150°C, then increased at 3°C/min to 280°C and remained at this temperature for 5.10 minutes, for a total run time of 60 minutes. The MSD was operated in selective ion mode (SIM) and the ions and time windows are described in O'Rourke *et al.* (2004).

Performance reference compound PCBs, DDT and its metabolites were identified by retention time and quantification of individual compounds was based on the response factors from well characterized standard mixtures that were run with each set of 8 samples, including the reference homogenate and blank. Detection limits were between 0.012 and 0.248 ng/g for PCBs and between 0.042 and 0.450 ng/g for DDT and its metabolites. Percent recoveries of the internal surrogate standards C^{13} PCBs 52 and 153 were all above 70% therefore no recovery corrections were made. Mean ± SE percent recovery for C^{13} PCB 52 was 80.83 ± 1.00% and for C^{13} PCB 153 was 82.44 ± 0.87%.

3.2.3 – Statistical Analysis

Least Squares Regression (LSR) analysis was used to determine elimination rate coefficients (k_2) of PRC PCBs, DDT and its metabolites. To determine whether differences in elimination rate coefficients occurred as a result of temperature, and among the relationships between k_2 and log K_{ow}, Analysis of Covariance (ANCOVA) was used. Finally, to determine overall differences in tadpole length, lipid content, or DDT, DDE and DDD concentrations, Analysis of Variance (ANOVA) was carried out. All statistical tests were performed using Systat 8.0 (SPSS, Inc.). Results were considered significant at a value of $p<0.05$.

3.3 – Results

Green frog tadpoles began dying when the experiment was initiated. Their deaths were most likely due to stress associated with the presence of bullfrog tadpoles in the same tank. It has been demonstrated that green frog tadpoles become stressed when simultaneously exposed to the release of chemicals by predators such as bullfrogs (Relyea, 2004). As a result, sampling of green frogs was stopped after day 8 of the experiment, and statistical comparisons of elimination rate coefficients between the two species could only be done for those chemicals that were eliminated within 8 days (PRC PCBs 43 and 61).

3.3.1 – Lipid Content and Tadpole Length

Analysis of Variance revealed that percent lipid did not decrease significantly over the course of the experiment for either of the PRC or PRC + DDT treatments in either species. Comparisons among treatments and between species revealed no significant differences in lipid content over time (ANCOVA, $p>0.10$). Therefore, all chemical concentrations are based on lipid concentration (ng/g lipid).

Tadpole length (ranged between 49 to 71 mm for green frogs and between 62 and 99 mm for bullfrogs) did not significantly decrease over time in either species in any of the treatments (ANOVA, $p>0.10$). Tadpole length also did not differ significantly over time between PRC control and PRC + DDT dosed treatment groups in either species (ANCOVA, $p>0.30$). No significant differences in tadpole length over time existed between species either (ANCOVA, $p>0.60$). Finally, temperature had no significant effects on tadpole length over time in either species (ANCOVA, $p>0.80$).

3.3.2 – PRC PCB Elimination

Performance Reference Compound PCB elimination rate coefficients are summarized in Table 3.3. There were no significant differences in elimination rate coefficients of PRC PCBs between PRC control and PRC + DDT dose groups (ANCOVA, $p>0.05$).

There were, however, some inconsistent yet significant temperature effects on PRC PCB elimination rate coefficients. In PRC PCB control green frogs, PRC PCB 43

was eliminated significantly faster at 18°C than at 25°C (k_2 = 1.278 compared to 0.918, ANCOVA, p=0.013). In DDT dose bullfrogs, PRC PCB 109 was eliminated significantly faster at 25°C than at 18°C (k_2 = 0.585 compared to 0.251, ANCOVA, p=0.025). It is not known why these two congeners behaved differently from the other congeners, although individual exposure differences might account for such observations.

There were also differences in elimination rate coefficients between species. Elimination rates were more rapid in green frog tadpoles than bullfrog tadpoles. At 18°C in the PRC PCB control group, PRC PCB 43 was eliminated significantly faster in green frogs than in bullfrogs (rates of 1.278 and 0.421, ANCOVA, p=0.0001). Largest differences in PRC PCB elimination rate coefficients in bullfrog tadpoles were as follows: at 18°C, k_2 for PRC PCB 23 in the control group was 0.352 compared to 0.078 day^{-1} in the DDT dose group. Smallest differences in coefficients were: in bullfrogs at 25°C k_2 for PRC PCB 43 was 0.402 in the control group compared to 0.368 in the DDT dose group; and in green frogs at 25°C k_2 for PRC PCB 61 was 0.882 in the control compared to 0.802 day^{-1} in the DDT dose group. When metabolic grouping was accounted for (Kannan *et al.*, 1995), there were no significant differences in elimination rate coefficients between species (ANOVA, p>0.95). Again, high individual variability might have confounded the measurement of accurate elimination rate coefficients.

3.3.3 – Dynamics of DDT and its Metabolites

Concentrations of DDT in tadpoles over time are summarized in Table 3.2. Mean concentrations of DDT in DDT-treated tadpoles on day 0 were 281,729 ± 154,572 ng/g in green frogs and 492,132 ± 111,037 ng/g in bullfrogs. The 18 and 25°C internal control tadpoles exhibited significant uptake of DDT (LSR, p=0.05 and p=0.025 respectively), therefore concentrations of DDT were control corrected in order to compensate for chemical recycling in the calculation of rate coefficients. Elimination rate coefficients of DDT and its metabolites are summarized in Table 3.4. At 25°C, DDE was being eliminated significantly from the green frogs, and at both 18 and 25°C from bullfrogs (LSR, p=0.05, 0.001 and 0.003 respectively). Sum DDTs also exhibited significant elimination in both the 18 and 25°C bullfrog groups (LSR, p=0.003, 0.034 respectively).

Elimination of DDT in bullfrogs was significantly greater at 18°C than at 25°C (0.297 versus 0.184, ANCOVA, p=0.0001). Elimination rates of DDE and DDD were also greater at 18°C in green frog tadpoles and bullfrog tadpoles respectively, although these were not significantly different. There were also no significant interspecific differences in elimination rates of DDT or its metabolites. Elimination rate coefficients for DDT, DDE and DDD are summarized in Figure 3.3.

3.3.4 – Relationship Between k_2 and log K_{ow}

When k_2 values for PRC PCBs were grouped by temperature treatment and species, significant relationships between k_2 and log K_{ow} were found (see Figure 3.2). The relationships for both temperature treatment groups in both green frogs and bullfrogs were significant (18°C green frog: p=0.007; 18°C bullfrog: p=0.025; 25°C green frog: p=0.025; and 25°C bullfrog: p=0.001). None of the slopes were significantly different from each other (ANCOVA, p>0.05).

3.4 – Discussion

3.4.1 – PRC PCB Elimination

The lack of consistent temperature effects in both green frog and bullfrog tadpoles is contradictory to the findings of Licht (1976) and Honkanen and Kukkonen (2006) who found that uptake and elimination rate coefficients were higher at higher temperatures. Higher temperatures should affect exposure dynamics in poikilotherms due to higher metabolic rates (Carey and Alexander, 2003; Reading, 2007) and increased growth rates of developing tadpoles, which are temperature-dependant (Beebee, 1995). The fact that an increase in toxicokinetics with temperature was not observed in this study suggests that tadpoles of these species can tolerate temperature variability in this upper range. However, the effects of temperature changes on amphibians are still an important factor to consider when exploring the mechanisms behind amphibian declines, as increased temperature can affect body condition with respect to lipid content (Reading and Clark, 1995; Reading, 2007), larval development (Reading and Clark, 1999) and thus survival. Also, studies that aim to quantify elimination rate coefficients at different temperatures are essential for the development of accurate life cycle models.

3.4.2 – Dynamics of DDT and its Metabolites

There were no significant differences in elimination rates of DDT or its metabolites between species. This observation suggests that metabolism of DDT and its metabolites did not differ among amphibian species. When examining the plot of DDT and DDD concentrations over time in bullfrog tadpoles at 25°C, an interesting pattern was observed, in that concentrations increased until day 8, and then decreased. Since tadpoles were not losing lipid during this time period, the bullfrog tadpoles must have been picking up the DDT from other tadpoles in the same tank. As to why the concentration of DDD was increasing, there are two possibilities: either bullfrog tadpoles were picking it up from other tadpoles in the same tank, or they were metabolizing DDE to form DDD. Since the concentration of DDD was decreasing after day 8, metabolism of DDE either slowed down or tadpoles were able to eliminate DDD from their bodies at this point in time.

3.4.3 – Differences in Kinetics Between Species

There were numerical differences in elimination rates between species, with elimination rates of PRC PCBs being more rapid in green frogs than bullfrogs. However, due to the fact that sampling could not be continued after day 8, no statistical comparisons could be made for PRC PCBs 68 to 205. The relationships between k_2 and log K_{ow} for both bullfrog tadpoles and those estimated for green frog tadpoles were similar (slopes from -0.105 up to -0.127). This similarity suggests that metabolic activity in green frog tadpoles was higher than that observed in bullfrog tadpoles for certain congeners, but not for all (e.g. PRC PCBs 23 and 43 at 18°C in the control group). However, when grouped by metabolic class, there were no significant differences in elimination rate coefficients between species. Alternatively, differences in elimination rate coefficients between green frog and bullfrog tadpoles are possibly due the combination of predatory cues (from bullfrog tadpoles) and chemical stress in the green frog tadpoles (Relyea, 2004). There were no significant differences in tadpole length or lipid weight over time between the two species thus it is not possible that allometric or capacity changes were affecting these observed interspecific differences. Since statistical testing could not be done to compare

most elimination rate coefficients between species, no conclusions could be made about the possibility of these phenomena.

The possibility that differences in metabolic activities exist among amphibian species is supported by Leney *et al.* (2006b) who found that leopard frog metamorphs had higher elimination rates of PCBs than green frog metamorphs. The findings of this experiment and those of Leney *et al.* indicate that choosing one amphibian species to be a representative of that class could be difficult. When selecting a species in order to protect the entire class of amphibians, then the species with the highest toxicokinetic and metabolic abilities should be chosen, because this species would theoretically be the most sensitive species to contaminants. Overall, a much greater understanding of amphibian toxicokinetics and metabolism is needed in order to understand the mechanisms relating amphibian decline to chemical exposure.

3.5 – Conclusions

Performance Reference Compound PCBs allowed for control of temperature effects on exposure dynamics. This was a novel approach, and was highly effective. Temperature (18 – 25°C) had no consistent effects on PRC PCB or DDT elimination, which indicated that tadpoles were tolerant of temperature changes in this range, but the effects of broader temperature ranges need to be tested. There is some indication of the possibility of metabolic differences between green frog and bullfrog tadpoles due to numerical differences in elimination rate coefficients of PRC PCB congeners and DDT, but these differences need to be verified since statistical testing could not be carried out on most congeners. This study provides an effective method of controlling for possible temperature effects on chemical elimination using PRC PCBs, thus providing better quantitative data for estimating chemical uptake.

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Table 3.1 Concentrations of PRC PCBs, DDT, DDE and DDD (mean ± SE, ng/g) in food fed to tadpoles at the beginning of the experiment. Log K_{ow} values are from Hawker and Connell (1988) and Mackay *et al.* (2006).

Chemical (log K_{ow})	Food	Concentration
PRC 23 (5.57)	PRC and DDT dosed	23148 ± 15169
	PRC dosed	32151 ± 14845
PRC 43 (5.75)	PRC and DDT dosed	3646 ± 2360
	PRC dosed	4616 ± 2037
PRC 61 (6.04)	PRC and DDT dosed	19391 ± 12538
	PRC dosed	19912 ± 8842
PRC 68 (6.26)	PRC and DDT dosed	5718 ± 3692
	PRC dosed	9022 ± 4046
PRC 109 (6.48)	PRC and DDT dosed	24211 ± 15499
	PRC dosed	28643 ± 12707
PRC 129 (6.73)	PRC and DDT dosed	19095 ± 12365
	PRC dosed	26994 ± 12495
PRC 173 (7.02)	PRC and DDT dosed	10159 ± 6512
	PRC dosed	12332 ± 5286
PRC 167 (7.27)	PRC and DDT dosed	8719 ± 5594
	PRC dosed	10904 ± 4723
PRC 198 (7.62)	PRC and DDT dosed	4496 ± 4391
	PRC dosed	5524 ± 3720
PRC 205 (8.00)	PRC and DDT dosed	2604 ±1697
	PRC dosed	4235 ± 2817
DDT (6.19)	PRC and DDT dosed	103082 ± 8404
	PRC dosed	4.360 ± 4.116
DDE (5.70)	PRC and DDT dosed	304 ± 18.6
	PRC dosed	2.305 ± 0.493
DDD (5.90)	PRC and DDT dosed	1512 ± 77.9
	PRC dosed	5.412 ± 4.347

Table 3.2 Concentrations of DDT (mean ± SE, ng/g lipid or body weight) in green frog and bullfrog tadpoles at each time point, in both the PRC control and DDT dose groups at 18°C and 25°C. A value of N/A (not applicable) indicates that tadpoles were not able to be sampled at that time point.

Lipid Weight				
Species	**Green Frog**			
Treatment	**PRC Control**		**DDT Dose**	
Temperature	**18°C**	**25°C**	**18°C**	**25°C**
Day 0	2856 ± 2145	2856 ± 2145	281,729 ± 154,572	281,729 ± 154,572
Day 8	33,187 ± 10796	0	84,874	56
Species	**Bullfrog**			
Treatment	**PRC Control**		**DDT Dose**	
Temperature	**18°C**	**25°C**	**18°C**	**25°C**
Day 0	90 ± 49	90 ± 49	492,132 ± 111,037	492,132 ± 111,037
Day 8	63,966 ± 60,018	18 ± 10	789,498 ± 459,910	184,705 ± 35,171
Day 25	108,614 ± 88,304	6453 ± 2030	211,383 ± 159,335	127,723 ± 88,339
Day 39	N/A	1061 ± 48	108,341 ± 68,367	N/A
Body Weight				
Species	**Green Frog**			
Treatment	**PRC Control**		**DDT Dose**	
Temperature	**18°C**	**25°C**	**18°C**	**25°C**
Day 0	3.75 ± 0.76	3.75 ± 0.76	642 ± 126	642 ± 126
Day 8	0.25 ± 0.31	37.55 ± 21.62	281	68 ± 46
Species	**Bullfrog**			
Treatment	**PRC Control**		**DDT Dose**	
Temperature	**18°C**	**25°C**	**18°C**	**25°C**
Day 0	2.22 ± 0.74	2.22 ± 0.74	4764 ± 1436	4764 ± 1436
Day 8	1026 ± 991	0.15 ± 0.05	6201 ± 3209	2038 ± 649
Day 25	1570 ± 1015	37 ± 14	3847 ± 2504	918 ± 632
Day 39	N/A	12 ± 10	289 ± 154	N/A

Table 3.3 Elimination rate coefficients (k_2, day^{-1}) and significance values (p-values) for PRC PCBs in both the PRC control and DDT dose treatment groups at 18°C and 25°C. An asterisk (*) indicates that the elimination rate coefficient is significant ($p<0.05$). Elimination rate coefficients for PRC PCBs 68-205 in green frogs are estimates because sampling could not occur after day 8 and these PRC congeners were not eliminated that quickly.

Species	**Bullfrog**							
Temperature	**18°C**				**25°C**			
Treatment	**Control**		**Dose**		**Control**		**Dose**	
PRC (log K_{ow})	**k_2**	**p-value**	**k_2**	**p-value**	**k_2**	**p-value**	**k_2**	**p-value**
23 (5.57)	0.352*	0.019	0.078*	0.008	0.192*	0.003	0.131	0.200
43 (5.75)	0.421*	0.0001	0.359*	0.021	0.402*	0.001	0.368*	0.004
61 (6.04)	1.608*	0.0001	1.354*	0.0001	1.608*	0.0001	1.354*	0.0001
68 (6.26)	0.015	0.335	0.029	0.068	0.026	0.244	0.017	0.393
109 (6.48)	0.456*	0.005	0.251*	0.001	0.178	0.145	0.585*	0.0001
129 (6.73)	0.032	0.125	0.024*	0.045	0.082	0.357	0.040	0.224
173 (7.02)	0.006	0.819			0.045	0.080	0.038	0.100
167 (7.27)			0.006	0.533	0.013	0.457	0.009	0.654
198 (7.62)					0.017	0.421	0.007	0.732
205 (8.00)					0.011	0.546	0.002	0.943
Species	**Green Frog**							
Temperature	**18°C**				**25°C**			
Treatment	**Control**		**Dose**		**Control**		**Dose**	
PRC (log K_{ow})	**k_2**	**p-value**	**k_2**	**p-value**	**k_2**	**p-value**	**k_2**	**p-value**
23 (5.57)	0.611	0.403	0.214	0.562	0.330	0.137	0.298*	0.015
43 (5.75)	1.278*	0.012	0.200	0.345	0.918	0.482	0.253*	0.021
61 (6.04)	1.545*	0.001	0.802	0.667	0.882	0.323	0.802	0.423
68 (6.26)	0.259	0.396	0.103	0.638	1.238	0.350		
109 (6.48)	0.496	0.176			0.270	0.427	0.209	0.433
129 (6.73)	0.823	0.296	0.140	0.091	1.106	0.267		
173 (7.02)	0.294	0.283			0.196	0.523		
167 (7.27)	0.034	0.537	0.091	0.699			0.066	0.768
198 (7.62)	0.058	0.334	0.107	0.615			0.029	0.896
205 (8.00)	0.026	0.611	0.106	0.643			0.048	0.824

Table 3.4 Elimination rate coefficients ($k_2 \pm$ SE, day^{-1}), coefficients of variation (R^2) and significance values (p-values) of DDT, DDE and DDD in green and bullfrogs from the DDT dose groups at 18 and 25°C. A blank space indicates that the particular chemical exhibited uptake and not elimination. An asterisk (*) indicates that the corresponding k_2 value is statistically significant ($p<0.05$).

Chemical & Species	Temperature Treatment	k_2	R^2	p-value
DDE				
Green Frog	18	0.341 ± 0.183	0.778	0.313
	25	0.161 ± 0.052	0.763	0.053
Bullfrog	18	0.162* ± 0.038	0.605	0.001
	25	0.203* ± 0.050	0.645	0.003
DDD				
Green Frog	18	0.562 ± 0.175	0.911	0.193
	25			
Bullfrog	18	0.127* ± 0.045	0.400	0.015
	25	0.099 ± 0.065	0.207	0.160
DDT				
Green Frog	18	0.474 ± 0.133	0.926	0.175
	25			
Bullfrog	18	0.297* ± 0.111	0.471	0.028
	25	0.184 ± 0.140	0.161	0.221
Σ DDT				
Green Frog	18	1.377 ± 0.491	0.887	0.218
	25	0.250 ± 0.107	0.646	0.101
Bullfrog	18	0.399* ± 0.110	0.523	0.003
	25	0.585* ± 0.207	0.500	0.022

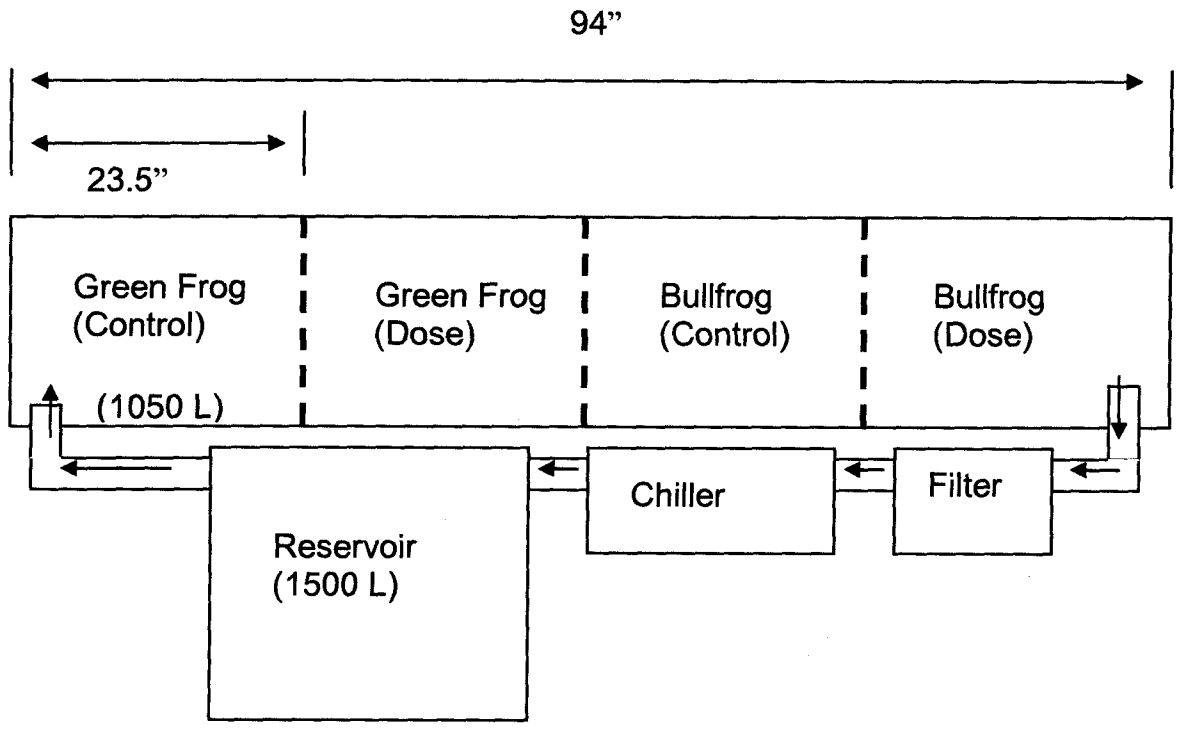
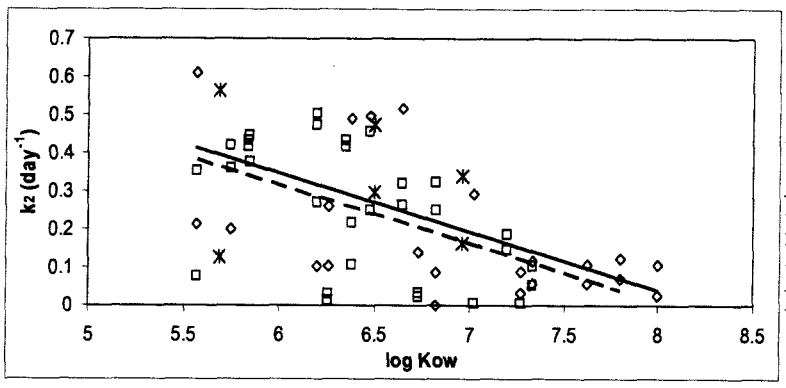


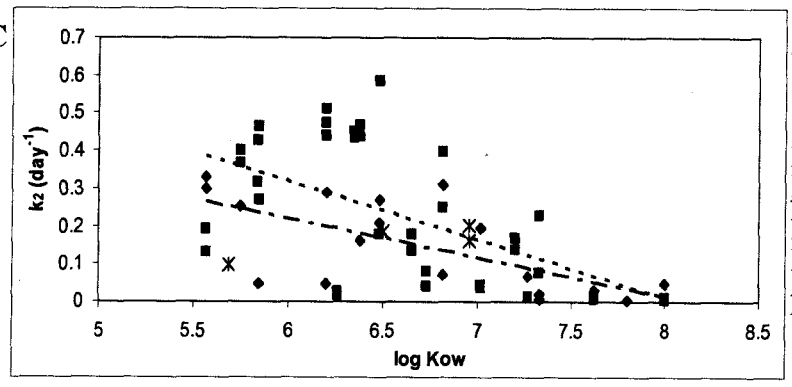
Figure 3.1 Tank layout of the 18°C treatment tank. The 25°C tank is identical, with the exclusion of the Chiller. One directional arrows (←—) indicate the direction of water flow. Dashed lines symbolize mesh used to separate treatment groups and species. Numbers in brackets indicate the volume of water present.

(a) 18°C



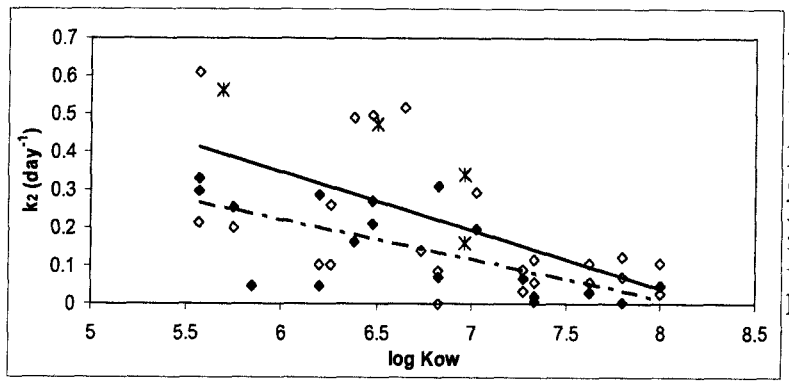
GF
$y = -0.127x + 1.070$
$R^2 = 0.255$
$p = 0.007$
BF
$y = -0.107x + 0.932$
$R^2 = 0.129$
$p = 0.025$

(b) 25°C



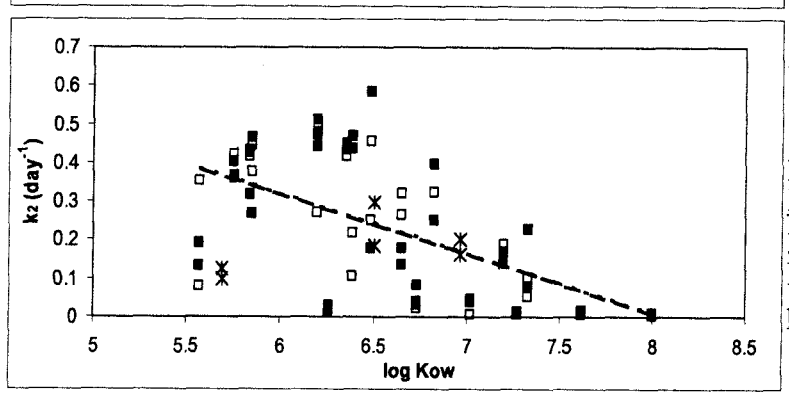
GF
$y = -0.105x + 0.850$
$R^2 = 0.438$
$p = 0.002$
BF
$y = -0.124x + 1.049$
$R^2 = 0.218$
$p = 0.001$

(c) GF



GF 18°C
$y = -0.127x + 1.070$
$R^2 = 0.255$
$p = 0.007$
GF 25°C
$y = -0.105x + 0.850$
$R^2 = 0.438$
$p = 0.002$

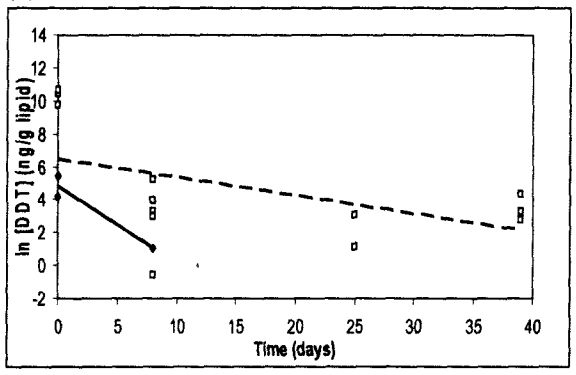
(d) BF



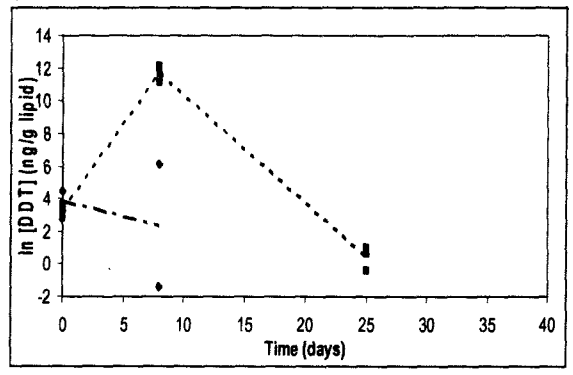
BF 18°C
$y = -0.107x + 0.932$
$R^2 = 0.129$
$p = 0.025$
BF 25°C
$y = -0.124x + 1.049$
$R^2 = 0.218$
$p = 0.001$

Figure 3.2 Regressions of elimination rate coefficients (k_2) against corresponding log K_{ow} values for DDT, its metabolites and PRC PCBs (18°C green frog, GF: ◊ and —; 18°C bullfrog, BF: □ and – – –; 25°C GF: ♦ and - · -; 25°C BF: ■ and - - -). Points for DDT and its metabolites are symbolized by an asterisk (*).

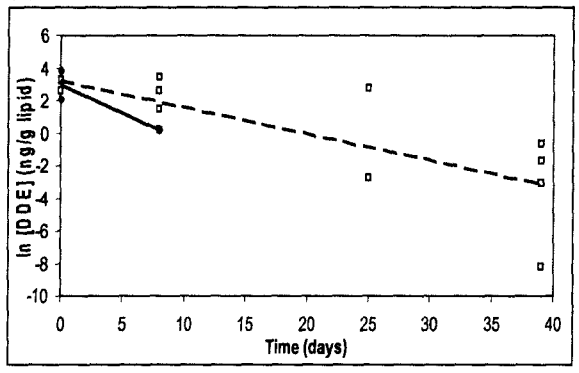
(a) 18°C DDT



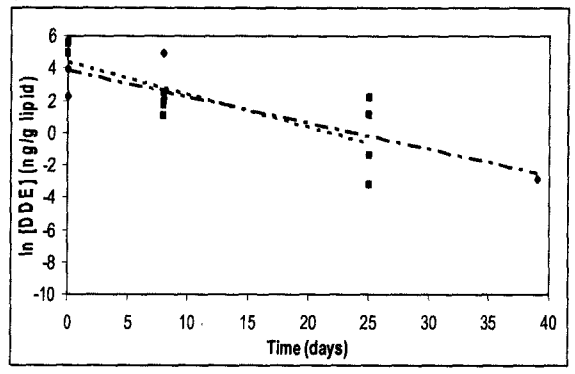
(b) 25°C DDT



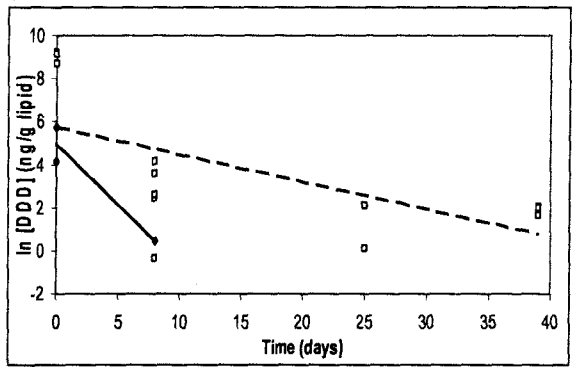
(c) 18°C DDE



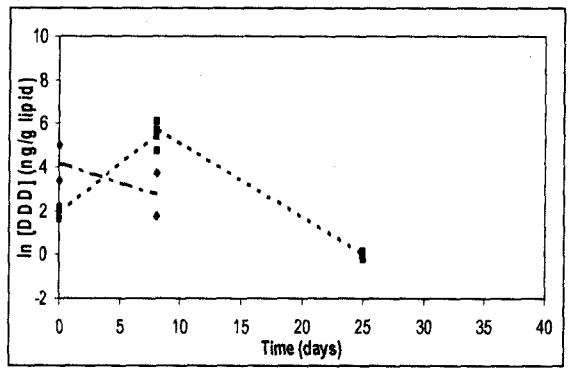
(d) 25°C DDE



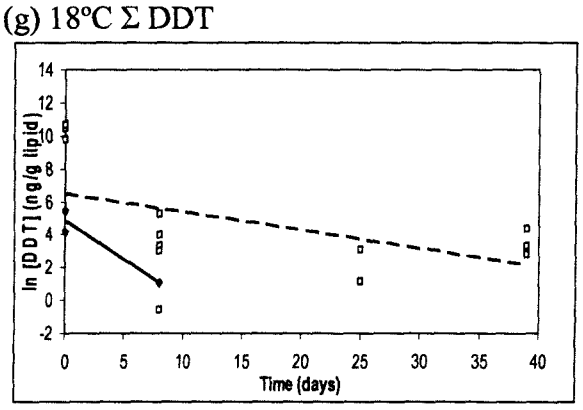
(e) 18°C DDD



(f) 25°C DDD



(g) 18°C Σ DDT



(h) 25°C Σ DDT

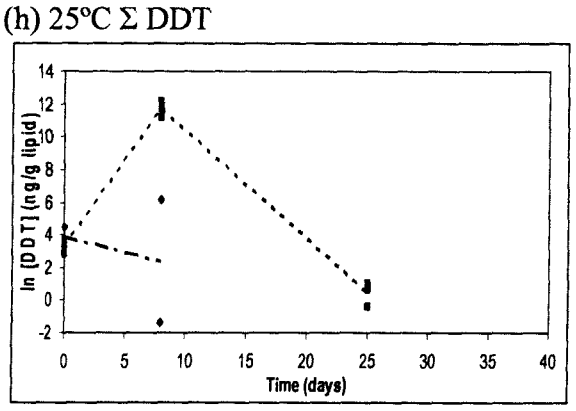


Figure 3.3 Exposure dynamics of DDT and its metabolites over time (18°C green frog: ◊ and —; 18°C bullfrog: □ and – – –; 25°C green frog: ♦ and – · –; 25°C bullfrog: ■ and ····).

CHAPTER 4 – GENERAL CONCLUSIONS

In this thesis, studies were carried out in order to determine *in situ* chemical exposure dynamics and to explore the effects of temperature on the toxicokinetics of DDT in amphibians during their tadpole and pre-metamorphic phases. The specific hypotheses tested and results of each chapter are summarized below. The outcomes of each experiment in relation to these hypotheses will also be addressed in this chapter.

4.1 – Chapter 2

Chapter 2 investigated the *in situ* chemical exposure dynamics of pre-metamorphic and metamorphic tadpoles. This involved deploying tadpoles in cages at various locations across Essex County in south-western Ontario, including an agricultural watershed, a municipal watershed and a reference site. The effectiveness of Performance Reference Compound polychlorinated biphenyls (PRC PCBs) as environmental tracers in tadpoles was also explored. The specific hypotheses tested were:

2.1) The elimination rate coefficients of PRC PCBs are not significantly different among sites.

2.2) The relationship between k_2 and log K_{ow} (as measured by the slope of the line) for PRC and Aroclor congeners will not differ for field and laboratory observations.

Hypothesis 2.1 was supported; ANCOVA revealed no significant differences in PRC PCB elimination rate coefficients among sites.

Hypothesis 2.2 was rejected: ANCOVA revealed that the slope for PRC PCBs developed in this experiment was significantly higher (-0.474) than the laboratory-derived relationship for PCBs (-0.182; Leney *et al.*, 2006).

4.2 – Chapter 3

Chapter 3 examined the effects of temperature on the toxicokinetics of DDT in green frog and bullfrog pre-metamorphic and metamorphic tadpoles. The hypotheses tested in Chapter 3 were as follows:

3.1) There are temperature effects on elimination rate coefficients of PRC PCBs and DDT.

3.2) There are no interspecific differences in elimination rate coefficients of PRC PCBs or DDT.

Hypothesis 3.1 was rejected. There were no consistent effects of temperature on elimination rate coefficients of PRC PCBs or DDT.

Hypothesis 3.2 was tentatively rejected. Comparing all elimination rate coefficients numerically, it was observed that some of those belonging to green frogs were greater than those for bullfrogs. When the k_2 versus log K_{ow} relationships were compared, however, there were no significant differences between species. Therefore, it was concluded that some PRC PCBs were being eliminated faster in green frog tadpoles than in bullfrog tadpoles, but not all.

4.3 – Implications and Future Research

The results of these studies have several important implications for amphibian populations. First, it was observed that tadpoles display rapid toxicokinetics and reach steady state within 23 days for most organochlorines in the environment. The *in situ* toxicokinetics observed in this thesis are much faster than would be predicted based on previous laboratory studies. These rapid toxicokinetics imply that laboratory-derived uptake and elimination rates are not environmentally relevant in spite of the common view that laboratory conditions are "ideal." The observation that tadpoles have the ability to come to steady state rapidly with chemicals in their environments elucidates some of the mechanisms behind amphibian declines as related to chemical exposure; scientists have evidently been underestimating dose in feral populations of tadpoles. Tadpoles would therefore more likely experience toxic effects, especially given the increase in chemical activity they can undergo during metamorphosis (Leney *et al.*, 2006). In order to remedy this underestimation of dose, more *in situ* experiments investigating environmental exposure dynamics with frequent sampling of tadpoles as related to inputs and assessments of sediment contamination need to take place. These kinds of studies will

help researchers develop models that allow an accurate portrayal of exposure dynamics in feral tadpole populations.

For adult frogs, however, the situation is more complex. Leney *et al.* (2006) demonstrated that adult frogs have slow elimination rates in comparison to tadpoles (Leney *et al.*, 2006). Since faster elimination rates translate to faster times to steady state, the findings of Leney *et al.* indicate that under natural conditions, there will be a time lag in adult frogs between chemical exposure and a body burden in the adult frog that reflects that exposure. This will make it difficult to verify life cycle models in feral populations of adult frogs.

This thesis also demonstrated that temperatures between 18 and 25°C do not have a significant effect on the kinetics of organic chemicals in either green frog or bullfrog tadpoles. This finding suggests that tadpoles are tolerant of temperature variation in this range. It has been shown previously that temperature does affect toxicokinetics in tadpoles at other ranges (15 and 21°C; Licht, 1976). Therefore, effects of temperature on toxicokinetics in frogs need to be explored in order to develop life cycle models that accurately reflect *in situ* chemical exposure dynamics.

A final observation made in this thesis was that elimination rate coefficients of some PRC PCBs were faster in green frog tadpoles as compared to bullfrog tadpoles. The possible reasons for this are: green frog tadpoles were being stressed by the simultaneous presence of predatory chemical cues from the bullfrog tadpoles and exposure to DDT (Relyea, 2004), or; green frog tadpoles have the capability to metabolize certain PRC PCB congeners, whereas bullfrog tadpoles lack this ability. Ponds will often contain many species of amphibians at once, so the possibility of predatory presence having an impact on toxicokinetics is one that needs to be explored. As well, if green frog tadpoles were metabolizing chemicals because of the presence of bullfrog tadpoles, then these chemicals were circulating in their blood streams where they could interact with receptor sites and cause toxicity; this may explain some of the mechanisms behind amphibian declines. Alternatively, if green frogs have the capability to metabolize some chemicals while bullfrogs do not, it may be difficult to choose one amphibian species as a representative of that class for ecosystem models of chemical exposure dynamics.

The studies in this thesis underlined the paucity of research on toxicokinetics and metabolic abilities in amphibians. Future studies should further explore temperature effects on amphibian toxicokinetics in ranges greater than between 18 and 25°C; differences in toxicokinetics and metabolic activity among amphibian species; the effects of predatory stress on toxicokinetics; the effects of exposure to multiple chemicals on toxicokinetics in amphibians; *in situ* contaminants in tadpoles; and *in situ* chemical exposure dynamics throughout the amphibian life cycle.

4.4 – References

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