BIOACCUMULATION, BIOTRANSFORMATION, AND TOXICITY OF FIPRONIL: ENANTIOMER-SPECIFIC CONSIDERATIONS

by

BRAD JOSEPH KONWICK

(Under the Direction of Marsha Black)

ABSTRACT

Fipronil is an increasingly used phenylpyrazole insecticide with a high probability to contaminate aquatic ecosystems, and is released into the environment as a racemic mixture (equal amounts of optical isomers called enantiomers) due to its chirality. Enantiomers can have different toxicological and biological activity; however, information on these differences, which is necessary for accurate risk assessment of chiral pesticides, is limited. We examined the acute toxicity of fipronil to the crustacean, *Ceriodaphnia dubia,* and indicated toxicity was significantly greater for the (+) enantiomer. In assessing the bioaccumulation potential of fipronil in rainbow trout (*Oncorhynchus mykiss*), rapid elimination via biotransformation was shown to be the dominant pathway, with a greater biotransformation rate of the (+) enantiomer, as indicated by changes in the enantiomeric composition of the fish. This thesis highlights the utility of chiral compounds to provide insights into biotransformation and toxicity processes with additional research needed on fipronil's enantiomer-specific activity.

INDEX WORDS: Chiral, Pesticides, Organochlorines, Enantioselective, Rainbow Trout, Daphnia, Metabolites, Biomagnification, Half-life, photolysis, LC50

BIOACCUMULATION, BIOTRANSFORMATION, AND TOXICITY OF FIPRONIL: ENANTIOMER-SPECIFIC CONSIDERATIONS

by

BRAD JOSEPH KONWICK

B.S., University of Georgia, 2002

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2005

BIOACCUMULATION, BIOTRANSFORMATION, AND TOXICITY OF FIPRONIL: ENANTIOMER-SPECIFIC CONSIDERATIONS

by

BRAD JOSEPH KONWICK

Major Professor: Marsha C. Black

Committee: Aaron T. Fisk
Kang Xia

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2005

ACKNOWLEDGEMENTS

I would like to thank my advisor, Marsha Black, and my committee members Aaron Fisk and Kang Xia for their grateful advise, support, and encouragement during the duration of my Master's study. Special thanks to Marsha Black and Aaron Fisk for their wealth of knowledge, invaluable discussions, and friendship concerning my research and future endeavors. None of this research would have been possible without the support of Wayne Garrison and Jimmy Avants who were instrumental in providing chemical analyses. Dr. Garrison was also influential in providing me the chiral background for conducting such exciting research. I would like to acknowledge the assistance of several individuals and lab mates who provided help in my research: Tricia Smith, Emily Rogers, Deanna Conners, Ted Henry, and Mark Rigglesford. Finally, I would like to thank my parents, David and Colleen Konwick, and especially Michelle Peterson for much appreciated moral support.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Overview

Many current-use pesticides and halogenated persistent pollutants are chiral (exist as mirror images called enantiomers), and were released into the environment as racemates (i.e., equal concentrations of each enantiomer). Because enantiomers can interact with biological processes in a stereo-specific manner, there is the potential for one enantiomer to elicit toxicity and be biotransformed in biota to a greater extent than its mirror image. The following research focuses on fipronil, a currently used chiral insecticide, and its enantioselective toxicity and bioaccumulation in aquatic species. Background information concerning this literature review will first be presented on notation, identification, and properties of chiral compounds in general. This is followed by a brief review of pesticides, the identification of several chiral pesticides of importance, and the significance of identifying their enantiomer-specific effects for environmental and regulatory risk assessment. A review of bioaccumulation will follow and the importance of chiral analysis in demonstrating stereo-specific biotransformation. Emphasis and concern over the use, toxicity, and environmental fate of fipronil is then presented, followed by a brief overview of persistent organochlorine pollutants that were additionally studied in the bioaccumulation aspect of this research. Finally, a brief statement of the research goals and objectives follows the literature review.

Stereo-Chemistry Background

There has been increasing study of stereo-chemistry since the discovery by Louis Pasteur in 1848 that tartaric acid exists as two asymmetric forms, the crystals of which rotated polarized light in different directions. When he combined the crystal solutions, no rotation of light was observed, and it was later hypothesized to be a result of the spatial tetrahedral arrangement of atoms by Nobel prize winner J.H. van't Hoff. Today, stereo-chemistry has evolved and a number of underlying principles are understood. Chemicals that are stereoisomers are generally recognized as atoms attached in the same order but differ in their arrangement in space. There are two basic types of stereoisomers: enantiomers and diastereomers. Enantiomers are stereoisomers that are mirror images of one another (Figure 1.1), while diastereomers are not mirror reflections of one another. Compounds that exist as enantiomers are called chiral, coming from the greek word *cheir*, meaning "hand." Hence, the typical example of chirality is the non-super-imposability of your left and right hands. The chemical and physical properties of chiral compounds are identical (Garrison et al. 1996, Buser and Muller 1995, Buser and Muller 1993), but one difference is in their rotation of polarized light as observed by Pasteur.

Chiral compounds are indicated as (+) and (-) based on their clockwise (dextrorotary) and counter-clockwise (levorotary) rotation of optical light, respectively. A mixture of the two compounds in equal amounts is called racemic, or a racemate, and results in no rotation of light. Another designation is by absolute configuration in space where you prioritize the lowest substituent atom or group to highest resulting in *R* (*rectus* meaning right, clockwise) and *S* (*sinister* meaning left, counter-clockwise) terminology of the enantiomer. This designation, however, does not tell us about the optical rotation of the enantiomer and the more conventional (+) and (-) designation should be used where possible.

To determine whether a chemical is chiral, there are certain elements of symmetry that need to be observed. The most common element of chirality is when the chemical possesses an asymmetric chiral center, such as a carbon atom with 4 different substituent groups; frequently termed a tetrahedral carbon. Alternatively, if the chemical lacks a plane of symmetry or lacks any *n*-fold (where *n* = even number) alternating axes of symmetry, then it is chiral. Even with these general rules of thumb, they may not be applicable in all cases. Chemicals that exhibit hindered rotation around a single bond are axial chiral and called atropisomers, as is the case for some polychlorinated biphenyls (Kaiser 1974). Also, a chemical can have more than one stereo center resulting in 2^n enantiomers for each *n* chiral center.

There is an abundance of molecules (proteins, amino acids, enzymes) that occur naturally that display the chiral configuration (Landoni et al. 1997). In many cases, because of their chiral stereo-specifity, enantiomers react or interact differently with these molecules. As a result, one enantiomer may behave quite differently in biological activity than its mirror image (Kodama et al. 2002, Williams 1996, Garrison et al. 1996). A dramatic example is the drug Neurosedyn, which was sold as a racemic mixture that caused birth defects. It was later determined that only one enantiomer of thalidomide, the active ingredient, was teratogenic (Blaschke et al. 1979). Today, single enantiomer drugs are now routinely synthesized or separated from their racemic mixtures and compose a large fraction of the total drug market. On the other hand, the great majority of chiral agrochemicals, such as pesticides, are produced and marketed as their racemates.

Chiral Pesticides

Pesticides are routinely applied in agricultural, industrial, and home use to control and prevent invasive insects, diseases, and unwanted plant growth. Insecticides are applied to prevent the colonization and control of nuisance pests, such as ticks and fire ants. Herbicides and fungicides are applied to eradicate weeds and prevent fungal invasions, respectively. In the United States (U.S.), approximately 900 million pounds of pesticides were applied in each of 2000 and 2001 (Donaldson et al. 2002); and with growing population and increased urbanization, demand for pesticides will only rise. The benefits of pesticide use include the protection of humans from hazardous pests and for aesthetic pleasures, but these benefits may not outweigh the potential risks to human and ecosystem health.

Pesticides that do not reach their target site often will move off-site to contaminate surface waters and present a threat to aquatic life. Surface water run-off after rainfall events and leaching in groundwater can move pesticides in dissolved forms or bound to particles to nearby water bodies (Richards and Baker 1993), where they are readily detected throughout U. S. streams (Larson et al. 1999). On the other hand, abiotic reactions, such as photolysis and hydrolysis, and microbial degradation usually break down current-use pesticides rapidly in the environment leading to their decreased persistence (Nowell et al. 1999). However, many current-use pesticides are found in aquatic biota despite this low environmental persistence (Nowell et al. 1999). The risks associated with the exposure to aquatic fauna to these current-use pesticides and their degradation products are largely unknown.

Of the current-use pesticides, approximately 25% are chiral (Williams 1996). These include some of the most frequently used pesticides (Donaldson et al. 2002) such as phenoxypropionic acid herbicides (e.g. dichlorprop and mecoprop) and organophosphorus

insecticides (e.g., malathion, terbufos). In addition, all of the popular pyrethroid insecticides that are currently used are chiral. And, of course, there are several other environmental pollutants that are chiral besides current-use pesticides. Many of the older chlorinated (OC) pesticides (e.g., *o,p'*-DDT, *o,p'*-DDD, α-HCH, and *cis*- and *trans*-chlordane) that are now banned are chiral. Of the possible 209 polychlorinated biphenyl (PCB) congeners, 19 are chiral molecules (Kaiser 1974). Furthermore, there is an increasing likelihood of chiral centers with the increasing complexity of new pesticide structures, such as conazole fungicides and phenylpyrazole insecticides.

Due to the high number of chiral pesticides and other pollutants and their frequent release into the environment as racemates, interest in environmental fate and exposure of these chemicals has been focused on for just over a decade. This was possible through the introduction of chiral gas chromatography (GC) and high pressure liquid chromatography (HPLC) leading to the analysis and discovery of non-racemic composition in various environmental media (König et al. 1989). It is known that enantiomers of pesticides and other environmental pollutants usually differ in their biological and toxicological properties through their differential interaction with biochemical processes or other naturally occurring chiral molecules (Kodama et al. 2002, Williams 1996, Garrison et al. 1996). This may lead to changes in effects (i.e. toxicity) and fate (i.e. metabolism) of enantiomers in aquatic species. However, abiotic processes (e.g., photolysis, hydrolysis, volatilization, etc.) are not stereo-specific resulting in no change to the enantiomeric composition of chiral pollutants after their release to the environment (Garrison et al. 1996, Buser and Müller 1995, Buser and Müller 1993). Towards this end, most studies have focused on chiral OC pesticides and PCBs and the different environmental fate of enantiomers as tracers for atmospheric/water movement (Leone et al.

2001, Jantunen et al. 2000, Jantunen et al. 1998, Bidleman et al. 1995), and the fate and kinetics of these compounds in aquatic species through chiral signatures (Wong et al. 2002, Moisey et al. 2001, Karlsson et al. 2000, Huhnerfuss et al. 1995, Kallenborn et al. 1991). Increasing attention, however, has been towards the enantioselective toxic effects (Polec et al. 1998), degradation (Lewis et al. 1999) and fate (bioaccumulation and biotransformation, see chapter three) of current-use pesticides, an area that has not received much attention for accurate risk assessment of these compounds.

Enantioselective Effects

It is of importance to determine whether the enantiomers of chiral compounds have selective toxicity and biological activity to evaluate the risks they pose in aquatic organisms. Regularly, one enantiomer is target-active, or is more target-active than the other to its intended species. The other enantiomer then is inactive or less active, and simply adds an unwanted pollution burden to the environment. However, there is limited information on enantiomer-specific pesticide effects, particularly within aquatic species. In some cases, manufacturers have determined the activity (toxicity) of separated enantiomers of new pesticides (Kurihara and Miyamoto 1998), but this is usually done as a prerequisite for marketing single-enantiomer products. It should be noted that these tests are relegated to those required for U.S. Environmental Protection Agency (EPA) registration for racemic pesticides and are often limited in scope. Therefore, it is rare to find effects data in the literature, but there are enough to demonstrate that enantioselective effects should be expected to differ.

For example, recent research has shown that for the pyrethroid insecticides, *cis*-bifenthrin and *cis*-permethrin, up to 94-97% of the observed toxicity of the racemate to daphnids

(*Ceriodaphnia dubia* and *Daphnia magna*) was due to the (+) enantiomer (Liu et al. 2005). For another pyrethroid insecticide, fenvalerate, a >400 fold difference in toxicity of its stereoisomers was shown in fathead minnows (*Pimephales promelas*) (Bradbury et al. 1987). In other studies, the *R* (+) enantiomer of malathion, an organophosphorus (OP) pesticide, is selective in acute toxicity to a variety of arthropods and rats (Polec et al. 1998). Similarly, the cholinesterase inhibition activity of several chiral OP pesticides is enantioselective (Rodriguez et al. 1997). The (-) enantiomer of *o,p*'-DDT has greater estrogenic activity than the (+) enantiomer (McBlain et al. 1976), which is significant considering that the (+) enantiomer was shown to be depleted in human fat (Muller and Buser 1995). Furthermore, all of the fungicidal action of metalaxyl resides with the *R* enantiomer, and the two *S* enantiomers of metolachlor, a herbicide with two chiral centers, are about 10 times more toxic to target weeds than its two *R* enantiomers (Spindler and Fruh 1998). While current-use pesticides usually have high specificity for their target species, there is a high probability for them to come into contact with non-target species, especially aquatic biota through run-off. It is then conceivable that effects for enantiomers will differ in non-target species, both environmental and human. Moreover, whether these effects are consistent among target and non-target species are important but rarely considered implications for risk assessment of these pesticides.

Despite the limited knowledge concerning enantiomer-specific effects, further research defining enantiomer activity in the environment needs to be developed for safer pesticides. The U.S. EPA has released comment to recognize the issue of chirality in pesticide registration (U.S. EPA 1999a), but is currently unable to consider enantiomers due to the lack of enantiomer-specific toxicity and fate information (US EPA 1999b). The availability of such information can then help in the decision making process on the isomeric form of chiral compounds released into

the environment. This will lower potential impacts on species and reduce the pollution load to the environment. For example, metolachlor is now manufactured to contain 86% of the active *S* enantiomers, and still has the same activity despite allowing a 40% reduction in the amount of herbicide applied (Spindler and Fruh 1998). With single enantiomer or enriched enantiomer pesticides growing in use, especially in Europe, argues for more development and research on the effects of pesticide enantiomers (Williams 1996). For accurate risk assessment of these chemicals, though, fate and exposure data in biota are needed in combination with effects information.

Bioaccumulation and Enantioselective Biotransformation

To assess the potential risk of contaminants, it is important to understand their fate and dynamics in aquatic species, in particular their bioaccumulation. Bioaccumulation is the net process by which a chemical increases in an aquatic organism exceeding that in its environment as a result of chemical uptake through all possible routes of exposure (e.g., water, food) (Borgå et al. 2004). The bioaccumulation factor (BAF) that is used to assess this increase in chemical concentration can be calculated by

$$BAF = [Chemical_{field\ exposed\ organism}] / [Chemical_{water}]_{dissolved}$$

Aquatic organisms may also accumulate chemicals directly from water. Referred to as bioconcentration, this is a process by which the chemical concentration exceeds that in water as a result of exposure to waterborne chemicals only. The bioconcentration factor (BCF) can be calculated using the equation

$$BCF = [Chemical_{organism}] / [Chemical_{water}]_{dissolved}$$

When the chemical concentration in an organism achieves a level that exceeds that in the organism's diet due to dietary accumulation, biomagnification occurs. The biomagnification factor (BMF) can then be calculated by

$$BMF = [Chemical_{organism}] / [Chemical_{food}]$$

Biomagnification occurs frequently for lipophilic contaminants (Connolly and Pedersen 1988) and in higher trophic organisms (Oliver and Niimi 1988) because diet is the only relevant exposure pathway for these chemicals as you increase in trophic level (Thomann and Connolly 1984). Concern then arises over contaminants that are observed to pass from organism to organism in a food web, progressively increasing or biomagnifying in concentration with trophic level. The extent of bioaccumulation plays a key role in determining toxicity or response in an organism due to the quantity which reaches the target organ or tissue. Hence, it is often used as the exposure facet in the dose-response relationship. Also, human consumption of a fish contaminated with toxic substances can result in high dosages of harmful chemicals. As a result, it is necessary to quantify bioaccumulation and biomagnification of contaminants in aquatic food webs.

A general approach for describing and quantifying bioaccumulation is to use mechanistic or empirical models. Empirical models routinely measure the concentration of the contaminant in the organism to that in the field (e.g., water or sediment) (Mackay and Fraser 2000). The empirical data are then used in establishing bioaccumulation correlations with the chemical's physical-chemical properties. A representative surrogate for describing the uptake of the chemical within organisms that is often used is the octanol-water partition coefficient (log K_{ow}), often referred to as the lipophilicity potential of the chemical. All chemicals with a positive log K_{ow} value will bioaccumulate to a certain extent by definition because a greater concentration

will partition in the biota compared to water. Generally, though, chemicals with a log K_{ow} values below 4 are not considered to biomagnify up the food chain, although some trophic transfer may occur. Conversely, many chemicals above a log K_{ow} of 4 will show some biomagnification, especially for persistent organic pollutants (Fisk et al. 1998). This is because with decreasing water concentration (Oliver and Niimi 1985) and increasing trophic position (Connoly and Pederson 1988), there is greater accumulation from food.

Mechanistic models, on the other hand, are based on quantifying the uptake and elimination process rates to the observed data. In general, there are three uptake and six possible elimination mechanisms for contaminants in organisms (Figure 1.2). Mechanistic models try to predict bioaccumulation by quantifying the chemical concentration and the uptake or loss rates (first-order rate constants (k) with units of time^{-1}) for each mechanism. For example, the bioaccumulation within an organism can then be expressed as followed:

$$dC/dt = k_{in}C_{food/water} - k_{out}C_{fish}$$

In addition, assumptions to include each process and their parameters can be made to suit your specific organism and chemical of study. However, one obvious trend that has been noticed is the need to include the process of biotransformation as a factor influencing bioaccumulation (Mackay and Fraser 2000), which unfortunately is difficult to quantify.

Biotransformation is a key component of a fish's elimination pathway for contaminants, by making them more water soluble or more easily to excrete through the addition of key functional groups. Elimination (e.g., following biotransformation) can ultimately influence the potential for a chemical to bioaccumulate (Borgå et al. 2004) and cause adverse effects in organisms, especially for current-use pesticides. Aquatic organisms such as fish and invertebrates are recognized to have a limited ability to biotransform contaminants compared to

birds and mammals due to their lower levels of cytochrome (CYP) P-450 1A and 2B isozymes (Norstrom et al. 1998, Stegeman and Kloeppper-Sams 1987, Kleinow et al. 1987). Additionally, field studies have indirectly implied biotransformation ability through a decrease in predator body residues compared to their prey (Norstrom et al. 1992), lower retention of contaminants expected to be metabolized based on structure-activity relationships (Boon et al. 1989), and lower contaminant concentrations compared to recalcitrant compounds such as polychlorinated biphenyl (PCB) 153 (Niimi 1996). However, these methods are qualitative and do not unequivocally provide evidence that *in vivo* biotransformation of contaminants in aquatic biota occurs, in particular providing quantitative rates of biotransformation. As a result, chiral analysis has been proposed for tracing enzymatic metabolism in aquatic species.

Chiral analysis is a new area of research that can be powerful in tracing chiral compounds in the environment because of their unique stereo-configuration. This is because enantiomers can be subjected to metabolic processes (e.g., enzyme mediated receptors, biochemical reactions) that act enantioselectively (Landoni et al. 1997, Tucker and Lennard 1990) and are unaffected by abiotic processes. A resulting change in the enantiomeric composition of aquatic species would imply biotransformation is occurring, a tracer that would not have otherwise been observed with conventional achiral analysis. Changes in the relative proportions of enantiomers in biota can be measured in enantiomeric ratios (ERs) or enantiomeric fractions (EFs) calculated from enantiomer peak areas by

$$EF = ER/(1 + ER) = 1 / (1 + 1 / ER)$$

Racemates (ER = 1) therefore would have an EF of 0.5, and pure single enantiomers (ER = 0 or ER = ∞) would have an EF of 0 or 1, respectively. Because the EF is a true fraction and can also

be applied more naturally in mathematical fate expressions, its notation is preferred (Harner et al. 2000).

Previous studies have detected non-racemic levels of chiral contaminants in aquatic invertebrate and vertebrate species (see e.g., Herzke et al. 2002, Moisey et al. 2001, Karlsson et al. 2000), which suggests that enzymatic transformation has occurred. Hoekstra et al. (2002) found non-racemic amounts of chiral PCBs in bowhead whales (*Balaena mysticetus)*, while their phytoplankton prey had racemic levels, and was the first confirmation that cetaceans could biotransform PCBs. Some comparisons are also made between chiral signatures measured in biota and those measured in sediment to determine differences in biotransformation among species. For example, enantiomeric fractions (EFs) of toxaphene in fish (*Fundulus* sp.) were non-racemic compared to racemic mixtures measured in sediment of a toxaphene contaminated marsh (Vetter and Maruya 2000). Likewise, bivalves and crayfish contained different EFs of PCBs compared to their contaminated sediment (Wong et al. 2001). As well, trends of enantiomeric signatures have been detected for different species suggesting that enzymes responsible for biotransforming chiral compounds differ in their stereo-selectivity. Chlordane isomers have enantioselectively accumulated among different species in seabirds (Fisk et al. 2001b) and ringed seals (Fisk et al. 2002). Together, these results underscore the ability of quantifying enantiomeric compositions within biota as a tool for investigating biotransformation.

Recent laboratory investigations have focused on quantifying the extent, or rate, of biotransformation through chiral analysis in controlled studies. Rainbow trout (*Oncorhynchus mykiss*) were able to enantioselectively eliminate α-HCH and PCB 95; however, selectivity was not observed for trans-chlordane or PCB 136 in a controlled bioaccumulation study (Wong et al. 2002). Calculated minimum biotransformation rates indicated that more than half of the

elimination was due to metabolism, which indicates aquatic organisms such as fish may be biotransforming these compounds to a greater extent than previously believed (Wong et al. 2002). As well, Vetter et al. (2001) removed *Fundulus* sp. from a toxaphene contaminated estuary and placed them under toxaphene free conditions. After 60 days, calculated EF residues in fish were 1.00 (i.e., complete elimination of one enantiomer) with one enantiomer being eliminated twice as fast as its mirror image. These results suggest that the assumption that fish have minimal biotransformation capacity is incorrect and controlled bioaccumulation studies are warranted, in particular with current-use pesticides where there is a dearth of data.

Fipronil: Use, Fate, and Toxicity

My research focuses on the current-use pesticide fipronil (Figure 1.3). Fipronil is phenylpyrazole-class insecticide first approved for use in the U.S. in 1996. A number of its commercial formulations are widely used, which most notably includes targeting rice water weevils (*Lissorhoptrus oryzophilus*), which feed on the root system of rice shoots (U.S. EPA 1997). Additional uses of fipronil include residential pest control of ticks and fleas for pets, and in turf grass management (e.g. fire ant control) (U.S. EPA 1996). Fipronil has been recognized as a disrupter of γ-aminobutyric acid (GABA) gated chloride channels in nerve cells leading to hyper-excitation and eventual mortality (Cole et al. 1993). Its toxicity is much higher (>500 fold) in invertebrates relative to mammals due to differences in binding between insect and mammalian GABA receptors (Gant et al. 1998, Hainzl et al. 1998). This has been attributed to its unique trifluoromethylsulfinyl group that is not present in other similar pesticides (Hainzl and Casida 1996). As a result, use of this insecticide is increasing worldwide due in part to restrictions in use and species resistance to organophosphorus and other pesticides (Gant et al.

1998, Hosie et al. 1995). Fipronil's chiral configuration is a result of the asymmetric sulfur atom resulting in two enantiomers that have been identified as *S* (+) and *R* (-). (Figure 1.3)

The environmental fate of fipronil is somewhat unique in that it undergoes extensive photolysis to a desulfinyl derivative in aquatic environments (Figure 1.4). This photo-extrusion of the sulfinyl group readily occurs with a half-life of < 0.5 days under ultraviolet fluorescent lamps (> 300 nm) (Ngim and Crosby 2001) and with a half-life of 3.6 days in saltwater mesocosms exposed to natural sunlight (Walse et al. 2004a, Walse et al. 2004b). As a result, the photo-degradation product may prove to be of greater concern because it is stable to further environmental degradation (Walse et al. 2004a, U.S. EPA 1997). In addition, fipronil can undergo biological oxidation or reduction to its respective sulfone and sulfide metabolites. The sulfide degradate is mainly formed in soils (Ngim and Crosby 2000) and sediment (Schlenk et al. 2001) and is likely a result of microbial transformation. Studies exposing fipronil to rats and mice indicate *in vivo* formation of fipronil sulfone as the main metabolite (Hainzl et al. 1998). Hydrolysis to the amide form of fipronil only represents a minor degradation pathway for fipronil in solution (Walse et al. 2004b). Thus, the long term effectiveness of fipronil is probably owed to the environmental persistence of fipronil's metabolites (of which only the hydrolysis amide product is chiral) due to their similar physical-chemical properties (e.g., water solubility, volatility) (Walse et al. 2004b, Schlenk et al. 2001).

Because of its present and frequent use, fipronil has been detected in surface waters at concentrations presenting a potential threat to aquatic organisms. Fipronil has been measured in 25% of the water samples collected by the National Ambient Water-Quality Assessment (NAWQA) at concentrations ranging from 0.01 and 0.07 µg/L (Sandstrom and Madsen 2003). In this study, the only one to date to look for metabolites, maximum concentrations of 0.04 µg/L

were detected for desulfinyl fipronil, whereas the other metabolites were found below 0.02 µg/L. In rice culture, an estimated peak water concentration of approximately 5.0 µg/L for fipronil and 1.4 µg/L for desulfinyl fipronil has been calculated (U.S. EPA 1997). A 2001 US EPA document has projected an average peak fipronil concentration of 1.7 µg/L in Southeastern U.S. waters when used for fire ant control (U.S. EPA 2001). The highest concentration of fipronil (5.29 µg/L) was measured adjacent to treated rice fields in Louisiana and it was detected 20 miles downstream at concentrations of 0.79 µg/L (Demcheck and Skrobialowski 2003). The lower presence of desulfinyl fipronil compared to fipronil may be due to partioning of these compounds to the sediment phase. As a result, both compounds will be reduced in the water column and because fipronil's photodecomposition is shown to be through direct photolysis (Walse et al. 2004a), formation of desulfinyl fipronil may be limited to an important degree.

Fipronil concentrations found in the environment are in the range to have caused toxicity and adverse effects in aquatic organisms. Invertebrates have been found to be the most sensitive to fipronil with median lethal concentrations (LC50s) of 0.14, 0.22 , and 0.32 µg/L for mysids (*Americamysis bahia*), black fly larvae (*Simulium vittatum),* and adult grass shrimp (*Palaemonetes pugio*), respectively (U.S. EPA 1996, Overmyer et al. 2005, Key et al. 2003). On the other hand, fish generally have LC50 values above 80 µg/L (U.S. EPA 1996). While there has not been much research on the acute effects of its metabolites, similar LC50 values compared to fipronil have been found in crayfish and bluegill for desulfinyl fipronil (Schlenk et al. 2001, U.S. EPA 1996). Furthermore, some of fipronil's other metabolites (sulfone and sulfide) have been shown to be more toxic than the parent compound in invertebrates (U.S. EPA 1997). For this reason, it is generally considered that fipronil's metabolites are equal or more toxic compared to fipronil. However, more research concerning this notion is needed as some

metabolites (desulfinyl fipronil) have been shown to be less toxic (Schlenk et al. 2001, U.S. EPA 1996, see chapter two).

Of particular concern are sub-lethal effects of fipronil that likely occur at environmental concentrations, but there have been limited investigations. For example, fipronil decreased reproduction in copepods (*Amphiascus tenuiremis)* at a concentration of 0.42 µg/L (Chandler et al. 2004) and was associated with male-specific infertility (Cary et al. 2004). Also, fipronil inhibited growth of larval rainbow trout at 6.6 µg/L and weight of daphnia at 0.63 µg/L (U.S. EPA 1997, U.S. EPA 1996). Since fipronil is chiral, identifying the enantiomer-specific effects and toxicity may alleviate the potential risks concerning the use of this pesticide, especially toward sensitive arthropod species. However, few studies have examined this as a potential means for protecting aquatic health.

Fipronil and its metabolites are likely to accumulate in biota due to their physical chemical properties. For fipronil (log K_{ow} = 4.01) (U.S. EPA 1996), previous work has shown that there is potential for accumulation in fish and aquatic invertebrates in aqueous exposures (Chaton et al. 2002, Chaton et al. 2001, U.S. EPA 1997). However, the kinetics (i.e., uptake, depuration, and biotransformation rates) of fipronil have not been adequately addressed for many of these studies. Concern over possible food web biomagnification that would occur through a dietary exposure have also not yet been explored for fipronil. Due to the similar physiochemical properties of fipronil's metabolites (log K_{ow} values 3.7 - 4.7 (Walse et al. 2004b), there is also concern over potential accumulation of these compounds in biota. Because fipronil has been shown to undergo biotransformation in other species (Roberts and Hutson 1999), metabolites formed biologically may result in potential greater exposure risks, especially those that are possibly more persistent and toxic. Consequently, predicting exposure to fipronil and other

current-use pesticides through bioaccumulation studies is important for assessing risks to aquatic organism health and in establishing safer criteria for their use.

Persistent Organic Pollutants

Since the bulk of chiral bioaccumulation research has been done on persistent organochlorine pollutants (POPs), of which some were included in the bioaccumulation aspect of my research (Chapter three), a brief background concerning their use and exposure concerns is merited. Technical pesticides (e.g., DDTs, chlordanes) and polychlorinated biphenyls (PCBs) were extensively used before the 1970s due to their unique chemical properties. These include low water solubility, low volatility, and low biological transformation in the environment. Unfortunately, as a result of these properties, many of these compounds still persist in the environment 25 years later despite bans on their production and use, thus they are often referred to as legacy contaminants. Although concentrations have been shown to be decreasing slowly with time (Jeremiason et al. 1994), it is estimated that close to 70% of the global production for some compounds (Hileman 1993) can still be found in the environment, posing a threat to fish and wildlife in many areas (Schmitt et al. 1999). These contaminants readily bioaccumulate (Buckman et al. 2004, Wiberg et al. 2000, Fisk et al. 1998) in aquatic organisms due to their high octanol-water partition coefficient values (i.e., lipophilic), and many have been shown to biomagnify (Hoekstra et al. 2003a, Hoekstra 2003b, Zaranko et al. 1997, Muir et al. 1988) in aquatic food webs. Thus, chronic exposure to these persistent pollutants in aquatic species continues to remain a concern. In addition, due to the extensive knowledge concerning the bioaccumulation behavior of organochlorine contaminants (see Borgå et al. 2004, Fisk et al.

2001a), they serve as useful comparisons for less studied chemicals, such as current-use pesticides.

Objectives of Research

The aim of the research in this thesis was to generate the first information about the enantiomer-specific effects and fate of the current-use pesticide fipronil. To this end, chapter two will focus on the enantioselective toxicity of fipronil to *Ceriodaphnia dubia*, a commonly used daphnid species in aquatic bioassays. This research will put into context the possible regulatory application of using fipronil in either a single or enriched enantiomer form, as well as examining the photolysis of fipronil and its effect on toxicity. Chapter three investigates the bioaccumulation of fipronil for assessing the exposure kinetics of this pesticide in fish. The influence of biotransformation (enantiomer-specific) on bioaccumulation will be evaluated in addition to looking how several organochlorine pesticides and PCBs are possibly affected by this stereo-specific process. Chapter four will put into context the ecotoxicological implications of my findings with future areas of research concerning fipronil and current-use pesticides in general.

References

Bidleman TF, Jantunen LMM, Falconer RL, Barrie LA, Fellin P. 1995. Decline of hexachlorocyclohexane in the arctic atmosphere and reversal of air-sea gas exchange. *Geophys Res Lett* 22: 219-222.

Blaschke G, Kraft HP, Fickentscher K, Kohler F. 1979. Chromatographic separation of racemic thalidomide and teratogenic activity of its enantiomers. *Arzneimittel-forschung* 29: 1640 -1642.

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

Borgå K, Fisk AT, Hoekstra PF, Muir DCG. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* 23: 2367-2385.

Bradbury SP, Symonik DM, Coats JR, Atchison GJ. 1987. Toxicity of fenvalerate and its constituent isomers to the fathead minnow, *Pimephales promelas*, and bluegill, *Lepomis macrochirus*. *Bull Environ Contam Toxicol* 38: 727-735.

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

Buser H-R, Müller MD. 1995. Isomer-selective and enantiomerselective determination of DDT and related compounds using chiral high-resolution gas chromatography/mass spectrometry and chiral high-performance liquid chromatography. *Anal Chem* 67: 2691-2698.

Buser H-R, Müller MD. 1993. Enantioselective determination of chlordane components, metabolites, and photoconversion products in environmental samples using chiral high-resolution gas chromatography and mass spectrometry. *Environ Sci Technol* 27: 1211-1220.

Cary TL, Chandler GT, Volz DC, Walse SS, Ferry JL. 2004. Phenylpyrazole insecticide fipronil induces male infertility in the estuarine meiobenthic crustacean *Amphiascus tenuiremis*. *Environ Sci Technol* 38: 522-528.

Chandler GT, Cary TL, Volz DC, Walse SS, Ferry JL, Klosterhaus SL. 2004. Fipronil effects on estuarine copepod (*Amphiascus tenuiremis*) development, fertility, and reproduction: a rapid life-cycle assay in 96-well microplate format. *Environ Toxicol Chem* 23: 117-124.

Chaton PF, Ravanel P, Tissut M, Meyran JC. 2002. Toxicity and bioaccumulation of fipronil in the nontarget arthropodan fauna associated with subalpine mosquito breeding sites. *Ecotoxicol Environ Safety* 52: 8-12.

Chaton PF, Ravanel P, Meyran JC, Tissut M. 2001. The toxicological effects and bioaccumulation of fipronil in larvae of the mosquito *Aedes aegypti* in aqueous medium. *Pestic Biochem Physiol* 69: 183-188.

Cole LM, Nicholson RA, Casida JE. 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic Biochem Physiol* 46: 47-54.

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

Demcheck DK, Skrobialowski SC. 2003. *Fipronil and degradation products in the rice-producing areas of the Mermentau River Basin, Louisiana, February-September 2000*. U.S. Geological Survey Fact Sheet, FS-010-03.

Donaldson D, Kiely T, Grube A. 2002. *Pesticide Industry Sales and Usage: 1998 and 1999 Market Estimates.* United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Report EPA-733-R-02-001, Washington DC, 35 p.

Fisk AT, Holst M, Hobson KA, Duffe J, Moisey J, Norstrom RJ. 2002. Persistent organochlorine contaminants and enantiomeric signatures of chiral pollutants in ringed seals (*Phoca hispida*) collected on the east and west side of the Northwater Polynya, Canadian Arctic. *Arch Environ Contam Toxicol* 42: 118-126.

Fisk AT, Hobson KA, Norstrom RJ. 2001a. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya food web. *Environ Sci Technol* 35: 732-738.

Fisk AT, Moisey J, Hobson KA, Karnovsky NJ, Norstrom RJ. 2001b. Chlordane components and metabolites in seven species of Arctic seabirds from the Northwater Polynya: relationships with stable isotopes of nitrogen and enantiomeric fractions of chiral components. *Environ Pollut* 113: 225-238.

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

Gant DB, Chalmers AE, Wolff MA, Hoffman HB, Bushey DF. 1998. Fipronil: Action at the GABA receptor. *Rev Toxicol* 2: 147-156.

Garrison AW, Schmitt P, Martens D, Kettrup A. 1996. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ Sci Technol* 30: 2449-2455.

Hainzl D, Cole LM, Casida JE. 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem Res Toxicol* 11: 1529-1535.

Hainzl D, Casida JE. 1996. Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity. *Proc Natl Acad Sci* 93: 12764-12767.

Harner T, Wiberg K, Norstrom RJ. 2000. Enantiomeric fractions are preferred to enantiomer ratios for describing chiral signatures in environmental analysis. *Environ Sci Technol* 34: 218-220.

Herzke D, Kallenborn R, Nygard T. 2002. Organochlorines in egg samples from Norwegian birds of prey: congener-, isomer-, and enantiomer specific considerations. *Sci Total Environ* 291: 59-71.

Hileman, B. 1993. Concerns broaden over chlorine and non-chlorinated hydrocarbons. *Chem. Eng. News*, April 19, p 11-20.

Hoekstra PF, O'Hara TM, Fisk AT, Borga K, Solomon KR, Muir DCG. 2003a. Trophic transfer of persistent orgnochlorines contaminants (Ocs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. *Environ Pollut* 124: 509-522.

Hoekstra PF, O'Hara TM, Karlsson H, Solomon KR, Muir DCG. 2003b. Enantiomer-specific biomagnification of α-hexachlorocyclohexane and selected chiral chlordane-related compounds within an Arctic marine food web. *Environ Toxicol Chem* 22: 2482-2491.

Hoekstra PF, Wong CS, O'Hara TM, Solomon KR, Mabury SA, Muir DCG. 2002. Enantiomer-specific accumulation of PCB atropisomers in the bowhead whale (*Balaena mysticetus*). *Environ Sci Technol* 36: 1419-1425.

Hosie AM, Baylis HA, Buckingham SD, Sattelle DB. 1995. Actions of the insecticide fipronil on dieldrin sensitive and resistant GABA receptors of *Drosophila melanogaster*. *Br J Pharmacol* 115: 909-912.

Hühnerfuss H, Pfaffenberger B, Gehrcke B, Karbe L, König WA, Landgraff O. 1995. Stereochemical effects of PCBs in the marine environment: seasonal variation of coplanar and atropisomeric PCBs in blue mussels (*Mytilus edulis*) of the German Bight. *Mar Pollut Bull* 30: 332-340.

Jantunen LMM, Bidleman TF, Harner T, Parkhurst WJ. 2000. Toxaphene and other organochlorine pesticides in Alabama air. *Environ Sci Technol* 34: 5097-5105.

Jantunen LMM, Bidleman TF. 1998. Organochlorine pesticides and enantiomers of chiral pesticides in Arctic Ocean water. *Arch Environ Contam Toxicol* 35: 218-228.

Jeremiason JD, Hornbuckle KC, Eisenreich SJ. 1994. PCBs in Lake Superior, 1978-1992: decreases in water concentrations reflect loss by volatilization. *Environ Sci Technol* 28: 903-914.

Kaiser KLE. 1974. Optical activity of polychlorinated biphenyls. *Environ Pollut* 7: 93-101.

Kallenborn R, Hühnerfuss H, König WA. 1991. Enantioselective metabolism of (±)-α-hexachlorocyclohexane in organs of the eider duck. *Angew Chem Int Ed Engl* 30: 320-321.

Karlsson H, Oehme M, Skopp S, Burkow IC. 2000. Enantiomer ratios of chlordane congeners are gender specific in cod (*Gadus morhua*) from the Barents Sea. *Environ Sci Technol* 34: 2126-2130.

Key PB, Chung KW, Opatkiewicz AD, Wirth EF, Fulton MH. 2003. Toxicity of the insecticides fipronil and endosulfan to selected life stages of the grass shrimp (*Palaemonetes pugio*). *Bull Environ Contam Toxicol* 70: 533-540.

Kleinow KM, Melancon MJ, Lech JJ. 1987. Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. *Environ Health Perspect* 71: 105-119.

Kodama S, Yamamoto A, Saitoh Y, Matsunaga A, Okamura K, Kizu R, Hayakawa K. 2002. Enantioseparation of vinclozolin by γ-cyclodextrin-modified micellar electrokinetic chromatography. *J Agric Food Chem* 50: 1312-1317.

König WA, Krebber R, Minschnick P. 1989. Cyclodextrines as chiral stationary phases in capillary gas chromatography. *J High Resol Chromatogr* 12: 732-738.

Kurihara N, Miyamoto J, eds. 1998. *Chirality in Agrochemicals*. John Wiley & Sons, Chichester.

Landoni MF, Soraci AL, Delatour P, Lees P. 1997. Enantioselective behaviour of drugs used in domestic animals: a review. *J Vet Pharmacol Ther* 20: 1-16.

Larson SJ, Gilliom, Capel PD. 1999. *Pesticides in Streams of the United States – Initial Results from the National Water-Quality Assessment Program.* United States Geological Survey, Water-Resources Investigations Report 98-4222, Sacramento CA, 99 p.

Leone AD, Amato S, Falconer RL. 2001. Emissions of chiral organochlorine pesticides from agricultural soils in the cornbelt region of the U.S. *Environ Sci Technol* 35: 4592-4596.

Lewis DL, Garrison AW, Wommack KE, Whittemore A, Steudler P, Melillo J. 1999. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* 401: 898-901.

Liu W, Gan J, Schlenk D, Jury WA. 2005. Eantioselectivity in environmental safety of current insecticides. *Proc Natl Acad Sci* xx: x-y.

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

McBlain WA, Lewin V, Wolfe FH. 1976. Differing estrogenic activities for the enantiomers of o,p-DDT in immature female rats. *Canadian J Physiol and Pharmacol* 54: 629-632.

Moisey J, Fisk AT, Hobson KA, Norstrom RJ. 2001. Hexachlorocyclohexane (HCH) isomers and chiral signatures of α-HCH in the Arctic marine food web of the Northwater Polynya. *Environ Sci Technol* 35: 1920-1927.

Muir DCG, Norstrom RJ, Simon M. 1988. Organochlorine contaminants in Arctic marine food chains: accumulation of specific polychlorinated biphenyls and chlordane compounds. *Environ Sci Technol* 22: 1071-1079.

Ngim KK, Crosby DG. 2001. Abiotic processes influencing fipronil and desthiofipronil dissipation in California, USA, rice fields. *Environ Toxicol Chem* 20: 972-977.

Ngim KK, Mabury SA, Crosby DG. 2000. Elucidation of fipronil photodegradation pathways. *J Agric Food Chem* 48: 4661-4665.

Niimi AJ. 1996. Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. *Sci Total Environ* 192: 123-150.

Norstrom RJ, Belikov S, Born, Garner GW, Malone B, Olpinski S, Ramsey MA, Schliebe S, Stirling I, Stishov MS, Taylor MK, Wiig O. 1998. Chlorinated hydrocarbon contaminants in polar bears from eastern Russia, North America, Greenland, and Svalbard: biomonitoring of arctic pollution. *Arch Environ Contam Toxicol* 35: 354-367.

Norstrom RJ, Muir DCG, Ford CA, Simon M, Macdonald CR, Beland P. 1992. Indications of P450 monooxygenase activities in beluga whale (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) from patterns of PCB, PCDD, and PCDF accumulation. *Mar Environ Res* 34: 267-272.

Nowell LH, Capel PD, Dileanis PD. 1999. Chapter 5 analysis of key topics – sources, behavior, and transport. In: *Pesticides in Stream Sediment and Aquatic Biota: Distribution, Trends, and Governing Factors*, CRC Press, Boca Raton FL, p 229-347.

Oliver BG, Niimi AJ. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ Sci Technol* 22: 388-397.

Oliver BG, Niimi AJ. 1985. Bioconcentration factors of some halogenated organics for rainbow trout: limitation in their use for prediction of environmental residues. *Environ Sci Technol* 19: 842-848.

Overmyer JP, Mason BN, Armbrust KL. 2005. Acute toxicity of imidacloprid and fipronil to a non-target aquatic insect, *Simulium vittatum* zetterstedt cytospecies IS-7. *Bull Environ Contam Toxicol* xx: x-y.

Polec I, Cieslak L, Sledzinski B, Ksycinska H. 1998. Simple synthesis of malathion and malaoxon enantiomers and isomalathion diastereoisomers: toxicity-configuration relationship. *Pestic Sci* 53: 165-171.

Richards RP, Baker DB. 1993. Pesticide concentration patterns in agricultural drainage networks in theLake Erie basin. *Environ Toxicol Chem* 12: 13-26.

Roberts TR, Hutson DH. 1999. Metabolic Pathways of Agrochemicals Part 2: Insecticides and Fungicides. The Royal Society of Chemistry, Cambridge, UK.

Rodriguez OP, Muth GW, Berkman CE, Kim K, Thompson CM. 1997. Inhibition of various cholinesterases with the enantiomers of malaoxon. *Bull Environ Contam Toxicol* 58: 171-176.

Sandstrom MW, Madsen JE. 2003. *Abstracts*, 24th SETAC Annual Meeting. Austin, Texas, November 8-13.

Schlenk D, Huggett DB, Allgood J, Bennett E, Rimoldi J, Beeler AB, Block D, Holder AW, Hovinga R, Bedient P. 2001. Toxicity of fipronil and its degradation products to *Procambarus* sp.: Field and laboratory studies. *Arch Environ Contam Toxicol* 41: 325-332.

Schmitt CJ, Zajicek JL, May TW, Cowman DF. 1999. Organochlorine residues and elemental contaminants in US freshwater fish, 1976-1986: National Contaminant Biomonitoring Program. *Rev Environ Contam Toxicol* 162: 43-104.

Spindler F, Früh T. 1998. Chiral acylanilides and chiral triazole-related fungicides, in Kurihara N and Miyamoto J (eds.) *Chirality in Agrochemicals*, John Wiley & Sons, Chichester, p155-157.

Stegeman JJ, Kloepper-Sams PJ. 1987. Cytochrome P-450 isozymes and monooxygenase activity in aquatic animals. *Environ Health Perspect* 71: 87-95.

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

Tucker GT, Lennard MS. 1990. Enantiomer specific pharmacokinetics. *Pharmacol Therap* 45: 309-329.

U.S. Environmental Protection Agency. 2001. *Fipronil environmental fate and ecological effects assessment and characterization for a Section 3 for broadcast treatment with granular product to control turf insects and fire ants* (addendum). Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA.

U.S. Environmental Protection Agency. 1999a. Pesticides; request for comment on pesticide registration proposal for isomeric active ingredients. *Fed Reg* 64: 22863-22865, April 28, 1999.

U.S. Environmental Protection Agency. 1999b. Response to public comments on the preliminary risk assessment for the organophosphate naled. Docket #34136, US EPA, Washington DC, USA.

U.S. Environmental Protection Agency. 1997. *Environmental fate and effects Section 3 registration decision for fipronil: Use on rice seed*, D235912. Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA.

U.S. Environmental Protection Agency. 1996. *New pesticide fact sheet*. EPA 737-F-96-005. Office of Pesticide Programs, Washington, DC, USA.

Vetter W, Smalling KL, Maruya KA. 2001. Interpreting nonracemic residues of chiral organochlorines using naturally contaminated fish. *Environ Sci Technol* 35: 4444-4448.

Vetter W, Maruya KA. 2000. Congener and enantioselective analysis of toxaphene in sediment and food web of a contaminated estuarine wetland. *Environ Sci Technol* 34: 1627-1635.

Walse SS, Morgan SL, Kong L, Ferry JL. 2004a. Role of dissolved organic matter, nitrate, and bicarbonate in the photolysis of aqueous fipronil. *Environ Sci Technol* 38: 3908-3915.

Walse SS, Pennington PL, Scott GI, Ferry JL. 2004b. The fate of fipronil in modular estuarine mesocosms. *J Environ Monit* 6: 58-64.

Wiberg K, Letcher RJ, Sandau C, Norstrom RJ, Tysklind M, Bidleman TF. 2000. The enantioselective bioaccumulation of chiral chlordane and α-HCH contaminants in the polar bear food chain. *Environ Sci Technol* 34: 2668-2674.

Williams A. 1996. Opportunities for chiral agrochemicals. *Pestic Sci* 46: 3-9.

Wong CS, Lau F, Clark M, Mabury SA, Muir DCG. 2002. Rainbow trout (*Oncorhynchus mykiss*) can eliminate chiral organochlorine compounds enantioselectively. *Environ Sci Technol* 36: 1257-1262.

Wong CS, Garrison AW, Smith PD, Foreman WT. 2001. Enantiomeric composition of chiral polychlorinated biphenyl atropisomers in aquatic and riparian biota. *Environ Sci Technol* 35: 2448-2454.

Zaranko DT, Griffiths RW, Kaushik NK. 1997. Biomagnification of polychlorinated biphenyls through a riverine food web. *Environ Toxicol Chem* 16: 1463-1471.

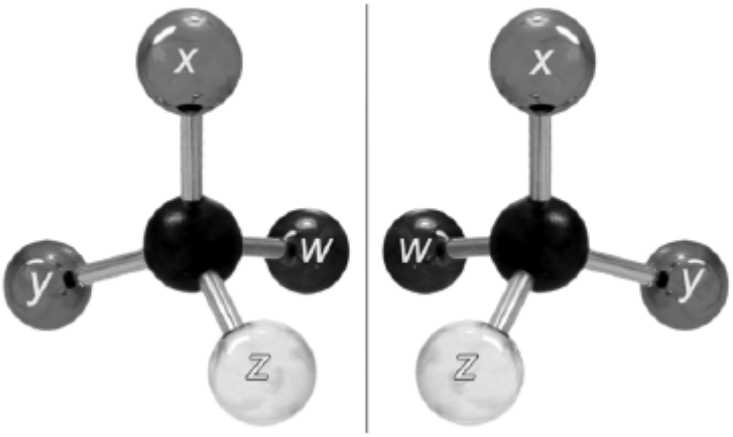

Figure 1.1. Enantiomers are non-super-imposable mirror images of each other.

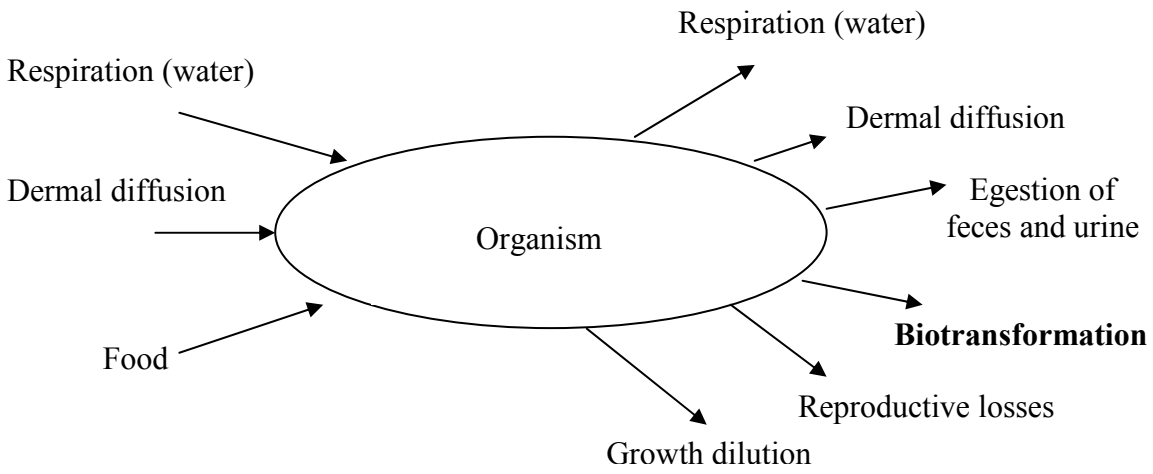

Figure 1.2. Uptake and elimination mechanisms of contaminants applicable to a general aquatic organism.

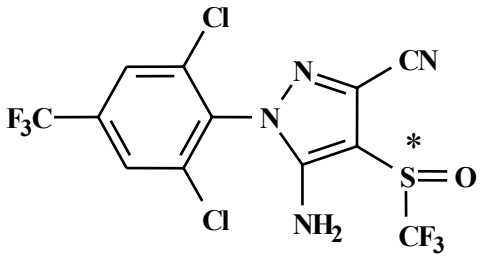

Figure 1.3. Molecular Structure of fipronil with * indicating asymmetric chiral center.

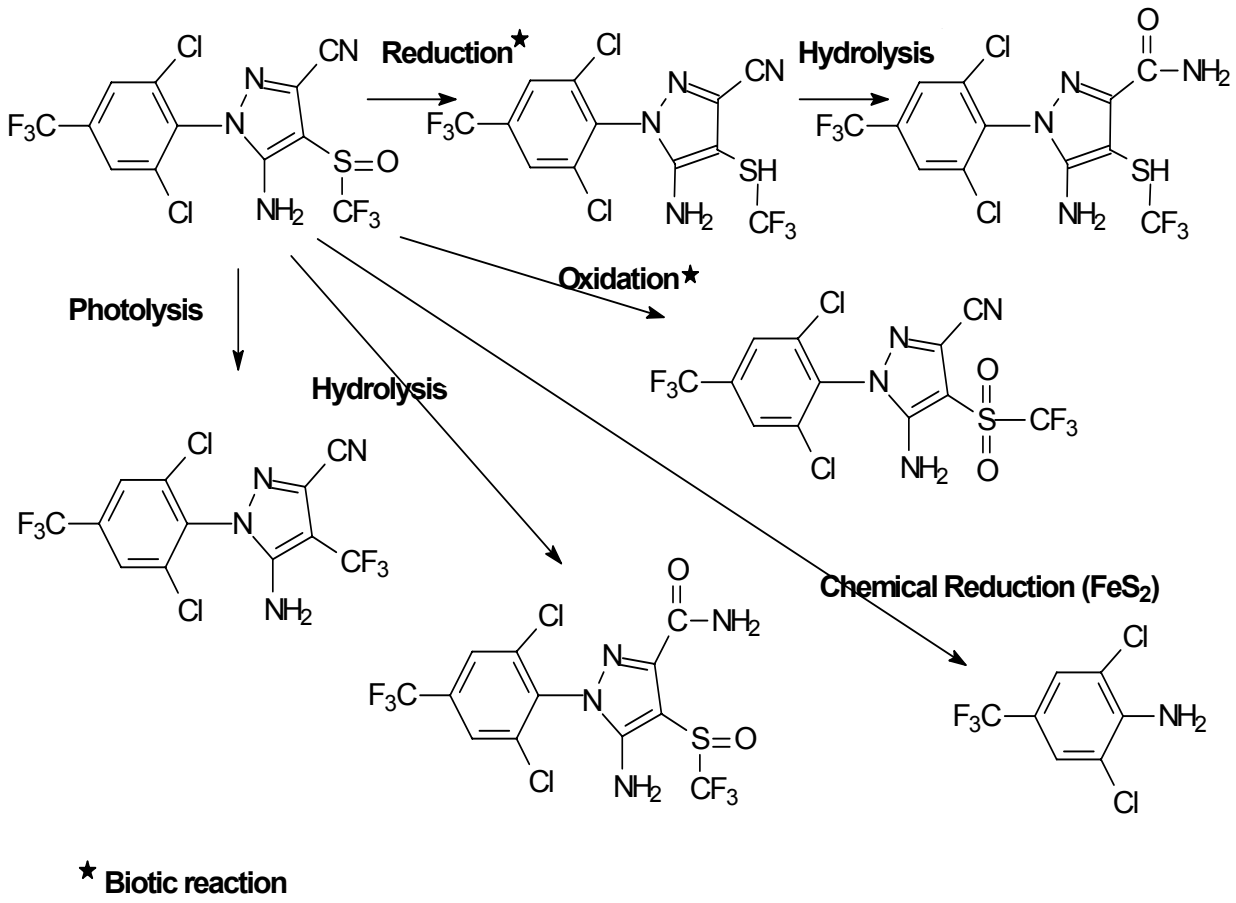

Figure 1.4. Schematic of the biotic and abiotic environmental degradation of fipronil.

CHAPTER 2

ACUTE ENANTIOSELECTIVE TOXICITY OF FIPRONIL AND ITS DESULFINYL PHOTOPRODUCT TO *CERIODAPHNIA DUBIA*[1]

[1]Konwick BJ, Fisk AT, Garrison AW, Avants JK, Black MC. Submitted to *Environmental Toxicology and Chemistry*

Abstract

Fipronil is a phenylpyrazole insecticide increasingly used in applications such as rice culture, turf grass management, and residential pest control, with a high probability to contaminate aquatic environments. As a chiral pesticide, fipronil is released to the environment as a racemic mixture (equal amounts of optical isomers called enantiomers). Enantiomers can have different toxicological and biological activity; however, information on these differences, which is necessary for accurate risk assessment of chiral pesticides, is limited. Here we examine the acute toxicity of fipronil enantiomers, the racemate, and its photoproduct (desulfinyl fipronil) to *Ceriodaphnia dubia.* The 48-h LC50 (concentration resulting in 50% mortality) values based on measured concentrations of each compound indicate the (+) enantiomer (LC50 = 10.3 ± 1.1 µg/L, mean ± SE) was significantly more toxic to *C. dubia* than either the (-) enantiomer (LC50 = 31.9 ± 2.2 µg/L) or racemate (LC50 = 17.7 ± 1.3 µg/L). To account for any potential loss of fipronil through photolysis, tests were performed under light (fluorescent) and dark exposure conditions, and no significant differences in toxicity were observed. Desulfinyl fipronil, the major photodegradation product, which is not chiral, was detected at <1% of each parent compound in test solutions after 48-h. Separate toxicity tests with desulfinyl fipronil found a >20 fold higher LC50 (355 ± 9.3 µg/L) compared to the fipronil racemate, suggesting lower adverse effects to *C. dubia* as a result of fipronil photolysis. The present results suggest selection of the (-) enantiomer in fipronil production for lower impacts to *C. dubia*; however, the consistency and relevancy of fipronil's enantiomer-specific activity at both acute and chronic levels of concern to additional target and non-target species needs further consideration.

Introduction

Fipronil (Figure 2.1) is a phenylpyrazole-class insecticide first approved for use in the U.S. in 1996. A number of its commercial formulations are widely used in rice culture, turf grass management, and residential pest control. Fipronil has been recognized as a disrupter of γ-aminobutyric acid (GABA) gated chloride channels in nerve cells leading to hyper-excitation and eventual mortality [1]. Its toxicity is much higher (>500 fold) in invertebrates relative to mammals due to differences in binding between insect and mammalian GABA receptors [2, 3]; this has been attributed to its unique trifluoromethylsulfinyl group that is not present in other similar pesticides [4]. As a result, use of this insecticide is increasing worldwide due in part to restrictions in use and species resistance to organophosphorus and other pesticides [2, 5].
The environmental degradation of fipronil is controlled in large part by photolysis in aquatic systems. This photo-conversion readily occurs ($t_{½} < 0.5$ d [6, 7]; $t_{½} < 3.6$ d) [8, 9]), resulting in extrusion of the sulfinyl group (Figure 2.1). Previous research has shown fipronil to be highly toxic to aquatic crustaceans [7, 10-12], with its desulfinyl photoproduct being equal or greater in toxicity within some species [4, 7], as well as being more environmentally persistent [9, 13]. Thus, it is important to consider both fipronil and its photoproduct when evaluating potential contamination of the aquatic environment.

Fipronil is one of the approximately 25% of current-use pesticides that are chiral (Figure 2.1) [14]. Chiral compounds exist as two non-superimposable mirror images called enantiomers, which are designated as (+) and (-) based on their rotation of plane-polarized light. The manufacture of chiral chemicals results in a mixture designated as racemic (±), which contains 50% of each enantiomer and is the form in which they are typically released into the environment. Enantiomers have identical physical-chemical properties and abiotic degradation

rates [15], but can have different toxicity, biological activity, and microbial degradation rates from each other [14-18]. Knowledge of the effects and persistence of individual enantiomers is critical for future regulation of chiral pesticides [19]. In fact, due to the enantiomer-specific activity and effects of some chiral pesticides, some European countries have revoked registrations of racemates in favor of registration of single enantiomers [14]. Also, the U.S. Environmental Protection Agency (EPA) has recognized the issue of chirality in pesticide registration [20], but is usually unable to consider individual enantiomers due to the lack of toxicity and fate information concerning them [21].

A starting point in understanding the environmental impact of potential contaminants in the environment, such as enantiomers of chiral pesticides, is to conduct standard freshwater aquatic toxicity tests with *Ceriodaphnia dubia* (Class Crustacea) [22]. Therefore, the objectives of the present study were to evaluate the differences in toxicity of the two fipronil enantiomers and the racemate to *C. dubia*; compare toxicity of the fipronil species (+, -, ±) under dark and light (fluorescent) conditions to determine whether possible photolysis within the exposure regimen influences toxicity; and measure the toxicity of the desulfinyl photoproduct to evaluate its toxicity to *C. dubia*. To our knowledge, this is the first study to examine the acute toxicity of fipronil in *C. dubia,* and the enantioselective toxicity of fipronil in any organism.

Materials and Methods

Culturing of Test Organisms

*Ceriodaphnia dubi*a were obtained from the US EPA (Region IV Ecological Services Laboratory, Athens, GA, USA) and stock cultures were maintained for a month prior to initiation of tests according to established protocol [22]. All cultures were maintained in an incubator (24-

26ºC) with a 16 h light: 8 h dark photoperiod. Moderately hard water (MHW; 20% Perrier® in Milli-Q water (volume/volume)) was used for all stock cultures and experiments. *C. dubia* were individually cultured in 30 ml polypropylene cups containing 15 ml MHW, with healthy third broods used to start new cultures every week. Water and food [100 µL YCT (Yeast, Cerophyll®, Tetramin®), 100 µL *Pseudokirchneriella subcapitata* [(3.0 X 10^7 cells/ml)] were replaced daily at approximately the same time. Daily records of *C. dubia* reproduction in stock cultures were kept to verify that only healthy neonates from third and fourth broods containing 10 or more neonates were used for toxicity tests. The range of water quality characteristics used in stock cultures and to initiate experiments were: dissolved oxygen (DO, YSI Model 55, Yellow Springs, OH, USA), 7.50-8.54 mg/L; pH (Orion model 720A, Beverly, MA, USA), 8.16-8.36; total hardness, 82-90 mg/L (as $CaCO_3$); total alkalinity, 68-80 mg/L (as $CaCO_3$).

Chemicals

Fipronil (± 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4[(trifluoromethyl)-sulfinyl]-1*H*-pyrazole-3-carbonitrile; 98% pure) was obtained from ChemService (West Chester, PA, USA). Desulfinyl fipronil was obtained from Aventis (Research Triangle Park, NC, USA; 97.8 % pure). Fipronil enantiomers were separated by Chiral Technologies (Exton, PA, USA). In brief, this process involved 3.0 g of racemic fipronil that was separated by HPLC on a CHIRALPAK®AS-H (Chiral Technologies) preparative column (3.0 cm i.d. X 25 cm length). Elution was by supercritical CO_2/Isopropyl alcohol (IPA): 90/10, column 30°C, and detection was by UV at 290 nm. Quality assurance HPLC of each separated enantiomer involved a CHIRALCEL®OD-H (Chiral Technologies) analytical column at 25°C with mobile phase of hexane/IPA: 85/15 at one ml/min. Under these conditions, peak one was the (-) enantiomer and

peak two the (+) enantiomer, as measured by a polarimeter (PDR-Chiral). Preparative yields were 1.47 g with a purity of 98.1% for peak one and 1.32 g with purity of 97.3% for peak two.

Test solutions

Stock solutions (1000 mg/L) of fipronil (+, -, ±) and desulfinyl fipronil were prepared in 100% pesticide grade acetone. Each stock solution was diluted with MHW to the following nominal concentrations for fipronil (+, -, ±): 5, 10, 20, 40, and 80 µg/L; and for desulfinyl fipronil: 220, 260, 300, 340 and 380 µg/L. An equal amount of acetone was used as a carrier solvent in all test solutions (including the vehicle control) for all toxicity tests (0.1%, v/v). Aqueous dilutions were always made on the same day of test initiation. All containers used in making solutions were pre-cleaned and wrapped in foil to eliminate any contamination or photolyzation. At the beginning and end of each test (n = 6 for (+,-, ±) fipronil tests and n = 3 for desulfinyl fipronil tests) (Table 2.1), composite samples (from three replicates, approximately 45 ml total) of the test waters from the highest (n = 2) and the lowest (n = 2) concentrations were collected in pre-cleaned amber jars for analysis of fipronil and desulfinyl fipronil.

Analysis of Fipronil and Desulfinyl Fipronil in Water

Fipronil and desulfinyl fipronil were extracted from water using solid phase extraction tubes (Supelco LC-18, 6 ml, 0.5 g) preconditioned with deionized water and methanol. Samples were gradually added to the tubes immediately following preconditioning at 5 ml/min using vacuum. The sorbent was then dried for 30 min. Samples were eluted with 3 X 1 ml of ethyl acetate using gravity flow. The eluant was evaporated under nitrogen to 500 µL and analyzed by gas chromatography/ mass spectrometry (HP 6890/5973) in selected ion mode using a BGB-172

chiral column (GB Analytik, AG, Anwil, Switzerland). Average recoveries of compounds spiked into water were 99 ± 0 % for fipronil (100 µg/L) and 130 ± 0.5 % for desulfinyl fipronil (250 µg/L) (n = 3 for each). Measured water concentrations from toxicity tests were not corrected according to recovery of these spiked compounds.

Toxicity bioassays

Methods for acute toxicity tests conformed to EPA guidelines [22]. *C. dubia* neonates less than 24 hours old and within 8 hours of the same age were used for tests that were initiated at the same time each day. Neonates were pipetted into 30 ml polypropylene plastic cups containing 15 ml MHW (control), a 0.1% (v/v) acetone solution (vehicle control), or fipronil compound dissolved acetone and diluted with MHW. *C. dubia* were not fed during acute toxicity tests. Two series of tests were conducted for each fipronil enantiomer and for the racemate, under normal culture photoperiod (16 h light: 8 h dark; fluorescent incubator light) and under dark conditions (no photoperiod) to eliminate photolysis (if any was observed) and its effect on fipronil toxicity. Three replicate toxicity tests were conducted for each compound under light and dark conditions. For each test, 15 neonates (3 cups: 5 neonates per cup) were exposed to each fipronil treatment level along with a control and vehicle control, and mortality was assessed after 48 h by immobilization after gentle probing with a pipet. Tests for desulfinyl fipronil were conducted in a similar manner except under only normal culture photoperiod (16 h light: 8 h dark). After 48 h, DO and pH were measured (as described above) and acceptable levels (DO, 7.80-8.38 mg/L; pH, 8.30-8.46) [22] were found in each of the test cups.

Quality assurance and quality control measures were employed for acute toxicity tests. For test acceptance, survival of control and vehicle control organisms was to exceed 90%. In

addition, concurrent toxicity tests with the reference toxicant copper sulfate ($CuSO_4$) were conducted in MHW, and the concentration resulting in 50% mortality (LC50) did not deviate by more than two standard deviations from the mean values computed for our laboratory [22].

Statistics

All LC50 values and statistics were determined based on measured water concentrations. The highest and lowest concentrations in each test were determined analytically and remaining test concentrations where determined by adding or subtracting the mean percent deviation (determined from measured high and low water concentrations) from nominal concentrations in each test. Based on this, average concentrations used for LC50 determinations for (+) fipronil exposures were 4.1, 8.1, 16.2, 32.4, and 64.8 µg/L; (-) fipronil exposures were 4.5, 9.0, 17.8, 35.7, and 71.9 µg/L; (±) fipronil exposures were 4.7, 9.3, 18.6, 37.2, and 74.4 µg/L; and for desulfinyl fipronil exposures were 213, 251, 290, 329 and 367 µg/L.

The 48-h LC50 (concentration resulting in 50% mortality) values for fipronil (+, -, ±) and desulfinyl fipronil were computed by the Trimmed Spearman-Karber Method (Version 1.5) [23]. LC50 values of the fipronil enantiomers and the racemate were tested for significant differences with an ANOVA followed by Tukey's multiple comparison ($\alpha = 0.05$) under light and dark exposures and also combined (light and dark). Logistic regression for the fipronil enantiomers and racemate (light and dark data combined) was used as an additional method to determine LC50 values and for comparing slopes of the dose-response relationships. Due to the dispersion of the data ($\hat{c} = 3.58$), the model was corrected according to Williams [24] before investigating differences in slope of fipronil enantiomers and racemate (χ^2, $p<0.05$). All statistical analyses were conducted with Statistical Analysis Software (Version 8.0, SAS Institute, Cary, NC, USA)

and were preceded by Levene's test to determine if the statistical assumptions of homogeneity of variance were violated.

Results and Discussion

Analysis of water samples collected at initiation and end of acute toxicity tests indicated no change over time (i.e., 0-h vs. 48-h) in measured low (5 µg/L) and high (80 µg/L) concentrations of fipronil enantiomers and racemate (*t*-test, $p > 0.10$) (Table 2.1). Measured concentrations of desulfinyl fipronil in the low test concentration (220 µg/L) did not vary over the start and end of the exposure (*t*-test, $p > 0.6$), however the high test concentration (380 µg/L) did differ (*t*-test, $p = 0.02$) (Table 2.1). It is unclear why this high concentration declined because the low concentration did not follow this same pattern and desulfinyl fipronil is suggested as being stable to further abiotic degradation [4]. However, the difference was minor (<10%) and could possibly be explained by the analytical method, where our recoveries were somewhat enhanced for this compound. After 48 h, in the fipronil (+,-, ±) exposures, concentrations of the desulfinyl photoproduct were detected at <1% of each parent compound in analyzed test concentrations; although the photoproduct was observed more often, and on average, at higher concentrations under light conditions (0.77 µg/L, 72% of samples) compared to dark conditions (0.10 µg/L, 33% of samples).

Mortality of *C. dubia* increased with increasing concentration for each fipronil stereoisomer with the (+) enantiomer having greater toxicity under both light and dark exposure conditions (Table 2.2). When the data were either tested combined or under dark conditions, the (+) enantiomer was significantly more toxic than either the racemate or (-) enantiomer; however, under light conditions this significance was not seen, although the (+) enantiomer still had

greater toxicity (Table 2.2). LC50 values for the (+) enantiomer and racemate of fipronil were not found to be statistically different between exposure conditions (i.e., light vs. dark) (*t*-test, $p > 0.10$). The LC50 values for the (-) enatiomer of fipronil were found to significantly different between the light and dark exposure (*t*-test, $p = 0.04$). However, the difference was minor (LC50s were 35.4 ± 2.6 in light and 28.4 ± 2.4 in dark, mean ± SE, $n = 3$), and considering they were more variable than the other fipronil stereoisomers (Table 2.2), an increase in test replicates may show no difference in toxicity with exposure conditions. Also, when comparing all LC50 values regardless of fipronil stereoisomer, exposure conditions (light and dark) did not significantly (*t*-test, $p > 0.10$) influence fipronil toxicity. Therefore, combined data (light and dark) would give a better approximation of the true toxicity and are referred to herein.

Additional testing on more target and non-target species, including sub-lethal chronic exposures, is needed to assess possible risk reduction before production of an enriched or single enantiomer formulation of fipronil is used. The relative difference in LC50 values of fipronil enantiomers found here was approximately 3 fold (10.3 ± 1.1 µg/L for (+) and 31.9 ± 2.2 µg/L for (-) enantiomer), while the toxicity of the racemate (17.7 ± 1.3 µg/L) was approximately midway between the toxicity of each enantiomer, suggesting possible additive effects. However, the biological relevance of whether these results, including any additive effects of enantiomers, holds true at chronic sub-lethal exposures are unknown. Furthermore, the relative toxicity of enantiomers is of importance to all organisms that could potentially come into contact with the stereoisomer, both target and non-target. In our study, *C. dubia* is a non-target organism with regard to fipronil effects, and additional tests on the enantiomer-specific activity to target organisms (i.e., rice water weevil, fire ants, etc.) as well as other non-target species are warranted.

The toxicity of the photoproduct, desulfinyl fipronil, was considerably lower compared to fipronil, and further impacts on *C. dubia* survival would be dependent on the rate of photolysis of fipronil. The estimated LC50 value for desulfinyl fipronil (355 ± 9.3 µg/L, mean ± 1 SE, $n = 3$) found here to *C. dubia* was approximately 11 fold less than the fipronil racemate (Table 2.2). Although limited photodegradation likely occurred during this study, based on the more frequent presence and higher concentrations of desulfinyl fipronil under light exposures (see above), these concentrations (< 0.77 µg/L) were well below the estimated LC50 value of desulfinyl fipronil to *C. dubia.* Therefore, any residual photolysis that occurred during the study likely had no effect on toxicity to *C. dubia*, which was the intent of this study and not to examine photolysis or photodegradation of fipronil. This is because the exposure conditions (i.e., incubator fluorescent light) used in this experiment did not provide the necessary irradiation [25] for fipronil's rapid photolysis. Consequently, ultraviolet (UV) or natural sunlight at the appropriate wavelength are needed to elucidate fipronil's photodegradation and the resulting toxicity to *C. dubia*. While fipronil's pathway to the desulfinyl derivative has been shown to be largely a result of direct photolysis in aqueous solutions [9], observations of lower environmental concentrations of desulfinyl fipronil compared to fipronil have been noted (see below). This can be due to association of fipronil with dissolved organic matter (DOM) [9], as fipronil has a high affinity for organic carbon, sediment, and soils ($\log K_{ow} = 4.01$) [7]. As a result, implications over whether the resulting photoconversion of desulfinyl fipronil occurs to a significant degree to afford any detrimental effects in aquatic fauna needs further investigation.

There are several possible reasons for the differences in toxicity among fipronil enantiomers, the racemate, and its photodegradation product. First, differential toxicity may be related to binding to different GABA receptor subunits among different species. Although study

of the GABA receptor is limited within aquatic species, its composition is inferred to be homologous among arthropods [26]. It is suggested that the β3 subunit is the target site for insecticide binding, however other subunits may alter the binding site thereby providing receptor selectivity and potency, as shown with fipronil and desulfinyl fipronil [27]. In addition, given that other receptors can be enantioselective [28, 29], differential GABA receptor binding of fipronil enantiomers is a possibility for the observed differential toxicity. Since metabolic conversion is generally stereospecific [30], another prospect is that one enantiomer is preferentially metabolized before reaching the intended target site (i.e., GABA receptor). Differences in toxicity of fipronil enantiomers and racemate may also be explained by dissimilar modes of action. Comparable slopes of dose-response curves typically indicate a similar mode of action of toxicants [31]. Here, fipronil enantiomers and racemate (Figure 2.2) were found to have similar slopes ($p > 0.05$ for all comparisons) but different intercepts ($p < 0.001$ for all comparisons) indicating that toxicity is likely a result of the same mechanism of action (i.e., GABA disruption).

In comparison to other pesticides, fipronil is one of the more toxic, but this toxicity varies greatly with different aquatic species (Table 2.3). Of the most commonly used pesticides in any sector (agricultural, industry, or home and garden) [32], fipronil's toxicity to *C. dubia* is among the top five of any pesticide based on available information in EPA's ECOTOX database (http://www.epa.gov/ecotox, accessed August 17, 2004). Only malathion, chlorpyrifos, diazinon, and carbaryl have shown the potential to be more acutely toxic to *C. dubia* than the fipronil racemate. In comparison to other species, *Daphnia magna* is the only other daphnid species to have a reported LC50 value for fipronil (190 µg/L) [7], approximately 10 times less toxic than the results found for here using *C. dubia*. On the other hand, mysids (*Americamysis*

bahia) [7], adult grass shrimp (*Palaemonetes pugio*) [11], and male adult copepods (*Amphiascus tenuiremis*) [12] are even more sensitive to fipronil than *C. dubia*, suggesting possible greater toxicity in estuarine organisms. For desulfinyl fipronil, there are no reported data for other daphnid species, but our results are in agreement with its reduced toxicity relative to fipronil in rainbow trout [7], mysids (*Mysidopsis bahia*) [7], and *Procambarus* sp. [10]. However, the desulfinyl photoproduct has been shown to be more toxic than fipronil to bluegill sunfish [7], houseflies and mice [4]. These interspecies differences in toxicity are not easily explained, however additional testing with a greater number of species may help reduce uncertainties in risks associated with fipronil use.

To assess the significance of fipronil in the environment, it is necessary to compare toxicity data with environmental levels. Fipronil has been measured in 25% of the water samples collected by the National Ambient Water-Quality Assessment (NAWQA). Concentrations of fipronil ranged between 0.01 and 0.07 µg/L, being more prevalent and of greater concentration than its desulfinyl photoproduct (1.61% of samples > 0.01 µg/L) (M.W. Sandstrom, USGS, Denver, CO, unpublished data). In rice culture, an estimated peak water concentration of approximately 5.0 µg/L for fipronil and 1.4 µg/L for desulfinyl fipronil has been calculated [13]. A 2001 US EPA document has projected an average peak fipronil concentration of 1.7 µg/L in Southeastern U.S. waters when used for fire ant control [33]. Concentrations reported for fipronil and desulfinyl fipronil in the environment are below those needed to produce acute toxicity in *C. dubia* (this study); but are in the range reported to be acutely toxic in mysids (*Americamysis bahia)* [7]. Additionally, environmental concentrations of fipronil are in the range to illicit sublethal effects in select organisms. For example, fipronil decreased reproduction in copepods (*Amphiascus tenuiremis)* at a concentration of 0.42 µg/L [12] and was

associated with male-specific infertility [34]. Thus, increasing concern and need for more research on fipronil's impacts on non-target organisms are warranted, especially study on chronic exposures. In light of the growing use of fipronil, identification of its enantiomer-specific effects on a variety of organisms may indicate that production and use of the single active enantiomer, or at least a product enriched in that enantiomer, would be prudent for protection of the environment.

Acknowledgement

The authors thank J. Peterson (University of Georgia) for assistance and advice with statistical analysis. This manuscript benefited from the comments of two anonymous reviewers.

References

1. Cole LM, Nicholson RA, Casida JE. 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic Biochem Physiol* 46: 47-54.
2. Gant DB, Chalmers AE, Wolff MA, Hoffman HB, Bushey DF. 1998. Fipronil: Action at the GABA receptor. *Rev Toxicol* 2: 147-156.
3. Hainzl D, Cole LM, Casida JE. 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem Res Toxicol* 11: 1529-1535.
4. Hainzl D, Casida JE. 1996. Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity. *Proc Natl Acad Sci* 93: 12764-12767.

5. Hosie AM, Baylis HA, Buckingham SD, Sattelle DB. 1995. Actions of the insecticide fipronil on dieldrin sensitive and resistant GABA receptors of *Drosophila melanogaster*. *Br J Pharmacol* 115: 909-912.
6. Ngim KK, Crosby DG. 2001. Abiotic processes influencing fipronil and desthiofipronil dissipation in California, USA, rice fields. *Environ Toxicol Chem* 20: 972-977.
7. U.S. Environmental Protection Agency. 1996. New pesticide fact sheet. EPA 737-F-96-005. Office of Pesticide Programs, Washington, DC.
8. Walse SS, Pennington PL, Scott GI, Ferry JL. 2004. The fate of fipronil in modular estuarine mesocosms. *J Environ Monit* 6: 58-64.
9. Walse SS, Morgan SL, Kong L, Ferry JL. 2004. Role of dissolved organic matter, nitrate, and bicarbonate in the photolysis of aqueous fipronil. *Environ Sci Technol* 38: 3908-3915.
10. Schlenk D, Huggett DB, Allgood J, Bennett E, Rimoldi J, Beeler AB, Block D, Holder AW, Hovinga R, Bedient P. 2001. Toxicity of fipronil and its degradation products to *Procambarus* sp.: Field and laboratory studies. *Arch Environ Contam Toxicol* 41: 325-332.
11. Key PB, Chung KW, Opatkiewicz AD, Wirth EF, Fulton MH. 2003. Toxicity of the insecticides fipronil and endosulfan to selected life stages of the grass shrimp (*Palaemonetes pugio*). *Bull Environ Contam Toxicol* 70: 533-540.
12. Chandler GT, Cary TL, Volz DC, Walse SS, Ferry JL, Klosterhaus SL. 2004. Fipronil effects on estuarine copepod (*Amphiascus tenuiremis*) development, fertility, and reproduction: A rapid life-cycle assay in 96-well microplate format. *Environ Toxicol Chem* 23: 117-124.

13. U.S. Environmental Protection Agency. 1997. Environmental fate and effects Section 3 registration decision for fipronil: Use on rice seed, D235912. Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA.
14. Williams A. 1996. Opportunities for chiral agrochemicals. *Pestic Sci* 46: 3-9.
15. Garrison AW, Schmitt P, Martens D, Kettrup A. 1996. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ Sci Technol* 30: 2449-2455.
16. Kodama S, Yamamoto A, Saitoh Y, Matsunaga A, Okamura K, Kizu R, Hayakawa K. 2002. Enantioseparation of vinclozolin by γ-cyclodextrin-modified micellar electrokinetic chromatography. *J Agric Food Chem* 50: 1312-1317.
17. Rodriguez OP, Muth GW, Berkman CE, Kim K, Thompson CM. 1997. Inhibition of various cholinesterases with the enantiomers of malaoxon. *Bull Environ Contam Toxicol* 58: 171-176.
18. Bradbury SP, Symonik DM, Coats JR, Atchison GJ. 1987. Toxicity of fenvalerate and its constituent isomers to the fathead minnow, *Pimephales promelas*, and bluegill, *Lepomis macrochirus*. *Bull Environ Contam Toxicol* 38: 727-735.
19. Lewis DL, Garrison AW, Wommack KE, Whittemore A, Steudler P, Melillo J. 1999. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* 401: 898-901.
20. U.S. Environmental Protection Agency. 1999. Pesticides; Request for comment on pesticide registration proposal for isomeric active ingredients. *Fed Reg* 64: 22863-22865, April 28, 1999.

21. U.S. Environmental Protection Agency. 1999. Response to public comments on the preliminary risk assessment for the organophosphate naled. Docket #34136, US EPA, Washington DC, USA.

22. U.S. Environmental Protection Agency. 1993. Methods for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms, 4th ed. EPA/600/4-90/027F. Cincinnati, OH, USA.

23. Hamilton MA, Russo R, Thurston RV. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci Technol* 11: 714-718.

24. Williams DA. 1982. Extra-binomial variation in logistic-linear models. *Applied Statistics* 31: 144-148.

25. Hirahara Y, Ueno H, Nakamuro K. 2001. Comparative photodegradation study of fenthion and disulfoton under irradiation of different light sources in liquid- and solid-phases. *J Health Sci* 47: 129-135.

26. Robinson TM, Olsen RW. 1988. *Comparative Invertebrate Neurochemistry*. Cornell University Press, Ithaca, NY, USA.

27. Ratra GS, Kamita SG, Casida JE. 2001. Role of human $GABA_A$ receptor β3 subunit in insecticide toxicity. *Toxicol Appl Pharmacol* 172: 233-240.

28. Abou-Zeid LA, El-Mowafy AM. 2004. Differential recognition of resveratrol isomers by the human estrogen receptor-α: molecular dynamics evidence for stereoselective ligand binding. *Chirality* 16: 190-195.

29. Adejare A, Deal SA, Day Jr. MS. 1999. Syntheses and β-adrenergic binding affinities of (R)- and (S)- fluoronaphthyloxypropanolamines. *Chirality* 11: 144-148.

30. Wong CS, Lau F, Clark M, Mabury SA, Muir DCG. 2002. Rainbow trout (*Oncorhynchus mykiss*) can eliminate chiral organochlorine compounds enantioselectively. *Environ Sci Technol* 36: 1257-1262.

31. Landis WG, Yu M-H. 2004. *Introduction to environmental toxicology: Impacts of chemicals upon ecological systems*. Lewis publishers, Boca Raton, FL, USA.

32. Donaldson D, Kiely T, Grube A. 2002. Pesticide industry sales and usage: 1998 and 1999 market estimates. EPA-733-R-02-001. US Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington DC, USA.

33. U.S. Environmental Protection Agency. 2001. Fipronil environmental fate and ecological effects assessment and characterization for a Section 3 for broadcast treatment with granular product to control turf insects and fire ants (addendum). Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA.

34. Cary TL, Chandler GT, Volz DC, Walse SS, Ferry JL. 2004. Phenylpyrazole insecticide fipronil induces male infertility in the estuarine meiobenthic crustacean *Amphiascus tenuiremis*. *Environ Sci Technol* 38: 522-528.

35. Biever RC, Hoberg JR, Jacobson B, Dionne E, Sulaiman M, McCahon P. 2003. ICON® rice seed treatment toxicity to crayfish (*Procambarus clarkii*) in experimental rice paddies. *Environ Toxicol Chem* 22: 167-174.

36. Shan Z, Wang L, Cai D, Gong R, Zhu Z, Yu F. 2003. Impact of fipronil on crustacean aquatic organisms in a paddy field-fishpond ecosystem. *Bull Environ Contam Toxicol* 70: 746-752.

Table 2.1. Measured concentrations (mean ± 1 SE) of fipronil enantiomers, racemate, and desulfinyl fipronil in high and low test solutions at beginning (0-h) and end (48-h) of *C. dubia* toxicity tests. Concentrations were not significantly different (t-test, $p > 0.10$) over the exposure period (0-h vs. 48-h) except for the high solution of desulfinyl fipronil ($p = 0.02$).

Fipronil Compound	*n*	Nominal Conc. (µg/L)	Measured Conc. (µg/L) (Time 0)	Measured Conc. (µg/L) (48-h)
(+) Enantiomer	6	5 (low)	3.5 ± 0.3	3.5 ± 0.2
	6	80 (high)	76.4 ± 1.7	72.5 ± 1.4
(-) Enantiomer	6	5 (low)	4.3 ± 0.2	4.1 ± 0.3
	6	80 (high)	76.8 ± 3.0	76.9 ± 3.1
Racemate	6	5 (low)	4.4 ± 0.3	4.4 ± 0.2
	6	80 (high)	79.7 ± 4.4	78.0 ± 1.5
Desulfinyl Fipronil	3	220 (low)	217.7 ± 2.9	210.3 ± 9.9
	3	380 (high)	379.0 ± 4.0	351.7 ± 4.4

Table 2.2. Acute toxicity (LC50) of fipronil enantiomers, racemate, and desulfinyl fipronil to *C. dubia* after 48-h exposure computed by either Trimmed Spearman-Karber (TSK) or Logistic Regression (LR) analysis. Tests were not significantly different under light and dark for the (+) enantiomer or racemate (*t*-test, $p > 0.40$), but were for the (-) enantiomer (*t*-test, $p = 0.04$). All reported values are mean ± 1 SE.

Fipronil Compound	48-h LC50 (µg/L)			
	TSK[a]			LR
	Light	Dark	Combined	
(+) Enantiomer	11.3 ± 2.0 A	9.4 ± 0.7 A	10.3 ± 1.1 A	11.7
(-) Enantiomer	35.4 ± 2.6 B	28.4 ± 2.4 C	31.9 ± 2.2 C	38.9
Racemate	17.9 ± 2.7 A	17.5 ± 0.7 B	17.7 ± 1.3 B	20.3
Desulfinyl Fipronil	355 ± 9.3	ND	-	ND

[a] = Different letters indicate significantly different LC50 values among fipronil stereoisomers under their respective exposure conditions (determined by ANOVA) (mean ± 1 SE, $n = 6$ tests)
ND = Not determined

Table 2.3. Acute toxicity (LC50) of fipronil and its desulfinyl photoproduct to aquatic species.

Species	LC50 Value (µg/L)		Source
	Fipronil	Desulfinyl Fipronil	
A. bahia (mysid)	0.14	1.5	[7]
P. pugio (adult grass shrimp)	0.32	-	[11]
A. tenuiremis (copepod)	3.5-13.0	-	[12]
P. clarkii (red swamp crayfish)	14.3	68.6	[10]
P. zonangulus (white river crayfish)	19.5	-	[10]
P. clarkii (red swamp crayfish)	180	-	[35]
M. rosenbergii (shrimp)	2.24	-	[36]
M. nipponensis (shrimp)	11.61	-	[36]
E. sinensis (crab)	22.57	-	[36]
D. magna (daphnid)	190	-	[7]
C. dubia (daphnid)	17.7	355	Current Study
L. macrochirus (bluegill sunfish)	83	20	[7]
C. variegatus (sheepshead minnow)	130	-	[7]
O. mykiss (rainbow trout)	246	>100,000	[7]

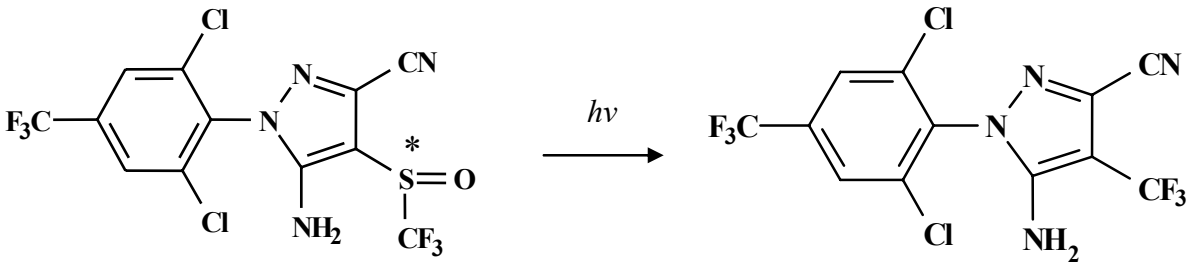

Figure 2.1. Structure of fipronil (left) with * indicating asymmetric chiral center. Fipronil degrades under environmental conditions to non-chiral desulfinyl fipronil (right) as the major photoproduct.

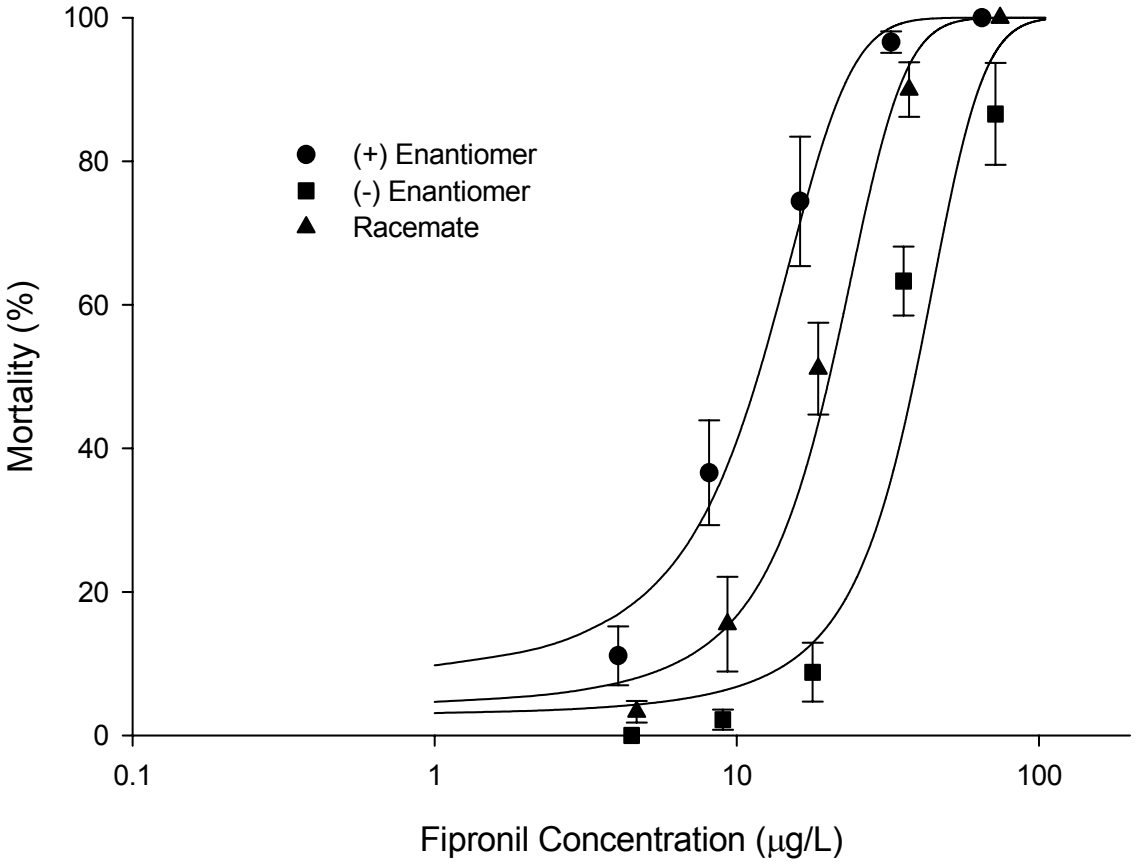

Figure 2.2. Dose-response mortality of *C. dubia* exposed to fipronil enantiomers and racemate. Each point represents the mean percent mortality ± 1 SE of 6 tests based on measured test concentrations. Fitted line represents logistic regression model of data ($r^2 = 0.59$). For model LC50 values see Table 1.

CHAPTER 3

BIOACCUMULATION AND ENANTIOSELECTIVE BIOTRANSFORMATION OF FIPRONIL AND SELECTED ORGANOCHLORINES BY RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)[2]

[2]Konwick BJ, Garrison AW, Black MC, Avants JK, Fisk AT. To be submitted to *Environmental Science and Technology*

Abstract

Dietary accumulation of the chiral current-use pesticide fipronil, selected chiral organochlorines [α-hexachlorocyclohexane (HCH), heptachlor epoxide (HEPX), polychlorinated biphenyls (PCBs) 84, 132, 174, *o,p'*-DDT, and *o,p'*-DDD], and the non-chiral organochlorines (*p,p'*-DDT, *p,p'*-DDD) were studied to determine the degree to which juvenile rainbow trout (*Oncorhynchus mykiss*) can bioaccumulate and enantioselectively eliminate these compounds. Fish rapidly accumulated all compounds during the 32-d uptake phase, which was followed by varying elimination with half-lives ranging from 0.6 d for fipronil to 77.0 d for PCB 174 during the 96-d depuration period. Similarly, biomagnification factors (BMFs) ranged from 0.04 for fipronil to 5.9 for PCB 174, and absorption efficiencies ranged from 23% for fipronil to 71% for HEPX. No evidence of significant enantiomer-specific biotransformation was observed for α-HCH, HEPX, PCB 132, PCB 174, *o,p'*-DDT, or *o,p'*-DDD. Enantiomeric fractions (EFs) determined in the fish for these compounds were similar (racemic) to those in the spiked food. However, fish preferentially eliminated the (+) enantiomer of fipronil and PCB 84 indicating that biotransformation was enantiomer-specific for these two compounds. These results are supported by previous research of PCB 84 metabolism and the formation in the fish of fipronil sulfone, a metabolite of fipronil, which had slightly greater persistence (half-life of approximately 2 days) within the fish. Most of the compounds fell on the same log K_{ow} – half-life relationship as 16 preselected PCB congeners that are recalcitrant in fish, suggesting that little to no metabolism was evident in this study. The notable exception was fipronil where biotransformation accounted for the majority (88%) of its elimination, supporting the use of this relationship as a mechanistic tool for quantifying biotransformation rates for other chemicals in fish.

Introduction

Fipronil is a phenylpyrazole-class insecticide first approved in 1996 for use on a number of crops in the U.S. Typical fipronil applications include rice culture, turf grass management, and residential pest control (1-4). Fipronil has been recognized as a disrupter of γ-aminobutyric acid (GABA) gated chloride channels in nerve cells, leading to hyper-excitation and eventual mortality (5-6). This toxicity is much greater in invertebrates than in mammalians due to their different GABA receptor binding affinities (6-7). The use of fipronil is expected to increase because of developing species resistance to organophosphate (OP) insecticides (8) and restrictions in OP use as a result of the Food Quality Protection Act (FQPA) of 1996 (9). Fipronil applications can impact aquatic environments at low concentrations (1-2, 10-11), thus there is potential for adverse effects on non-target species. In addition, fipronil's degradation products, which are similar in potency (4, 7, 12) and more environmentally stable (1, 12, 13), may lead to long-term effects on non-target species.

To assess the potential risk of contaminants, such as current-use pesticides (e.g., fipronil), it is important to understand their environmental accumulation in aquatic biota. Typically, the bioaccumulation potential for current-use pesticides is considered minimal because of their low octanol-water partition coefficients (log K_{ow}), a surrogate for describing chemical accumulation in biota due to their lipophilicity. However, fipronil's log K_{ow} (4.01) (3, 14) is in the range of some persistent organic chemicals (e.g., α-HCH, β-HCH) that have been shown to bioaccumulate and biomagnify in food webs (15-17), thus indicating the potential concern for bioaccumulation in aquatic biota. On the other hand, many current-use pesticides are readily metabolized by cytochrome P450s and other enzymes due to their presence of structural groups that are susceptible to biotransformation reactions (18). Biotransformation is a key component of an

organisms' elimination pathway for contaminants, by making them more water soluble and more easily to excrete through the addition of key functional groups. Ultimately, the elimination rate is what determines whether a chemical will bioaccumulate and cause adverse effects in organisms (19); however, biotransformation is rarely quantified but can have considerable influence on this elimination (20). Unfortunately, methods to quantitatively assess biotransformation are limited, especially for fish, which are believed to have limited biotransformation ability. This is inferred from fish having lower concentrations and activities of cytochrome P450 enzymes compared to mammals and birds (21-22). Also, when modeling contaminant movement through food webs, biotransformation for fish is assumed to be negligible (23-24).

Investigations focusing on chiral analysis have been used recently to study the biotransformation dynamics of contaminants. Approximately 25% of current-use pesticides are chiral (25), in addition to many legacy pesticides (e.g., DDTs, chlordanes) and some polychlorinated biphenyls (PCBs) (26). Chiral compounds exist as two non-superimposable mirror images called enantiomers, which are designated as (+) and (-) based on their rotation of plane-polarized light. The manufacturing of chiral chemicals results in a mixture designated as racemic (±), which contains 50% of each enantiomer and is the form in which they are typically released into the environment. Enantiomers have identical physical-chemical properties (25, 27); however, relative abundances of enantiomers can change after being subject to biochemical metabolic processes (e.g., enzyme mediated receptors, reactions) due to their symmetry dependence (28-29). Previous research has suggested that often the enantiomeric composition in biota is altered, providing a tracer for enantioselective biotransformation and elimination (30-31). For example, non-racemic residues have implied the ability for fish to biotransform some

chiral organochlorine (OC) contaminants to an important degree for the first time (28-29). Determining the biotransformation potential of OC contaminants due to their environmental persistence for assessing future trends of these compounds has been the focus of most chiral studies to date. However, chiral analysis of current-use pesticides in biota can achieve these same goals, yet has not received much attention.

In this paper, we report the dietary accumulation and biotransformation of racemic fipronil and selected OCs in juvenile rainbow trout (*Oncorhynchus mykiss)*. Exposure to OCs was performed to expand on the existing data to which OC compounds rainbow trout have been shown to eliminate enantioselectively (29). Biotransformation potential was determined by monitoring changes in enantiomeric signatures and through calculating inferred biotransformation rates from log K_{ow} to half-life relationships. Briefly, this relationship is based on the premise that by direct partitioning alone (log K_{ow}), we can model a recalcitrant chemical's half-life ($t_{1/2}$) (32). Non-recalcitrant chemicals whose half-life (determined experimentally) that then fall below this relationship are suggested to be due to biotransformation (32). This model has successfully been applied previously in providing biotransformation rates for polychlorinated alkanes and PCBs in juvenile rainbow trout (33-34). This research will expand on this relationship to lower log K_{ow} chemicals (e.g., current-use pesticides). Because of the extensive knowledge regarding fate and bioaccumulation of contaminants within rainbow trout, it was chosen as the test species. To our knowledge, this is the first experiment to determine the toxicokinetics of fipronil in fish via dietary exposure and its presumed biotransformation among any species as a result of its chiral configuration.

Materials and Methods

Chemicals and Food Preparation

Fipronil, heptachlor epoxide (HEPX), α-hexachlorocyclohexane (α-HCH), *trans/cis*-chlordane, *o,p'*-dichlorodiphenyltrichloroethane (DDT), *p,p'*-DDT, *o,p'*-dichlorodiphenyldichloroethane (DDD), and *p,p'*-DDD were obtained from ChemService (West Chester, PA). Polychlorinated biphenyls (PCBs) 84 and 65 were obtained from AccuStandard (New Haven, CT), and PCBs 174 and 132 were obtained from Ultra Scientific (North Kingston, RI). The purities of all chemical standards were ≥ 98%. All solvents (Ultra Resi-Analyzed®) were obtained from J.T. Baker (Phillipsburg, NJ).

A stock solution of fipronil in methanol (1000 μg/mL) and remaining organochlorine compounds in hexane (100 μg/mL) were diluted in hexane (1:100) and mixed with the commercial trout food (Zeigler, Gardner, PA) to give a nominal concentration of 10 μg/g of fipronil and 1 μg/g for each organochlorine in the feed. The solvent was then slowly evaporated to dryness in a rotary evaporator, followed by air drying the food for 48 hours before being stored in amber jars at 8°C. Control food was treated in an identical manner but without addition of the target compounds. Five days elapsed between the making of the spiked trout food and the initiation of the experiment. The trout food consisted of 38% protein, 14% lipid, and 3% fiber. The concentrations of fipronil and organochlorines were determined in spiked and control food using the same technique described below to determine concentrations in rainbow trout tissue (Table 3.1).

Experiments

Juvenile rainbow trout (initial weights 10 ± 0.5 g, mean ± SE) were collected from Lake Burton Fish Hatchery (GA) and randomly assigned to one of three 800 liter fiberglass aquaria (45 fish per tank) with recirculating dechlorinated tap water, chilled to 12°C (Frigid Units, Toledo, OH). Fish were maintained on a 12 h light: 12 h dark photoperiod. One tank of fish was exposed to all of the compounds listed above (MIX treatment), one tank was exposed to fipronil by itself (FIP treatment), and the final tank served as a control. Fish were exposed to the spiked food for 32 days (uptake) followed by 96 days of clean food (depuration). The daily rate of feeding was 1.5% of the mean weight of the rainbow trout, corrected for weight gain after each sampling day. All food was consumed within minutes of feeding. Three fish were randomly sampled from each treatment on days 2, 4, 8, 16, and 32 of the uptake phase, and days 34, 36, 40, 48, 64, and 128 of the depuration phase. Sampled fish were separated into liver, gastrointestinal (GI) tract (including stomach and contents, spleen, pyloric caeca, intestines and adipose tissue associated with these organs), and carcass (whole fish minus GI tract and liver) (32). GI tract was removed thereby not confounding target analytes that remained in undigested food. Only carcass results were used in calculating bioaccumulation parameters and enantiomeric fractions.

Chemical Analysis

Tissue samples were weighed, stored frozen at -8°C, and then freeze-dried. For analysis, samples were homogenized/extracted in dichloromethane (DCM): hexane (1:1 by volume) using a polytron (PowerGen 125, Fisher Scientific). The whole fish carcass was homogenized, except for the last sampling day, on which only 10-12 g of carcass fillet was extracted due to the large size of the fish. Polychlorinated biphenyl 65 was added to samples as a surrogate recovery

standard just before extraction. Samples were extracted twice; the extracts were then combined and evaporated to 10 ml. One ml of the extract was used to determine lipids gravimetrically. The remaining extract was then evaporated to 2 ml and applied to gel permeation chromatography (GPC) columns to remove lipids (32). The GPC columns (inner diameter 29.5 mm, length 400 mm, reservoir 500 ml) were packed with 60 g (dry weight) of 200-400 mesh Bio-Beads® S-X3 (Bio-Rad Laboratories, Hercules, CA). The column was eluted with 340 ml of DCM: hexane (1:1 by volume); the first 140 ml contained the lipids and was discarded. The next 200 ml eluate contained the target analytes and was rotary evaporated to 1 ml before analysis.

Analysis of the cleaned fish tissue extracts for the compounds was by chiral capillary gas chromatography with mass spectrometric detection (GC-MS) using a Hewlett-Packard 6890 GC with a 5973 mass selective detector, or with electron capture detection (ECD) using a Hewlett Packard 5890 GC. The GC column in both cases was a BGB 172 (BGB Analytik AG, Switzerland, sales@bgb-analytik.ch) containing a chiral phase composed of 20% tert-butyldimethylsilylated-β-cyclodextrin; column length was 30 m long, ID was 0.25 mm, and film thickness was 0.25 μm. GC column temperature programs are given in Table 3.2 (footnote d). For the GC-MS, injection was splitless at 250^{o} and the carrier gas was helium at a flow rate of 1.5 mL/min; electron voltage was 70 eV. All GC-MS detection was by selected ion monitoring; ions are given in Table 3.2. For the GC-ECD, carrier gas was helium with a column flow of 2 mL/min; detector temperature was 350^{o}C.

Two chromatographic runs were necessary for unambiguous detection and quantitation of each target analyte (Table 3.2). The enantiomers of *cis*-and *trans*-chlordane and PCB 174 were most efficiently separated from each other and from other analytes using column program B;

these analyses were on the GC with the ECD detector so as to allow simultaneous runs for other analytes with the GC-MS system. SIM ions for GC-MS detection were generally 2 isomer peaks of the parent ion chlorine isotope cluster. The quality of chromatography was assessed by measurement of the GC resolution between each enantiomer of a pair using the mixture of analyte standards. Table 2 gives mean resolution values as well as their standard errors, where here R = 1.5 represents approximate baseline resolution of enantiomers. The lowest resolution was with the PCBs; the lowest of these was PCB 174 with R = 0.78, which resulted in separation of about the top 1/2 of the 2 enantiomer peaks. PCB 174 was actually observed on both chromatographic programs – it apparently has ions in common with some of the SIM ions used in the MS runs – but its enantiomers separate from each other best on program B, where the ECD detector was used. In addition to enantiomer separation, the use of 2 chromatographic conditions resulted in complete separation of the individual analytes from each other, with one minor exception: there was a small overlap between the second eluting enantiomers of *cis*- and *trans*-chlordane. The resolution between these 2 peaks was about 1.0, but this overlap did not significantly interfere with peak area measurements. It was realized after most of the analyses were completed that fipronil sulfone, the most likely metabolite of fipronil, should have been analyzed for. So, separate GC-MS runs with selected ions for the sulfone were made using program A. These ions were different from any used for other program A analytes (Table 3.2), so only fipronil sulfone was detected.

Calculation of concentrations of target analytes in the fish extracts was based on the GC peak area (or sum of enantiomer areas) compared to the peak areas of each analyte in a mixture of standards of all analytes run on the same day. Typically, three mixed standards at a concentration of 1 mg/L of each analyte were analyzed with a batch of 10 fish samples. These

extract concentrations were corrected by comparison to the recovery of the surrogate standard (PCB 65), 10 μg of which had been added to each whole fish carcass just before extraction; recovery of the surrogate standard ranged from 35 to 77%. Detection limits at three times the signal to noise ratio ranged from 30 ng/g for fipronil to 3 ng/g for *o,p'*-DDD.

Enantiomer fraction (EF) and enantiomer ratio (ER) values (35) for each analyte were calculated from concentration data using

$$(1) \quad EF = ER / (1 + ER) = 1 / (1 + 1/ ER)$$

Even though elution orders are known as determined by spiking each racemic standard with one of its pure enantiomers, EF values are calculated as first peak over sum of both peaks for all analytes to avoid confusion, being that the first eluting enantiomer is (+) in five of the chiral analytes and (-) in the other five (Table 3.2). Notice that EF values for standards were all near racemic (between 0.49 for fipronil and 0.51 for *o,p'*-DDD) except for *o,p'*-DDT, which is 0.48 (Table 3.2). This deviation has been observed in earlier research (36). Often, *o,p*'-DDT and *p,p*'-DDT standards contain small amounts of *o,p*'-DDD and *p,p*'-DDD respectively, perhaps because of degradation of the DDT in storage. If this degradation is biological in nature, EF values would deviate from the nominal racemic value of 0.50. Thermal degradation in the GC system would not result in a change in EF. At any rate, these ER and EF values were taken as baseline values for the analyte standards.

Data analysis

Growth rates were determined by fitting all fish weight data to an exponential model (ln fish weight = $a + bt$; where a is a constant, b is the growth rate, and t is time in days) (32). As growth dilution can cause differences in concentration between individual fish, all concentrations

were corrected for growth by multiplying the fish concentrations by a factor of $(1 + bt)$, where b is the growth rate. Depuration rate (k_d) constants were determined by fitting the depuration data to a first order decay curve (ln concentration = $a + k_d t$; where a is a constant and t is time in days) (32). Half-life ($t_{1/2}$) values were calculated using $\ln 2/k_d$. Steady state biomagnification factors (BMF_{ss}) were predicted from the equation $BMF = C_{fish}/C_{food}$ where C_{fish} is the average concentration at steady state in the fish and C_{food} is the average concentration in the food, normalized to lipid content. Steady state was assumed only when a significant increase in fish concentrations was not observed over three consecutive time intervals and did not increase thereafter (33). If steady state was not detected, BMFs were calculated from the equation $BMF = \alpha F/k_d$ where absorption efficiency (α) was determined by fitting the data to the integrated form of the following kinetic rate equation for constant dietary exposure using iterative nonlinear regression (32)

$$(2) \qquad C_{fish} = (\alpha F C_{food}/k_d) \times [1 - \exp(-k_d t)]$$

where F is the feeding rate ($F = 0.015$ g food/g of fish/d, lipid normalized), C_{fish} is the concentration in the fish (lipid normalized), C_{food} is the concentration in the food (lipid normalized), and t is time (d).

Differences between whole body and liver growth rate constants among treatments were examined by testing the homogeneity of slopes in an analysis of covariance. Student's t test was used to compare growth rate constants at the 0.05 level of significance. Tukey's honestly significant difference test ($p < 0.05$) was used to compare percent lipid and liver somatic indices of treatments to control fish (Systat, Ver 9, SPSS, Chicago, IL).

Biotransformation of each compound was examined using two methods. The first compared the $t_{½}$s of the compounds of interest with those of 16 known recalcitrant PCBs in

juvenile rainbow trout (32). In this study, the 16 recalcitrant PCB congeners examined represent congeners with maximum chlorine substitution in the *meta* and *para* positions of the biphenyl rings. Therefore, these congeners should have minimal biotransformation and the slowest elimination of all the PCB congeners in fish (37). If compounds fall below the log K_{ow} to half-life relationship (32-34), biotransformation is suggested as there is likely no other elimination pathway for the compound. Furthermore, the difference in the half-lives determined experimentally to that of the recalcitrant model allows for calculation of a quantitative rate of biotransformation (32 -34). Biotransformation was also assessed by comparing EFs in fish among contaminants to EFs in food and standards with an analysis of variance by a Tukey's aposteriori test using Systat ($\alpha = 0.05$).

Results and Discussion

Fish health and effects

Exposure to fipronil and organochlorines indicated no significant differences in lipid percentages or liver somatic indices on any day of the two treatments in comparison to control fish (Table 3.3). Although, it was observed that liver somatic indices declined over the course of the experiment in each, which is similar to previous experiments (32-33). No significant differences were found in liver growth rates among treatments; however, there was a significant decrease in the whole fish growth rate for the MIX treatment in comparison to control treatment. This reduced fish growth rate was not expected as coloration of MIX treatment fish was consistent with control fish and there was no mortality. Furthermore, there was no reduced growth rate in the FIP treatment in comparison to control treatment indicating this compound was not responsible for this growth effect. Reduced growth was not observed in previous

experiments when rainbow trout were exposed to a greater number of organochlorine compounds than in our study (33), suggesting that this reduced growth is not likely a result of a toxicological effect. Because fish size and lipid content increased over the course of the experiment, concentrations of each analyte were growth and lipid corrected, accordingly.

Bioaccumulation parameters

All compounds were detected in treated fish after 4 days of exposure to the spiked food and accumulated rapidly during the uptake phase of the experiment (Figure 3.1). Only fipronil in both treatments and α-HCH appeared to reach steady state by day eight of the experiment. For the remaining compounds, concentrations increased throughout the uptake portion of the experiment (Figure 3.1). None of the compounds were detected in control fish on any collection day.

The most rapid depuration rate was observed for fipronil, resulting in a half-life of 0.61 d and 0.56 d in the FIP and MIX treatments, respectively (Table 3.4); and was not detected after day 34 in either treatment. There are no similar studies with which to compare our dietary depuration values for fipronil in fish; however, in an aqueous exposure, fipronil was completely (>96%) eliminated after a 14 day depuration in bluegill indicating an estimated half-life of 7 d (1). Thus, it would seem that fipronil is more persistent in fish exposed via the water, which may be due to the fact that greater concentrations are generally accumulated from water for hydrophobic chemicals and therefore result in longer depuration (19). Of the OCs, α-HCH had the most rapid depuration rate resulting in a half-life of 3.85 d (Table 3.4) and was not detected after day 36. This depuration is similar to bioaccumulation experiments of guppies and zebrafish, where rapid elimination (half-lives of 2 - 4 days) of α-HCH was found (38-39), but

approximately 10 days lower than found in a similar experiment using larger sized rainbow trout (half-life of 13 d) (29). The depuration rates of the remaining OC compounds were considerably lower resulting in half-lives ranging from 26.65 d for HEPX and *p,p*'-DDT to 81.53 d for PCB 174 (Table 3.4). The variability in the half-lives is likely due to a number of variables such as biotransformation (discussed later), and varying log K_{ow} values of the compounds. Generally, half-lives of hydrophobic compounds increase with log K_{ow} to a maximum half-life with a log K_{ow} near 7.0 (32). In this study, most of the compounds adhered to this increasing half-life to log K_{ow} relationship with the exception of fipronil, which had a considerably lower half-life in comparison to α-HCH, both of which are similar in log K_{ow}. This rapid depuration of fipronil is likely due to additional elimination pathways of this compound in the fish, such as biotransformation (discussed below).

Absorption efficiencies in this experiment are similar to other studies for organochlorines and PCBs. Absorption of contaminants into fish tissue from food is an important mechanism of bioaccumulation for contaminants with log K_{ow} values ≥ 4 (33). In this study, the absorption efficiency ranged from 42% (*o,p*'-DDD) to 71% (HEPX) for the OC compounds, with the exception being the DDTs (Table 3.4). The elevated absorption efficiency for the DDT compounds (Table 3.4) is likely a result of the lower concentrations detected in the food (Table 3.1). It is likely that the DDTs were broken down biologically in the food because concentrations were not analyzed for each analyte until after the uptake phase of the experiment. As a result, a constant dietary exposure at these concentrations in the food for the DDTs would need an elevated absorption efficiency to reach the concentrations we detected in the fish. With the exception of the DDTs, absorption efficiencies for the OCs found here were similar to other studies in rainbow trout for PCBs and OCs (32-34). The reduced absorption efficiencies for

fipronil in both treatments (Table 3.4) are consistent with previous studies showing less hydrophobic chemicals having smaller absorption efficiencies (32-33). However, fipronil and α-HCH have essentially the log K_{ow} value (Table 3.4), yet fipronil's absorption efficiency is about twice as low as α-HCH indicating that it is either being biotransformed or eliminated before being absorbed into the fish tissue.

For the majority of the OCs, biomagnification within aquatic food webs would occur, based on BMFs > 1. BMF_{calc} values derived from absorption efficiencies were all greater than one, except for fipronil (0.02) and α-HCH (0.24), ranging from 2.4 for *o,p*'-DDD to 9.9 for *p,p*'-DDT (Table 3.4). Due to the unexpected high absorption efficiencies of the DDTs likely resulting in inaccurate BMF_{calc} values, a second set of BMFs were determined assuming an absorption efficiency of 50% (BMF_{equil}), which is typically observed in similar studies with OCs (32-33). This resulted in good agreement with BMF_{calc} values for the other OC compounds (Table 3.4) and most likely represents the true approximation of the BMF potential for the DDT compounds. In addition, BMF values calculated at steady state (BMF_{ss}) for fipronil and α-HCH were in agreement with our other BMF methods indicating that these compounds would not biomagnify in the food web (Table 3.4). This is in contrast to field studies where α-HCH has been shown to biomagnify within Arctic marine food webs (15-17). The extremely low BMF of fipronil (Table 3.4) would indicate no biomagnification for this compound in this study (BMF < 1), and would again suggest that the fish are bioprocessing this compound. This is evident from the order of magnitude difference in the BMFs of fipronil compared to α-HCH (similar in log K_{ow}) (Table 3.4).

Enantiomer Fractions, Biotransformation, and Metabolite Formation

Calculated enantiomeric fractions (EFs) showed that relative abundance of fipronil enantiomers changed quickly over time in this study (Figure 3.1). After two days, the (-) enantiomer is more prominent indicating a greater enantioselective biotransformation rate of the (+) enantiomer. This EF trend continued throughout the uptake phase of the experiment before a possible slight increase in EF during the depuration phase, perhaps due to variable metabolism of individual organisms. It was observed that fipronil EFs in both treatments showed selective biotransformation of the (-) enantiomer, and were significantly different compared to EFs of fipronil in food or standards on day 16 for the FIP treatment and day 4 for the MIX treatment (ANOVA, $p < 0.05$).

The detection of any metabolites in fipronil exposed fish would confirm that biotransformation was occurring throughout the experiment. Analysis for its known metabolite from rat studies, the oxidation product fipronil sulfone (12, 14), indicated the presence of this compound within the fish carcass tissue (Figure 3.1), and followed similar trends in both treatments. It was detected concurrently with the parent compound starting on day two, and at higher concentrations than fipronil throughout the study. From this data, we were able to establish a depuration half-life of roughly two days for fipronil sulfone indicating a slightly greater persistence in the fish compared to fipronil. It should be noted that low concentrations of fipronil sulfone were detected in the food exposed to rainbow trout (Table 3.1), a result of its presence in the standard used to spike the food. However, concentrations of fipronil sulfone detected in the food were approximately 35 times lower than fipronil. The BMF of fipronil sulfone (4.8 to 7.2) calculated from our fish food and fish tissue concentrations (BMF_{ss}) is

unlikely for this low log K_{ow} compound that is similar in persistence to fipronil, which has a calculated BMF of approximately 0.05. A BMF of this magnitude means fipronil sulfone would biomagnify to the extent of some of the higher chlorinated PCBs (33), such as PCB 174 in this study. However, fipronil sulfone's rapid half-life compared to PCB 174 clearly distinguishes that this is not the case. Therefore, the high concentrations of fipronil sulfone found in our exposed fish are the direct result of biotransformation of fipronil and do not come from the spiked food.

Unlike the EFs found for fipronil, chiral signatures for the majority of the OCs were racemic over the course of the experiment. The EFs of PCB 174, α-HCH, and HEPX were consistently racemic throughout the experiment, although there was some slight variability in data, indicating metabolism was not enantiomer-specific for these compounds (Figure 3.1). Furthermore, there was no significant difference in EFs of fish on any sampling day when compared to EFs of food and analyte standards. This is consistent with previous research showing that α-HCH was not biotransformed enantioselectively by rainbow trout (29), and that near racemic levels of PCB 174 were detected in carp and suckers (40). On the other hand, our results counter the findings of non-racemic levels of α-HCH and HEPX found in marine mammals and seals, respectively (15, 41-42).

Likewise, the EFs of *o,p'*-DDT and *o,p'*-DDD in fish were not significantly different than food EFs on any sampling day indicating non-selective metabolism (Figure 3.1). However, significant differences occurred on several sampling days when compared to the analyte standard EFs. The EFs of the food deviated from the EFs of the standards in these and other compounds in this study, as a likely result of the biological breakdown of these compounds over time. Microbially, this degradation process can occur rapidly for some of these compounds (36), and

likely proceeds in a stereo-specific manner as suggested by previous research (43-45). For this reason, we chose to compare the EFs of the OC analytes in the fish to those in the food (see Figure 3.1), as this likely represents the enantiomeric composition of the chemical the fish were exposed to. It is possible that the fish may be biotransfoming these compounds (α-HCH, HEPX, PCB 174, *o,p'*-DDT, *o,p'*-DDD) nonenantioselectively, as has been observed in the biotransformation of *o,p'*-DDT by plants (36). However, this is unclear due to the fact that we did not analyze for degradation products of these compounds or they were already included within this study (e.g., *o,p'*-DDD). Moreover, because EFs did not change for neither *o,p'*-DDT nor its degradation product (*o,p'*-DDD) compared to food EFs, this would suggest that no biotransformation and/or bioformation of this degradation product occurred in the fish. These results are in contrast to the unequal concentrations of *o,p'*-DDD enantiomers that have been found previously in fish (46).

For PCB 84 and 132, there appeared to be some stereo-selective biotransformation, in particular for the case of PCB 84. The EFs of PCB 84 were racemic throughout the uptake phase of the experiment; however, EFs increased significantly starting on day 36, and continued in this trend throughout the duration of the experiment (Figure 3.1). These results indicate that the fish were selectively biotransforming the (+) enantiomer of PCB 84, and is consistent with the enantiomer-specific (+) distribution of this congener in mice (47). In the case of PCB 132, there were no significant differences with EFs in fish compared to those in food throughout the study; however, there was a trend of decreasing EF, which was statistically different on the last sampling day (day 128) (Figure 3.1). It is possible that there is a lag time before subtle metabolic differences of enantiomers can be seen in EFs, which is supported by a similar study in that enantiomer fractions deviated greater from racemic as depuration time increased (29).

Whether the fish were enantioselectively biotransforming PCB 132 is hard to establish, but is supported by the non-racemic composition of this congener in bivalves; however racemic quantities were detected in other biota (40). Moreover, biotransformation of these compounds is consistent with previous research concerning PCB metabolism. For fish to metabolize a PCB congener via cytochrome enzymes, it is believed that adjacent *ortho, meta* (CYP1A) or *meta, para* (CYP2B) positions on the biphenyl ring not be substituted with chlorine atoms (48-49). Both congeners (PCB 84, 132) have a pair of vicinal hydrogen atoms in the *ortho,meta* positions, with PCB 84 having two pairs of vicinal hydrogen atoms in the *meta, para* positions compared to one for PCB 132. Therefore, the likelihood of metabolism would be greater for PCB 84 compared to PCB 132, which is supported by the enantioselective bioprocessing of these congeners in this study.

The changes in EFs shown for fipronil and PCB 84 (perhaps for PCB 132 if the experiment was carried out for a longer time period) are most likely due to biotransformation by the rainbow trout. Enantioselective uptake is unlikely because the transfer from GI tract into the body through mixed micelle vesicles for hydrophobic compounds is a passive transport process that is not considered to be enantioselective (50-52). If uptake was enantioselective, EFs would have deviated from racemic during this phase of the experiment; however, this was not apparent for the OCs. In contrast, fipronil did deviate from racemic during the uptake phase, but this was clearly a result of the biotransformation of this compound to its sulfone metabolite throughout the study (Figure 3.1), and was predicted by the non-racemic EFs. It is possible that bacteria in the GI tract could metabolize the compounds (29), thus altering the EFs before they are absorbed into the body. A previous study that was conducted in a similar manner showed that analyte EFs were altered in the liver, the organ where most biotransformation occurs, before they entered the

GI tract, thus likely ruling out this possibility (29). Likewise, elimination of these compounds, such as excretion through feces or the gills, is also considered a passive process and is not expected to be stereo-specific (52-54). Therefore, the observed EFs suggest that enantioselective metabolism was taking place, which started early in the case of fipronil but after the uptake phase for PCB 84, and proceeded in such a way that residues were non-racemic throughout the remainder of the experiment for these two compounds.

The majority of the chemicals analyzed fell on the same log K_{ow} to half-life relationship developed from recalcitrant PCBs (32) (Figure 2.3). No observed differences between the K_{ow}-half-life regression relationship and our experimental half-lives indicated little to no metabolism (negligible biotansformation rates) for PCB 174, *o,p'*-DDT, *o,p'*-DDD, HEPX, and α-HCH (Table 3.4). This is in agreement with these compounds having limited biotransformation through calculated EFs. However, PCB 84, which showed enantioselective biotransformation through non-racemic EFs, adhered to the log K_{ow} – half-life relationship, as well as PCB 132 (Figure 3.2). It is possible that this achiral relationship for assessing biotransformation does not detect subtle differences of enantiomer metabolism *in vivo*, which can only be obtained from chiral analysis. Another factor at play may be temperature, which has already been shown to influence this relationship (33); however, the temperature and other experimental elements in this study were consistent with the Fisk et al. (32) experiment. Additional studies are needed to assess the degree of which this relationship can predict biotransformation in conjunction with altered EFs. It is interesting to note that *p,p'*-DDT fell below the log K_{ow} – half-life relationship, indicating that it may be metabolized to a limited degree in the fish (Figure 3.2). Consequently, it degradation product, *p,p'*-DDD was similarly positioned above the relationship suggesting that any biotransformation of *p,p'*-DDT may have resulted in the formation of *p,p'*-DDD in the fish

leading to its longer half-life. Unfortunately, these compounds are not chiral and we are unable to compare changes in the log K_{ow} – half-life relationship with any altered enantiomeric compositions.

Another consideration is that this log K_{ow} – half-life relationship was developed for PCBs with log K_{ow} values primarily between 5.5 and 8.0 (32). For this study, the relationship was extrapolated down to include compounds with lower log K_{ow} values, even though no previous experimental data for validation were available, which is indicated from the increasing 95% confidence intervals below log K_{ow} of 5 (Figure 3.2). Based on the determined half-life of α-HCH, which has previously been shown to have little to no biotransformation (29), it would seem that this relationship holds true even at these lower log K_{ow} values. Therefore, assuming this relationship is correct at lower log K_{ow} values, we were able to determine fipronil's biotransformation rate, which accounted for approximately 88% of its elimination in both treatments (Table 3.4). Furthermore, this biotransformation was accurately depicted in non-racemic EFs earlier, and strengthens the log K_{ow} – half-life relationship at these lower log K_{ow} values. For fipronil sulfone, there was minimal biotransformation calculated from the log K_{ow} – half-life relationship (Table 3.4), which is consistent with this compound not undergoing further metabolism to an important degree from previous research (7,12,14). As a result, elimination of this compound from fish may serve as a potential source to the environment.

This study shows that the use of chiral compounds can provide insights into biotransformation processes. Through measurement of EFs, we were able to demonstrate the biological metabolism of fipronil and possibly some PCBs (84 and perhaps 132) by fish; although, it is difficult to say for certain that metabolism occurred for the PCBs because no biological metabolites were analyzed for in the fish unlike that for fipronil. However, for the

majority of the OCs there was no indication of enantiomer-specific metabolism. These biotransformation processes would not have been otherwise observed with traditional achiral analysis, and suggests that fish may have a greater ability to metabolize OCs than previously thought. Due to the increasing complexity of current-use pesticides leading to the increasing likelihood of chiral centers, similar studies are warranted in quantifiying biotransformation processes of these compounds. Furthermore, development of the log K_{ow} – half-life relationship at lower log K_{ow} values may serve as a mechanistic tool for predicting biotransformation in fish for a variety of contaminants, such as current-use pesticides.

Acknowledgement

We would like to thank M. Rigglesford for maintenance and culturing of rainbow trout during this experiment. Support for B. Konwick was provided by a National Network for Environmental Management System Fellowship and the Interdisciplinary Toxicology Program at the University of Georgia.

References

(1) U.S. Environmental Protection Agency. *Environmental fate and effects Section 3 registration decision for fipronil: Use on rice seed,* D235912. Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA, 1997.

(2) U.S. Environmental Protection Agency. *Fipronil environmental fate and ecological effects assessment and characterization for a Section 3 for broadcast treatment with granular product to control turf insects and fire ants* (addendum). Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC, USA, 2001.

(3) U.S. Environmental Protection Agency. *New pesticide fact sheet*. EPA 737-F-96-005. Office of Pesticide Programs, Washington, DC, USA, 1996.

(4) Schlenk, D.; Huggett, D.B.; Allgood, J.; Bennett, E.; Rimoldi, J.; Beeler, A.B.; Block, D.; Holder, A.W.; Hovinga, R.; Bedient, P. Toxicity of fipronil and its degradation products to *Procambarus* sp.: Field and laboratory studies. *Arch. Environ. Contam.Toxicol.* **2001**, 41, 325-332.

(5) Cole, L.M.; Nicholson, R.A.; Casida, J.E. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic. Biochem. Physiol.* **1993**, 46, 47-54.

(6) Gant, D.B.; Chalmers, A.E.; Wolff, M.A.; Hoffman, H.B.; Bushey, D.F. Fipronil: action at the GABA receptor. *Rev. Toxicol.* **1998**, 2, 147-156.

(7) Hainzl, D.; Cole, L.M.; Casida, J.E. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* **1998**, 11, 1529-1535.

(8) Hosie, A.M.; Baylis, H.A.; Buckingham S.D.; Sattelle D.B. Actions of the insecticide fipronil on dieldrin sensitive and resistant GABA receptors of *Drosophila melanogaster*. *Br. J. Pharmacol.* **1995**, 115, 909-912.

(9) Overmyer, J.P.; Mason, B.N.; Armbrust, K.L. Acute toxicity of imidacloprid and fipronil to a non-target aquatic insect, *Simulium vittatum* zetterstedt cytospecies IS-7. *Bull. Environ. Contam. Toxicol.* **2005**, 74, in press.

(10) Demcheck, D.K.; Skrobialowski, S.C. *Fipronil and degradation products in the rice-producing areas of the Mermentau River Basin, Louisiana, February-September 2000*. U.S. Geological Survey Fact Sheet, FS-010-03, Reston, VA, 2003.

(11) Sandstrom, M.W.; Madsen, J.E. *Abstracts*, 24th SETAC Annual Meeting. Austin, Texas, November 8-13, 2003.

(12) Hainzl, D.; Casida, J.E. Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity. *Proc. Natl. Acad. Sci.* **1996**, 93, 12764-12767.

(13) Walse, S.S.; Morgan, S.L.; Kong, L.; Ferry, J.L. Role of dissolved organic matter, nitrate, and bicarbonate in the photolysis of aqueous fipronil. *Environ. Sci. Technol.* **2004**, 38, 3908-3915.

(14) Roberts, T.R.; Hutson, D.H. *Metabolic Pathways of Agrochemicals Part 2: Insecticides and Fungicides*. The Royal Society of Chemistry, Cambridge, UK, 1999.

(15) Hoekstra, P.F.; O'Hara, T.M.; Karlsson, H.; Solomon, K.R.; Muir, D.C.G. Enantiomer-specific biomagnification of α-hexachlorocyclohexane and selected chiral chlordane-related compounds within an Arctic marine food web. *Environ. Toxicol. Chem.* **2003**, 22, 2482-2491.

(16) Hop, H.; Borgå, K.; Gabrielsen, G.W.; Kleivane, L.; Skaare, J.U. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. *Environ. Sci. Technol.* **2002**, 36, 2589-2597.

(17) Moisey, J.; Fisk, A.T.; Hobson, K.A.; Norstrom, R.J. Hexachlorocyclohexane (HCH) isomers and chiral signatures of α-HCH in the Arctic marine food web of the Northwater Polynya. *Environ. Sci. Technol.* **2001**, 35, 1920-1927.

(18) Nowell, L.H.; Capel, P.D.; Dileanis, P.D. *Pesticides in Stream Sediment and Aquatic Biota: Distribution, Trends, and Governing Factors.* CRC Press, Boca Raton, FL, 2001.

(19) Borgå, K.; Fisk, A.T.; Hoekstra, P.F.; Muir, D.C.G. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. *Environ. Toxicol. Chem.* **2004**, 23, 2367-2385.

(20) Mackay, D.; Fraser, A. Bioaccumulation of persistent organic chemicals: mechanisms and models. *Environ. Pollut.* **2000**, 110, 375-391.

(21) Stegeman, J.J.; Kloepper-Sams, P.J. Cytochrome P-450 isozymes and monooxygenase activity in aquatic animals. *Environ. Health Perspect.* **1987**, 71, 87-95.

(22) Kleinow, K.M.; Melancon, M.J.; Lech, J.J. Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. *Environ. Health Perspect.* **1987**, 71, 105-119.

(23) Thomann, R.V. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci. Technol.* **1989**, 23, 699-707.

(24) Gobas, F.A.P.C. A model for predicting the bioaccumulation of hydrophobic organic compounds in aquatic food-webs: Application to Lake Ontario. *Ecol. Modell.* **1993**, 69, 1-17.

(25) Williams, A. Opportunities for chiral agrochemicals. *Pestic. Sci.* **1996**, 46, 3-9.

(26) Kaiser, K.L.E. Optical activity of polychlorinated biphenyls. *Environ. Pollut.* **1974**, 7, 93-101.

(27) Garrison, A.W.; Schmitt, P.; Martens, D.; Kettrup, A. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ. Sci. Technol.* **1996**, 30, 2449-2455.

(28) Vetter, W.; Smalling, K.L.; Maruya, K.A. Interpreting nonracemic residues of chiral organochlorines using naturally contaminated fish. *Environ. Sci. Technol.* **2001**, 35, 4444-4448.

(29) Wong, C.S.; Lau, F.; Clark, M.; Mabury, S.A.; Muir, D.C.G. Rainbow trout (*Oncorhynchus mykiss*) can eliminate chiral organochlorine compounds enantioselectively. *Environ. Sci. Technol.* **2002**, 36, 1257-1262.

(30) Fisk, A.T.; Holst, M.; Hobson, K.A.; Duffe, J.; Moisey, J.; Norstrom, R.J. Persistent organochlorine contaminants and enantiomeric signatures of chiral pollutants in ringed seals (*Phoca hispida*) collected on the east and west side of the Northwater Polynya, Canadian Arctic. *Arch. Environ. Contam. Toxicol.* **2002**, 42, 118-126.

(31) Fisk, A.T.; Moisey, J.; Hobson, K.A.; Karnovsky, N.J.; Norstrom, R.J. Chlordane components and metabolites in seven species of Arctic seabirds from the Northwater Polynya: relationships with stable isotopes of nitrogen and enantiomeric fractions of chiral components. *Environ. Pollut.* **2001**, 113, 225-238.

(32) Fisk, A.T.; Norstrom, R.J.; Cymbalisty, C.D.; Muir, D.C.G. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ. Toxicol. Chem.* **1998**, 17, 951-961.

(33) Buckman, A.H.; Brown, S.B.; Hoekstra, P.F.; Solomon, K.R.; Fisk, A.T. Toxicokinetics of three polychlorinated biphenyl technical mixtures in rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **2004**, 23, 1725-1736.

(34) Fisk, A.T.; Tomy, G.T.; Cymbalisty, C.D.; Muir, D.C.G. Dietary accumulation and quantitative structure-activity relationships for depuration and biotransformation of short

(C_{10}), medium (C_{14}), and long (C_{18}) carbon-chain polychlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **2000**, 19, 1508-1516.

(35) Harner, T.; Wiberg, K.; Norstrom, R.J. Enantiomeric fractions are preferred to enantiomer ratios for describing chiral signatures in environmental analysis. *Environ. Sci. Technol.* **2000**, 34, 218-220.

(36) Garrison, A.W.; Nzengung, V.A.; Avants, J.K.; Ellington, J.J.; Jones, W.J.; Rennels, D.; Wolfe, N.L. Phytodegradation of *p,p'*-DDT and the enantiomers of *o,p'*-DDT. *Environ. Sci. Technol.* **2000**, 34, 1663-1670.

(37) Niimi, A.J.; Oliver, B.G. Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). *Can. J. Fish Aquat. Sci.* **1983**, 40, 1388-1394.

(38) Butte, W.; Fox, K.; Zauke, G.-P. Kinetics and bioaccumulation and clearance of isomeric hexachlorocyclohexanes. *Sci. Total Environ*. **1991**, 109/110, 377-382.

(39) Yamato, Y.; Kiyonaga, M.; Watanabe, T. Comparative bioaccumulation and elimination of HCH isomers in short-necked clam (*Venerupis japonica*) and guppy (*Poecilia reticulate*). *Bull. Environ. Contam. Toxicol.* **1983**, 31, 352-359.

(40) Wong, C.S.; Garrison, A.W.; Smith, P.D.; Foreman, W.T. Enantiomeric composition of chiral polychlorinated biphenyl atropisomers in aquatic and riparian biota. *Environ. Sci. Technol.* **2001**, 35, 2448-2454.

(41) Wiberg, K.; Letcher, R.J.; Sandau, C.D.; Norstrom, R.J.; Tysklind, M.; Bidleman, T.F. The enantioselective bioaccumulation of chiral chlordane and α-HCH contaminants in the polar bear food chain. *Environ. Sci. Technol.* **2000**, 34, 2668-2674.

(42) Wiberg, K.; Oehme, M.; Haglund, P.; Karlsson, H.; Olsson, M.; Rappe, C. Enantioselective analysis of organochlorine pesticides in herring and seal from the Swedish marine environment. *Mar. Pollut. Bull.* **1998**, 36, 345-353.

(43) Faller, J.; Huhnerfuss, H.; Konig, W.A.; Krebber, R.; Ludwig, P. Do marine bacteria degrade α-hexachlorocyclohexane stereoselectively? *Environ. Sci. Technol.* **1991**, 25, 676-678.

(44) Zipper, C.; Bunk, M.; Zehnder, A.J.B.; Kohler, H.-P.E. Enantioselective uptake and degradation of the chiral herbicide dichlorprop [(*RS*)-2-(2,4-Dichlorophenoxy)propanoic acid] by *Sphingomonas herbicidovorans* MH. *J. Bacteriol.* **1998**, 180, 3368-3374.

(45) Law, S.A.; Bidleman, T.F.; Martin, M.J.; Ruby, M.V. Evidence of enantioselective degradation of α-hexachlorocyclohexane in groundwater. *Environ. Sci. Technol.* **2004**, 38, 1633-1638.

(46) Garrison, A.W.; Nzengung, V.A.; Avants, J.K.; Ellington, J.; Wolfe, N.L. Determining the environmental enantioselectivity of *o,p'*-DDT and *o,p'*-DDD. In *Organohalogen Compounds*; Proceedings of Dioxin '97, August 25-29, Indianapolis, IN, 1997.

(47) Lehmler, H.-J.; Price, D.J.; Garrison, A.W.; Birge, W.J.; Robertson, L.W. Distribution of PCB 84 enantiomers in C57BL/6 mice. *Fresenius Environ. Bull.* **2003**, 12, 254-260.

(48) Kleinow, K.M.; Haasch, M.L.; Williams, D.E.; Lech, J.J. A comparison of hepatic P450 induction in rat and trout (*Oncorhynchus mykiss*): Delineation of the site of resistance of fish to Phenobarbital-type inducers. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* **1990**, 96, 259-270.

(49) Brown, J.F.J.; Metabolic alterations of PCB residues in aquatic fauna: Distributions of cytochrome P4501A- and P4502B-like activities. *Mar. Environ. Res.* **1992**, 34, 261-266.

(50) Gobas, F.A.P.C.; Wilcockson, J.B.; Russell, R.W.; Haffner, G.D. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ. Sci. Technol.* **1999**, 33, 133-141.

(51) Drouillard, K.G.; Norstrom, R.J. Dietary absorption efficiencies and toxicokinetics of polychlorinated biphenyls in ring doves following exposure to Aroclor® mixtures. *Environ. Toxicol. Chem.* **2000**, 19, 2707-2714.

(52) Landoni, M.F.; Soraci, A.L.; Delatour, P.; Lees, P. Enantioselective behaviour of drugs used in domestic animals; a review. *J. Vet. Pharmacol. Therap.* **1997**, 20, 1-16.

(53) Thomann, R.V. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci. Technol.* **1989**, 23, 699-707.

(54) Barber, M.C. A review and comparison of models for predicting dynamic chemical bioconcentration in fish. *Environ. Toxicol. Chem*. **2003**, 22, 1963-1992.

(55) Walse, S.S.; Pennington, P.L.; Scott, G.I.; Ferry, J.L. The fate of fipronil in modular estuarine mesocosms. *J. Environ. Monit.* **2004**, 6, 58-64.

(56) Hawker, D.W.; Connell, D.W. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol*. **1988**, 22, 382-387.

(57) Mackay, D.; Shiu, W.Y.; Ma, K.C. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals*. Lewis Publishers, Boca Rotan, FL, 1997.

Table 3.1. Concentrations (μg/g wet wt) and Enantiomeric Fractions (EFs) of Fipronil and Organochlorines (mean ± SE) in Control and Treated Food (n = 3).

Treatment	Compound	Concentration in food (μg/g wet wt)	Food EF
Control	All[a]	ND[a]	-
FIP	Fiponil	7.68 ± 0.18	0.497 ± 0.001
	Fipronil sulfone	0.19 ± 0.01	NC
MIX	Fipronil	12.27 ± 0.52	0.500 ± 0.000
	Fipronil sulfone	0.41 ± 0.01	NC
	HEPX	0.71 ± 0.03	0.455 ± 0.008
	o,p'-DDD	0.70 ± 0.03	0.554 ± 0.001
	p,p'-DDD	0.87 ± 0.04	NC
	o,p'-DDT	0.40 ± 0.05	0.510 ± 0.004
	p,p'-DDT	0.42 ± 0.04	NC
	PCB 174	0.92 ± 0.08	0.533 ± 0.001
	PCB 132	0.77 ± 0.06	0.502 ± 0.001
	PCB 84	0.87 ± 0.02	0.502 ± 0.003
	α-HCH	0.87 ± 0.07	0.505 ± 0.002
	trans-chlordane	0.88 ± 0.02	0.495 ± 0.001
	cis-chlordane	0.87 ± 0.02	0.502 ± 0.001

[a] None of the compounds were detected in control food

NC = Not chiral

Table 3.2. Gas Chromatograph Data and Enantiomeric Fraction (EF) Values for the Mixture of Standard Compounds.

Analyte[a]	Column/Detector[c]	Column Program[d]	SIM Ions[f]	Enantiomer Resolution[g]	Resolution SE[h]	OR Signal[i]	ER(mean)[j]	EF(mean)[k]	EF (SE)[l]
α-HCH	GC-MS	A	181,183	1.14	0.081	+	0.97	0.49	0.004
PCB 65[b]	GC-MS	A	290,292	NC		NC			
PCB 84	GC-MS	A	324,326	1.32	0.052	-	0.98	0.49	0.005
Fipronil	GC-MS	A	367,369	2.2	0.07	-	0.95	0.49	0.014
Fipronil Sulfone	GC-MS	A[e]	383,385	NC		NC			
PCB 132	GC-MS	A	360,362	1.49	0.04	-	0.99	0.50	0.00
o,p'-DDD	GC-MS	A	235,237	2.48	0.15	-	1.02	0.51	0.005
HEPX	GC-MS	A	353,355	3.61	0.214	+	0.98	0.49	0.005
p,p'-DDD	GC-MS	A	235,237	NC		NC			
o,p'- DDT	GC-MS	A	235,237	4.8	0.157	+	0.91	0.48	0.005
p,p'-DDT	GC-MS	A	235,237	NC		NC			
trans-chlordane	GC-ECD	B	NA	4.69	0.115	-	1.01	0.50	0.00
cis-chlordane	GC-ECD	B	NA	1.36	0.095	+	0.98	0.49	0.005

PCB 174	GC-ECD	B	NA	0.78	0.054	+	0.99	0.50	0.0

[a] Target analytes in order of elution within column program type

[b] PCB 65 was used as the recovery standard

[c] BGB-172 column with MS or ECD detector

[d] Column A: 150-220°C at 1.5°/min, Column B: 150-220°C at 0.5°/min, Column C: 150-190°C at 0.3°/min, then 190-200°C at 5°/min

[e] Fipronil sulfone was analyzed using program A but with a separate GC run

[f] Ions used for selective ion monitoring

[g] Mean GC resolution (R) between enantiomers, $n = 5$: $R = 2(t^1-t^2)/(w^1+ w^2)$, where t = elution time, w = width at peak base, and 1 and 2 are enantiomer peaks

[h] Standard error of resolution, $n = 5$

[i] Sign of optical rotation of first eluting enantiomer

[j] Mean enantiomer ratio: ER = area peak 1/area peak 2, $n = 5$

[k] Mean enantiomer fraction: EF = area peak 1/sum of areas of both peaks, $n = 5$

[l] Standard error of EF, $n = 5$

NA = not applicable

NC = not chiral

Table 3.3. Growth parameters (mean ± SE) of juvenile rainbow trout exposed to fipronil and organochlorines.

Treatment	Growth rate d^{-1} [a]	Lipid (%) [b]	LSI (%) [c]	Mortality (%)
Control	0.018 ± 0.002 (0.77) A	3.5 ± 0.2	1.66 ± 0.07	0
MIX	0.014 ± 0.001 (0.76) B	3.7 ± 0.2	1.68 ± 0.08	0
FIP	0.015 ± 0.001 (0.78) A,B	3.3 ± 0.3	1.62 ± 0.07	0

[a] Growth rates were calculated using the equation ln weight = $a + b \times$ time (in days), where b is the growth rate (coefficient of determination in parentheses). Significant differences ($p < 0.05$) are indicated by capital letters.

[b] Lipid percentage is the average of all fish in each treatment.

[c] Liver somatic index (LSI) calculated using the equation 100 × liver weight (g) / body weight (g) and is the average of all fish in each treatment.

Table 3.4. Dietary bioaccumulation parameters (± SE) for fipronil and organochlorines using rainbow trout carcass data

Treatment	Compound	Log K_{ow}[a]	Depuration rate (d^{-1})[b]	Biotrans-formation rate (d^{-1})[c]	$t_{1/2}$ (d)[d]	Absorp. Eff. (%)[e]	BMF^{f}_{ss}	BMF^{g}_{calc}	BMF^{h}_{equil}
FIP	Fipronil	4.0	1.144 ± 0.050 (0.99)	1.006	0.61± 0.03	23 ± 2	0.04	0.02	0.04
	Fipronil sulfone	3.7	0.293 ± 0.009 (0.99)	0.064	2.37 ± 0.07	-	4.78	-	-
MIX	Fipronil	4.0	1.230 ± 0.076 (0.99)	1.091	0.56 ± 0.03	28 ± 4	0.05	0.02	0.04
	Fipronil sulfone	3.7	0.374 ± 0.038 (0.92)	0.145	1.85 ± 0.18	-	7.20	-	-
	HEPX	5.4	0.026 ± 0.002 (0.80)	0.003	26.7 ± 2.1	71 ± 5	-	2.6	1.8
	o,p'-DDD	6.1	0.017 ± 0.003 (0.65)	0.004	40.8 ± 7.2	42 ± 3	-	2.4	2.8
	p,p'-DDD	5.5	0.016 ± 0.002 (0.85)	-0.005	43.3 ± 5.4	67 ± 4	-	4.0	3.0
	o,p'-DDT	5.7	0.019 ± 0.004 (0.52)	0.002	36.5 ± 7.7	139 ± 10	-	6.9	2.5
	p,p'-DDT	6.0	0.026 ± 0.006 (0.54)	0.011	26.7 ± 6.2	269 ± 36	-	9.9	1.8
	PCB 174	7.1	0.009 ± 0.002 (0.61)	<0.001	77.0 ± 17.1	54 ± 3	-	6.4	5.9
	PCB 132	6.6	0.012 ± 0.001 (0.78)	0.002	57.8 ± 4.8	69 ± 4	-	5.5	4.0
	PCB 84	6.0	0.017 ± 0.002 (0.84)	0.003	40.8 ± 4.8	57 ± 3	-	3.2	2.8

α-HCH	3.9	0.180 ± 0.035 (0.84)	0.017	3.85 ± 0.75	45 ± 13	0.29	0.24	0.26
trans-chlordane	6.1	0.003 ± 0.003 (0.10)	-	231 ± 231	102 ± 28	-	32.3	15.9
cis-chlordane	5.9	0.004 ± 0.003 (0.11)	-	173.3 ± 130	93 ± 28	-	22.0	11.9

[a] Log K_{ow} values for fipronil and fipronil sulfone taken from (14) and (55) respectively, PCB log K_{ow} values were taken from (56), and remaining log K_{ow} values were selected from (57).

[b] Depuration rate constants (k_ds) were calculated using the model ln concentration = $a + b \times$ time for the 96 day elimination period (coefficient of determination (r^2) for the model is shown in parentheses).

[c] Biotransformation rate = measured depuration rate – minimum depuration rate. Minimum depuration rates = 0.693/half-life (d), where half-lives were determined from the equation log half-life = -3.7 + (1.5 * log K_{ow}) – (0.1-log K_{ow}^2) (32), which assumes no biotransformation.

[d] Half-lives ($t_{1/2}$) were calculated from the equation $t_{1/2} = 0.693/k_d$.

[e] The absorption efficiency (α) was determined by equation 2.

[f] Biomagnification factors at steady state (BMF_{ss}) = C_{fish} (lipid, growth corrected)/C_{food} (lipid corrected).

[g] BMF_{calc} is derived from the equation $BMF = \alpha F/k_d$.

[h] BMF_{equil} calculated assuming α is 0.5.

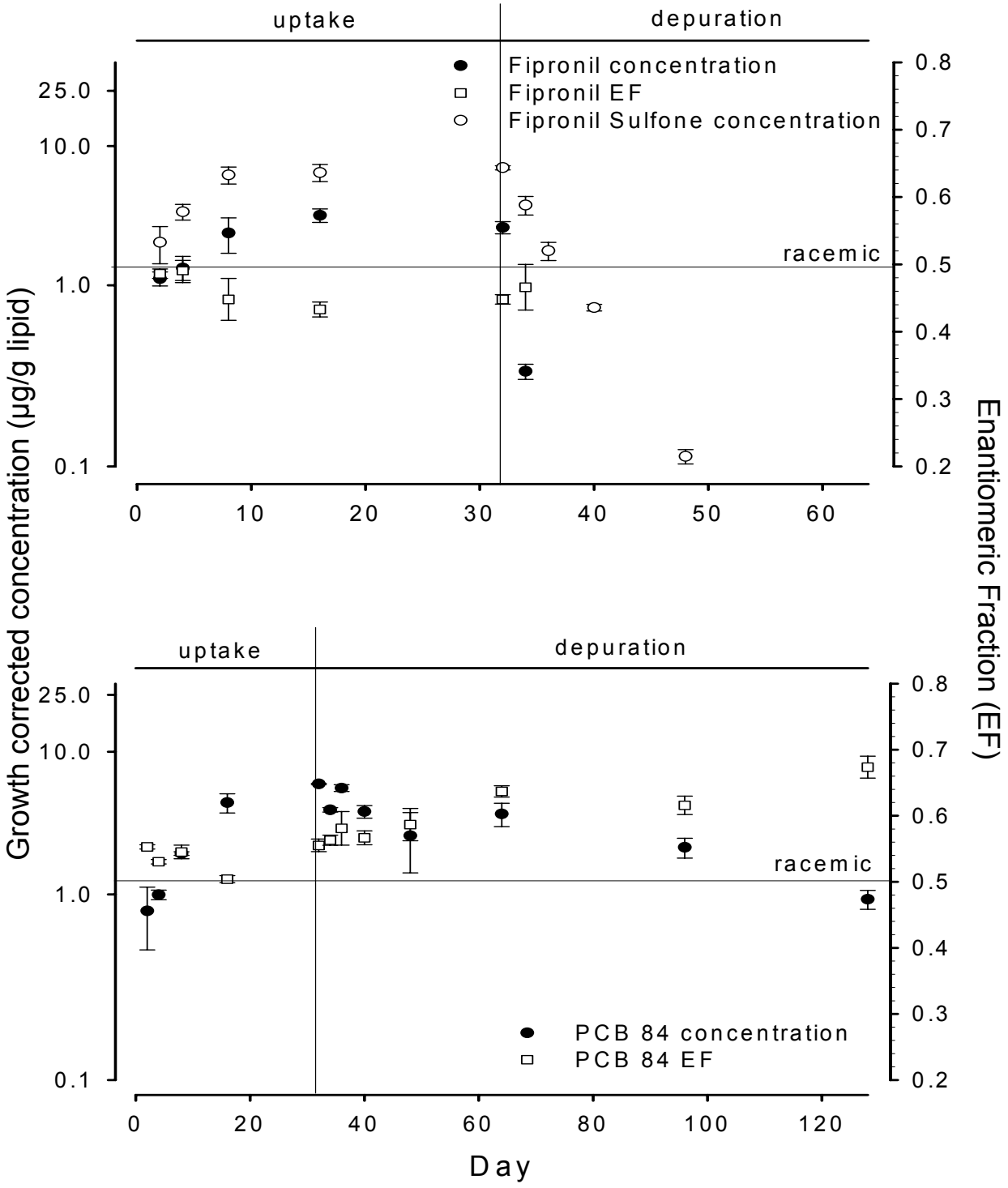

Figure 3.1. Concentrations and enantiomeric fractions (EFs) of fipronil (from FIP treatment), PCB 84, *o,p'*-DDT, and PCB 132 in juvenile rainbow trout over time. Each point represents the mean ± SE (if larger than symbol used) of three fish sampled at that time point.

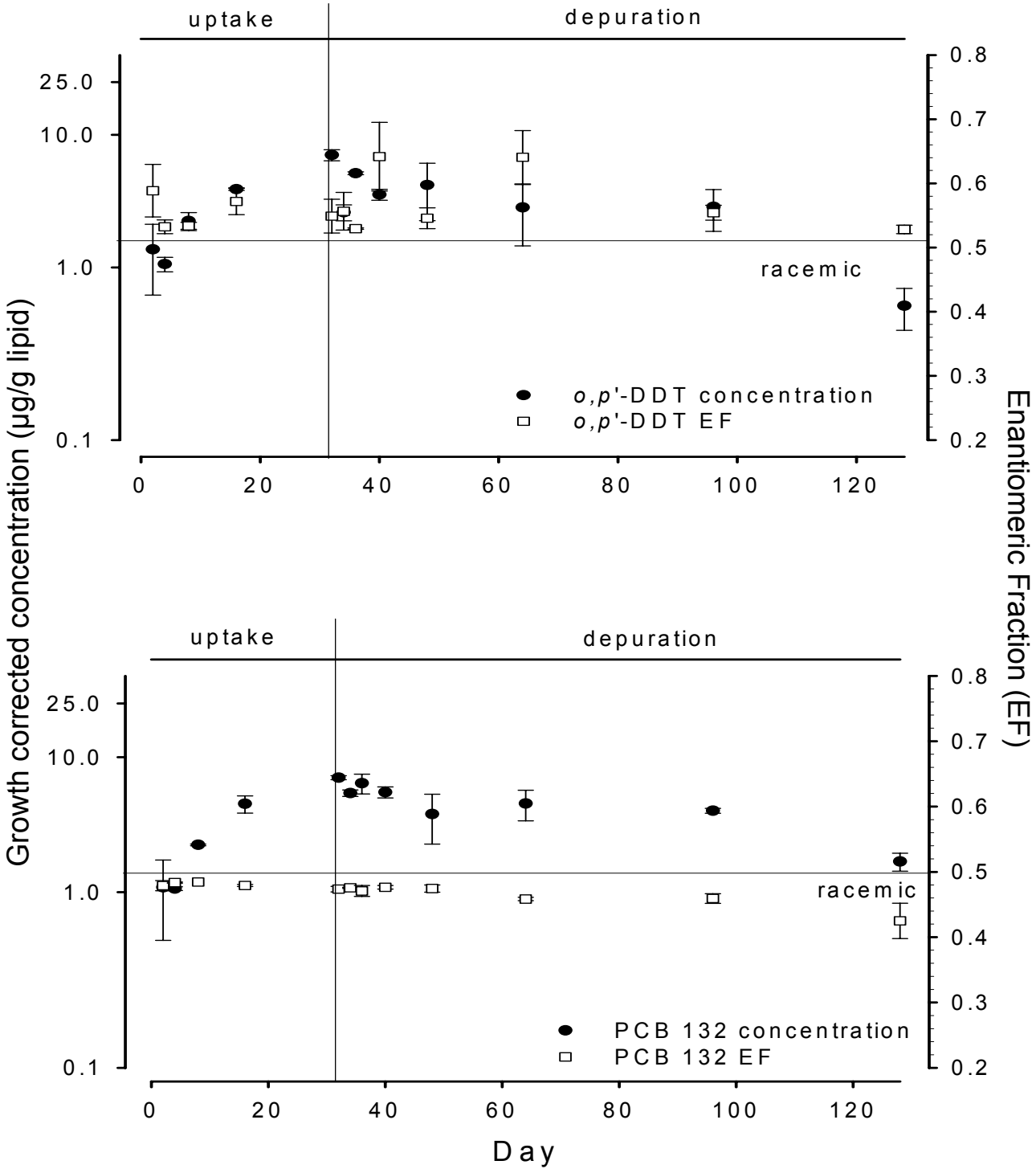

Figure 3.1. (continued) Concentrations and enantiomeric fractions (EFs) of fipronil (from FIP treatment), PCB 84, *o,p'*-DDT, and PCB 132 in juvenile rainbow trout over time. Each point represents the mean ± SE (if larger than symbol used) of three fish sampled at that time point.

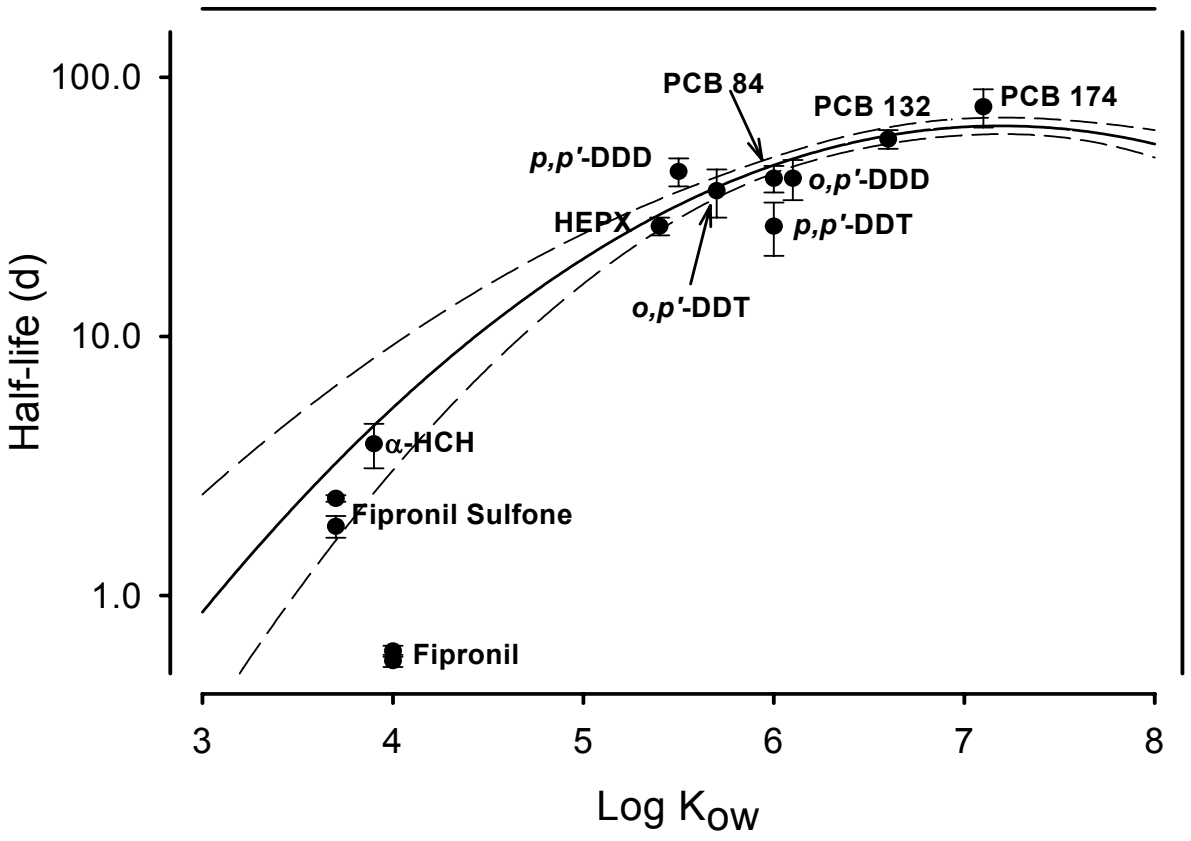

Figure 3.2. Half-life of compounds in juvenile rainbow trout from this study versus log octanol/water partition coefficient (K_{ow}). The quadratic regression (solid line) and 95% confidence intervals (dashed lines) were taken from Fisk et al. (32), which represents a series of recalcitrant PCBs not metabolized by juvenile rainbow trout.

CHAPTER 4

CONCLUDING REMARKS

Protecting water quality and aquatic fauna against xenobiotics, such as pesticides, will be a priority for future generations of water resource managers. Despite the introduction of less persistent pesticides that are usually broken down rapidly by biotic and abiotic reactions, pesticide usage will continue to rise as a result of population growth, resulting in the presence of these chemicals in surface waters along with the threats they pose to ecosystem health. Due to the increasing complexity of newly developed pesticides, many (25%) have the element of chirality (i.e., contain optically active enantiomers) (Williams 1996). A possible management option for the beneficial protection of the environment is to identify the fate and toxicity of individual enantiomers of chiral pesticides. Therefore, instead of the common practice of releasing the racemate (equal concentrations of enantiomers) of a pesticide, an individual enantiomer which is less persistent and toxic to non-target organisms may be marketed and used.

Even though enantiomers of chiral pesticides are alike in their physical-chemical properties, they can behave quite differently in their toxicity, metabolism, and degradation (Garrison et al.1996, Williams 1996). Because current legislation for registration of pesticides rarely considers these differences of individual enantiomers, the resulting approval and use of the racemate may be more harmful to the environment due to an unwanted pollution load. For example, one enantiomer of a pesticide may persist longer in the environment (degraded at a

slower rate) leading to its potential contamination of water resources. In a worse case scenario, this enantiomer may also exhibit greater toxicity to non-target species once entering the aquatic environment. These points underline the importance of determining both the fate and effects of pesticide enantiomers before their registration and use. Likewise, if a pesticide is bioaccumulative, it is of concern to identify the exposure of individual enantiomers within biota. One enantiomer may be more detrimental in sub-lethal effects because of greater accumulated concentrations due to its slower metabolism or elimination. While the U.S. has been lagging in addressing these issues of pesticide chirality, some countries in Europe have revoked registrations of racemates in favor of registration of single enantiomers due to the their unwanted effects and fate (Williams 1996).

The studies contained in this thesis investigated the exposure and toxicity of the current-use pesticide fipronil. In assessing the acute toxicity of fipronil (Chapter 2), significant differences were found between enantiomers; however, the difference was minor [LC50 = 10.3 ± 1.1 μg/L for (+) and 31.9 ± 2.2 μg/L for (-)]. Moreover, the degree to which toxicity is enantiomer-specific in other species is largely unknown. Recent research, though, has suggested that the toxicity of the (+) enantiomer holds true in other invertebrate species (J. Overmyer, personal communication), but whether the toxicity of this enantiomer is consistent in other biota, such as fish, is yet to be determined. An often important point that needs further consideration is whether the sub-lethal effects of fipronil enantiomers hold true at concentrations that are likely to be found in the environment. Because of fipronil's high toxicity to arthropods at environmental levels, further work would be significant in determining whether adverse effects (i.e., both lethal and sub-lethal) can be alleviated with the use of a single or enriched enantiomer concerning this pesticide.

In assessing the bioaccumulation potential of fipronil (Chapter 3), elimination via biotransformation was shown to be the dominant pathway. This was indicated in the non-racemic enantiomeric composition of fipronil in the fish, with a greater biotransformation rate of the (+) enantiomer. Due to the rapid clearance of fipronil, it was determined that no biomagnification of this pesticide would occur in higher trophic species. As a result, if other current-use pesticides are similar in persistence in fish, any changes in the enantiomeric composition will be of little importance because of the short exposure period for biota to the chemical. That is unless one enantiomer is extraordinary more toxic or causes adverse effects to a significant degree. Therefore, due to the persistence and bioaccumulation of organochlorines in fish, determining biotransformation ability of these pollutants is better served for predicting accurate future trends of these compounds. Also, because invertebrates exposed in aqueous solution to fipronil have indicated a high accumulation potential for this pesticide (Chaton et al. 2002, Chaton et al. 2001), it would interesting to determine both the degree of bioaccumulation and biotransformation (through chiral analysis) of fipronil in lower trophic species. Such findings could indicate that lower trophic organisms may have a greater metabolic capacity, contrary to the general notion of increasing biotransformation ability as you increase in trophic level. Furthermore, a study determining bioaccumulation and stereo-specific metabolism in invertebrates may help establish a link for discerning the different toxicity of individual fipronil enantiomers, which would be evident from one enantiomer being metabolized before reaching the target site.

This research provided the first evaluation of the enantiomer-specific toxicity, bioaccumulation, and biotransformation of fipronil. There is considerable more research concerning fipronil's chirality before any production of a single enantiomer formulation is

warranted. However, it highlights the utility of chiral compounds to provide insights into biotransformation and toxicity processes. Additionally, chiral analysis allows you to note the whole picture concerning the fate and toxicity of chiral pesticides instead of just part, which would have been the case in using solely achiral techniques. As was recently noted at a conference concerning chirality, it is time to develop strategies for predictive capability for enantioselectivity so that a science-based approach can be made toward production of single-enantiomer pesticides for alleviating the environment of unnecessary chemicals (Garrison et al. 2004).

References

Chaton PF, Ravanel P, Tissut M, Meyran JC. 2002. Toxicity and bioaccumulation of fipronil in the nontarget arthropodan fauna associated with subalpine mosquito breeding sites. *Ecotoxicol Environ Safety* 52: 8-12.

Chaton PF, Ravanel P, Meyran JC, Tissut M. 2001. The toxicological effects and bioaccumulation of fipronil in larvae of the mosquito *Aedes aegypti* in aqueous medium. *Pestic Biochem Physiol* 69: 183-188.

Garrison AW, Ekman D, Kenneke J, Mazur C. 2004. *Abstracts*, 25th SETAC Annual Meeting, Portland, Oregon, November 14-18.

Garrison AW, Schmitt P, Martens D, Kettrup A. 1996. Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis. *Environ Sci Technol* 30: 2449-2455.

Williams A. 1996. Opportunities for chiral agrochemicals. *Pestic Sci* 46: 3-9.