Bull. Environ. Contam. Toxicol. (2005) 74:872–879

DOI: 10.1007/s00128-005-0662-7

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Imidacloprid and Fipronil to a Nontarget Aquatic Insect, *Simulium vittatum* Zetterstedt cytospecies IS-7

J. P. Overmyer,[1] B. N. Mason,[2] K. L. Armbrust[3]

[1] University of Georgia, Department of Entomology, 413 Biological Sciences Building, Athens, GA 30602, USA
[2] Redan High School, 5247 Redan Road, Stone Mountain, GA 30088, USA
[3] Mississippi State University, Mississippi State Chemical Laboratory, Post Office Box CR, Mississippi State, Mississippi 39762, USA

Received: 7 December 2004/Accepted: 14 February 2005

The Food Quality Protection Act (FQPA) of 1996 (U. S. Environmental Protection Agency, http://www.epa.gov/oppfead/fqpa) has forced the restriction and cancellation of many widely-used organophosphate insecticides. They are being replaced with synthetic pyrethroids and relatively new chemical products, such as phenylpyrazoles and neonicotinoids, for use in agriculture and domestic settings. Two chemicals gaining increased use are the phenylpyrazole insecticide fipronil and the neonicotinoid imidacloprid. These are considered safer because of their high insecticidal activity and low mammalian toxicity. In addition, they can be applied at very low rates reducing potential impacts on the environment. However, due to increased usage of these insecticides in agriculture and around the home, the risk of contamination of nearby waterways may be increased through accidental spills, drift or runoff. Imidacloprid is susceptible to runoff when applied to lawns especially if irrigated or if rain occurs within 48 h of application (Armbrust and Peeler 2002) and fipronil has been detected in water samples from rivers receiving ricefield tailwater (Demcheck and Skrobialowski 2003). Very little toxicity data exists for these chemicals on non-target organisms, especially those inhabiting aquatic systems. Thus, it is important to assess the concentrations at which these chemicals are toxic to non-target aquatic organisms.

Fipronil is used in agriculture for controlling pests of rice, corn and cotton. It is used around the home for controlling fleas, ticks, fire ants and termites. Fipronil elicits its toxicity by binding to γ-aminobutyric acid (GABA) receptors disrupting chloride ion control of neuronal signaling (Ecobichon 1996; Hainzl et al. 1998). Fipronil dissipates rapidly in alkaline water (hydrolysis $T_{1/2}$ = 2.4 (pH 12), 770 (pH 9) @ 22°C) (Bobe et al. 1998) and is likely to partition to sediments in aquatic systems (Log K_{ow} = 4.0) (Roberts and Hutson 1999). The two major degradation products of fipronil are fipronil sulfone and desulfinyl fipronil, each possessing high insecticidal activity (Hainzl et al. 1998).

Imidacloprid is used in agriculture as a seed dressing or is applied to soil or foliage to control insect pests of corn, cotton, potatoes, rice, vegetables and fruit. Around the home, imidacloprid is used for control of fleas, ticks and grubs. Imidacloprid elicits its toxicity by acting as an agonist and as a blocker of the post-synaptic nicotinic receptor disrupting normal nerve impulses (Iwaya and

Correspondence to: J. Overmyer

Kagabu 1998). Imidacloprid is slightly persistent in water (aerobic sediment/water DT50, 30-162 d) and is likely to remain in the water column in aquatic systems (Log K_{ow} = 0.57) (Roberts and Hutson 1999). The primary degradation product of imidacloprid is 6-chloronicotinic acid, which eventually degrades into carbon dioxide (Hellpointer 1994).

The purpose of this study was to establish baseline toxicity data for imidacloprid and fipronil using laboratory-reared black fly larvae, *Simulium vittatum* Zetterstedt cytospecies IS-7, a non-target aquatic insect. The toxicity of these chemicals was assessed using the median lethal concentrations (LC50s) which were determined using an acute 48-h orbital shaker toxicity test.

MATERIALS AND METHODS

Black fly larvae (Diptera: Simuliidae) for this investigation were obtained from the University of Georgia (Athens, GA, USA) *Simulium vittatum* IS-7 colony being reared under the methods outlined by Gray and Noblet (1999). Fifth instar larvae were used in all toxicity tests.

Analytical-grade standards of imidacloprid and fipronil were purchased from Chemservice (Westchester, PA, USA). Standards were certified to be ≥ 98% pure. Stock solutions of all standards were prepared by dissolving a weighed amount of insecticide into 100 ml of pesticide-grade acetone. The resulting solutions were stored at 4°C in amber bottles with Teflon®-lined caps.

Moderately-hard reconstituted test water was prepared using the method of Weber (1993). Temperature, pH, dissolved oxygen, conductivity, alkalinity, and hardness were measured before and after each test except alkalinity and hardness, which were measured pre-test only (Table 1). Temperature, dissolved oxygen, and conductivity were measured using a YSI 85 water quality meter (Yellow Springs, OH, USA) and pH was measured with a Corning 440 pH meter (Corning, NY, USA). Alkalinity and hardness concentrations were determined through titration with 0.2 N H_2SO_4 and 0.1 M ethylenediaminetetraacetic acid (EDTA), respectively (APHA 1995). Testing was conducted in the laboratory at 20°C (± 0.5) and a l6:8-h light:dark photoperiod.

An orbital shaker toxicity test was used to determine the 48-h LC50 values for imidacloprid and fipronil to *S. vittatum* IS-7 using the method described by Overmyer et al. (2003) with slight modifications. Modifications implemented were the addition of 5 ml of food suspension to 140 ml of moderately-hard water to increase the turbidity in the flask to 5 NTU. Previous studies have shown that the addition of food to the test water at this concentration does not affect the bioavailability of insecticides (Overmyer and Noblet 2003).

Table 1. Mean values ± standard deviation for water quality parameters measured before and after acute orbital shaker toxicity tests.

DO (mg/L)	pH	Temperature (°C)	Conduct. (μS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
[a] 8.9 ± 0.3	7.3 ± 0.2	20.2 ± 0.1	273.1 ± 7.6	66.7 ± 1.12	92.0 ± 2.0
[b] 8.8 ± 0.2	7.7 ± 0.1	19.9 ± 0.7	275.1 ± 2.3		

[a] Pretesting values
[b] Posttesting values

Prior to addition of insecticide to the flasks, six insecticide concentrations were prepared in 50-ml and 200-ml volumetric flasks for imidacloprid and fipronil respectively, by spiking test water with insecticide stock solution. The maximum volumes of stock solution used for preparation of the dosing solutions were 145.2 and 200 μl for imidacloprid and fipronil, respectively which produced concentrations well below the water solubility limits for the chemicals (Roberts and Hutson 1999). The contents were then emptied into 250-ml amber bottles until treatment. Approximately 200 ml and 300 ml of spiked water was collected before and after each test for analysis of imidacloprid and fipronil concentrations, respectively, and stored in amber bottles at -20°C until analysis.

Six insecticide concentrations and two controls, a test-water and a carrier (acetone) control, were tested on one shaker with five flasks per concentration and control, bringing the final totals to 40 flasks and 600 larvae. The carrier control for each insecticide had an acetone content equivalent to the maximum volume of stock insecticide used in preparation of the treatment solutions for the chemical being tested. Each of the three insecticides was tested on a separate shaker and replicated three times for each insecticide.

All data were adjusted for control mortality before statistical analysis using Abbott's formula (Abbott 1925). Mortality in the controls was < 4% for all tests. Data were analyzed using the maximum likelihood method fitting normal (probit) and logistic models to the data (Newman 1995). The model with the best fit across all repetitions was used for determining the LC50 for the specific insecticide.

Concentrations of imidacloprid and fipronil were analytically determined from water samples collected before and after the 48-h toxicity tests. Imidacloprid was extracted from 200-ml aliquots using C_{18} solid phase extraction and analyzed using the methods of Baskaran et al. (1997). Fipronil was extracted in a similar manner from 300-ml aliquots and analyzed using the methods of Ngim and Crosby (2001). Percent recoveries of imidacloprid and fipronil from spiked water samples were > 92.5%. Detection limits were 0.1 and 0.03 μg/L for imidacloprid and fipronil, respectively.

RESULTS AND DISCUSSION

Fipronil was more toxic to *S. vittatum* IS-7 than imidacloprid in this study. Over the three repetitions, median lethal concentrations of fipronil ranged between 0.29 and 0.19 μg/L using the average of the initial and final analytically determined concentrations with a probit model (Table 2). Fipronil was stable in the test water over the 48-h experiment (Table 3). Although the concentrations of fipronil detected in the test water at the end of the experiment were greater than the initial concentrations for four of the six concentrations used, the difference was not significant (paired t-test, $p > 0.10$).

The LC50 for imidacloprid was between 9.54 and 6.75 μg/L using the average of the initial and final analytically determined concentrations with a logistic model (Table 2). Like fipronil, imidacloprid was stable in the test water over the 48-h experiment (Table 3). Concentrations of imidacloprid were slightly lower in test water collected after the experiment than the initial concentrations. However, like fipronil these differences were not significant (paired t-test, $p > 0.10$) and likely due to slight degradation of the chemical over the course of the experiment.

Although mortality (lack of movement of larva) was the endpoint measured in this study, concentrations causing abnormal behavior and muscle control (i.e. impaired movement in the abdominal and thoracic segments or inability to attach to the pan with anal hooks during counting) were much lower for both chemicals. Although not quantified, abnormal behavior and muscle control were observed in larvae exposed to fipronil at all concentrations tested. Thus, the no observed effects concentration (NOEC) for fipronil to *S. vittatum* IS-7 larvae is likely to be < 0.05 μg/L. A NOEC < 0.05 μg/L could be detrimental to black fly and other aquatic insect populations considering that fipronil concentrations have been reported in agricultural watersheds ranging from 0.83-5.29 μg/L (Demcheck and Skrobialowski 2003). Similar effects were observed, but not quantified, in larvae exposed to imidacloprid at concentrations > 4 μg/L. However, all larvae appeared normal after exposure to imidacloprid at approximately 2 μg/L. Thus, the NOEC for imidaclorid to *S. vittatum* IS-7 is likely between 2 and 5 μg/L, approximately two to three times less than the reported LC50.

Both fipronil and imidacloprid have been shown to be more toxic to aquatic insects than other aquatic invertebrates. A comparison of LC50 values for aquatic

Table 2. Median lethal concentration (LC50) values for *Simulium vittatum* IS-7 larvae exposed to imidacloprid and fipronil in a 48-h acute orbital shaker toxicity test.

Insecticide	Concentration	LC50 (μg/L)	FL	Slope
Fipronil [a]	Nominal	0.34	0.29 – 0.38	2.42 ± 0.22
		0.34	0.29 – 0.41	1.77 ± 0.15
		0.42	0.34 – 0.52	1.37 ± 0.13
	Analytical	0.19	0.16 – 0.23	2.25 ± 0.21
		0.19	0.16 – 0.21	2.43 ± 0.20
		0.29	0.22 – 0.38	1.21 ± 0.17
Imidacloprid [b]	Nominal	6.96	6.53 – 7.37	10.40 ± 0.98
		6.91	6.42 – 7.41	7.99 ± 0.78
		8.24	7.50 – 9.20	6.30 ± 0.74
	Analytical	8.25	7.56 – 8.87	10.27 ± 1.08
		6.75	6.04 – 7.41	6.36 ± 0.73
		9.54	8.71 – 10.57	6.48 ± 0.77

LC50 values reported are the results of three separate tests and are accompanied by the fiducial limits (FL) and slope ± standard error. Analytical concentrations used in the analysis were the average of the initial and final concentrations detected in water samples.

[a] LC50 vlaues determined using a Probit model
[b] LC50 values determined using a Logistic model

insects and other aquatic invertebrates shows that insects are more sensitive to these chemicals, in some instances, by several orders of magnitude (Table 4). It is interesting to note that all of the insect species used for testing in the literature and this study are members of the order Diptera. It would be of interest to determine whether these chemicals are as toxic or more toxic to other orders of insects such as the Ephemeropterans, Plecopterans and Trichopterans which are considered to be three of the most sensitive to environmental contaminants (Plafkin et al. 1989).

Differences in sensitivity to toxicants among classes of aquatic invertebrates may be an issue affecting the perceived environmental impacts of the more recently developed insecticides such as fipronil and imidacloprid considering that these chemicals are highly selective to insects and are widely used in broadcast applications. The use of aquatic invertebrate toxicity data other than data derived from using aquatic insects as the basis for risk assessments and developing water quality criteria such as total maximum daily loads (TMDLs) may underestimate potential impacts on aquatic insect communities. Thus, incorporation of aquatic insect data into assessments of current and future insecticides that are highly selective may be necessary.

Table 3. Concentrations of fipronil and imidacloprid detected from water samples in a 48-h orbital shaker toxicity test.

Insecticide	Nominal Conc. (μg/L)	Initial Conc. (μg/L)	Final Conc. (μg/L)
Fipronil	2.00	1.19 ± 0.39	1.29 ± 0.37
	1.00	0.55 ± 0.13	0.66 ± 0.12
	0.50	0.30 ± 0.09	0.32 ± 0.05
	0.25	0.14 ± 0.03	0.17 ± 0.01
	0.13	0.10 ± 0.02	0.07 ± 0.01
	0.06	0.05 ± 0.00	0.05 ± 0.00
Imidacloprid	12.00	14.24 ± 1.35	12.90 ± 1.60
	10.00	11.19 ± 0.69	10.09 ± 0.34
	8.00	9.52 ± 0.71	9.71 ± 1.01
	6.00	7.25 ± 1.12	6.39 ± 0.74
	4.00	4.89 ± 0.20	4.17 ± 0.45
	2.00	2.28 ± 0.20	2.17 ± 0.26

Initial concentrations were determined from spiked water samples at the time of dosing. Final concentrations were determined from water samples collected at the end of the experiments. Initial and final concentrations are reported as the average ± standard error of the three repetitions.

Table 4. Median lethal concentration (LC50) values for aquatic insects and other aquatic invertebrates exposed to fipronil or imidacloprid in laboratory toxicity tests.

Insecticide	Organism (Class)	LC50 (μg/L)	Reference
Fipronil	*Simulium vittatum* IS-7 (Insecta)	0.31-0.18	Current Study
	Chironomus crassicaudatus (Insecta)	0.42	Ali et al. 1998
	Aedes taeniorhynchus (Insecta)	0.43	Ali et al. 1998
	Anopheles quadrimaculatus (Insecta)	0.43	Ali et al. 1998
	Culex nigripalpus (Insecta)	0.87	Ali et al. 1998
	Procambarus clarkia (Malacostraca)	14.30 [a]	Shlenck et al. 2001
	Procambarus zonangulus (Malacostraca)	19.50 [a]	Shlenck et al. 2001
	Ceriodaphnia dubia (Branchiopoda)	36.20-12.10	Konwick et al. 2003
	Daphnia sp. (Branchiopoda)	190.00 [b]	USEPA 1996
Imidacloprid	*Simulium vittatum* IS-7 (Insecta)	9.45 - 6.74	Current Study
	Aedes aegypti (Insecta)	45.00	Song et al. 1997
	Daphnia magna (Branchiopoda)	17360.00	Song et al. 1997

All tests were 48-h unless specified.
[a] 96-h LC50
[b] EC50, duration not reported

Acknowledgement. We thank the organizers of the Young Scholars Program at the University of Georgia for providing financial assistance for second author B. Mason throughout the duration of this work.

REFERENCES

Abbott WS (1925) A method for computing the effectiveness of an insecticide. J Econ Entomol 18:265-267

Ali A, Nayar JK, Gu WD (1998) Toxicity of a phenyl pyrazole insecticide, fipronil, to mosquito and chironomid larvae in the laboratory. J Am Mosquito Contr 14: 216-218

American Public Health Association, American Water Works Association, Water Environment Federation (1995) Standard Methods for the Examination of Water and Wastewater, 19th ed, APHA, AWWA, WEF, Washington D. C., pp 2-25-2-38

Armbrust KL, Peeler HB (2002) Effects of formulation on the run-off of imidacloprid from turf. Pest Manag Sci 58: 702-706

Baskaran S, Kookana RS, Naidu R (1997) Determination of the insecticide imidacloprid in water and soil using high-performance liquid chromatography. J Chromatogr 787: 271-275

Bobe A, Meallier P, Cooper JF, Coste CM (1998) Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). J Agric Food Chem 46: 2834-2839

Demcheck DK, Skrobialowski SC (2003) Fipronil and degradation products in the rice-producing areas of the Mermentau River basin, Louisiana, February-September 2000. Untied States Geological Survey fact sheet FS-010-03, Baton Rouge, LA

Ecobichon DJ (1996) Toxic effects of pesticides. In: Klassen CD (ed) Casarett & Doull's toxicology: the basic science of poisons. McGraw-Hill, New York, NY pp 643-689

Gray EW, Noblet R (1999) Large scale laboratory rearing of black flies. In: Maramorosch K, Mahmood F (eds) Maintenance of Human, Animal, and Plant Pathogen Vectors. Oxford and IBH Publishing Co. New Delhi. pp 85-105.

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

Hellpointer E (1994) Degradation and Translocation of Imidacloprid (NTN 33893) Under Field Conditions on a Lysimeter. Miles Report No. 106426, Miles Inc., Agricultural Division, Kansas City, MO pp. 1-71

Iwaya K, Kagabu S (1998) Biological properties of the chloronicotinyl insecticide imidacloprid: high selectivity and safer use in practice. Rev Toxicol 2: 121-132

Konwick BJ, Fisk AT, Garrison AW, Black MC (2003) Enantioselective toxicity of fipronil to *Ceriodaphnia dubia.* Twenty-fourth annual Society of Environmental Toxicology and Chemistry Meeting, Austin, TX, USA

Newman MC (1995) Lethal and other quantal responses to stressors. In: Adriano DC (ed) Quantitative Methods in Aquatic Ecotoxicology, Lewis, Boca Raton, FL pp 119-176

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

Overmyer JP, Armbrust KL, Noblet R (2003) Susceptibility of black fly larvae (Diptera: Simuliidae) to lawn care insecticides individually and as mixtures. Environ Toxicol Chem 22: 1582-1588

Overmyer JP, Noblet R (2003) Influences of a laboratory diet and natural seston on the bioavailability of carbaryl, chlorpyrifos and malathion to black fly larvae (Diptera: Simuliidae) in an acute toxicity test. Arch Environ Contam Toxicol 45: 209-215

Plafkin JL, Barbour MT, Porter KD, Gross SK, Hughes RM (1989) Rapid bioassessment protocols for use in streams and rivers, benthic macroinvertebrates and fish. EPA/440/4-89/001, United States Environmental Protection Agency, Office of Water, Washington DC

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

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

Song MY, Stark JD, Brown JJ (1997) Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environ Toxicol Chem 16: 2494-2500

United States Environmental Protection Agency (1996) New Pesticide Fact Sheet: Washington D.C., U. S. EPA Office of Prevention, Pesticides and Toxic Substances, EPA 737-F-96-005

Weber CI (1993) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F, US Environmental Protection Agency, Washington, DC, pp 33-39