and is the major residue in carrots resulting from carbofuran treatment, it appears to be a logical candidate for toxicity testing.

Acid hydrolysis of the angelic acid ester conjugate of 3-hydroxycarbofuran in 0.25 N HCl is expected to produce 3-hydroxycarbofuran. The development of a quantitative procedure for this compound in its unaltered, conjugated form is necessary to determine the amount of this compound which may be present. The angelic acid ester conjugate is the major carbofuran metabolite in carrots, but the extent of its occurrence in other crops is unknown.

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A Method for Determining Fenamiphos and Its Sulfoxide and Sulfone in Plants and Soil

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A method was developed for the individual determination of residues of fenamiphos (Nemacur) and its sulfoxide and sulfone in raspberry fruit, carrots, and a sandy loam soil. Separation of the two metabolites was achieved on silica gel, and cleanup was improved on charcoal. Direct gas chromatographic detection was accomplished by using a flame photometric detector. Recoveries were calculated on samples fortified with 0.01, 0.1, and 1.0 ppm of each of the three compounds and ranged from 75.0 to 106.7%.

Fenamiphos (Nemacur, ethyl 3-methyl-4-(methylthio)-phenyl (1-methylethyl)phosphoramidate) is currently under investigation for postplanting control of the lesion nematode, *Pratylenchus penetrans* (Cobb), the dagger nematode, *Xiphinema bakeri* (Williams), common pests in raspberry plantings in British Columbia, and the carrot rust fly, *Psila rosae* (Fab.).

Fenamiphos dissipates very rapidly (Waggoner and Khasawinah, 1974) and its thiooxidation products, fenamiphos sulfoxide and sulfone, have been identified as the major metabolities in both soils and plants (Waggoner, 1972). Published residue methods determine either the parent compound only (Sagredos and Eckert, 1977) or the sulfone as the total residue after permanganate oxidation of both the parent compound and the sulfoxide (Thornton, 1971). So that the degradation of fenamiphos can be followed, a new residue method for the individual determination of the parent compound and its sulfoxide and sulfone in soil, raspberries, and carrots has been developed and is presented in this paper.

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EXPERIMENTAL SECTION

Apparatus. A Microtek MT 220 gas chromatograph equipped with a flame photometric detector was used. Peak quantitation was performed by a Hewlett-Packard Model 3385A reporting computing integrator.

Reagents. Silica gel (Davidson Chemical Division, W. R. Grace and Co., Grade 923, 100–200 mesh) and Hyflo Super Cel were used as received. Nuchar C was acid-washed as previously described (Brown, 1975) and a 2:5 mixture of charcoal–Whatman CF11 cellulose powder was prepared. All solvents were distilled in glass. Anhydrous

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Na_2SO_4 was heated overnight at 230 °C prior to use. Analytical standards of fenamiphos (94.3%) and fenamiphos sulfoxide and fenamiphos sulfone (both >95%) were supplied by Chemagro, Kansas City, MO.

Sample Preparation and Fortification. Untreated silt loam soil from Abbotsford, B.C., containing 21.5% moisture by weight, was sieved to pass a 10-mesh screen. Two hundred grams of soil was weighed into an amber screw-capped jar, and then a 20-g portion was removed to a beaker. The soil in the beaker was moistened with ethyl acetate, and then appropriate volumes of the 100 μg/mL stock solutions of fenamiphos, its sulfoxide, or its sulfone in ethyl acetate were added. The solvent was evaporated off in a fume hood, and the soil was mixed frequently while drying. When no trace of solvent remained, the soil was returned to the jar which was then tumbled for 2 h to ensure adequate mixing. The fortified soil samples were stored at 10 °C for a minimum of 72 h before extraction.

Raspberries, harvested the previous summer and stored frozen at −18 °C, and fresh carrots, scrubbed and coarsely shredded in a Braun vegetable shredder, were used in this study. Raspberries were thawed, and both raspberries and shredded carrots were fortified in a 400-mL Sorval Omni-Mix container by pipetting appropriate volumes of the 100 μg/mL stock solutions directly onto the plant tissue and then equilibrating them at room temperature for 0.5 h.

Sample Extraction. *Extraction of Plant Tissue.* Fifty grams of fortified raspberries or carrots was homogenized with 70 mL of acetone in a Sorval Omni-Mix for 3 min with the container immersed in an ice water bath. The homogenate was filtered through a Büchner funnel lined with glass fiber filter paper into a 1-L separatory funnel. The filter cake was homogenized twice more with 70-mL portions of acetone, and all extracts were combined. After the addition of 550 mL of distilled water, 50 mL of saturated aqueous sodium chloride solution, and 50 mL of chloroform, the funnel was shaken for 1 min. When the phases had separated, the lower chloroform layer was drained into a 250-mL Erlenmeyer flask. The aqueous phase was extracted twice more with 25 mL of chloroform and then discarded. The combined chloroform extract was dried over anhydrous Na_2SO_4, transferred to a 500-mL round-bottom flask, and evaporated just to dryness in a rotary vacuum evaporator at 35 °C. The residues were dissolved in 10 mL of a 1:1 hexane–acetone mixture for cleanup.

Extraction of Soil. Twenty-five grams of fortified soil was weighed into a beaker, and then 5 g of Hyflo Super Cel was mixed into it. Six grams of water was added to the mixture to increase the moisture content to ~50% (w/w) and then mixed thoroughly by hand. After equilibration at room temperature for 30 min, the sample was transferred to a Soxhlet extractor plugged with a loosely packed wad of glass wool at the bottom. The added soil sample was held in place with a small wad of glass wool on the top. The extractor was then attached to a 300-mL boiling flask, and the soil was extracted for 8 h with 225 mL of acetone at a rate of ~2 cycles/h. Reflux was stopped when a minimum amount of solvent (~75 mL) was in the boiling flask. The extract in the flask was transferred to a 1-L separatory funnel for the extraction of fenamiphos and its sulfoxide and sulfone as previously described for plant tissues.

Column Cleanup. Two columns (11 mm i.d. × 300 mm) were plugged at the bottom with a small wad of glass wool and a 1-cm layer of anhydrous Na_2SO_4 was added. Column I was packed with 3 g of silica gel; column II was packed with a 3-cm layer of Nuchar C–cellulose; both were topped with a 1-cm layer of Na_2SO_4 and a small wad of glass wool. Column I was positioned so that its effluent dripped into column II. Both columns were prewashed with 10 mL of 1:1 hexane–acetone. An aliquot of the extract representing 10 g of sample fortified at the 1.0- and 0.1-ppm levels or 25 g of sample fortified at the 0.01-ppm level was applied to column I. After the sample had entered the packing, columns I and II were eluted with 30 mL of 2:1 acetone–hexane. This eluate, which had passed through both columns, was collected in a 100-mL round-bottom flask as fraction 1 and contained fenamiphos and fenamiphos sulfone. After completion of the first elution, column I was repositioned so that its eluate emptied directly into a second 100-mL round-bottom flask, omitting column II. Column I was then eluted with 40 mL of acetone, and this fraction, containing fenamiphos sulfoxide, was designated fraction 2. To each fraction was added 0.25 mL of 1% OV-101 in ethyl acetate as a keeper solution, and both fractions were evaporated just to dryness in a rotary vacuum evaporator at 35 °C. The residues were dissolved in a known volume of ethyl acetate for gas chromatographic analysis.

Gas Chromatographic Analysis. Standards were made up in ethyl acetate, fenamiphos, and fenamiphos sulfone in one solution and fenamiphos sulfoxide in the other. Samples fortified at 0.01 ppm were reconstituted in 1 mL and analyzed against 0.25 μg/mL standards, whereas those fortified at 0.1 or 1.0 ppm were prepared in 1 or 10 mL and quantified against 1.0 μg/mL standards.

The GC column used was a 122 cm × 2 mm i.d. glass column packed with 3% OV-3 on 60–80-mesh Gas-Chrom Q and operated at 220 °C with a nitrogen flow of 30 mL min^{-1}. The flame photometric detector was operated at 165 °C with a hydrogen flow at 50–60 mL min^{-1} and an air flow at 80–100 mL min^{-1}. The inlet temperature was maintained at 230 °C.

RESULTS AND DISCUSSION

Initially the soil samples were spiked with all three compounds. They were left for 4 days in the refrigerator for the pesticides to equilibrate because it was felt that extracting the soil immediately after fortification gave no true indication of the ability of the method to recover adsorbed compounds. However, oxidation of fenamiphos to the sulfoxide occurred during equilibration but not from the sulfoxide to the sulfone. It was therefore necessary to fortify two batches of soil, batch 1 with fenamiphos and the sulfone and batch 2 with the sulfoxide only. The percentage recovery of sulfoxide from equilibrated soil was determined by analyzing batch 2. Batch 1 which contained both fenamiphos and fenamiphos sulfone was then analyzed for all three compounds. On the basis of the mean recovery determined previously, the sulfoxide found in batch 1 was converted to the equivalent amount of fenamiphos and designated f_{eq}. The amount of fenamiphos actually present in the soil at the time of extraction would be the amount originally added minus f_{eq}. The fenamiphos residue recovered from batch 1 was then compared with the amount actually present to determine the percentage recovery. Since the sulfone is relatively stable, no adjustment was necessary to determine its recovery. In contrast, no significant oxidation was observed in plant tissues probably because analyses were begun within 1 h of fortification.

Although it has been shown that oxidation of fenamiphos in soil is rapid (Waggoner and Khasawinah, 1974), it was interesting to find in this study that oxidation was more rapid in soil fortified at lower levels. Nearly 50%

Table I. Percent Recovery of Fenamiphos and Its Sulfoxide and Sulfone from Plant Tissue and Soil

fortification level	% recovery ± SD (n = 3)		
	fenamiphos	FSO	FSO_2
	Raspberry		
1.0 ppm	84.4 ± 5.4	82.5 ± 2.9	88.7 ± 1.2
0.1 ppm	80.0 ± 6.7	80.8 ± 6.5	83.9 ± 2.0
0.01 ppm	97.6 ± 2.2	77.1 ± 3.5	86.4 ± 6.6
	Carrot		
1.0 ppm	79.2 ± 9.1	80.7 ± .8	88.8 ± 2.8
0.1 ppm	80.2 ± 6.9	91.6 ± 7.2	99.5 ± 3.0
0.01 ppm	78.7 ± 1.5	90.0 ± 2.5	84.9 ± 6.1
	Soil		
1.0 ppm	89.2 ± 2.7	76.0 ± 3.4	92.6 ± 5.1
0.1 ppm	106.7 ± 11.8	75.0 ± 2.2	81.0 ± 7.6
0.01 ppm[a]	94.1 ± 19.6	85.8 ± 4.7	79.9 ± 9.6

[a] Actual fortification was 0.02 ppm.

of the fenamiphos in the soil fortified at 0.1 ppm had oxidized to the sulfoxide in 5 days, but over 50% had oxidized in 4 days in the soil fortified at 0.01 ppm. In light of this rapid degradation, it was evident that fortifying soils at one level actually resulted in assessing a recovery at about half the level applied. Therefore, to determine more accurately the recovery of fenamiphos at the 0.01-ppm level, 0.02 ppm of fenamiphos was initially added to the soil.

The effect of soil moisture content on extraction efficiency was investigated. Twenty-five-gram aliquots of air-dried soil which contained 7.0–7.5% moisture were fortified with 1 ppm of all three compounds, thoroughly mixed with 0, 20, 40, or 60% (w/w) distilled water and 5 g of Hyflo Super Cel, and then analyzed by the described method. Recovery was very poor for air-dried soil with no additional water but improved significantly with increasing soil moisture content up to 40%. Extraction efficiency remained unchanged from 40 to 60% moisture content. Therefore, all soils were adjusted to contain 50% moisture for the recovery studies.

Although a GC column which would separate the sulfoxide and the sulfone satisfactorily was never found, such a separation was achieved by column chromatography with silica gel. The sulfoxide was retained when the column was eluted with 2:1 acetone–hexane but desorbed with 100% acetone. Further cleanup of the acetone–hexane fraction was necessary, and it was achieved on a separate Nuchar C–cellulose column. Since most impurities were already eluted from the silica gel with the acetone–hexane mixture, a clean fraction containing the sulfoxide was obtained in the second elution with acetone. Further cleanup of the acetone fraction was unsuccessful because all other adsorbents investigated (Nuchar C, Norit A, Al_2O_3, and Florisil) retained the sulfoxide so strongly that even the most polar solvents cannot dislodge it. However, the described cleanup was sufficient even when 25 g of sample was applied to the cleanup columns.

The 3% OV-3 GC column proved to be satisfactory for the analysis of fenamiphos and its sulfone and sulfoxide. Usually one 3-μL injection of peppermint extract prior to use was sufficient to condition the column for the whole day. Other substances or even a dirty extract may accomplish the same goal. Column longevity was found to be ~1 month for continuous daily use. However, this is not a limitation as a column can be repacked easily and it is ready for use after overnight conditioning at 230 °C.

Detector response to the standard solutions was linear to both peak area and peak height. However, linearity was not affected by broadening in sample peaks if calculations were made using peak area. This made a computing integrator very useful if not mandatory. Five nanograms of fenamiphos gave a 50–60% full-scale deflection peak when the electrometer was set at 3.2×10^{-8} afs and the integrator attenuation was 2^3. Five nanograms of fenamiphos sulfone gave a similar size peak at 3.2×10^{-8} afs with the integrator attenuation at 2^1 while 5 ng of fenamiphos sulfoxide gave a peak of 30% fsd at the same setting. Retention times for fenamiphos, the sulfoxide, and the sulfone were 1.6, 3.7, and 3.8 min, respectively. Percentage recoveries were calculated by comparing areas under sample and standard peaks.

Percentage recoveries for fortified samples of raspberry, carrot, and soil are given in Table I. Each mean percentage with its SD was derived from three separate analyses. Better than 75% recoveries of all three compounds were obtained for both soil and plant material.

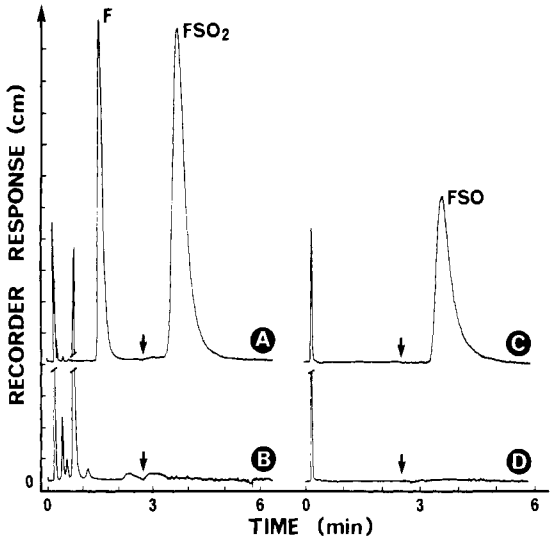

Figure 1. (A) 5 ng each of fenamiphos (F) and fenamiphos sulfone (FSO_2) and (C) fenamiphos sulfoxide (FSO) standards at 3.2×10^{-8} afs; fraction 1 (B) and fraction 2 (D) of a 10-g untreated raspberry sample, separated on the silica gel column. The arrows indicate change of integrator attenuation from 2^3 to 2^1.

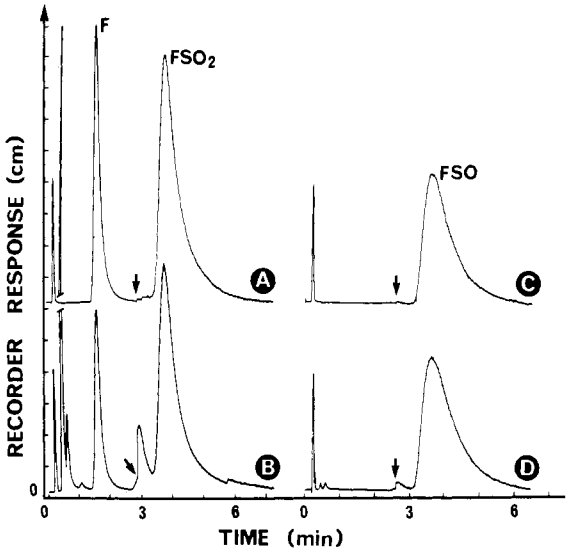

Figure 2. (A) 1.25 ng each of fenamiphos (F) and fenamiphos sulfone (FSO_2) and (C) fenamiphos sulfoxide (FSO) standards at 3.2×10^{-8} afs; fraction 1 (B) containing fenamiphos (F) and the sulfone (FSO_2) and fraction 2 (D) containing fenamiphos sulfoxide (FSO) of the extract of soil fortified at 0.01 ppm, separated on the silica gel column. The arrows indicate change of integrator attenuation from 2^1 to 2^0.

The recovery was sufficiently reproducible so that a correcting factor for 100% recovery can be applied confidently to the analysis of field-treated samples if required. Figure 1 shows chromatograms of 5 ng each of fenamiphos, the sulfoxide, and the sulfone, and of a raspberry blank of 25 g of tissue/mL of extract. Figure 2, run at a higher attenuation, shows 1.25 ng of each compound and their recovery from soils fortified at the 0.1-ppm level which gave the most interference. Since 100 pg of fenamiphos or 300 pg of fenamiphos sulfoxide or fenamiphos sulfone gives peaks 4–5 times the noise level using the FPD with attenuation at 1.6×10^{-8} afs, the limit of detection of the described method may well be below 0.01 ppm for soil and plant tissue.

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Determination of Seventeen *s*-Triazine Herbicides and Derivatives by High-Pressure Liquid Chromatography

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Mixtures of simazine, atrazine, ametryne, prometryne, deethylsimazine, deethylatrazine, hydroxysimazine, hydoxyatrazine, hydoxyprometryne, *N*-ethyl- and *N*-isopropylammeline, *N*-ethyl- and *N*-isopropylammelide, melamine, ammeline, ammelide, and cyanuric acid in aqueous solution were separated and determined in a single analysis with a detection limit of 30–400 pmol. The *s*-triazines were detected by a UV detector after elution from a reversed-phase high-pressure liquid chromatography column using a phosphate buffer–methanol gradient at 2 °C.

The *s*-triazine herbicides are widely used, accounting for 28% of herbicide manufacture in 1974 (Kühle, 1976), and *s*-triazines have many other industrial uses [e.g., Cook and Hütter (1981a)]. However, no single method is available for the routine identification and quantification of these compounds, especially the more polar derivatives like ammeline, ammelide, and their N-alkylated derivatives. The available techniques all apply only to limited ranges of compounds. Paper chromatography and thin-layer chromatography require many solvent systems for the desired separations and offer poor and time-consuming quantification [reviews by Fishbein (1970, 1975); see also Loos and Kearney (1978)]. Gas chromatography has been used extensively, but derivatization is essential for many compounds and there seems to be no universal derivatizing agent; furthermore, derivatization is seldom quantitative, and several columns are required to cover the whole range of compounds (Fishbein, 1970, 1975; Lusby and Kearney, 1978; Muir and Baker, 1978; Stoks and Schwartz, 1979; Muir, 1980). Low-pressure column chromatography, which has poor separative properties and is time consuming, has been used occasionally (Plaisted and Thornton, 1964). High-pressure liquid chromatography (HPLC) of *s*-triazines overcomes many difficulties arising from low volatility, low solubility, and chemical inertness. Thus, e.g., Smolková and Pacáková (1978) separated 19 *s*-triazine herbicides on a CN-bonded silica column [see also Lawrence and Turton (1978)]. Lawrence and Leduc (1978), Ramsteiner and Hörmann (1979), and Muir (1980) showed partial separation of *N*-alkylammelines and five "hydroxyparents" (hydroxysimazine, hydroxyatrazine, hydroxyprometryne, etc.) on silica columns. Demian et al. (1979) used a reversed-phase column to determine hydroxyparents. Each of these methods covers only limited ranges of compounds.

We report here a simple method to identify and quantify chloro- and (methylthio)-*s*-triazine herbicides, dealkylatrazines, hydroxyparents, *N*-alkylammelines, *N*-alkylammelides, melamine, ammeline, ammelide, and cyanuric acid in aqueous solutions by reversed-phase HPLC of underivatized samples.

EXPERIMENTAL SECTION

Apparatus. HPLC was done using jacketed stainless steel analytical columns (25 cm × 4.6 mm inner diameter) containing a reversed-phase packing of 5-μm mean particle diameter (LiChrosorb RP-18; Merck, Darmstadt, FRG). The mobile phase was delivered through a dynamic mixer (Altex, Berkeley, CA) by two pumps (Altex Model 110) which were controlled by a gradient programmer (Altex Model 420), and samples were applied to the column by using a high-pressure sample injector with pneumatic actuator (Model 7010/70-01; Rheodyne, Berkeley, CA) connected to an automatic sampler (ASI 45; Kontron, Zürich, Switzerland). The sample loop of the injector was normally 20 μL. The eluate from the column passed through a UV detector (Uvikon LCD 725; Kontron) coupled to an integrator with printer/plotter (C-R1A; Shimadzu, Kyoto, Japan). The HPLC column was maintained at 2 °C by passing cooling fluid from a cryostat through the jacket.

Mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6 mass spectrometer by using direct probe insertion and electron impact ionization at 70 eV. UV spectra were obtained by using a Uvikon 820 spectrophotometer (Kontron).

Chemicals. The *s*-triazines used, their abbreviations, sources, and Chemical Abstracts Service Registy Numbers are given in Table I. The identity of each triazine was

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