Bioaccumulation, Biotransformation, and Metabolite Formation of Fipronil and Chiral Legacy Pesticides in Rainbow Trout

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To assess the fate of current-use pesticides, it is important to understand their bioaccumulation and biotransformation by aquatic biota. We examined the dietary accumulation and enantioselective biotransformation of the chiral current-use pesticide fipronil, along with a mixture of selected chiral [α-hexachlorocyclohexane (α-HCH), heptachlor epoxide (HEPX), polychlorinated biphenyls (PCBs) 84, 132, 174, *o,p′*-DDT, and *o,p′*-DDD] and nonchiral (*p,p′*-DDT, *p,p′*-DDD) organochlorine compounds in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish rapidly accumulated all compounds, as measured in the carcass (whole body minus liver and GI tract) during the 32 d uptake phase, which was followed by varying elimination rates of the chemicals (half-lives ($t_{1/2}$s) ranging from 0.6 d for fipronil to 77.0 d for PCB 174) during the 96 d depuration period. No biotransformation was observed for α-HCH, HEPX, PCB 174, *o,p′*-DDT, or *o,p′*-DDD based on consistent enantiomeric fractions (EFs) in the fish and their $t_{1/2}$s falling on a log K_{ow} − log $t_{1/2}$ relationship established for recalcitrant contaminants in fish. *p,p′*-DDT and PCBs 84 and 132 were biotransformed based on the former's $t_{1/2}$ position below the log K_{ow} − log $t_{1/2}$ relationship, and the PCBs change in EF. Fipronil was rapidly biotransformed, based on a change in EF, a $t_{1/2}$ that fell below the log K_{ow} − log $t_{1/2}$ relationship, which accounted for 88% of its elimination, and the rapid formation of fipronil sulfone, a known metabolite. Fipronil sulfone was found to persist longer ($t_{1/2} \sim 2$ d) than its parent compound fipronil ($t_{1/2} \sim 0.6$ d) and needs to be considered in fate studies of fipronil. This research demonstrates the utilities of the log K_{ow} − log $t_{1/2}$ relationship as a mechanistic tool for quantifying biotransformation and of chiral analysis to measure biotransformation in fish.

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Introduction

To assess the potential risk of contaminants, such as current-use pesticides (e.g., fipronil), it is important to understand their accumulation and fate in aquatic biota. However, there have been few studies that have addressed this issue for nonpersistent compounds, likely due to a combination of the low octanol−water partition coefficients (log K_{ow}) and short environmental persistence of these chemicals (*1*−*2*). Furthermore, models that describe bioaccumulation based on the physical-chemical properties of these chemicals may not be accurate. This is because many current-use pesticides are readily biotransformed (*1*−*2*), which if rates are unknown, confounds efforts to use chemical-physical properties to infer bioaccumulation. Unfortunately, methods to estimate biotransformation of contaminants are limited, especially for fish (*3*−*4*). Although bioaccumulation may be minimal for current-use pesticides, it is still important to measure accumulation, assess biotransformation, and track the formation of any metabolites that may have detrimental effects (*5*).

Approximately 25% of current-use pesticides are chiral (*6*), in addition to several legacy pesticides (e.g., *o,p′*-DDT, chlordanes) and some PCBs (*7*). Chiral compounds exist as two nonsuperimposable 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 racemic (±) mixture, containing 50% of each enantiomer, the form in which they are typically released into the environment. Enantiomers have identical physical-chemical properties (*8*); however, relative abundances of enantiomers can change after enzymatic metabolic processes (*9*−*11*). As a result, the enantiomeric composition in biota has been used as a tracer for biotransformation (*9*). For example, nonracemic residues have indicated, for the first time, that fish can biotransform a number of chiral organochlorines (OCs) (*10*−*11*).

Another method for determining rates of biotransformation has been proposed based on a curve-linear relationship developed between log K_{ow} and $t_{1/2}$ for a series of recalcitrant contaminants in juvenile rainbow trout (*12*−*13*). Nonrecalcitrant chemicals, whose $t_{1/2}$ (determined experimentally) fall below this curve-linear relationship, are suggested to be biotransformed, whereas those chemicals that fall on or near this relationship would show little to no biotransformation (*12*−*13*). This model has been used to generate biotransformation rates for polychlorinated alkanes and PCBs in juvenile rainbow trout (*13*−*14*) with potential application to less-persistent chemicals.

Fipronil is a chiral, phenylpyrazole-class insecticide first approved in 1996 for use on a number of crops in the U.S., including rice culture, turf grass management, and residential pest control (*15*−*16*). Fipronil use is expected to increase due to species resistance and restrictions on organophosphate (OP) insecticides (*17*−*18*). Fipronil is more toxic to invertebrates than mammals (*19*) and can impact aquatic environments at low concentrations (*15*, *20*). In addition, fipronil's degradation products, which are suggested to have similar toxic potential (*16*, *19*) and are more environmentally stable (*21*), increase the threat of fipronil to the environment. While fipronil's log K_{ow} value (4.01) (*1*) is in the range of some persistent OCs shown to bioaccumulate in food webs (*22*−*23*), there is little information on its accumulation and biotransformation in aquatic organisms.

To address fipronil bioaccumulation, as well as to test the utility of chiral analysis and the log K_{ow} − log $t_{1/2}$ relationship

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in assessing biotransformation, juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed to fipronil and a series of legacy organochlorines (OCs) incorporated into their diet. The OCs were included to validate the log K_{ow} − log $t_{1/2}$ relationship for this study, for expansion of this relationship to lower log K_{ow} chemicals, and to increase the existing information on the enantioselective biotransformation capacity of fish. A metabolite of fipronil, fipronil sulfone, was also monitored throughout the experiment to further assess biotranformation of the parent compound. To our knowledge, this is the first experiment to determine the toxicokinetics of fipronil, or fipronil sulfone, in fish via dietary exposure and its enantioselective biotransformation for any species.

Materials and Methods

Chemicals and Food Preparation. Fipronil, heptachlor epoxide (HEPX), α-hexachlorocyclohexane (α-HCH), *o,p*′-DDT, *p,p*′-DDT, *o,p*′-DDD, and *p,p*′-DDD were obtained from ChemService (West Chester, PA). 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).

Fipronil (1000 μg/mL in methanol) and the OCs (100 μg/mL in hexane) were added to 1 L of hexane and mixed with 500 g of the commercial trout food (Zeigler, Gardner, PA; 38% protein, 15% lipid, 3% fiber) in a round-bottom flask. The solvents were slowly evaporated to dryness in a rotary evaporator, followed by air-drying the food for 48 h, and then stored in amber jars at 8 °C. Control food was treated in an identical manner but without the addition of the contaminants. The concentrations of fipronil and OCs (Table 1) were determined in spiked and control food by using the technique described below for fish tissue.

Experimental Protocol. Juvenile rainbow trout (Lake Burton Fish Hatchery, GA; initial weights 10.2 ± 0.5 g, mean ± SE) were haphazardly assigned to one of three 800-L fiberglass aquaria (45 fish per tank) with recirculating, dechlorinated tap water chilled to 12 °C and carbon-filtered to remove any contaminant residues in the water. 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 only (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), at 1.5% of the mean weight of the rainbow trout, corrected for weight gain after each sampling day. Three fish were randomly sampled from each treatment on days 2, 4, 8, 16, and 32 of the uptake phase and on 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 liver and GI tract to avoid analytes in the undigested food) and frozen until analysis. Only carcass results were used in calculating bioaccumulation parameters and enantiomer fractions (EFs).

Chemical Analysis. Extraction and cleanup of samples followed established methods for quantifying OCs in fish (*12*). PCB 65 was added to samples as a recovery standard prior to extraction. Tissue samples (whole carcass, except the last sampling day, on which 10−12 g of carcass fillet was extracted due to the large sample size) were freeze-dried and homogenized/extracted in dichloromethane (DCM)/hexane (1:1 by volume) by using a polytron (PowerGen 125, Fisher Scientific). Samples were extracted twice; the extracts were then combined, centrifuged, and evaporated to 10 mL. One mL of the extract was used to determine lipids gravimetrically. Lipids were removed (first 140-mL fraction) from the

TABLE 1. Concentrations and EFs in Food (n = 3), and Contaminant Bioaccumulation Parameters in Rainbow Trout Carcass Following Dietary Exposure[a]

treatment	compound	food concentrated (μg/g wet wt)[b]	food EF	log K_{ow}[c]	depuration rate k_d (d^{-1})[d]	biotransformation rate (d^{-1})[e]	depuration $t_{1/2}$ (d)[f]	absorption efficiency (%)[g]	BMF^h_{ss}	BMF^i_{calcd}	BMF^j_{equil}
FIP	fipronil	7.68 ± 0.18	0.50 ± 0.001	4.0	1.144 ± 0.050 (0.99)	1.006	0.61 ± 0.03	23 ± 2	0.04	0.02	0.04
	fipronil sulfone	0.19 ± 0.01	NC	3.7	0.293 ± 0.009 (0.99)		2.37 ± 0.07		4.78		
MIX	fipronil	12.27 ± 0.52	0.50 ± 0.000	4.0	1.230 ± 0.076 (0.99)	1.091	0.56 ± 0.03	28 ± 4	0.05	0.02	0.04
	fipronil sulfone	0.41 ± 0.01	NC	3.7	0.374 ± 0.038 (0.92)		1.85 ± 0.18		7.20		
	α-HCH	0.87 ± 0.07	0.51 ± 0.002	3.9	0.180 ± 0.035 (0.84)		3.85 ± 0.75	45 ± 13	0.29	0.24	0.26
	HEPX	0.71 ± 0.03	0.46 ± 0.008	5.4	0.026 ± 0.002 (0.80)		26.7 ± 2.1	71 ± 5		2.6	1.8
	o,p′-DDT	0.40 ± 0.05	0.51 ± 0.004	5.7	0.019 ± 0.004 (0.52)		36.5 ± 7.7	139 ± 10		6.9	2.5
	o,p′-DDD	0.70 ± 0.03	0.55 ± 0.001	6.1	0.017 ± 0.003 (0.65)		40.8 ± 7.2	42 ± 3		2.4	2.8
	p,p′-DDT	0.42 ± 0.04	NC	6.0	0.026 ± 0.006 (0.54)	0.011	26.7 ± 6.2	269 ± 36		9.9	1.8
	p,p′-DDD	0.87 ± 0.04	NC	5.5	0.016 ± 0.002 (0.85)	−0.006	43.3 ± 5.4	67 ± 4		4.0	3.0
	PCB 84	0.87 ± 0.02	0.50 ± 0.003	6.0	0.017 ± 0.002 (0.84)		40.8 ± 4.8	57 ± 3		3.2	2.8
	PCB 132	0.77 ± 0.06	0.50 ± 0.001	6.6	0.012 ± 0.001 (0.78)		57.8 ± 4.8	69 ± 4		5.5	4.0
	PCB 174	0.92 ± 0.08	0.53 ± 0.001	7.1	0.009 ± 0.002 (0.61)		77.0 ± 17.1	54 ± 3		6.4	5.9

[a] Values missing indicate the parameter was not calculable. [b] None of the compounds were detected in control food. [c] Log K_{ow} values for fipronil and fipronil sulfone taken from (*1*) and (*44*), respectively, PCB log K_{ow} values were taken from (*45*), and remaining log K_{ow} values were selected from (*46*). [d] 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). [e] 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) (*12*), which assumes no biotransformation. A biotransformation rate was only calculated when the SE of the $t_{1/2}$ did not overlap the 95% CI of the regression. [f] Half-lives ($t_{1/2}$) were calculated from the equation $t_{1/2} = 0.693/k_d$. [g] The absorption efficiency (α) was determined by eq 2. [h] Biomagnification factors at steady state (BMF_{ss}) = C_{fish} (lipid, growth corrected)/C_{food} (lipid corrected). [i] BMF_{calc} is derived from the equation BMF = $\alpha F/k_d$. [j] BMF_{equil} calculated from BMF = $\alpha F/k_d$, assuming α is 0.5.

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remaining extract by using gel permeation chromatography (GPC) columns packed with 60 g (dry weight) of 200−400 mesh Bio-Beads S−X3 (Bio-Rad Laboratories, Hercules, CA) (*12*). The GPC eluate was reduced to 1 mL prior to analysis by GC-MS.

All analytes were quantified by a Hewlett-Packard (HP) 5973 mass spectrometer (MS) linked to a 6890 gas chromatograph (GC) equipped with a chiral column, with the exception of PCB 174, which was quantified by an electron capture detector (ECD) coupled to a HP 5890 GC. The GC column, in both cases, was a 30-m BGB 172 (BGB Analytik AG, Switzerland) containing a chiral phase composed of 20% *tert*-butyldimethylsilylated-β-cyclodextrin. All GC-MS detection was by selected ion monitoring (SIM); ions were generally 2 isomer peaks of the parent ion chlorine isotope cluster. All extract concentrations were corrected to PCB 65 recovery, which averaged 57 $\pm$ 5% (mean $\pm$ SE) over all samples. Detection levels (three times the signal-to-noise ratio) ranged from 30 ng/g for fipronil to 3 ng/g for *o,p′*-DDD based on fish sample weight.

EFs (*24*) for each chiral analyte were calculated using:

$$EF = [E_1]/([E_1] + [E_2]) \quad (1)$$

where $[E_1]$ and $[E_2]$ are the concentrations of the first and second eluting enantiomers on a given chiral column. Even though elution orders were determined by spiking each racemic standard with one of its pure enantiomers, EF values were calculated as the first peak over the sum of both peaks for all analytes to avoid confusion. The first eluting enantiomer was (+) for α −HCH, HEPX, *o,p′*-DDT, PCB 174, and fipronil and (−) for PCB 132, 84, and *o,p′*-DDD. Mean EF values for standards were all near racemic (between 0.48 for *o,p′*-DDT and 0.51 for *o,p′*-DDD).

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) (*12*). As growth dilution can significantly reduce concentrations and estimated elimination rates (*12*), all concentrations were corrected for growth by multiplying the fish concentrations by a factor of $(1 + bt)$. Depuration rate (k_d) constants were determined by fitting the concentration data obtained during depuration to a first-order decay curve (ln concentration $= a + k_d t$, where a is a constant, and t is time in days). 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 assuming steady state in the fish, and C_{food} is the average concentration in the food; both concentrations were calculated based on lipid content. Steady state was assumed only when concentrations did not continue to increase over three consecutive sampling intervals in the fish. If steady state was not reached, 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 (*12*):

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

where F is the feeding rate ($F = 0.015$ g food/g of fish/d, lipid basis), C_{fish} is the concentration in the fish (lipid basis), C_{food} is the concentration in the food (lipid basis), 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. Tukey's honestly significant difference (HSD) 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 by using two methods. The first was achiral and quantitative in that it produced biotransformation rates by comparing the $t_{1/2}$ of each compound in this study with those of 16 known recalcitrant PCBs in juvenile rainbow trout (as identified in (*12*)). These 16 recalcitrant PCB congeners had maximum chlorine substitution in the meta and para positions of the biphenyl rings and, thus, should have no significant biotransformation, the slowest elimination, and highest $t_{1/2}$ (which will vary with congener log K_{ow}) of all PCB congeners (*25*). Contaminants of the same log K_{ow} value with a depuration rate greater than that established from the log K_{ow} − log $t_{1/2}$ regression relationship (and thus a shorter $t_{1/2}$), determined from the depuration rates of the recalcitrant PCBs in Fisk et al. (*12*), are suggested to be biotransformed. Subtracting this minimal regression depuration rate based on the contaminant's log K_{ow} from the experimentally determined depuration rate provides an estimate of biotransformation rate (*13*). Compounds with biotransformation rates that approach zero (positive or negative) are assumed to be recalcitrant. Biotransformation was deemed to be significant for a contaminant when the mean plus standard error of its $t_{1/2}$ fell below the 95% confidence intervals of the log K_{ow} − log $t_{1/2}$ regression. The second biotransformation method was chiral and qualitative and was based on comparing contaminant EFs in fish to EFs in food and standards with an analysis of variance by a Tukey's a posteriori test using Systat (α = 0.05). If significant changes were seen in EFs of a contaminant in the fish, the first method described above was used to identify the biotransformation rate for the more depleted enantiomer. In addition, we monitored for a known metabolite, fipronil sulfone, of fipronil for confirmation of biotransformation regarding this contaminant.

Results and Discussion

Fish Health and Effects. Exposure to fipronil and the OCs did not appear to influence the health of the rainbow trout, as no significant differences were found in lipid percentages, liver somatic index (LSI), or liver growth rates among treatments, and no mortality or signs of stress (e.g., coloration change) were observed. However, the whole fish growth rate of the MIX treatment was lower than the control (Table S1, Supporting Information), although both are in the range reported for similar size rainbow trout (*12*−*13*).

Bioaccumulation Parameters. All compounds were detected in treated fish on the first collection day (day 2) after exposure to the spiked food, and accumulation was rapid during the uptake phase of the experiment (Figure 1). Only fipronil and α-HCH appeared to reach steady state during the uptake phase, which is consistent with their shorter $t_{1/2}$. For the remaining compounds, concentrations increased throughout the uptake portion of the experiment failing to achieve steady state (Figure 1). Similar uptake and elimination curves were found for those OCs not in Figure 1. None of the compounds were detected in control fish on any collection day.

Fipronil was rapidly eliminated by the rainbow trout, having the highest depuration rate among the studied contaminants, with $t_{1/2}$s of 0.61 $\pm$ 0.03 and 0.56 $\pm$ 0.03 d in the FIP and MIX treatments, respectively (Table 1). It was not detected in fish beyond 4 days after cessation of exposure in either treatment. There are very limited data for which to compare these $t_{1/2}$s. In an aqueous exposure, fipronil was completely (>96%) eliminated by bluegill (*Lepomis macrochirus*) within 14 days; however, there was no $t_{1/2}$ reported and concentrations were not determined on other days, with a reported bioconcentration factor (BCF) of 321 in whole fish (*15*).

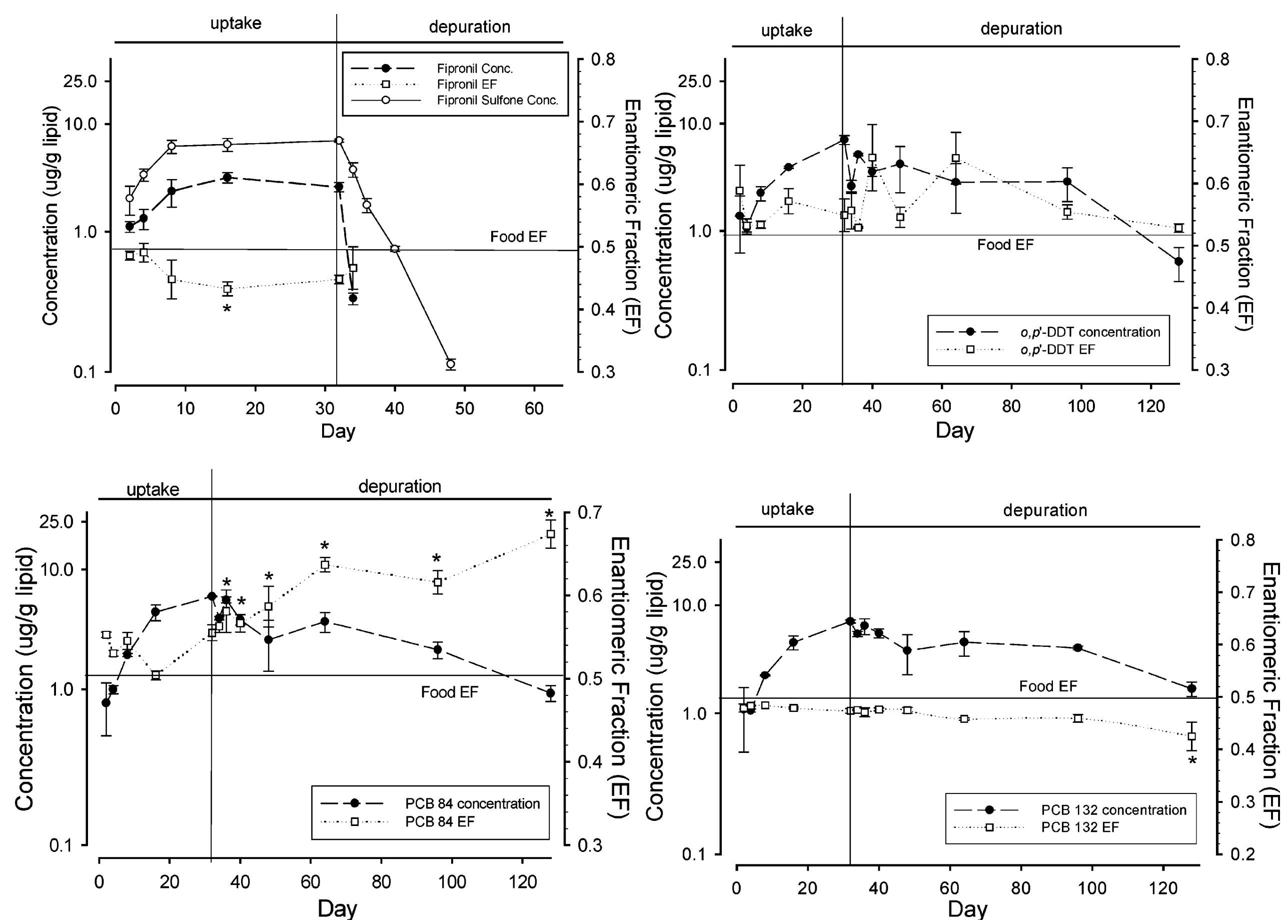

FIGURE 1. Concentrations (dashed lines) and enantiomeric fractions (EFs) (dotted lines) of fipronil and fipronil sulfone (from FIP treatment), PCB 84, PCB 132, and *o,p′*-DDT (from MIX treatment) in juvenile rainbow trout carcass over time. Each point represents the mean ± SE (if larger than symbol used) of concentrations or EF of three fish sampled at that time point. No symbols are present if the chemical was found below detection limits. An asterisk indicates significantly ($p < 0.05$) different EFs in fish on an individual sampling day compared to the food EF. Similar uptake and elimination curves were found for those OCs not shown.

Of the OCs, α-HCH had the highest depuration rate, resulting in a $t_{1/2}$ of 3.85 ± 0.75 d (Table 1). This $t_{1/2}$ is similar to those reported for α-HCH in guppies (*Poecilia reticulate)* and zebrafish (*Danio rerio*) ($t_{1/2}$s of 2−4 days) (*26−27*), but approximately 10 days faster than reported for larger-sized (∼45 g initial weight) rainbow trout ($t_{1/2}$ of 13 d) (*10*). Previous research has shown $t_{1/2}$s to increase with fish size (*12*). The $t_{1/2}$ of the remaining OC compounds were considerably longer, ranging from ∼27 d for HEPX and *p,p′*-DDT to 77 d for PCB 174 (Table 1), are similar to those reported for other OCs in juvenile rainbow trout, and increased with log K_{ow}, consistent with other studies (*12−14*).

There was a wide range of absorption efficiencies in this experiment, although most fell between 40 and 70%, consistent with past studies with OCs in small fish (Table 1) (*12−14*). Absorption efficiencies for the DDT compounds exceeded 100%, which is not realistic or easily explained, and may be related to DDT breakdown in storage (*28*), which would underestimate the concentration in the food (Table 1). Low absorption efficiencies for fipronil (Table 1) are consistent with previous studies showing less-persistent chemicals having small absorption efficiencies due to confounding of this parameter by rapid elimination (*29*).

Many of the OCs in this study should biomagnify within aquatic food webs based on BMFs > 1 (Table 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. Because of the confounded absorption efficiencies (see above), a second set of BMFs were determined by assuming an absorption efficiency of 50% (BMF_{equil}), which is typically observed in similar studies with OCs (*12−14*). BMF_{equil} values agreed with those for the other OC compounds (Table 1) and in other DDT studies (*30*). In addition, the BMF values calculated at steady state (BMF_{ss}) for fipronil and α-HCH were in agreement with the other BMF determination methods in this study, indicating that these compounds would not biomagnify in aquatic food webs (Table 1). However, field studies have shown α-HCH to biomagnify within Arctic marine food webs (*22−23*), which may be due to the large size of the fish and colder temperatures in these studies.

Biotransformation of Fipronil. Fipronil was rapidly biotransformed by the rainbow trout with EFs, indicating relative abundance of fipronil enantiomers changing quickly over time (Figure 1). After 2 days, and throughout both exposures, the (−) enantiomer of fipronil was more prominent, indicating a greater enantioselective biotransformation rate of the (+) enantiomer. The detection of fipronil sulfone, a known metabolite in rodents and fish (*1*, *15*), on the first sampling day and at higher concentrations throughout the uptake phase (Figure 1) confirmed rapid biotransformation of fipronil. It should be noted that low concentrations of fipronil sulfone, about 3% of fipronil concentrations, were detected in the spiked food (Table 1) due to its presence in the fipronil standard. However, the presence of fipronil sulfone in the fipronil-exposed fish is considered to be insignificant because BMFs of fipronil sulfone (4.8 to 7.2) calculated from steady-state concentrations in the food were unrealistic based on its $t_{1/2}$ and were similar to PCB 174 in this study, which had a much longer $t_{1/2}$.

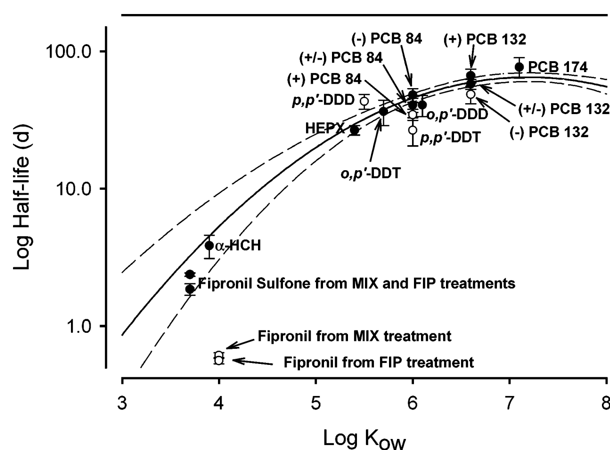

FIGURE 2. Log half-life of compounds in juvenile rainbow trout from this study versus log K_{ow}. The quadratic regression (solid line) and 95% confidence intervals (dashed lines) were taken from Fisk et al. (*12*), which represents the regression of the log of the half-life of 16 recalcitrant PCBs and log K_{ow} in juvenile rainbow trout. Compounds with open circles fell below or above the relationship, indicating they are being biotransformed or bioformed, respectively.

The position of fipronil below the log K_{ow} − log $t_{1/2}$ relationship indicated the rapid biotransformation of this chemical (Figure 2), consistent with metabolite-formation and EF results. The biotransformation rate of fipronil was estimated to account for the majority (approximately 88%) of its elimination in both treatments (Table 1). Furthermore, the $t_{1/2}$s and resulting biotransformation rates of the individual enantiomers of fipronil did not deviate more than 16% in either treatment, indicating rapid biotransformation of both enantiomers, although greater for the (+) enantiomer based on EF data. The inclusion of OCs in the spiked food did not alter fipronil bioaccumulation parameters (Table 1), and thus, enzyme induction by these OCs was likely not significant.

Bioaccumulation and Biotransformation of Fipronil Sulfone. Fipronil sulfone was found to be more recalcitrant ($t_{1/2}$ three times greater) in fish, and thus has a greater bioaccumulation potential than its parent compound, fipronil (Table 1). The $t_{1/2}$ of fipronil sulfone fell on the log K_{ow} − log $t_{1/2}$ relationship, indicating little biotransformation of this metabolite, as suggested by research in mammals (*1*). This result, however, warrants caution because the log K_{ow} − log $t_{1/2}$ relationship has not been established for chemicals under a log K_{ow} of approximately 5.5. For this study, the relationship was extrapolated down to these lower log K_{ow} values, as indicated by the increasing 95% confidence intervals. However, the determined $t_{1/2}$ of α-HCH (log K_{ow} ∼ 4), which has previously been shown to have little to no biotransformation in fish (*10*), fell on the log K_{ow} − log $t_{1/2}$ relationship, indicating that the relationship is holding at these lower K_{ow}s. Clearly, risk assessment of fipronil in aquatic systems must also consider fipronil sulfone.

Bioaccumulation and Biotransformation of the Other OCs. Most of the other OCs studied showed little or no biotransformation. EFs for a majority of the OCs (PCB 174, α-HCH, and HEPX) were racemic throughout the experiment, suggesting no enantioselective biotransformation. This is consistent with previous research showing that α-HCH was not biotransformed enantioselectively by rainbow trout (*10*) and that near-racemic levels of PCB 174 and HEPX were detected in fish (*31*, *32*). Likewise, the EFs of *o,p*′-DDT and *o,p*′-DDD in fish were not significantly different than in food on any sampling day, indicating nonselective biotransformation (Figure 1). However, significant differences occurred on several sampling days when compared to the analyte standard EFs for these two compounds as a likely result of their biological breakdown (*28*, *33*) in a stereospecific manner, as suggested by previous research with other OCs (*34*). It should be noted that fish may be biotransforming these chiral compounds (α-HCH, HEPX, PCB 174, *o,p*′-DDT, *o,p*′-DDD) in a nonenantioselective fashion, as reported for *o,p*′-DDT in plants (*28*). However, this could not be confirmed based on the second method (see below) for assessing biotransformation.

PCBs 84 and 132 were enantioselectively biotranformed, although slowly, based on EFs in the fish. The EFs of PCB 84 in fish were racemic throughout the uptake phase of the experiment but increased significantly starting on day 36 (day 4 of depuration) (Figure 1). Thus, the fish were selectively biotransforming the (+) enantiomer of PCB 84, consistent with that seen in mice (*35*). In the case of PCB 132, there were no significant differences with EFs in fish to those in food throughout the study; however, there was a trend of decreasing EF (biotransformation of (−) PCB 132), which was statistically significant on the last sampling day (day 128) (Figure 1).

Biotransformation of PCBs 84 and 132 would indicate that CYP 2B-like activity is present in fish and may play a role in bioaccumulation. To biotransform a PCB congener via cytochrome (CYP) enzymes, it is believed that adjacent ortho, meta (via CYP1A) or meta, para (via CYP2B) positions on the biphenyl ring must not be substituted with chlorine atoms (*36*−*37*). Both congeners (PCB 84, 132) have vicinal hydrogen atoms in the meta, para positions, with PCB 84 also having vicinal hydrogen atoms in the ortho, meta positions, consistent with our EF results. In addition, PCB 174, which does not have any adjacent vicinal hydrogen atoms on the biphenyl ring, did not show any biotransformation based on EFs.

The log K_{ow} − log $t_{1/2}$ regression relationship indicated little to no biotransformation of PCB 174, *o,p*′-DDT, *o,p*′-DDD, HEPX, and α-HCH, and is in agreement with unaltered EFs for these compounds (Table 1, Figure 2). In agreement with past studies, we illustrate that unmetabolized OCs adhere to this curve-linear relationship, in part validating the use of this model. PCBs 84 and 132, which showed enantioselective biotransformation through nonracemic EFs, adhered to the log K_{ow} − log $t_{1/2}$ relationship. However, looking at individual enantiomers of these two compounds, we see shorter $t_{1/2}$s for the more depleted enantiomers (Figure 2) resulting in significant biotransformation rates of 0.005 d^{-1} for (+) PCB 84 and 0.003 d^{-1} for (−) PCB 132. Thus, the biotransformation seen for these two PCBs is almost completely a result of these individual enantiomers. It is possible that this achiral relationship (log K_{ow} − log $t_{1/2}$) for assessing biotransformation does not detect subtle differences in enantiomer biotransformation, showing the sensitivity of chiral analysis for this purpose.

It is interesting to note that *p,p*′-DDT fell below the log K_{ow} − log $t_{1/2}$ relationship, indicating that it was being biotransformed slowly in the fish (Table 1). Its degradation product, *p,p*′-DDD, was similarly positioned above the relationship, suggesting that any biotransformation of *p,p*′-DDT (negative biotransformation rate, Table 1) may have resulted in the formation of *p,p*′-DDD in the fish, leading to a longer than expected $t_{1/2}$. Although biotransformation of DDT to the metabolites DDD or DDE by organisms is often indicated (*38*), experiments showing this biotransformation pathway are lacking. In fact, greater accumulation rates for some DDT metabolites (i.e., DDE) have been observed in aquatic food webs, which have been attributed to the formation of the metabolite via biotransformation of DDT (*39*).

The changes in EFs shown for fipronil and PCBs 84 and 132 are most likely due to biotransformation as opposed to enantioselective uptake or elimination or biotransformation

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in the gut. 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 stereospecific (*40*, *41*). The results of this study support this because EFs would have deviated from racemic immediately upon exposure; however, this was not apparent for the OCs. Although fipronil did deviate from racemic during the uptake phase, this deviation is a result of biotransformation, supported by the presence of the fipronil sulfone metabolite; however, breakdown by gut flora is also a possibility. Likewise, elimination of hydrophobic compounds, such as excretion through feces or the gills, is also considered a passive and nonstereospecific process (*42*, *43*).

This study shows the utility of using chiral analysis to provide insight into the biotransformation of contaminants. Through measurement of EFs, we were able to demonstrate the biotransformation of fipronil and two PCBs (84 and 132) by fish. These biotransformation processes would not have been observed with traditional achiral analysis, and our results suggest that fish have a greater ability to metabolize OCs than previously thought. On the other hand, the majority of the OCs examined showed no indication of enantiomer-specific biotransformation. Because of the increasing likelihood of chiral centers with the increasing complexity of current-use pesticides, similar studies are warranted to quantify biotransformation processes of these more modern, less persistent chemicals. Our results also highlight the value of the log K_{ow} − log $t_{1/2}$ relationship as a mechanistic tool for quantifying biotransformation for a variety of contaminants such as current-use pesticides in fish.

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Supporting Information Available

Further information regarding lipid percentages, LSI, and whole fish growth rates among investigated treatments. This material is available free of charge via the Internet at http://pubs.acs.org.

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