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Availability of Fenamiphos and its Metabolites to Soil Water[1]

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Abstract: Field and greenhouse experiments were conducted to determine the extent to which fenamiphos and its degradation products, fenamiphos sulfoxide and fenamiphos sulfone, are available to contact nematodes in the soil. Water extraction provided a relative measure of each chemical's availability to the soil water where the chemicals could contact nematodes, and methanol extraction provided a relative measure of the total amount of each chemical present in the soil. Only small amounts of fenamiphos and fenamiphos sulfone could be extracted by water, even when much larger amounts were present in the soil. In contrast, virtually all of the fenamiphos sulfoxide present in the soil was extractable by water several days after nematicide application. Three days after fenamiphos (3EC) was applied at 6.7 kg a.i./ha to field plots, 6.4% of the fenamiphos, 14.4% of the fenamiphos sulfone, and 100% of the fenamiphos sulfoxide present in the soil was extracted by water. In greenhouse experiments with soil from these field plots, a 15G formulation of fenamiphos containing 98.7% fenamiphos and 1.3% fenamiphos sulfoxide was added to the soil. After an initial period of 3–4 days, the sulfoxide which formed by oxidation of fenamiphos became completely available for water extraction, whereas fenamiphos remained relatively unextractable by water. Fenamiphos sulfoxide is much more available to soil water, thus available for contact with nematodes, than are fenamiphos or fenamiphos sulfone. Based on this availability in water, it seems likely that fenamiphos sulfoxide is the major component for controlling nematodes.

Key words: fenamiphos, fenamiphos sulfone, fenamiphos sulfoxide, metabolite, nematicide, nematode, soil solution, water.

Fenamiphos (Ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl)phosphoramidate), an organophosphorus nematicide with low volatility, is quickly oxidized in soil to fenamiphos sulfoxide, which is then oxidized more slowly into fenamiphos sulfone (8,9,13,14). Fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone have nematicidal properties (19), but their relative contributions to the overall nematicidal effect depend on their ability to come into contact with nematodes (22). Compounds that are not dissolved in the soil water will make no direct contribution to the overall nematicidal effect. The solubility of fenamiphos has been documented (20), but the solubilities of fenamiphos sulfoxide and fenamiphos sulfone have not been reported. Relative availabilities of the three compounds to the soil water can be extrapolated from reports of differential mobility in soil, which demonstrates that fenamiphos sulfoxide is more mobile than fenamiphos or fenamiphos sulfone (9,18) and is therefore apparently more available to the soil water.

Most tests of fenamiphos nematicidal efficacy do not examine the effects of fenamiphos sulfoxide and fenamiphos sulfone (5,6,12,15,16). Because these compounds are products of the degradation sequence when fenamiphos is applied to soil, indirect evidence (19) must be used to examine the relative contributions of the three compounds to the nematicidal effect. The question of which of these compounds contacts nematodes and causes the nematicidal effect is still to be resolved. The purpose of this study was to determine the relative availability of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone to the soil water.

Materials and Methods

Stability in water: A 3 μg/ml (3 ppm) solution of technical-grade fenamiphos in distilled water was prepared and 15 ml of the solution was placed into each of 14 glass petri dishes to determine if fenami-

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phos samples would degrade in water. The experimental design was a split-plot in time with whole-plots kept in light or dark (dishes covered with aluminum foil) and sample times treated as sub-plots. Treatments were replicated seven times in randomized-complete blocks.

Two-ml samples were collected from each petri dish at 0, 7, and 14 days after the start of the test. Concentrations of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone were determined by HPLC analysis. Petri dishes were sealed with Parafilm M (3M corp., Greenwich, CT) 2 days after beginning the test to retard evaporation. Split-plot analysis of variance with appropriate contrasts (17) was used to compare light and dark exposure at each of the sample collection times.

Analysis of formulation: Ten mg fenamiphos a.i. (67 mg fenamiphos 15G) was placed into each of four 10-ml flasks, to which 100 ml of methanol was added. The flasks were shaken by hand twice per day for 7 days. Samples of the methanol were collected at 7 days. Samples were diluted 1:100 with methanol for determination of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone concentrations by HPLC analysis.

Greenhouse experiment: Three runs of a greenhouse experiment were conducted with Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults) (84% sand, 9% silt, 7% clay; 1% organic matter; pH 6.0–6.7) to determine how much of the fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone present in the soil was available to the soil water. Soil was collected on 15 October and 8 December 1992 from field plots to which fenamiphos had last been applied on 18 June 1992. Soil was collected in December before fenamiphos application. Fenamiphos was applied to field plots through chemigation (in 3 mm water) at 6.7 kg a.i./ha before planting cotton (12 June 1991), before planting wheat (10 December 1991, 8 December 1992), and again before planting peanut and cotton (18 June 1992). All plots in the field from which soil was taken were planted with cotton in June 1991 and wheat in December 1991 and 1992. In 1992, two-thirds of the plots were planted with cotton and one-third were planted with peanut. Soil for this study was collected from both cotton and peanut plots. Soil for the three runs was passed through a sieve with 850-μm-openings to remove rocks and debris. At the time of collection, soil contained no residual levels of fenamiphos, fenamiphos sulfoxide, or fenamiphos sulfone.

Fenamiphos 15G (227 mg) was mixed with 11.34 kg of soil (≤5% moisture) at the beginning of each test to obtain a 3 mg/kg (ppm) a.i. concentration. Treatments were replicated six times in randomized complete blocks, which were part of a larger experiment to study the degradation of fenamiphos in soil. The experiment was repeated three times. A 2 × 7 factorial arrangement of treatments was used with extraction method (water or methanol) as one factor and sample time (0, 1, 2, 4 or 5, 7, 9, and 14 days) as the other factor. Extraction was done at 1–2 hours after addition of fenamiphos to the soil on day 0. Each experimental unit consisted of seven 266-ml polystyrene cups, each containing 100 cm^3 fenamiphos-treated soil. Cups were covered with four layers of moistened cheesecloth to impede evaporation. Water was added to the soil to achieve ca. 20% moisture (field capacity) without leaching. Average daily temperatures in the greenhouse were 17.4 C minimum and 31.6 C maximum for the first run, 17.7 C minimum and 26.9 C maximum for the second run, and 18.9 C minimum and 27.3 C maximum for the third run.

At each sampling time, soil was removed from the cups and thoroughly mixed. Then, 50 g of soil was placed into a flask for methanol extraction and 50 g of soil from the same cup was placed into another flask for water extraction. For methanol extraction, the 50 g soil samples were mixed with 50 ml methanol and the flasks were shaken on a platform-type shaker for 2 hours. Contents of the flasks were allowed to settle for 5 minutes before being

passed through a Büchner funnel with glass fiber filter paper (1.2-um-pores) under 500-mm-Hg vacuum. The filtrate was then passed through a 0.45-μm-pore polyvinyl difluoride syringe filter and collected in high pressure liquid chromatography (HPLC) vials. For water extraction, 50 ml distilled water was added to the flasks which were shaken by hand for 2 minutes before filtering as described for methanol extraction.

High pressure liquid chromatography analysis was used to determine fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone concentrations. The chromatography system used a C18 column, 55% acetonitrile–45% water mobile phase with a 1.3 ml/min flow rate, 225 nm wavelength with a 15 nm bandwidth for fenamiphos sulfoxide and fenamiphos sulfone (ca. retention times of 1.8 and 2.3 minutes), and a 250 nm wavelength with a 15 nm bandwidth for fenamiphos (ca. retention time of 4.4 minutes). Calibration standards included mixtures of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone solutions with concentrations of each compound at 0, 2.5, or 5.0 ppm.

General linear models procedures were used to fit least squares regression curves to the data sets. All differences reported herein are significant at the $P \leq 0.05$ level unless otherwise indicated.

Field experiment: Soil samples were collected from field plots used for the greenhouse tests 3 days after fenamiphos 3EC was applied at 6.7 kg a.i./ha through chemigation in 3 mm water (8 December 1992). Approximately 500 cm^3 of soil was randomly collected from each of five plots to which fenamiphos had been applied. Soil was collected 3–10 cm deep. Fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone were extracted from samples containing ca. 5% moisture as described for the greenhouse experiment. For each of the three chemicals, analysis of variance and Fisher's LSD (17) were used to compare methanol extraction to water extraction to determine what proportion of each compound was available to the soil water.

Release from formulation: Ten mg fenamiphos a.i. (67 mg fenamiphos 15G) was placed into each of three 5 × 35 mm stainless steel cylinders, which were then filled with sand (fine silica sand rinsed three times with methanol and dried) to hold the nematicide granules tightly in place. A disk of Whatman No. 1 filter paper was placed at the outflow end of the cylinder, which was held in a Perkin Elmer HPLC cartridge column holder. Peristaltic pumps moved distilled water past the formulation at 2 ml/30 minutes. Water was collected in 2-ml HPLC vials at a rate of 1 vial every 30 minutes for 24 hours. Three replicates were used and the test was repeated twice.

Results

Stability in water: Fenamiphos sulfoxide and fenamiphos sulfone were not detected in any samples. No differences were measured between fenamiphos concentrations in samples kept in the light and samples kept in the dark at 0, 7, or 14 days after fenamiphos solution was put into petri dishes.

Analysis of formulation: It was determined from methanol extraction that 66.7 mg of the 15G formulation used for this test contained 1,437.5 μg fenamiphos (98.7% of nematicidal compounds present) and 18.75 μg fenamiphos sulfoxide (1.3% of nematicidal compounds present). Fenamiphos sulfone was not detected. The total nematicidal content (1,456.25 μg) was 46% higher than expected.

Greenhouse experiment: Water extracted little or no fenamiphos, even when methanol extraction proved that fenamiphos was present (Fig. 1). Measurement of fenamiphos sulfoxide concentration could not be obtained at 0 days in the first run because the peaks were obscured in the HPLC analysis. Regression equations for the concentrations of fenamiphos and fenamiphos sulfoxide recovered by methanol and water extractions are as follows: (M = methanol extraction, W = water extraction, F = fenamiphos, FS = f. sulfoxide, X = days) Run 1—MF = 2.66 –

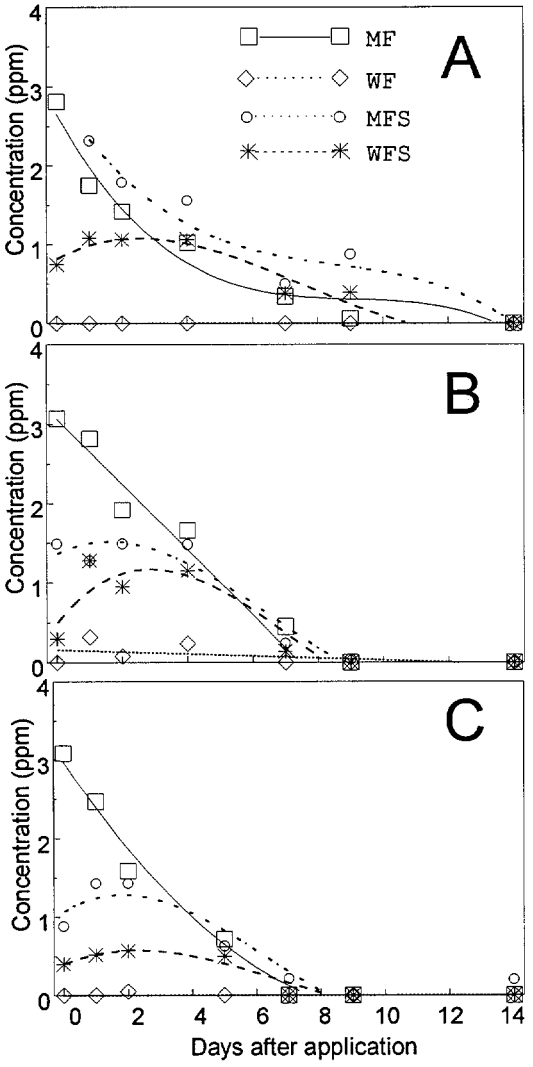

FIG. 1. Concentration of fenamiphos and fenamiphos sulfoxide extracted from soil by both methanol and water extractions for 14 days after incorporation of fenamiphos 15G. Data points are the means of six replicates, and lines represent regression curves. A, B, and C represent three replicate runs of the experiment. MF = methanol extraction of fenamiphos, WF = water extraction of fenamiphos, MFS = methanol extraction of fenamiphos sulfoxide, WFS = water extraction of fenamiphos sulfoxide.

$0.75X + 0.081X^2 - 0.0030X^3$, $R^2 = 0.74$; WF = 0; MFS = $2.93 - 0.65X + 0.070X^2 - 0.0027X^3$, $R^2 = 0.84$; WFS = $0.81 + 0.22X - 0.053X^2 + 0.0024X^3$, $R^2 = 0.86$; Run 2—MF = $3.07 - 0.41X$, $R^2 = 0.79$; WF = $0.159 - 0.0128X$, $R^2 = 0.19$; MFS = $1.36 + 0.207X - 0.074X^2 + 0.0038X^3$, $R^2 = 0.88$; WFS = $0.49 + 0.52X - 0.11X^2 + 0.0053X^3$, $R^2 = 0.73$; Run 3—MF = $2.97 - 0.607X + 0.028X^2$, $R^2 = 0.86$; WF = 0; MFS = $1.068 + 0.25X - 0.081X^2 + 0.0042X^3$, $R^2 = 0.83$; WFS = $0.402 + 0.16X - 0.043X^2 + 0.0021X^3$, $R^2 = 0.77$.

Significant main factor effects were detected for extraction method and sample time in addition to a significant extraction method × sample time interaction in each of the three runs for fenamiphos and fenamiphos sulfoxide. The interaction occurred because water extracted as much fenamiphos sulfoxide as methanol extracted beginning 2, 1, and 5 days after pesticide application in the first, second, and third runs, respectively, but water extracted less fenamiphos sulfoxide than methanol before these times. Water extracted no measurable quantities of fenamiphos sulfone even when methanol extracted small but measurable amounts (data not shown).

Field experiment: Fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone were extracted in measurable quantities by both methanol and water (Fig. 2). Only 6.4% of the fenamiphos and 14.4% of the fenamiphos sulfone present in the soil were available to the soil water.

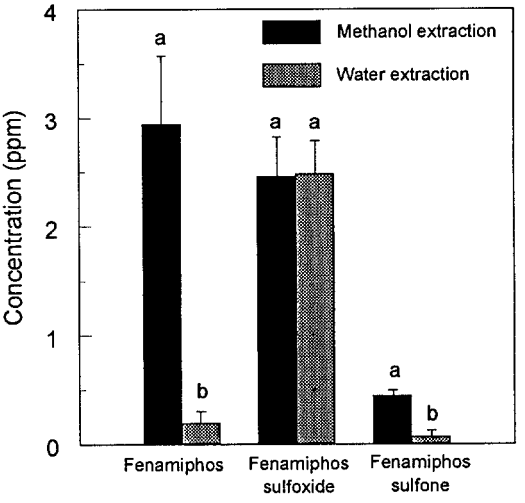

FIG. 2. Concentration of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone extracted from field soil 3 days after fenamiphos 3EC application. Error bars represent one standard error of the mean of five replicates. For each of the three chemicals, separate LSD comparisons ($P \leq 0.05$) between extraction techniques are shown.

Release from formulation: In the assay that used stainless steel cylinders, the highest concentrations of the three compounds were measured in samples collected during the first 30 minutes of each test (Fig. 3). Only trace concentrations of fenamiphos sulfone were detected ($\leq$0.65 ppm) during the first few hours of the experiment, and these levels may have resulted from fenamiphos sulfoxide oxidation during the experiment, since no fenamiphos sulfone was detected in methanol extracts from the formulation. Release curves from the 15G formulation demonstrated reproducible, quasi-exponential release of fenamiphos and a much faster initial release of fenamiphos sulfoxide. A mean of 72% of the nematicidal content of the formulation was released during the 24-hour runs of this test.

Discussion

The shapes of the release curves lead us to the conclusion that most of the fenamiphos sulfoxide and all of the fenamiphos sulfone is at or near the surface of the formulation granules, whereas fenamiphos is distributed more evenly throughout the granules. When water first began to flow past the granules, pesticide molecules from near the surface were released into the water. As these molecules were removed, molecules from the interior of the granules diffused toward the surface, where they were released into the water. The differential solubilities of the three compounds also may have influenced the rate at which they were released from the granules. Almost all of the fenamiphos sulfone (98.1%) and nearly two-thirds (64.1%) of the fenamiphos sulfoxide was released during the first 4 hours of the 24-hour test, which suggests that fenamiphos near the surface of the granules was oxidized during storage to form a small amount of fenamiphos sulfoxide, some of which was oxidized further to form fenamiphos sulfone. Since the granules continued to release fenamiphos sulfoxide throughout the runs, it appears that small amounts of fenamiphos sulfoxide are present throughout the granules.

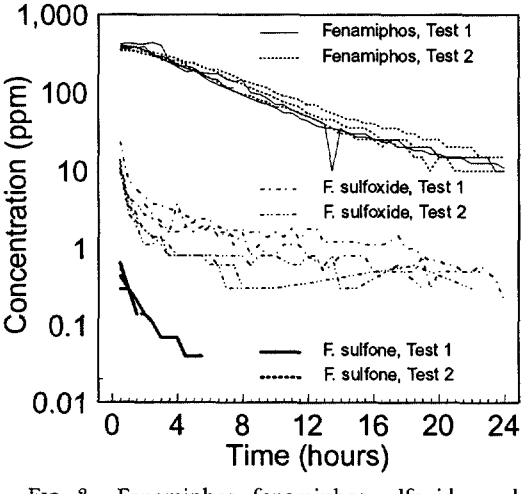

FIG. 3. Fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone release rates from a 15G formulation of fenamiphos into distilled water during a 24-hour period. Concentrations represent the amount released per ml of water during a 30 minute time period. Each replicate is presented for each run (test).

The two extraction solvents used in these experiments, methanol and water, give insight into how much of each compound present in the soil is available to soil water, where it can act as a nematicidal agent. Methanol extraction provided a measure of the total amount of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone present in the soil (including nematicide not yet released from the formulation granules), whereas water extracted only the nematicide that was already in the soil water plus the amount that could desorb into the extraction solvent and was therefore most available to affect nematodes. Methanol extraction does not distinguish between fenamiphos still retained by the formulation, fenamiphos that has been released but is not dissolved in the soil water, and fenamiphos that is dissolved in the soil water. This distinction is not made in this study because the methanol solvent was used to extract all of the nematicide present in the soil and thereby served as a positive check for the water extractions.

These data make it clear that even when there is much more fenamiphos than fenamiphos sulfoxide in the soil, fenamiphos sulfoxide will be much more available

to the soil water than fenamiphos. The soil used in these experiments contained relatively low levels of organic matter, but the relatively high water availability of fenamiphos sulfoxide (indicative of a low K_{oc}) indicates that soils with higher organic matter contents should show similar results.

If complete equilibration occurred during the water extraction (1,21) and methanol extracted all of the compound present, then soil adsorption coefficients (K_d) may be calculated as follows: K_d = ([concentration in methanol) − (concentration in water]/(concentration in water). These K_d values may then be used to calculate soil organic carbon sorption coefficients (K_{oc}) since $K_{oc} = K_d$/(fraction of organic carbon in the soil). Soil adsorption coefficients (K_d) are direct measures of the relative affinities of a pesticide for water and the soil surface for a particular soil; K_{oc} values "normalize" K_d values for the amount of organic carbon present in the soil (20). Data from the field experiment provide K_{oc} estimates of 1,740 for fenamiphos and 0 for fenamiphos sulfoxide (at 1.43% organic matter [0.84% organic carbon]). This method of calculation overestimates K_{oc} for fenamiphos and underestimates K_{oc} for fenamiphos sulfoxide according to previously reported values (7). If equilibrium had not been reached or if organic matter in the samples was higher than the 1.43% we measured, then K_{oc} would be overestimated. If methanol did not extract all of the compound present, then K_{oc} would be underestimated. Regardless of any overestimation or underestimation that may have occurred, the differences in estimates in values of K_{oc} (and therefore the availability of these compounds to the soil water) observed in this study were large.

The differences in availability of fenamiphos, fenamiphos sulfoxide, and fenamiphos sulfone to the soil water may influence the relative contributions of each to the overall nematicidal efficacy of the pesticide. Since only the portion of a chemical that is readily available to the soil water is likely to have an effect on nematodes (22), fenamiphos sulfone probably contributes little to the control of nematodes because it is formed in small amounts and only a small portion of what is formed is available to the soil water. The relative contributions of fenamiphos and fenamiphos sulfoxide to the control of nematodes would depend on their relative toxicities as well as their availability to the soil water. The three compounds have nematicidal properties (19), but their relative toxicities have not been reported. Even if fenamiphos sulfoxide is less toxic than fenamiphos, it may contribute more than fenamiphos to the overall nematicidal effect, since our data indicate that it is available to the soil water at much higher concentrations and for longer periods of time.

Aldicarb and fenamiphos follow similar degradation pathways in the soil, with both compounds forming first a sulfoxide and then a sulfone (10). Aldicarb sulfoxide is a potent cholinesterase inhibitor in insects (2), whereas aldicarb sulfone is a weak cholinesterase inhibitor in insects (11). This degradation sequence in aldicarb and fenamiphos involves changes in a thiomethyl group, leaving the remainder of the molecule unchanged. In aldicarb, the carbamate group is retained by the sulfoxide and the sulfone, thereby allowing these compounds to retain their acetylcholinesterase-inhibiting properties (4). It seems reasonable that fenamiphos sulfoxide and fenamiphos sulfone also should retain their acetylcholinesterase-inhibiting properties, thereby remaining nematicidal.

Because fenamiphos sulfoxide is more available to the soil water and is more mobile in the soil than fenamiphos, fenamiphos sulfoxide would leach deeper into the soil (18). It should, therefore, be more uniformly distributed throughout the root zone than fenamiphos. Fenamiphos sulfoxide would then be in a physical position to be an effective nematicidal agent. Our research (3) indicated that the accelerated degradation of fenamiphos sulfoxide in field plots treated previously with fenamiphos could account for the reduction of nematicidal efficacy in those plots. This ev-

idence demonstrated the important contribution of fenamiphos sulfoxide to the overall nematicidal effect of the pesticide.

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