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

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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;
chlordane) 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 orga-
chloinches (OCs) (10–11).

Another method for determining rates of biotransfor-
mation has been proposed based on a curve-linear relation-
ship 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 experimen-
tally) 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 alka-
nes 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, turfgrass management, and residential
pest control (15–16). Fipronil use is expected to increase
due to species resistance and restrictions on organophos-
phate (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 environ-
moment. 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 $K_{ow}$–$t_{1/2}$ relationship

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in assessing biotransformation, juvenile rainbow trout (Onchorynchus 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 biotransformation 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, N.J.).

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. Food was constituted to remove any contaminant residues in the water. Fish were exposed to the spiked food for 32 days (uptake), followed by 96 days of clean food (degradation), 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 degradation 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 anesthetics 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 140–150 mL fraction) from the

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**TABLE 1. Concentrations and EFs in Food (p = 3), and Contaminant Bioaccumulation Parameters in Rainbow Trout Carcass Following Dietary Exposure**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μg/g wet wt)</th>
<th>EF a</th>
<th>BMF b</th>
<th>log $K_{ow}$ c</th>
<th>$t_{1/2}$ d</th>
<th>$k_{d}$ e</th>
<th>$K_{ow}$ f</th>
<th>$t_{1/2}$ g</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIP</td>
<td>1.23 ± 0.02</td>
<td>0.50 ± 0.07</td>
<td>2.0 ± 2</td>
<td>1.091</td>
<td>2.0 ± 4</td>
<td>0.02</td>
<td>3.7 ± 0.69</td>
<td>0.04</td>
</tr>
<tr>
<td>MIX</td>
<td>1.29 ± 0.07</td>
<td>0.50 ± 0.07</td>
<td>2.0 ± 2</td>
<td>1.091</td>
<td>2.0 ± 4</td>
<td>0.02</td>
<td>3.7 ± 0.69</td>
<td>0.04</td>
</tr>
<tr>
<td>o,p'-DDT</td>
<td>1.29 ± 0.07</td>
<td>0.50 ± 0.07</td>
<td>2.0 ± 2</td>
<td>1.091</td>
<td>2.0 ± 4</td>
<td>0.02</td>
<td>3.7 ± 0.69</td>
<td>0.04</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>1.29 ± 0.07</td>
<td>0.50 ± 0.07</td>
<td>2.0 ± 2</td>
<td>1.091</td>
<td>2.0 ± 4</td>
<td>0.02</td>
<td>3.7 ± 0.69</td>
<td>0.04</td>
</tr>
</tbody>
</table>

a Values missing indicate the parameter was not calculated. *b None of the compounds were detected in control food. *c Log $K_{ow}$ values for fipronil and fipronil sulfone were taken from (11) and (44), respectively. *d PCBs were not quantifiable in control food. *e $K_{ow}$ values were taken from (42) and (43). Log $K_{ow}$ values were selected from literature, and $t_{1/2}$ values were calculated from the log $K_{ow}$ and $k_{d}$. *f $K_{ow}$ and $t_{1/2}$ values were calculated from the log $K_{ow}$ and $k_{d}$. *g $K_{ow}$ and $t_{1/2}$ values were calculated from the log $K_{ow}$ and $k_{d}$.
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 chro-
matograph (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-butylidimethylsilylated-β-cycloextrin. All GC-MS detec-
tion 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 ± 5% (mean ± 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:

$$EF = \frac{[E_1]}{([E_1] + [E_2])} \tag{1}$$

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 enan-
tomer 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 (In 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
date ($k_d$) constants were determined by fitting the concen-
tration data obtained during depuration to a first-order decay
curve (In 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$_w$) were
predicted from the equation BMF$ = C_{stead}/C_{food}$, where $C_{stead}$ 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_{p_b}$, where
absorption efficiency ($\alpha$) 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):

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

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
days.

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 biomagnification 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 biotrans-
formation, 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 $t_{1/2}$) were
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 contami-
ant’s log $K_{ow}$ from the experimentally determined depu-
ration rate provides an estimate of biomagnification rate
(13). Compounds with biomagnification rates that approach
zero (within the range of detection) are assumed to be racemic.
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
achiral and qualitative and was based on comparing con-
taminant EFs in fish to EFs in food and standards with an
analysis of variance by a Tukey’s a posteriori test using Systat
($\alpha = 0.05$). 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,
organ weights (liver, kidney, muscle), or liver growth rates among
control 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 ± 0.03 and 0.56 ± 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 macro-
chirus) 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).
Of the OCs, α-HCH had the highest depuration rate, resulting in a $t_{1/2}$ of 3.85 ± 0.75 days (Table 1). This $t_{1/2}$ is similar to those reported for α-HCH in guppies (Poecilia reticulata) and zebrafish (Danio rerio) ($t_{1/2}$ 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 days) (10). Previous research has shown $t_{1/2}$ to increase with fish size (12). The $t_{1/2}$ of the remaining OC compounds were considerably longer, ranging from ~27 days for HEPX and p,p′-DDT to 77 days for PCB 174 (Table 1), 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 α,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$_{equl}$), which is typically observed in similar studies with OCs (12–14). BMF$_{equl}$ 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 $t_{1/2}$.
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.
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 $1/2$ 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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