SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 9, pp. 2350–2355, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

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

BRAD J. KONWICK,† AARON T. FISK,‡ ARTHUR W. GARRISON,§ JIMMY K. AVANTS,§ and MARSHA C. BLACK*†
†Department of Environmental Health Science, ‡Warnell School of Forest Resources,
University of Georgia, Athens, Georgia 30602, USA
§U.S. Environmental Protection Agency, National Exposure Research Laboratory, Athens, Georgia 30605

(*Received* 13 *September* 2004; *Accepted* 14 *March* 2005)

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 median lethal concentration (LC50) values based on measured concentrations of each compound indicate the (+) enantiomer (LC50 = 10.3 ± 1.1 μg/L, mean ± standard error [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 nontarget species needs further consideration.

Keywords—Pesticide Chiral Enantiomer Median lethal concentration Cerio daphnia

INTRODUCTION

Fipronil (Fig. 1) is a phenylpyrazole-class insecticide first approved for use in the United States in 1996. A number of its commercial formulations are used widely 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 hyperexcitation 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 organophosphorous and other pesticides [2,5].

The environmental degradation of fipronil is controlled in large part by photolysis in aquatic systems. This photoconversion readily occurs ($t_{1/2}$ < 0.5 d [6,7]; $t_{1/2}$ < 3.6 d [8,9]), resulting in extrusion of the sulfinyl group (Fig. 1). Previous research has shown fipronil to be highly toxic to aquatic crustaceans [7,10–12], with its desulfinyl photoproduct being equal to or greater in toxicity within some species [4,7], as well as being more persistent environmentally [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 (Fig. 1) [14]. 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 mixture designated as racemic (±), which contains 50% of each enantiomer and is the form in which they typically are 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 (U.S. EPA) has recognized the issue of chirality in pesticide registration [20], but usually is 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

* To whom correspondence may be addressed (mblack@uga.edu).

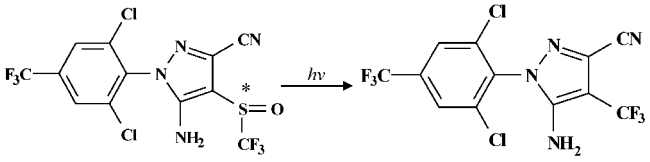

Fig. 1. Structure of fipronil (left) with * indicating asymmetric chiral center. Fipronil degrades under environmental conditions to nonchiral desulfinyl fipronil (right) as the major photoproduct.

acute toxicity of fipronil in *C. dubia*, and the enantioselective toxicity of fipronil in any organism.

MATERIALS AND METHODS

Culturing of test organisms

Ceriodaphnia dubia were obtained from the U.S. EPA (Region IV Ecological Services Laboratory, Athens, GA), and stock cultures were maintained for a month before initiation of tests according to established protocol [22]. All cultures were maintained in an incubator (24–26°C) with a 16:8-h light: dark photoperiod. Moderately hard water ([MHW]; 20% Perrier® in Milli-Q® water [v/v]; Bedford, MA, USA) was used for all stock cultures and experiments. *Ceriodaphnia dubia* were cultured individually in 30-ml polypropylene cups containing 15 ml of MHW, with healthy third broods used to start new cultures every week. Water and food (YCT; 100 μl yeast, cereal leaves [alfalfa], TetraMin®; Tetra, Blacksburg, VA, USA), and 100 μl *Pseudokirchneriella subcapitata* (3.0×10^7 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 (YSI Model 55, Yellow Springs, OH, USA), 7.50 to 8.54 mg/L; pH (Orion model 720A, Beverly, MA, USA), 8.16 to 8.36; total hardness, 82 to 90 mg/L (as $CaCO_3$); total alkalinity, 68 to 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 high-performance liquid chromatography on a CHIRALPAK®AS-H (Chiral Technologies) preparative column (3.0 cm i.d. × 25 cm length). Elution was by supercritical CO_2/isopropyl alcohol: 90/10, column 30°C, and detection was by ultraviolet at 290 nm. Quality assurance high-performance liquid chromatography of each separated enantiomer involved a CHIRALCEL®OD-H (Chiral Technologies) analytical column at 25°C with mobile phase of hexane/isopropyl alcohol: 85/15 at 1 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.

Table 1. Measured concentrations (mean ± 1 standard error) of fipronil enantiomers, racemate, and desulfinyl fipronil in high- and low-test solutions at beginning (0 h) and end (48 h) of *Ceriodaphnia 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 concn. (μg/L)	Measured concn. (μg/L) (time 0)	Measured concn. (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	3	220 (low)	217.7 ± 2.9	210.3 ± 9.9
Fipronil	3	380 (high)	379.0 ± 4.0	351.7 ± 4.4

Test solutions

Stock solutions (1,000 mg/L) of fipronil (+, −, ±) and desulfinyl fipronil were prepared in 100% pesticide-grade acetone. Each stock solution was diluted with MHW to the 5, 10, 20, 40, and 80 μg/L and 220, 260, 300, 340, and 380 μg/L nominal concentrations for fipronil (+, −, ±) and desulfinyl fipronil, respectively. 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 always were made on the same day of test initiation. All containers used in making solutions were precleaned 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 1), composite samples (from three replicates, ~45 ml total) of the test waters from the highest ($n = 2$) and the lowest ($n = 2$) concentrations were collected in precleaned 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; Bellefonte, PA, USA) preconditioned with deionized water and methanol. Samples gradually were added to the tubes immediately following preconditioning at 5 ml/min using vacuum. The sorbent then was dried for 30 min. Samples were eluted with 3 × 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) (Palo Alto, CA, USA) 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 U.S. EPA guidelines [22]. *Ceriodaphnia dubia* neonates less than 24 h old and within 8 h 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 of MHW (control), a 0.1% (v/v) acetone solution (vehicle control), or fipronil compound dissolved in acetone and diluted

Table 2. Acute toxicity (median lethal concentration [LC50]) of fipronil enantiomers, racemate, and desulfinyl fipronil to *Ceriodaphnia dubia* after a 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 standard error (SE)

	48-h LC50 (μg/L)			
	TSK[a]			
Fipronil compound	Light	Dark	Combined	LR
(+) 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[b]	—	ND

[a] Different letters indicate significantly different LC50 values among fipronil stereoisomers under their respective exposure conditions (determined by analysis of variance; mean ± 1 SE, $n = 6$ tests).
[b] ND = not determined.

with MHW. *Ceriodaphnia 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 (16:8-h light: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/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 pipette. Tests for desulfinyl fipronil were conducted in a similar manner except under only normal culture photoperiod (16:8-h light:dark). After 48 h, dissolved oxygen and pH were measured (as described above) and acceptable levels (dissolved oxygen, 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 LC50 value 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 were 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 (Ver 1.5) [23]. The LC50 values of the fipronil enantiomers and the racemate were tested for significant differences with an analysis of variance followed by Tukey's multiple comparison ($\alpha = 0.05$) under light and dark and also combined (light and dark) exposures. 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 (Ver 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 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 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 possibly could 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). When the data were tested either 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). The 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 be 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), 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 nontarget species, including sublethal 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 threefold (10.3 ± 1.1 μg/L for (+) and 31.9 ± 2.2 μg/L for (−) enantiomer), and 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 sublethal exposures is unknown. Furthermore, the relative toxicity of enantiomers is of importance to all organisms that potentially could come into contact with the stereoisomer, both target and nontarget. In our study, *C. dubia* is a nontarget 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 nontarget 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). Although limited photodegradation likely occurred, based on the more frequent presence and higher concentrations of desulfinyl fipronil under light studies (see above), these concentrations (<0.77 μg/L) were well below the estimated LC50 value of desulfinyl fipronil to *C. dubia*. As a result, the exposure conditions (i.e., incubator fluorescent light) used in this experiment were not efficient in providing the necessary irradiation [25] for fipronil's rapid photolysis, and ultraviolet or natural sunlight would have been a better exposure scenario to elucidate fipronil's photodegradation and resulting toxicity to *C. dubia*. Although fipronil's pathway to the desulfinyl derivative has been shown to be a result largely 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 [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.

Several possible reasons explain 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. Because metabolic conversion generally is stereospecific [30], another prospect is that one enantiomer is metabolized preferentially before reaching the intended target site (i.e., GABA receptor). Differences in toxicity of fipronil enantiomers and racemate also may 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 (Fig. 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 likely is a result of the same mechanism of action (i.e., GABA disruption).

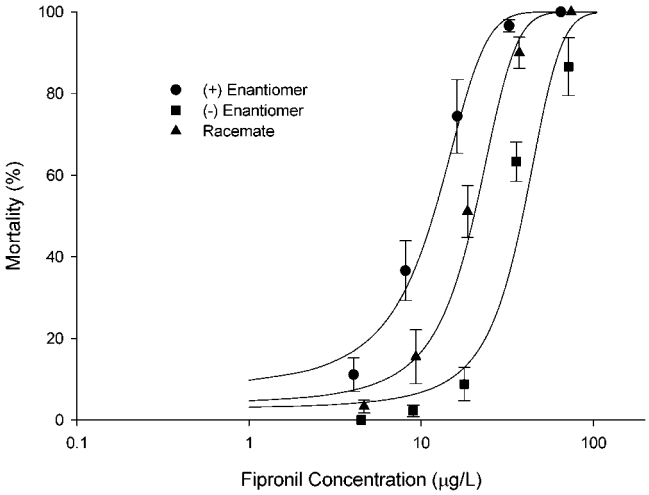

Fig. 2. Dose-response mortality of *Ceriodaphnia dubia* exposed to fipronil enantiomers and racemate. Each point represents the mean percent mortality ± 1 standard error of six tests based on measured test concentrations. Fitted line represents logistic regression of model of data ($r^2 = 0.59$). For model median lethal concentration values see Table 1.

In comparison to other pesticides, fipronil is one of the more toxic, but this toxicity varies greatly with different aquatic species (Table 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 the U.S. EPA's ECOTOX database (http://www.epa.gov/ecotox). 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 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],

Table 3. Acute toxicity (median lethal concentration [LC50]) of fipronil and its desulfinyl photoproduct to aquatic species

Species	LC50 value (μg/L)		Source
	Fipronil	Desulfinyl fipronil	
Mysidopsis bahia (mysid)	0.14	1.5	[7]
Palaemonetes pugio (adult grass shrimp)	0.32	—	[11]
Amphiascus tenuiremis (copepod)	3.5–13.0	—	[12]
Procambarus clarkii (red swamp crayfish)	14.3	68.6	[10]
Procambarus zonangulus (white river crayfish)	19.5	—	[10]
Procambarus clarkii (red swamp crayfish)	180	—	[35]
Macrobrachium rosenbergii (shrimp)	2.24	—	[36]
Machrobrachium nipponensis (shrimp)	11.61	—	[36]
Eriocheir sinensis (crab)	22.57	—	[36]
Daphnia magna (daphnid)	190	—	[7]
Ceriodaphnia dubia (daphnid)	17.7	355	Current study
Lepomis macrochirus (bluegill sunfish)	83	20	[7]
Cyprinodon variegatus (sheepshead minnow)	130	—	[7]
Oncorhynchus mykiss (rainbow trout)	246	>100,000	[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. 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, U.S. Geological Survey, 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 U.S. 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 (*A. 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 nontarget 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. This manuscript has been reviewed and approved for publication by the U.S. EPA, but does not necessarily reflect U.S EPA policy.

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. *Reviews in Toxicology* 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 USA* 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 lifecycle 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. Report D235912. Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC.
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.
21. U.S. Environmental Protection Agency. 1999. Response to public comments on the preliminary risk assessment for the organophosphate naled. Docket 34136. Office of Pesticide Programs, Washington, DC.
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.
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, 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 MS Jr. 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, 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. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, DC.
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). Internal Memorandum. Odenkirchen E, Hetrick J. Environmental Fate and Effects Division, Office of Pesticide Programs, Washington, DC.
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.