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Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity

(insecticide action/environmental persistence)

DOMINIK HAINZL AND JOHN E. CASIDA*

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720-3112

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ABSTRACT **Fipronil is an outstanding new insecticide for crop protection with good selectivity between insects and mammals. The insecticidal action involves blocking the γ-aminobutyric acid-gated chloride channel with much greater sensitivity of this target in insects than in mammals. Fipronil contains a trifluoromethylsulfinyl moiety that is unique among the agrochemicals and therefore presumably important in its outstanding performance. We find that this substituent unexpectedly undergoes a novel and facile photoextrusion reaction on plants upon exposure to sunlight, yielding the corresponding trifluoromethylpyrazole, i.e., the desulfinyl derivative. The persistence of this photoproduct and its high neuroactivity, resulting from blocking the γ-aminobutyric acid-gated chloride channel, suggest that it may be a significant contributor to the effectiveness of fipronil. In addition, desulfinylfipronil is not a metabolite in mammals, so the safety evaluations must take into account not only the parent compound but also this completely new environmental product.**

There is a continuing need for novel and selective insecticides acting on sensitive nerve targets because of the development of resistant pest strains and changing toxicological standards. This need is met in part by fipronil, the first phenylpyrazole introduced for pest control (1–4). Fipronil at low dosage provides long-term protection against major lepidopterous and orthopterous pests on crops and coleopterous larvae in soil (5, 6). It is registered for use to control pests of corn, cotton and rice in several parts of the world.† The mode of action involves blocking the γ-aminobutyric acid (GABA)-gated chloride channel that is also the target for cyclodiene insecticides such as endosulfan and dieldrin (5, 7). This is consistent with the observation that *Drosophila* resistant to cyclodiene insecticides due to an alanine-302 to serine replacement in their GABA receptor (8) have a modified binding site that also confers lower sensitivity to fipronil (9, 10). A distinct advantage of fipronil is that it is one of the most selective of the insecticidal blockers of the GABA-gated chloride channel with a favorable safety factor between insects and mammals (4, 7).

The safe use of an agrochemical depends on its toxicological properties and its distribution and persistence in the environment with consideration also of any unusual photoproducts and metabolites that might be formed. Fipronil contains a trifluoromethylsulfinyl substituent that is not present in any other agrochemical and, therefore, may contribute to its remarkable potency in the field. However, we find that this substituent is photolabile and by an unusual rearrangement-elimination reaction leads to a new active agent that probably contributes to this effectiveness and might be important for the safety evaluation of fipronil (see Fig. 1).

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MATERIALS AND METHODS

Chemicals. (±)-5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole-3-carbonitrile (fipronil) was provided by Rhône Poulenc Ag Co. (Research Triangle Park, NC). Reduction of fipronil with titanium dichloride in ether or oxidation with potassium permanganate in aqueous acetone gave the known (3) sulfide and sulfone derivatives, respectively. 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1*H*-pyrazole-3-carbonitrile (detrifluoromethylsulfinylfipronil) was synthesized according to Buntain *et al*. (3). 1-[2,6-Dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole (fipronil analog without the amino and carbonitrile substituents used for comparative photochemistry studies) was previously described (7).

Analytical Methods. GC-MS was performed with the Hewlett-Packard Model 5985 system, an HP-1701 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), helium as the carrier gas, and a temperature program of 150–250°C at 10°C/min and holding at 250°C for 16 min. Retention times were 13.95 min for fipronil and 11.55, 13.48, 13.70, and 20.90 min for the desulfinyl, detrifluoromethylsulfinyl, sulfide, and sulfone derivatives, respectively. GC with an electron capture detector (ECD) utilized the Hewlett-Packard 5480A instrument, equipped with the same column as above, to achieve the increased sensitivity required for analysis of extracts of fat and feces. HPLC (Hewlet–Packard Series 1050 System) with a reversed-phase C_{18} column (Ultrasphere 5 μm, 25 cm × 4.6 mm i.d.) was performed to determine polar metabolites in the aqueous fraction of the mouse feces and tissue homogenates. A methanol/water gradient was used as mobile phase with 290 nm as the monitoring wavelength. NMR (Bruker WM 300) spectra for ^{1}H, ^{13}C, and ^{19}F were recorded for solutions in C^2HCl_3 ($CDCl_3$) or acetone-d_6 with hexafluorobenzene as the internal standard (0 ppm) for ^{19}F NMR. X-ray analysis was made with a Siemens R3m/V diffractometer equipped with an Enraf-Nonius low temperature apparatus using graphite monochromated Mo Kα radiation.

Solution Photochemistry. The test compound was irradiated for 1 h at 25°C as a 200 ppm solution in anhydrous methanol or methanol containing 3% (vol/vol) water. Photolyses were carried out in a Rayonet reactor equipped with four RPR-3000 lamps that emit a broad band centered at 300 nm. The transmission cutoff of the glass tubes used was 280–290 nm.

Abbreviations: GABA, γ-aminobutyric acid; ECD, electron capture detector; [^{3}H]EBOB, 4′-ethynyl-4-*n*-[^{3}H]propylbicycloorthobenzoate.

*To whom reprint requests should be addressed at: Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, 115 Wellman Hall, University of California, Berkeley, CA 94720-3112. e-mail: ectl@nature.berkeley.edu.

†Gant, D. B., Chalmers, A. E. & Wolff, M. A., Eighth International Union of Pure and Applied Chemistry International Congress on Pesticide Chemistry, July 5, 1994, Washington, DC, abstr. 193 (and associated poster).

Each compound designated as a photoproduct was not found in "dark" controls.

Photoproducts on Plants. Fipronil was applied to leaves on corn and sweet pea plants and a pear tree in portions of 100 μg in 10 μl of acetone on areas of approximately 1 cm^2 ($\approx$100 times the normal use level). The studies took place in August (pear and pea) or November (corn) in Berkeley, CA. Corn plants in this experiment were also treated in the same way with four fipronil derivatives (desulfinyl, detrifluoromethylsulfinyl, sulfide, and sulfone). After sunlight exposure, an acetone rinse (600 μl) of the treated portion of the leaf was analyzed by GC-MS. A similar study with sunlight irradiation was made with fipronil applied to silica gel plates, paper, and Pyrex. Samples protected from light were used as dark controls.

Metabolism and Persistence in Mice. Metabolism studies involved male albino Swiss-Webster mice (20–23 g) (Simonsen Laboratories, Gilroy, CA) treated i.p. with fipronil or desulfinylfipronil at 2 mg/kg body weight with dimethyl sulfoxide as the carrier vehicle. The mice were killed 7 h later for analysis of brain, liver, and kidney. Feces samples from similarly treated animals were collected for 24 h. Analyses involved homogenization of tissues or feces in 10 mM phosphate buffer (pH 7.4) and extraction with ethyl acetate to recover apolar compounds for analysis by GC-MS and GC-ECD. Possible metabolites in the aqueous fraction were analyzed by reversed phase HPLC with a diode-array UV detector. To study persistence, the mice were treated i.p. with five daily doses of fipronil, desulfinylfipronil, or DDT at 1 mg/kg with dimethyl sulfoxide as above and then killed at day 6 or 27. The fat was homogenized in dimethyl formamide, and the extract recovered by centrifugation was subjected to cleanup by partitioning with hexane. Analysis of the dimethyl formamide phase involved GC-ECD. Recovery values for standards exceeded 95% for all three compounds.

Toxicity Studies. Toxicity was determined as the LD_{50} for organisms 24 h after treatment. The test compound was administered topically to adult female houseflies and i.p. to mice as above using acetone and dimethyl sulfoxide, respectively, as the carrier vehicles (7). Piperonyl butoxide, when used, was applied to the houseflies at 250 μg/g 1 h before the toxicant.

Binding Assays. 4′-Ethynyl-4-*n*-[^{3}H]propylbicycloorthobenzoate ([^{3}H]EBOB) is the radioligand of choice for the noncompetitive blocker site of the GABA-gated chloride channel (11). For binding assays, housefly head and mouse brain membranes, prepared as described (11), were suspended in 10 mM phosphate buffer (pH 7.5) containing 200 mM NaCl (referred to as assay buffer). Incubation mixtures consisted of 0.78 nM [^{3}H]EBOB (final concentration) in assay buffer (0.5 ml) and candidate inhibitors (introduced in 5 μl dimethyl sulfoxide) to which was added the membrane preparation (0.2 mg of protein) in assay buffer (0.5 ml). The mixtures were incubated for 90 min at 37°C (mouse) or 70 min at 22°C (housefly) and then filtered on Whatman GF/C (mouse) or GF/B (housefly) glass fiber filters followed by three 5.0-ml rinses with ice cold assay buffer and liquid scintillation counting. Specific binding was considered to be the difference between total ^{3}H bound with radioligand only and nonspecific ^{3}H bound on addition of 5 μM unlabeled EBOB. For determining the IC_{50} values, the candidate-unlabeled inhibitor was added to the radioligand before introducing the membrane preparation and incubation.

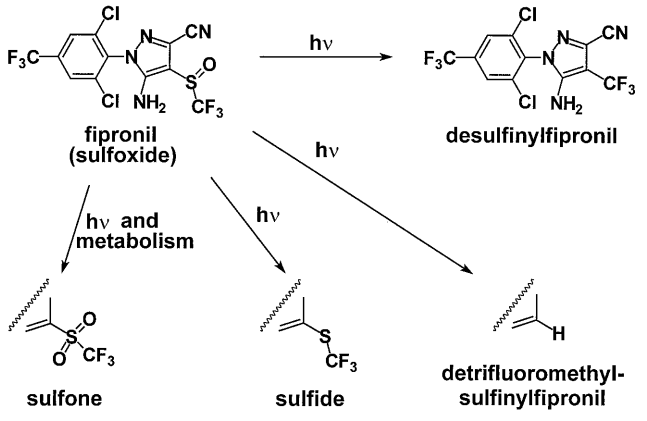

FIG. 1. Fipronil degrades photochemically (hν) under environmental conditions to the desulfinyl derivative as the major photoproduct and the detrifluoromethylsulfinyl, sulfone, and sulfide compounds as minor products. Metabolism of fipronil in mice yields the sulfone but not the other derivatives.

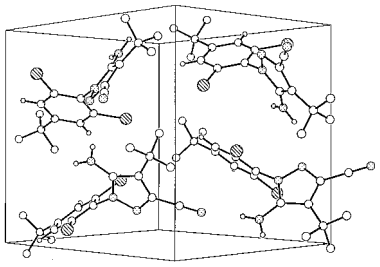

FIG. 2. Crystal structure of desulfinylfipronil (shown as a unit cell), which possesses an orthorhombic system with a $Pna2_1$ space group ($Z = 4$) and an angle of 75.3° between the phenyl and pyrazole ring planes. Surprisingly, the crystal structure contains two molecules of the compound with different distortions of the phenyl group, one chair-like and the other boat-like.

RESULTS AND DISCUSSION

Identification of Photoproducts. Our initial studies considered the structure of the photoproducts formed in methanol alone or methanol with 3% water irradiated at 300 nm (Fig. 1). The major photoproduct in aqueous methanol was a derivative of *m/z* 48 less than fipronil itself (molecular mass of 437 Da). This M-48 photoproduct is equivalent in mass to loss of the sulfinyl moiety and was identified as the new desulfinylfipronil not only by GC-MS and NMR (see below) but also by x-ray crystallography (Fig. 2). There was also one primary photoproduct of fipronil in anhydrous methanol, in this case of *m/z* 321 (M-116), identified by NMR and MS analyses as the detrifluoromethylsulfinyl derivative (see below).

The photoproducts isolated by silica gel thin-layer chromatography with hexane/ethyl acetate mixtures were identified by GC-MS and ^{1}H, ^{13}C, and ^{19}F NMR. Fipronil (for comparison): 437 Da; m.p. 201–202°C; NMR ($CDCl_3$): ^{1}H δ 7.83 (singlet, 2H, aryl), 5.14 (singlet, 2H, NH_2); ^{19}F δ −99.43 (phenyl-CF_3), −87.97 (pyrazole-CF_3). Desulfinylfipronil: 389 Da; m.p. 189–190°C; NMR ($CDCl_3$): ^{1}H δ 7.82 (singlet, 2H,

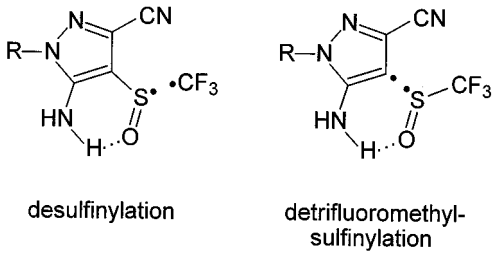

FIG. 3. Partial structures of proposed initial diradical intermediates in photodecomposition of fipronil. R, 2,6-dichloro-4-trifluoromethylphenyl.

Table 1. Comparison of the neurotoxicity of fipronil and its desulfinyl and detrifluoromethylsulfinyl photoproducts

Compound	Assay and species				
	LD_{50}, mg/kg			Receptor IC_{50}, nM	
	Housefly	Housefly (+ PB)	Mouse	Housefly	Mouse
Fipronil	0.13	0.020	41	6.3 ± 1.3	1010 ± 20
Desulfinyl	0.058	0.045	23	5.4 ± 1.0	97 ± 4
Detrifluoromethylsulfinyl	>150	20	>100	235 ± 18	>10,000

The LD_{50} values at 24 h are reproducible within 2.0-fold for houseflies and 1.3-fold for mice in repeated experiments. Twelve mice and several hundred houseflies were used for each determination. The receptor IC_{50} values are given as mean ± SEM (n = 3). PB, piperonyl butoxide.

aryl), 4.30 (singlet, 2H, NH_2); ^{19}F δ −99.39 (phenyl-CF_3), −106.17 (pyrazole-CF_3). Detrifluoromethylsulfinylfipronil: 321 Da; m.p. 117–118°C; NMR: ^1H ($CDCl_3$) δ 7.76 (singlet, 2H, aryl), 6.03 (singlet, 1H, vinyl), 3.81 (singlet, 2H, NH_2); ^{19}F ($CDCl_3$:acetone-d_6, 2:1) δ −99.86 (phenyl-CF_3).

Photolysis Reactions. Formation of desulfinylfipronil is accelerated 3-fold by adding 1% hydrogen peroxide to the aqueous methanol possibly due to hydroxyl radicals generated upon irradiation. Studies of the photochemistry of analogs establish that the sulfoxide (sulfinyl) moiety is required, i.e., the sulfide and sulfone analogs of fipronil are much more stable and do not undergo an analogous photoextrusion reaction. Photodegradation with extrusion of SO or loss of the trifluoromethylsulfinyl moiety does not occur with a fipronil analog lacking the amino and carbonitrile group.

Fipronil probably exists in the cyclic hydrogen-bonded form allowing interaction of the sulfinyl and amino moieties, as noted previously in ortho-substituted arylsulfoxides (12) (Fig. 3). Formation of the radical pair shown in Fig. 3 (*Left*), proposed in the direct photolysis of aryl and alkenyl methyl sulfoxides (12, 13), may be the first step in extrusion of SO from fipronil to give desulfinylfipronil in a reaction analogous to that established before only for a few (three- and four-membered) cyclic sulfoxides (14). Fipronil may form the initial radical pair shown on the right as well, although previously reported only in dialkyl sulfoxides (14), leading to the detrifluoromethylsulfinyl product.

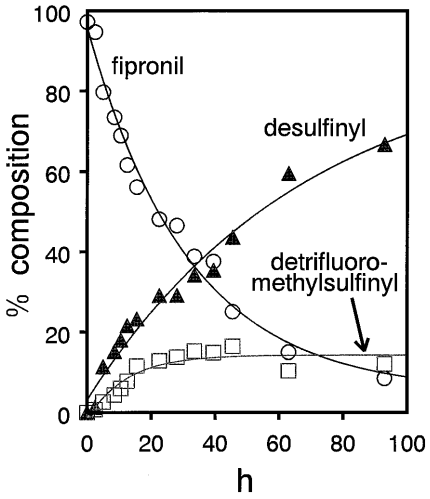

FIG. 4. Photodegradation of fipronil on corn leaves to the desulfinyl and detrifluoromethylsulfinyl derivatives. Time refers to the hours of exposure to sunlight. The results (n = 3) are given as percent composition of the recovered compounds without correction for volatility losses. Although not shown, fipronil sulfide and sulfone made up 7% of the composition at 93 h. Controls involving covered "dark" spots of the individual compounds revealed volatility losses over 93 h of 11%, 4%, and 21% for fipronil and its desulfinyl and detrifluoromethylsulfinyl derivatives, respectively.

Photoproducts on Plants and Other Surfaces. The relevance of the photodegradation of fipronil was considered as thin films on different surfaces, including plants exposed to sunlight. Irradiation on silica gel, paper, or Pyrex gave essentially one product, desulfinylfipronil, with trace levels of two by-products of *m/z* 421 and 453 identified as the sulfide and sulfone derivatives of fipronil, respectively, by comparison with authentic standards. Photochemical desulfinylation was also the major degradation reaction of fipronil on corn, pea, and pear foliage. Desulfinylfipronil comprised 45% of the total residues on pea and pear leaves after 12 h of exposure to August sunlight and 67% on corn leaves after 93 h of exposure to November sunlight, in the latter case with 13% detrifluoromethylsulfinylfipronil (Fig. 4). In the same experiment the individually applied photoproducts (desulfinyl, detrifluoromethylsulfinyl, sulfide, and sulfone) were very stable, and the products determined by GC-MS after 93 h were >95% of the compound applied. Thus, desulfinylfipronil is likely to be a major persisting residue on on foliage-treated crops.

Metabolism and Persistence in Mice. The fate of fipronil was also examined in mice to determine if desulfinylfipronil is a metabolite as well as a photoproduct (Fig. 1). Consistent with an earlier report for armyworms,‡ the major and almost exclusive metabolite of fipronil in mice is the sulfone that appears in brain, liver, kidney, fat, and feces. No polar metabolite was detected in the aqueous extracts of these samples. Significantly, there is also no conversion of fipronil to desulfinylfipronil in mice, making it even more important, as a major environmental photoproduct but not a metabolite, to define the residues and persistence of desulfinylfipronil in mammals. Accordingly, fipronil, desulfinylfipronil, and DDT were introduced into the fat of mice by five daily i.p. doses of 1 mg/kg. The fat levels were 22–24 ppm for each compound at day 6 with fipronil appearing only as its sulfone derivative and no metabolites evident with desulfinylfipronil and DDT. These residues, determined by GC-ECD, dissipated at different rates with levels at day 27 of 3.8 ± 0.2, 3.2 ± 0.3, and 0.8 ± 0.1 ppm (mean ± SEM, n = 4) for DDT, fipronil sulfone, and desulfinylfipronil, respectively. DDT is known to be preferentially stored in fat (15), and the sulfone and desulfinyl derivatives of fipronil are also retained at this site. The presence of desulfinylfipronil in mice was also evident by analysis of brain, liver, and kidney at 7 h and feces at 24 h after treatment in which it alone was detected by GC and HPLC with no trace of any apolar or polar transformation product.

Neurotoxicity of Fipronil and Its Photoproducts. The neurotoxic potency of fipronil, assayed in houseflies and mice both as a toxicant and chloride channel blocker, is maintained or increased on photodegradation to the desulfinyl derivative, whereas the detrifluoromethylsulfinyl compound is much less active or inactive (Table 1). Fipronil and the desulfinyl product

‡Brookhart, G. & Bushey, D., Eighth International Union of Pure and Applied Chemistry International Congress on Pesticide Chemistry, July 5, 1994, Washington, DC, abstr. 189.

are very similar in toxicity with LD_{50} values within about 2-fold of each other for both houseflies and mice. The cytochrome P450 oxidase inhibitor piperonyl butoxide increases the insecticidal activity of fipronil by 7-fold without affecting that of the desulfinyl derivative, suggesting that the latter compound is not readily metabolized. Importantly, fipronil and the desulfinyl derivative are both much more potent in houseflies than in mice as toxicants and in competing with [^{3}H]EBOB at the noncompetitive blocker site of the $GABA_A$ receptor. However, whereas fipronil and desulfinylfipronil are equipotent at the insect GABA receptor, the desulfinyl compound is 10-fold more potent than fipronil at the mammalian chloride channel, narrowing the intrinsic selectivity between the insect and mammal.

Concluding Remarks. Fipronil has outstanding insecticidal activity and provides long-term crop protection (3–5). This may be due in part to the combined action of the parent compound and the sulfone derivative, which is similar in potency to that of fipronil, consistent with its formation as the principal metabolite. Another factor may be the novel desulfinylated photoproduct reported here for the first time (to our knowledge). The toxicity and persistence of desulfinylfipronil indicate that this photoproduct should be considered along with the metabolites in residue and toxicology evaluations.

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1. Moffat, A. S. (1993) *Science* **261,** 550–551.
2. Perrior, T. R. (1993) *Chem. Ind. (London)* 883–887.
3. Buntain, I. G., Hatton, L. R., Hawkins, D. W., Pearson, C. J. & Roberts, D. A. (1988) Eur. Patent 0,295,117 A1, 40 pp.
4. Tomlin, C., ed. (1994) *The Pesticide Manual* (Crop Protection Publications/British Crop Protection Council, Farnham, U.K.), p. 463.
5. Colliot, F., Kukorowski, K. A., Hawkins, D. W. & Roberts, D. A. (1992) *Brighton Crop Prot. Conf. Pests Dis.* **1,** 29–34.
6. Burris, E., Leonard, B. R., Martin, S. H., White, C. A., Graves, J. B., Shaw, R. & Scott, W. P. (1994) *Proc. Beltwide Cotton Conf.* **2,** 838–844.
7. Cole, L. M., Nicholson, R. A. & Casida, J. E. (1993) *Pestic. Biochem. Physiol.* **46,** 47–54.
8. ffrench-Constant, R. H., Steichen, J. C., Rocheleau, T. A., Aronstein, K. & Roush, R. T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1957–1961.
9. Cole, L. M., Roush, R. T. & Casida, J. E. (1995) *Life Sci.* **56,** 757–765.
10. Hosie, A. M., Baylis, H. A., Buckingham, S. D. & Sattelle, D. B. (1995) *Br. J. Pharmacol.* **115,** 909–912.
11. Cole, L. M. & Casida, J. E. (1992) *Pestic. Biochem. Physiol.* **44,** 1–8.
12. Khait, I., Lüdersdorf, R., Muszkat, K. A. & Praefcke, K. (1981) *J. Chem. Soc. Perkin Trans. 2*, 1417–1429.
13. Guo, Y. & Jenks, W. S. (1995) *J. Org. Chem.* **60,** 5480–5486.
14. Still, I. W. J. (1988) in *The Chemistry of Sulfones and Sulfoxides*, eds. Patai, S., Rappaport, Z. & Stirling, C. J. M. (Wiley, New York), pp. 873–887.
15. Morgan, D. P. & Roan, C. C. (1972) *Nature (London)* **238,** 221–223.