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Fipronil Mobility and Transformation in Undisturbed Soil Columns

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Abstract The downward movement of fipronil and its two toxic metabolites i.e. sulfone and desulfinyl were studied in undisturbed soil columns. Mobility behavior of two different formulations of fipronil viz granular and suspension concentrate were also studied. The undisturbed columns containing sandy loam soil were leached with water equivalent to 400 mm rainfall. Results revealed that although majority of the fipronil (~80%) remained in top 0–5 cm layer, substantial amount (~15%) moved to 5–10 cm depth. In case of metabolites sulfone and desulfinyl >90% of the residues remained in 0–5 cm core indicating low mobility of these metabolites in comparison to fipronil. Results of mobility behavior of fipronil in granular and SC formulations revealed low mobility in granular formulation. Sulfide was detected as the major degradation product in both the formulations and was found to be distributed throughout the column. A little amount of sulfone (0.1% of the total recovered) was also detected upto 10 cm depth.

Keywords Mobility · Fipronil · Sulfone · Desulfinyl · Intact column · Formulations

Fipronil (5-amino-3-cyano-1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-4-trifluoromethyl sulfinyl pyrazole) is a member of the phenyl pyrazoles group, discovered by Rhone-Poulenc Ag Company (now Bayer Crop Science) in 1987. It has been found effective for the control of various insect pests of rice, citrus, cotton, mango, sugarcane, corn and sunflower etc. (Hadjmohammadi et al. 2006). Though very effective against numerous insect pests, fipronil is found to be toxic to various non target organisms such as bees, freshwater invertebrates and birds (Varrio et al. 2009). Fipronil form number of toxic metabolites in environment. Fipronil-sulfone, one of its oxidative metabolite was found to be 3.3 times more toxic to bluegill sunfish and 6.6 times more toxic to freshwater invertebrates than fipronil (Gunasekara et al. 2007). Another major metabolite, fipronil desulfinyl, formed under light conditions is reported to be 10 times more toxic than the parent molecule (USEPA 1998). Thus, while studying the environmental fate like dissipation or mobility of parent molecule it is important to account for the formation of toxic metabolites and also to study their mobility in soil to determine its ultimate impact on the environment.

Fipronil is reported to have low to moderate sorption capacity in soil (Ying and Kookana 2006). Leaching studies showed that 31%–37% of surface soil-applied fipronil moved into the 6–12 cm layer (Gunasekara et al. 2007). Among the metabolites desulfinyl, sulfide and sulfone derivatives were reported to have higher sorption coefficients (Lin et al. 2009; Ying and Kookana 2006). Another adsorption/desorption study carried out with fipronil sulfide suggested low mobility of sulfide metabolite in soil (Burr 1997).Transformation studies of fipronil carried out in the urban stream sediments from California revealed that under facultative conditions, fipronil sulfide and sulfone were the major metabolites whereas only fipronil sulfide was detected in anaerobic samples (Lin et al. 2008). Fipronil in tropical soil of Brazil is reported to be dissipated with the formation of sulfone (predominant metabolite), sulfide and amide (Masutti and Mermut 2007).

Presently fipronil is registered in India for rice, cabbage and chilly. Other than registered uses farmers are also using

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fipronil in sugarcane cultivation as termiticide at a very high dose. A recent study by Janhit Foundation (India) in association with International POP's elimination project has revealed fipronil contamination in ground water and river water in sugarcane growing belt of Ghaziabad and Meerut districts (Anonymous 2006a). US Geological survey data has also reported fipronil and its degradation products in several water bodies in US (Anonymous 2006b). No information is available on the leaching behavior of fipronil or its metabolites in agricultural soil under Indian tropical conditions. It has been reported that intact columns maintain much of the natural soil structure and therefore better approximate the field movement of water and solute compared to columns with repacked soil (Ghosh and Singh 2009). Therefore the present investigation was designed to obtain comparative information on the leaching behavior of fipronil (formulation and technical material) and two of its toxic metabolites (sulfone and desulfinyl) in intact (undisturbed) soil columns of a sandy loam soil of northern India. In addition, degradation of fipronil was also studied in intact soil columns.

Materials and Methods

The intact soil cores [25 cm (l) × 5 cm (i.d)] were collected from the fields of Indian Agricultural Research Institute, New Delhi, by pushing PVC columns into the field. Dry weight of the soil packed in column was ~1.1 kg. The bulk density of the columns was 2.24 g/cm^3. Texture, pH, organic carbon (%) and EC (dS/m) of the soil in columns were determined to be sandy loam, 8.2, 0.36 and 0.23, respectively.

Analytical grade fipronil was purchased from Sigma–Aldrich (purity 97.5%). Different metabolites of fipronil were synthesized chemically from analytical grade fipronil. Fipronil sulfone and sulfide were prepared by the method described by Schlenk et al. (2001). For the synthesis of fipronil desulfinyl method reported by Hainzl and Casida (1996) was used with some modifications. Purity of the compounds was checked by injecting in GLC and was characterized by comparing GC-MS fragmentation pattern (Sulfone: *m/z* 383, 335, 255, 241; Desulfinyl: *m/z* 388, 333, 281, 213; Sulfide: *m/z* 351, 327, 255) and melting point (Sulfone: 222–223°C; Desulfinyl: 118–119°C; Sulfide: 170–171°C) with literature values (Spencer et al. 2004; Schlenk et al. 2001; Hainzl and Casida 1996). Chemical structures of fipronil and its metabolites are shown in Fig. 1.

Leaching studies were carried out under laboratory conditions in intact (undisturbed) soil columns, separately with analytical grade fipronil and its two toxic metabolites fipronil desulfinyl and fipronil sulfone. Leaching behavior

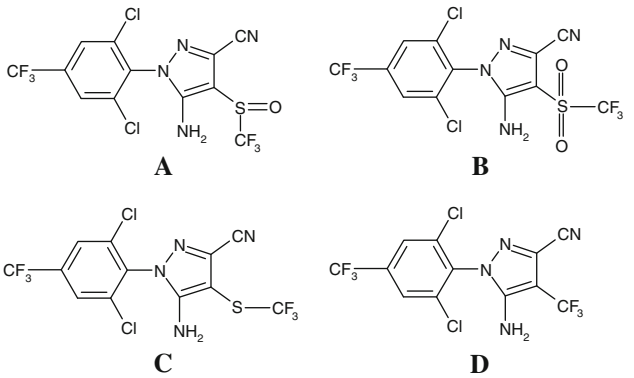

Fig. 1 Chemical structures of **a** fipronil **b** sulfone **c** sulfide **d** desulfinyl

of two formulations of fipronil i.e. 5% SC formulation and 0.3% granular formulation were also studied. Degradation of fipronil was studied with formulation treated soil columns. Standard solutions of fipronil, fipronil desulfinyl and fipronyl sulfone in acetone (each ~1,000 µg mL^{-1}) were used for fortification of soil. Granular formulation was used as such whereas aqueous solution of SC formulation (~1,000 µg mL^{-1}) was used for fortification of soil.

For leaching studies, soil (20 g) was fortified with 1,500 µg of fipronil. For fortification, 1.5 mL of 1,000 µg mL^{-1} standard solution of fipronil was added to the soil. Soil was mixed with glass rod and left undisturbed. Similarly fortification of soil was done with fipronil sulfone and fipronil desulfinyl, separately. For formulation treatment, 20 g soil was fortified with the aqueous solution of SC formulation containing 1,500 µg active ingradient. In case of 0.3% granular formulation, 20 g of soil was fortified with 500 mg of formulation (containing 1,500 µg of active ingredient).

The lower ends of the columns containing intact soil cores, collected from the fields, were covered with perforated poly sheet and dipped overnight into water and the water was allowed to rise by capillary action. This is done to bring the column soil at field capacity moisture level and also to reduce the variation in moisture content among the columns. Next day the columns were rested on Buchner funnels fitted with glass wool to prevent passing of soil particles in the leachate. Leaching behavior of analytical grade fipronil, fipronil desulfinyl, fipronil sulfone, 5% SC formulation and 0.3% Granular formulation were studied in separate columns by spreading 20 g of earlier fortified soil containing 1,500 µg a.i. of fipronil on top of the column. The study was carried out in duplicate and each column was leached with 800 mL of water (equivalent to 400 mm rainfall). A constant water head of 2 cm was maintained above the columns to simulate paddy field conditions. Four leachate fractions of 200 mL each were collected, filtered, diluted with 50 mL of saturated sodium chloride solution

and extracted with dichloromethane (3 × 50 mL). The dichloromethane layers were combined, dried over anhydrous sodium sulfate and concentrated. At the end of the leaching experiment, soil columns were cut horizontally into five cores of 5 cm each. Each core was extracted with aqueous acetone separately by dipping and shaking method. Acetone from the extract was removed by rotary evaporator; aqueous phase was diluted with 50 ml saturated sodium chloride solution and processed as per leachate samples.

Finally the residues obtained from different experiments were dissolved in *n*-hexane and analyzed for the fipronil/metabolite residue using GLC-ECD.

The GLC conditions for analysis of fipronil and its metabolites (fipronil desulfinyl, fipronil sulfide and fipronil sulfone) were standardized for proper resolution of all the components and also for determining the interfering co-extractives, if any, from soil and water matrix. Equipment used was Varian CP-3800 GLC equipped with electron capture detector, column-CP-SIL 5CB (25 m × 0.25 mm i.d., 0.25 μ film thickness), carrier gas-Nitrogen (IOLAR I grade), flow rate 2 ml min^{-1}(through column), make up flow 27 ml min^{-1}, detector 350°C, injector 275°C, column oven programmed as 200°C for 6 min, temperature increased @ 20°C/min to 260°C, hold for 2 min (total run time: 11 min). Under these conditions fipronil and its metabolites desulfinyl, sulfide and sulfone were eluted at 3.69, 2.43, 3.50 and 5.19 min, respectively. A representative GLC chromatogram showing well resolved peaks of the fipronil and its metabolites is presented in Fig. 2.

Linearity range of fipronil and its metabolites sulfone, sulfide and desulfinyl in ECD detector was 0.001–1.0 μg mL^{-1} with 2 μL injection volume. Limit of detection (LOD) of fipronil and its metabolites were found to be 0.001 μg $mL^{-1}$and limit of quantification (LOQ) for water and soil samples were found to be 0.005 μg mL^{-1} and 0.01 μg g^{-1}, respectively.

Recovery studies of fipronil and metabolites from aqueous matrix were carried out at 0.05 and 0.005 μg mL^{-1} fortification level using dichloromethane as extracting solvent. At 0.05 μg mL^{-1} level the recoveries for fipronil varied from 83.8% to 84.8% with a mean of 84.3% and %RSD of 0.49 and at 0.005 μg mL^{-1} level the recoveries varied from 81.7% to 85.8% with a mean of 83.8% and %RSD of 2.05. Mean recoveries of fipronil desulfinyl, sulfide and sulfone metabolites at 0.05 μg mL^{-1} fortification levels were 90.7%, 96.2% and 87.3% with the % RSD of 1.70, 1.00 and 1.48, whereas at 0.005 μg mL^{-1} level the recoveries were 90.0%, 92.5% and 85.5% with the % RSD of 1.76, 2.46 and 1.08, respectively. Recovery from soil samples were carried out at 0.05 and 0.01 μg g^{-1} fortification levels. At 0.05 μg g^{-1} level the recoveries for fipronil varied from 92.1% to 94.9% with a mean of 93.3% (%RSD 1.25) and at 0.01 μg g^{-1} level the recoveries varied from 85.6% to 87.8% with a mean of 86.6% (%RSD 1.04). Mean recoveries of fipronil desulfinyl, sulfide and sulfone metabolites were 92.6%, 85.4% and 89.4% with %RSD of 1.87, 1.37 and 1.12 at 0.05 μg g^{-1} whereas at 0.01 μg g^{-1} level the recoveries were 89.7%, 85.6% and 82.1% with %RSD of 2.55, 0.94 and 0.37, respectively.

Results and Discussion

On leaching the columns with water equivalent to 400 mm rainfall, residues of fipronil and its two metabolites were found to be distributed throughout the column. Even the lower most soil core (0–25 cm) showed presence of fipronil (0.3%), desulfinyl (0.8%) and sulfone (0.2%) in the respective columns. Traces of residues were also detected

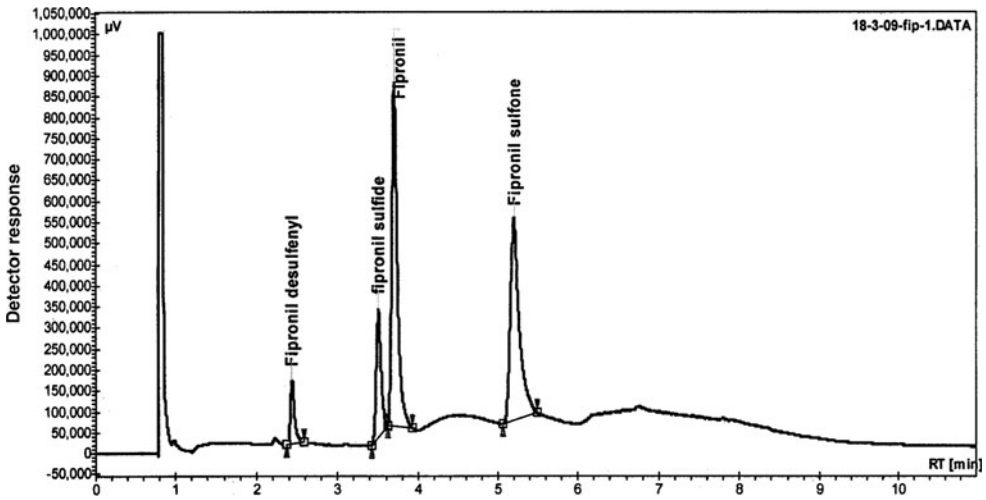

Fig. 2 GLC chromatogram of fipronil and its metabolites namely desulfinyl, sulfide and sulfone

Table 1 Leaching behavior of fipronil, fipronil sulfone and fipronil desulfinyl in intact column

Treatment	Mean residues in soil (μg) Column depth (cm)					Residues in leachate (μg)
	0–5	5–10	10–15	15–20	20–25	
Fipronil	402.38 (79.8)	78.85 (15.6)	19.42 (3.9)	2.11 (0.4)	1.75 (0.3)	Traces[a]
Fipronil sulfone	213.7 (89.9)	10.92 (4.6)	7.06 (2.9)	5.84 (2.5)	0.084 (0.2)	Traces[a]
Fipronil desulfinyl	336.9 (93.4)	12.96 (3.6)	4.78 (1.3)	3.31 (0.9)	2.86 (0.8)	Traces[a]

[a] Residues below quantifiable level (<0.005 μg mL^{-1}); figures in parenthesis show percent of total recovered

in leachate fractions (Table 1). In case of fipronil ~79.8% of the recovered amount was present in 0–5 cm core and ~15.6% in 5–10 cm core, whereas in case of sulfone and desulfinyl ~90% of the residues were detected in 0–5 cm layer. Results revealed lesser downward movement of metabolites compared to fipronil. Bobe et al. (1998) have also observed that fipronil and its degradates did not move beyond the 10 cm soil depth, except for fipronil-amide which is the most polar and water soluble breakdown product. Reduced leaching of metabolites in comparison to fipronil could be explained in terms of higher sorption coefficients of fipronil desulfinyl and sulfone than fipronil (Lin et al. 2009, Ying and Kookana 2006). Mobility of sulfone and desulfinyl were almost comparative. Traces of fipronil detected in the leachate may have come from the natural channel and cracks present in the undisturbed soil core. Mukherjee and Kalpana (2006) have also concluded from their adsorption desorption study that fipronil is moderately mobile in Indian tropical soils. Sharma et al. (2008) in a study on persistence and vertical distribution of termiticide fipronil in modified ground board test found fipronil to persist beyond 56 months and fipronil residues were found upto depth of 60 cm.

Results of the analysis further revealed that out of 1,500 μg added, 505 μg of fipronil, and 238 μg of sulfone and 361 μg of desulfinyl were recovered from the respective columns (Table 1).

Overall low recoveries of fipronil and the metabolites could be due to the degradation of these chemicals in the soil environment. Trend observed in recovered amount i.e. fipronil > desulfinyl > sulfone revealed that microbial population present in the soil environment in the intact column degrade sulfone fastest followed by desulfinyl and fipronil.

Leaching behavior of 5% SC formulation and 0.3% Granular formulation of fipronil were studied and compared with analytical grade fipronil in intact columns. The columns were leached with 800 mL of water (400 mm equivalent rainfall). Results revealed that out of 1,500 μg added only 421.9–735.9 μg was recovered from different soil cores (Table 2). The overall low recovery of fipronil could again be attributed to the microbial degradation in the soil column. In analytical grade treatment residues were found to be distributed throughout the column with the major amount (~80%) present in 0–5 cm layer. In case of formulations the residues were confined to 15 cm depth only. Comparison of mobility behavior of fipronil in granular and SC formulation revealed higher mobility in SC formulation treatment. This may be attributed to the higher water solubility of SC formulation leading to more downward movement. Increased mobility of EC formulation of isazofos as compared to granular formulation has earlier been reported (Bowman 1992). Traces of residues detected in leachate fractions may have come from the cracks and natural channels present in the intact soil cores.

Formation of degradation products from formulation materials were studied in intact columns. Both the formulation showed same pattern in metabolite formation. Parent molecule fipronil was detected upto 10–15 cm soil depth. Sulfide was the major transformation product detected and was found to be present throughout the column (Table 3). Its concentration ranged from 0.5% to 3.9% of the

Table 2 Leaching behavior of analytical grade fipronil and two of its formulations in intact column

Treatment	Mean residues in soil (μg) Column depth (cm)					Residues in leachate (μg)
	0–5	5–10	10–15	15–20	20–25	
Fipronil	402.38 (79.8)	78.85 (15.6)	19.42 (3.9)	2.11 (0.4)	1.75 (0.3)	Traces[a]
0.3% Granular formulation	650.07 (88.3)	85.9 (11.7)	ND	ND	ND	Traces[a]
5% SC formulation	360.35 (85.4)	58.77 (13.9)	2.74 (0.7)	ND	ND	Traces[a]

[a] Residues below quantifiable level (<0.005 μg mL^{-1}); figures in parenthesis show percent of total recovered

Table 3 Transformation of fipronil in undisturbed soil column

Soil depth (cm)	Amount recovered (μg)					
	(5% SC formulation)			(0.3% Granular formulation)		
	Fipronil	Sulfide	Sulfone	Fipronil	Sulfide	Sulfone
0–5	360.3 (78.1)	18.0 (3.9)	0.9 (0.2)	650.1 (83.3)	11.4 (1.5)	–
5–10	58.8 (12.7)	3.3 (0.7)	0.4 (0.1)	85.9 (11)	8.2 (1.0)	0.6 (0.1)
10–15	2.7 (0.6)	3.7 (0.8)	–[a]	–	7.2 (0.9)	–
15–20	–	10.9 (2.4)	–	–	11.8 (1.5)	–
20–25	–	2.4 (0.5)	–	–	5.3 (0.7)	–

[a] Residues below quantifiable level (<0.01 μg g^{-1}); figures in parenthesis show percent of total recovered

recovered amount in different soil cores. Partial anaerobic conditions prevailing inside the column may have facilitated the formation of sulfide metabolite, which is the reduction product of fipronil. A small amount of sulfone (0.1% of the total recovered) was also detected till 10 cm depth.

Results of preliminary study revealed low to moderate potential of fipronil to contaminate ground water. Two toxic metabolites of fipronil i.e. sulfone (oxidation product) and desulfinyl (photo product) were found to be less mobile than the parent molecule. Somewhat increased mobility of fipronil was observed in case of suspension concentrate formulation in comparison to granular formulation treatment. In both the formulation treatments, sulfide was the major metabolite isolated from the soil cores along with the little amount of sulfone. Further studies on mobility and degradation of fipronil and its toxic metabolites need to be carried out in soils of various agro ecological regions.

References

Anonymous (2006a) Establishing the prevalence of POPs pesticide residues in water, soil and vegetable samples and creating awareness about their ill effects. Report published by Janhit Foundation, India, pp 1–14. (http://www.ipen.org)

Anonymous (2006b) U.S. Geological Survey report. (http://infotrek.er.usgs.gov)

Bobe A, Cooper JF, Coste CM, Muller MA (1998) Behavior of fipronil in soil under Sahelian plain field conditions. Pestic Sci 52:275–281

Bowman BT (1992) Mobility and persistence of isazofos in granular and microencapsulated formulations in two soils, using field lysimeters. Pestic Sci 36:181–188

Burr CM (1997) [14C]-M&B 45950: adsorption/desorption to and from four soils and one sediment. Data package ID No. 169043. DPR No. 52062-241

Ghosh RK, Singh N (2009) Leaching behaviour of azoxystrobin and metabolites in soil columns. Pest Manag Sci 65:1009–1014

Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS (2007) Environmental fate and toxicology of fipronil. J Pestic Sci 32:189–199

Hadjmohammadi MR, Nikou SM, Kamel K (2006) Determination of fipronil residue in soil and water in the rice fields in north of Iran by RP-HPLC method. Acta Chim Slov 53:517–520

Hainzl D, Casida JE (1996) Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. Proc Natl Acad Sci 93:12764–12767

Lin K, Haver D, Oki L, Gan J (2008) Transformation and sorption of fipronil in urban stream sediments. J Agric Food Chem 56: 8594–8600

Lin K, Haver D, Oki L, Gan J (2009) Persistence and sorption of fipronil and degradates in urban streams and sediments. Environ Toxicol Chem 28:1462–1468

Masutti CSM, Mermut AR (2007) Degradation of fipronil under laboratory conditions in a tropical soil from sirinha′em pernambuco, Brazil. J Environ Sci Health 42:33–43

Mukherjee I, Kalpana (2006) Sorption of fipronil in tropical soils. Bull Environ Contam Toxicol 76:334–340

Schlenk D, Huggett DB, Allgood J, Bennett E, Rimoldi J, Beeler AB, Block D, Holder AW (2001) Toxicity of fipronil and degradation products to Procambarus sp.: field and laboratory studies. Arch Environ Contam Toxicol 41:325–332

Sharma KK, Kalpana, Sharma V, Gupta P, Jaya M, Kumar A, Singh B (2008) Persistence and vertical distribution of termiticide fipronil in modified ground board test. Environ Monit Assess 137:79–84

Spencer SW, Pennington PL, Scott GI, Ferry JL (2004) The fate of fipronil in modular esturine mesocosms. J Environ Monit 6:58–64

USEPA (1998) Office of prevention, pesticides and toxic substances. Washington, DC 20460, USA, pp 90

Varrio P, Gyori J, Vilagi I (2009) In vitro effects of fipronil on neuronal excitability in mammalian and molluscan nervous systems. Ann Agric Environ Med 16:71–77

Ying GG, Kookana RS (2006) Persistence and movement of fipronil termiticide under slab and trenching treatments. Environ Toxicol Chem 25:2045–2050