Fate of a Phenylpyrazole in Vegetation and Soil under Tropical Field Conditions

H. Fenet,[†] E. Beltran,[†] B. Gadji,[‡] J. F. Cooper,*[,†] and C. M. Coste[§]

Laboratoire de Chimie Analytique, Faculté de Pharmacie, Avenue C. Flahault, 34060 Montpellier, France; Ceres Locustox, B.P. 3300, Dakar, Senegal; and Centre de Phytopharmacie, Avenue de Villeneuve, 66000 Perpignan, France

The fate of fipronil, a phenylpyrazole insecticide, and its metabolites under tropical conditions was studied in soil and in vegetation after treatment for locust control. Two different plots were treated with a formulation of fipronil at doses of 5 and 10 g of active ingredient ha^{-1}, respectively. Vegetation and soil at depths of 0−5 and 5−20 cm were sampled for up to 2 months after treatment. After extraction and purification on fipronil immunoaffinity cartridges, residues were analyzed by gas chromatography using electron capture and mass detectors. In soil, a rapid initial decrease of fipronil was observed with a rapid formation of the sulfone and the photodegradate; the amide and the sulfide were not detected. In vegetation, a rapid initial decrease of fipronil was also observed with a rapid formation of mostly the sulfone; the photodegradate and the sulfide were also detected but at much lower concentrations. The metabolites resulting from the degradation of fipronil were similar in both soil and vegetation, but their relative concentrations were different.

Keywords: *Fipronil; degradation; soil; vegetation*

INTRODUCTION

Fipronil, 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile, a phenylpyrazole insecticide discovered in 1987 by Rhône-Poulenc Agro, was introduced in 1993. It is an insecticide that acts as a potent blocker of the GABA-regulated chloride channel (*1*). It is active against a wide range of insect pests of crops, notably rice insects, thrips, and termites, and has been recommended for use in locust control (*2*).

Degradation studies in soils are essential for the evaluation of the persistence of pesticides and their breakdown products. Data on the rate of degradation are extremely important as they permit prediction of the levels likely to remain in soil and allow assessment of the potential risk associated with exposure. Many factors such as soil properties (clay and organic matter contents, pH, and bulk density) as well as soil temperature and water content influence pesticide fate and transport. Laboratory and field studies on different soil types (loam, silt loam, clay loam, sand, etc.) under temperate conditions have enabled Rhône-Poulenc to establish that fipronil can be metabolized via reduction to the sulfide, oxidation to the sulfone, and hydrolysis to the amide (Figure 1). In the presence of sunlight, a photodegradate also forms via sulfoxide extrusion (*3*). The sulfide, sulfone, and photodegradate product are known to act at the GABA receptor site and are biologically active, whereas the amide elicits no reaction at the GABA receptor site and is not considered to be a biologically active metabolite (*4*).

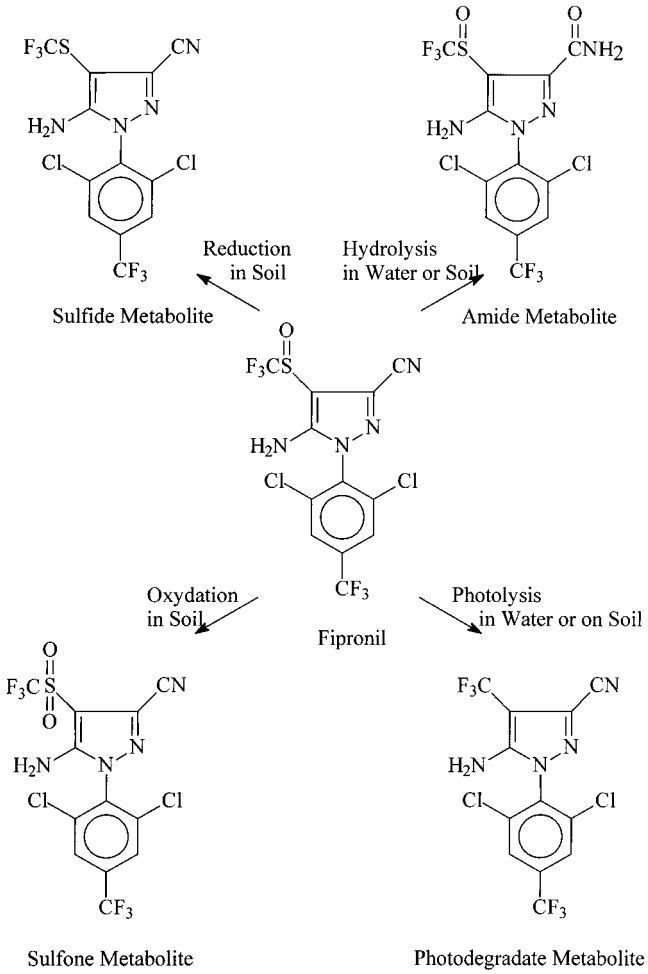

Figure 1. Degradation pathways of fipronil in water and soil.

Fipronil is used in locust control in tropical regions, but few data are available on its environmental fate

* Author to whom correspondence should be addressed [telephone 33 (0)4 67 54 45 20; fax 33 (0)4 67 54 45 25; e-mail jcooper@balard.pharma.univ-montp1.fr].

[†] Laboratoire de Chimie Analytique.

[‡] Ceres Locustox.

[§] Centre de Phytopharmacie.

10.1021/jf000996x CCC: $20.00
Published on Web 02/02/2001

Table 1. Characteristics of Soil Used (0−5 cm)

clay (%)	4.00	organic matter (%)	0.31
fine silt (%)	3.30	organic carbon (%)	0.18
coarse silt (%)	5.80	CEC[a] (mequiv/100 g)	2.43
fine sand (%)	46.20	pH (H_2O)	7.56
coarse sand (%)	40.70		

[a] Cation exchange capacity.

under these conditions, which are high temperature and low relative humidity. Previous work in Niger (*5*) has shown that in soil fipronil degrades rapidly into the four metabolites with low leaching of both fipronil and its main metabolites. Few data are available on the fate of fipronil in vegetation. The objective of the present study was to determine in parallel the fate of fipronil and its metabolites in soil and in vegetation after treatment for locust control.

MATERIALS AND METHODS

Treatment and Sampling. Two 0.5 ha plots were chosen at Richard Toll (northern Senegal). These plots had never been treated with fipronil. Soil properties are summarized in Table 1; the soil was very sandy with low organic matter content. Meteorological data were recorded throughout the experimental period—temperatures varied from 18 to 40 °C, and the relative humidity varied from 18 to 26%; there was no rainfall. Vegetation covered 70−90% of the soil and consisted of grasses (Poaceae) and shrub (Bossia). One plot was treated with 0.5 L of fipronil 10 g L^{-1}, corresponding to 10 g ha^{-1}, and the other plot was treated with 0.5 L of fipronil 5 g L^{-1}, corresponding to 5 g ha^{-1}. The formulation (ADONIS UL) was applied using a battery-powered rotary sprayer (micro-ULVA). Sampling of soil was carried out using a methodology based on the FAO method (*6*). Sampling was scheduled at 0 and 8 h and 1, 2, 4, 7, 15, 30, and 60 days after treatment. The sampling procedure was designed in such a manner as to obtain a homogenized sample representative of the treated plot. A composite soil sample (1.5−2 kg) was prepared, consisting of several subsamples taken at various points along the diagonals of the rectangular plot (10−12 subsamples). Subsamples from the same layer (0−5 and 5−20 cm) were taken with a corer to avoid cross-contamination between the layers. The portion of soil sampled was free of vegetation to avoid potential overlap of foliage reducing concentration. The samples were homogenized and sieved through a 2 mm mesh. A composite sample of vegetation (300 g) was prepared consisting of several subsamples taken at various points along the diagonals of the rectangular plots (10−12 subsamples). Sampling was performed at 0 and 8 h and 1, 2, 4, 7, 15, 30, and 60 days after treatment. The upper part of vegetation (10 cm) was collected, cut into small pieces, and homogenized. The vegetative growth was considered to be negligible as the study was performed during an arid period. Samples were stored at −18 °C until analysis. Soil water content was determined for all samples. The values ranged from 0.25 to 0.5%.

Materials. All solvents used were of analytical grade or pesticide residue analysis grade. Fipronil (purity = 99.9%), photodegradate [5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4- trifluoromethylpyrazole-3-carbonitrile, purity = 97.8%], sulfide [5-amino-1-(2,6-dichloro-α,α,α- trifluoro-*p*-tolyl)-4-trifluoromethylthiopyrazole-3-carbonitrile, purity = 98.8%]; sulfone [5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfonylpyrazole-3-carbonitrile, purity = 99.9%], and amide [5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carboxamide, purity = 99.8%] were supplied by Rhône Poulenc Agro. Stock solutions were prepared at 2 mg L^{-1} in acetone and stored at 4 °C. A range of standard solutions (0.01−0.4 mg L^{-1} in hexane) was prepared for chromatographic analysis and stored at 4 °C. Silica cartridges and reversed-phase octadecyl cartridges for solid phase extraction (SPE) were obtained from Supelco and Varian, respectively. The fipronil immunoaffinity cartridges were supplied by Rhône-Diagnostics.

Analytical Procedure. Soil samples (50 g) were extracted with acetonitrile/acetone (70:30). The extracts were dried by passage through anhydrous sodium sulfate on a glass microfiber filter. The filtrate was evaporated just to dryness and the residue taken up with petroleum ether prior to the cleanup step performed on a silica cartridge. After a silica cartridge had been conditioned with petroleum ether/acetone (70:30) and petroleum ether, the extract was placed on the cartridge, which was then eluted with petroleum ether/acetone (70:30). This eluate was evaporated to dryness and the residue taken up with 10 mL of water/methanol (90:10) before the cleanup was performed on the fipronil immunoaffinity cartridge. The vegetation samples (5 g) were extracted with acetonitrile. The extracts were filtered. The filtrate was evaporated to dryness and the residue taken up with 5 mL of acetonitrile prior to the cleanup step performed on a C_{18} cartridge. The extract was transferred to a 50 mL volumetric flask and made up to 50 mL with water. After an octadecyl cartridge had been conditioned with acetonitrile followed by water, the extract was applied to the cartridge, which was then eluted with water/acetonitrile (15:85). This eluate was transferred to a 50 mL volumetric flask and made up to 50 mL with acetonitrile. Twenty milliliters was evaporated to dryness and the residue taken up with 10 mL of water/methanol (90:10) before the cleanup was performed on a fipronil immunoaffinity cartridge. After the fipronil immunoaffinity cartridge had ben conditioned with water, the extract (soil or vegetation) was applied to the cartridge. The cartridge was washed with water, and the fipronil and its metabolites were then eluted with 2 mL of methanol. The eluate was evaporated to dryness under nitrogen and the residue taken up in 2 mL of toluene. No interference with fipronil and its metabolites was observed when the immunoaffinity cartridges, which are specific to fipronil and its metabolites (Figure 2), were used.

Instrumental Analysis. Each extract was analyzed by gas chromatography, with an electron capture and/or a mass detector to confirm the positive results. The chromatographic conditions were as follows: GC Varian 3350 with an electron capture detector (ECD); injector temperature, 250 °C; detector temperature, 300 °C; column, HP-1701, 15 m, 0.25 mm i.d., 0.25 μm film thickness; initial oven temperature, 70 °C for 1 min, raised at 15 °C min^{-1} to 220 °C, held for 19 min; carrier gas, nitrogen at a flow rate of 1.8 mL min^{-1}; GC HP 5890 connected to a mass spectrometric detector HP MSD 5971A; electron impact mode, 70 eV; splitless injection mode; injector temperature, 250 °C; detector temperature, 280 °C; column, SPB 5, 30 m, 0.25 mm i.d., 0.25 μm film thickness; initial oven temperature, 80 °C for 1 min, raised at 50 °C min^{-1} to 242 °C, held for 16 min; solvent delay, 3 min; carrier gas, helium 5.5 at a flow rate of 1.2 mL min^{-1}. Retention times and characteristic *m*/*z* ions of fipronil and its degradation products are presented in Table 2. An ECD chromatogram of an extract of a sample of treated vegetation (day 0, 8 h, 10 g ha^{-1}) and its confirmation by GC-MS are presented in Figures 2 and 3.

Recovery Studies. To determine method efficiency, untreated soil and vegetation samples were fortified with known amounts of analytical standards dissolved in toluene (4 and 20 μg kg^{-1} for soils and 20 and 80 μg kg^{-1} for vegetation). Each sample was analyzed in duplicate by GC-ECD and GC-MS. The mean recoveries are presented in Table 3.

The limit of quantification was defined for GC-ECD as the sample concentration required to give a signal-to-noise ratio of 10:1. For soil samples, it was evaluated at 0.2 μg kg^{-1} for fipronil, the photodegradate, and the sulfide and at 0.4 μg kg^{-1} for the sulfone and the amide. For vegetation samples, the limit of quantification was evaluated at 1 μg kg^{-1} fipronil, the photodegradate, and the sulfide and at 2 μg kg^{-1} for the sulfone and the amide.

RESULTS AND DISCUSSION

The pattern of formation and decline of fipronil and its metabolites in the 0−5 cm layer is presented in Figure 4, parts a and b, for soils treated at 5 and 10 g

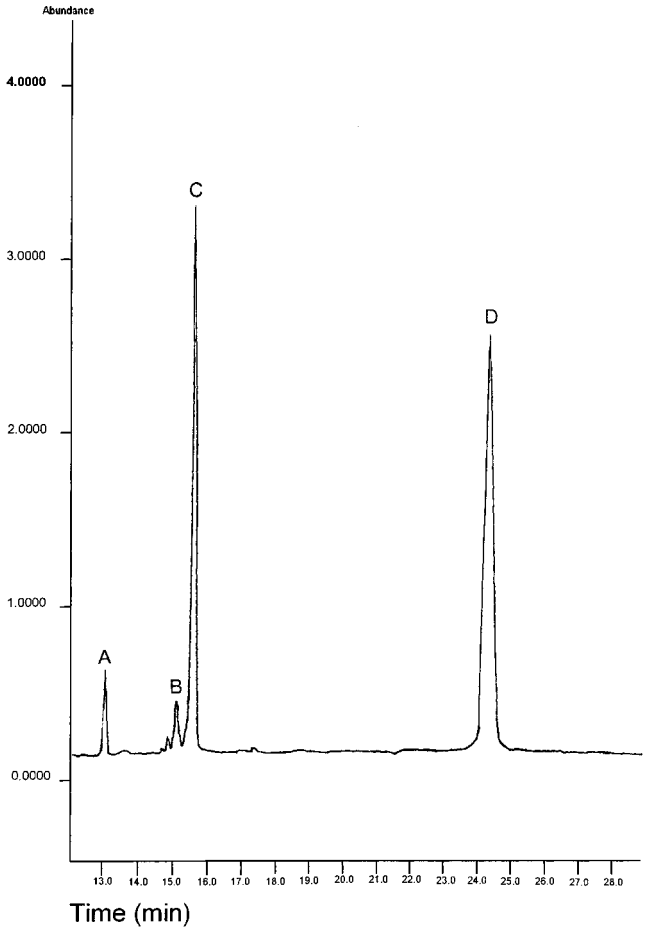

Figure 2. ECD chromatogram of an extract of a sample of treated vegetation (day 0, 8 h, 10 g ha^{-1}): determination of the photodegradate (A), the sulfide (B), fipronil (C), and the sulfone (D).

Table 2. GC-ECD and GC-MS Retention Times and Characteristic *m/z* Ions for Fipronil and Each Metabolite

compound (mol wt)	t_r (min) GC-ECD	t_r (min) GC-MS	MS, *m/z* (amu)
fipronil, $C_{12}H_4C_{12}F_6N_4OS$ (437)	15.5 ± 0.2	8.7 ± 0.2	351−367
photodegradate, $C_{12}H_4C_{12}F_6N_4$ (389)	13.0 ± 0.2	7.4 ± 0.2	333−388
sulfide, $C_{12}H_4C_{12}F_6N_4S$ (421)	15.0 ± 0.2	8.5 ± 0.2	351−420
sulfone, $C_{12}H_4C_{12}F_6N_4O_2S$ (453)	24.5 ± 0.2	10.0 ± 0.2	382−452
amide, $C_{12}H_6C_{12}F_6N_4O_2S$ (455)	23.4 ± 0.2	13.1 ± 0.2	255−385

ha^{-1}, respectively. Fipronil levels fell with time from 8.25 μg kg^{-1} (10 g ha^{-1}) and 4.75 μg kg^{-1} (5 g.ha^{-1}) to 1 μg kg^{-1} for both treated soils after 60 days. The decline of fipronil is rapid during the first 15 days, and the corresponding data fitted well with a pseudo-first-order kinetics. The estimated first-order half-lives of fipronil varied from 95 h (10 g ha^{-1}) to 130 h (5 g ha^{-1}). During a field study in Niger, Bobe et al. (*5*) estimated the half-life of fipronil to be 36 h, presumably because of higher soil moisture.

The degradation of fipronil (80−90% of the applied dose) was concomitant with the appearance of two metabolites: the photodegradate and the sulfone in a similar range. The levels of these two metabolites increased until the fourth day after treatment. During the first 4 days, the mass balance was correct, the

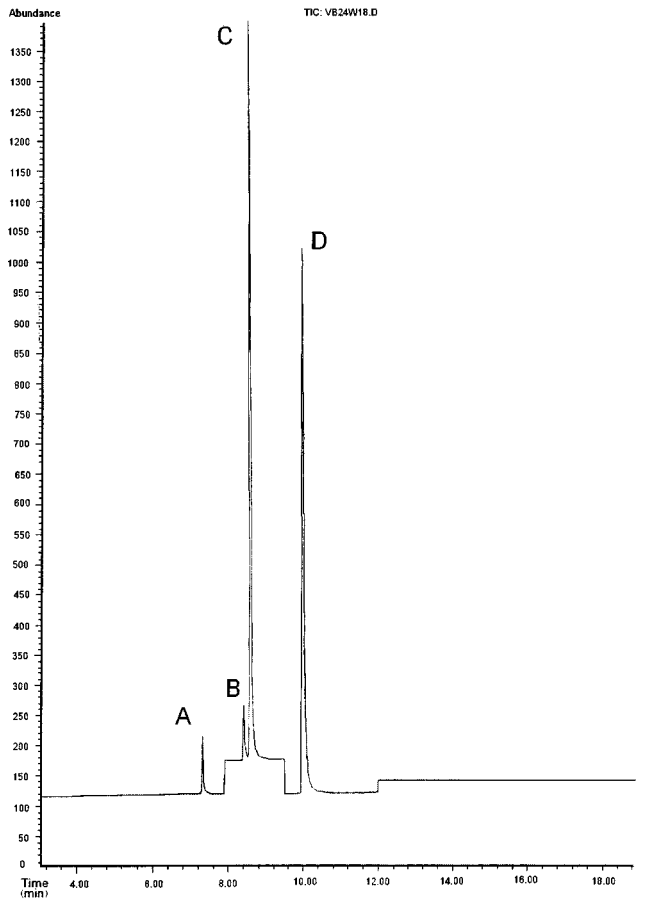

Figure 3. GC-MS chromatogram (SIM total ion chromatogram) of an extract of a sample of treated vegetation (day 0, 8 h, 10 g ha^{-1}): determination of the photodegradate (A), the sulfide (B), fipronil (C), and the sulfone (D).

Table 3. Recovery Study—Fortification Levels of Fipronil and Each Analyte: 4 and 20 μg kg^{-1} for Soils and 20 and 80 μg kg^{-1} for Vegetation

	fortification, μg kg^{-1}	% fipronil	% photodegradate	% sulfide	% sulfone	% amide
soils	4	90	85	105	70	75
	20	115	110	105	85	65
vegetation	20	100	120	90	90	85
	80	110	100	100	110	110

metabolites (photodegradate and sulfone) appearing in quantities equivalent to the amount of fipronil disappearing. After the fourth day, their concentration decreased to reach 0.5−1 μg kg^{-1} at day 14. These results were in accordance with previous work by Bobe et al. (*5*) under Sahelian conditions (Niger), which revealed that the two major metabolites formed in the soil were the photodegradate and the sulfone. After this rapid degradation, the process seemed to stabilize, with the metabolites and the fipronil disappearing very slowly.

The photodegradate detected from the first day after treatment is formed photochemically when fipronil is not protected from light by the soil. The transformation of fipronil to the photodegradate slowed a few days after treatment, which could have been due to a screening effect of soil particles and adsorption of fipronil by the soil (*7*−*9*). The sulfone detected after treatment is formed by oxidation, which can result from biological or chemical transformation. However, the biological

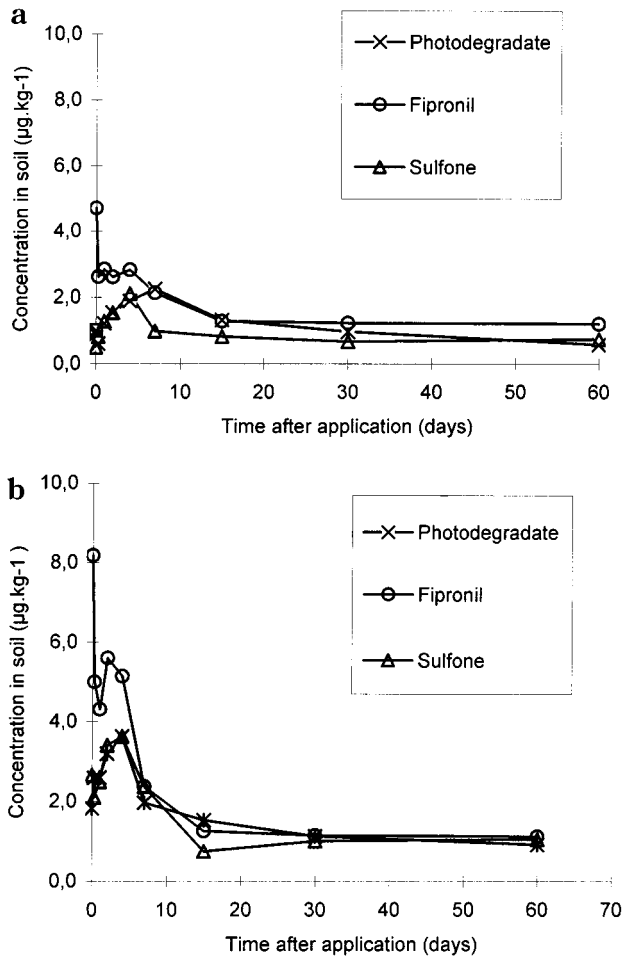

Figure 4. Behavior of fipronil and its metabolites detected in soil treated at (a) 5 g ha^{-1} and (b) 10 g ha^{-1}.

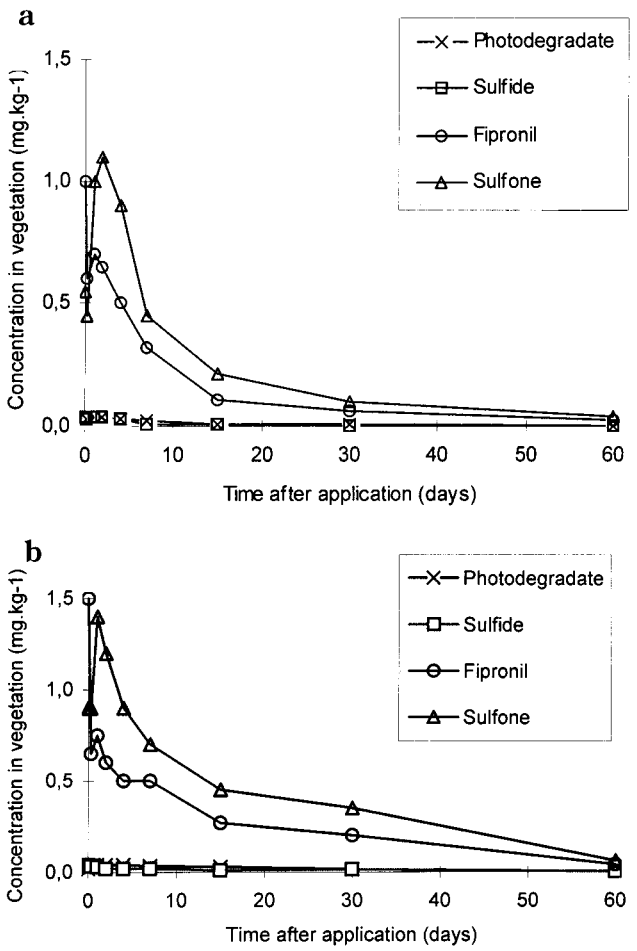

Figure 5. Behavior of fipronil and its metabolites detected in vegetation treated at (a) 5 g ha^{-1} and (b) 10 g ha^{-1}.

activity of the Senegal soil was probably very low, and the major degradation process was probably chemical transformation.

The sulfide and amide metabolites were not detected in the soil (0−5 cm) even 60 days after treatment. The sulfide results from a reduction; the Senegal soil was very dry and very sandy, which does not favor the reduction process. The nitrile group of fipronil in the soil was transformed to an amide group by chemical or biological hydrolysis. In acid soil, the amide metabolite results more from biological activity than chemical hydrolysis (*3*). The conditions did not favor chemical hydrolysis, and the microbiological activity of the soil, given its organic matter content, was certainly very low. This could explain the nonappearance of this metabolite. As expected, no fipronil or any of its metabolites were detected in the 5−20 cm layer after 60 days.

The pattern of formation and decline of fipronil and its metabolites in the vegetation is presented in Figure 5, parts a and b, for plots treated at 5 and 10 g ha^{-1}, respectively. Fipronil levels fell rapidly from 1.5 mg kg^{-1} (10 g ha^{-1}) and 1.0 mg kg^{-1} (5 g ha^{-1}) to <0.05 mg kg^{-1} for the two treated plots after 60 days. This degradation was accompanied by an increase in a major metabolite, that is, the sulfone. In vegetation, sulfone concentration increased from ∼0.5 mg kg^{-1} just after treatment to ∼1 mg kg^{-1} 2 days after treatment. After the first 2 days, the levels of the sulfone metabolite decreased to reach values close to 0.05 mg kg^{-1} at day 60. Two other metabolites were detected—the photodegradate and the sulfide. Their concentrations were low, ranging from 0.002 to 0.04 mg kg^{-1}. Their levels increased from day 0 to day 1−2 and decreased after the first 2 days to reach values close to the limit of quantification. The amide metabolite was not detected.

In vegetation, the concentration of the sulfone was much higher than that of the photodegradate, whereas in soil the concentrations of these two metabolites were similar. In vegetation, the photodegradate appeared after foliar exposure because the fipronil was not protected from the light. However, this transformation was not the major degradation process—this being the transformation of fipronil into sulfone. The well-aerated leaf surface with higher levels of water would probably have induced the photo-oxidation of fipronil to the sulfone rather than the photolyte. It could also be envisaged that the fipronil penetrates the plant where a bio-oxidation could occur.

In vegetation and soil samples just after treatment, high levels of sulfone compared to fipronil were observed. The transformation of fipronil into sulfone was probably very rapid during the application period.

CONCLUSION

The metabolites resulting from the degradation of fipronil were similar in both soil and vegetation, but their relative concentrations were different. In soil, the rapid degradation of fipronil was followed by the appearance of two major metabolites—the photodegradate and the sulfone, whereas in vegetation it was followed

by the appearance of one major metabolite, that is, the sulfone. The process of fipronil degradation was studied in soil, but few data were available on the process in vegetation. Further studies have been planned to investigate the degradation of fipronil in vegetation.

ACKNOWLEDGMENT

We thank Rhône-Poulenc Agro for their assistance throughout this study.

LITERATURE CITED

(1) Cole, L. M.; Nicholson, R. A.; Casida, J. E. Action of phenylpyrazole insecticide at the GABA-gated chloride channel. *Pestic. Biochem. Physiol.* **1993**, *46*, 47−54.
(2) Balança, G.; De Visscher, M. N. Impacts on nontarget insects of a new insecticide compound used against the desert locust [*Schistocerca gregaria* (Forskal 1775)]. *Arch. Contam. Toxicol.* **1997**, *32*, 58−62.
(3) Bobe, A.; Meallier, P.; Cooper, J. F.; Coste, C. M. Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). *J. Agric. Food Chem.* **1998**, *46*, 2834−2839.
(4) Dange, M. RPA 200766, Acute Oral LD50 in the rat; Rhône Poulenc Agro, internal report, study SA93016, 1993.
(5) Bobe, A.; Cooper, J. F.; Coste, C. M.; Muller, M. A. Behaviour of fipronil in soil under Sahelian plain field conditions. *Pestic. Sci.* **1998**, *52*, 275−281.
(6) FAO. *Pesticide Residue Trials To Provide Data for the Registration of Pesticides and Establishment of Maximum Residue Limit*; FAO: Rome, Italy, 1986.
(7) Hebert, V. R.; Miller, G. C. Depth dependence of direct and indirect photolysis on soil surfaces. *J. Agric. Food Chem.* **1990**, *38*, 913−918.
(8) Mathew, R.; Khan, S. U. Photodegradation of metolachlor in water in the presence of soil mineral and organic constituents. *J. Agric. Food Chem.* **1996**, *44*, 3996−4000.
(9) Nag, S. K.; Dureja, P. Phototransformation of triadimefon on glass and soil surfaces. *Pestic. Sci.* **1996**, *48*, 247−252.

Received for review August 8, 2000. Accepted December 20, 2000.

JF000996X