Determination of Residues of Nemacur and Its Metabolites in Plant and Animal Tissues

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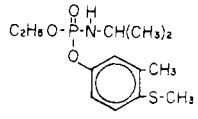
A specific gas chromatographic procedure is described for the determination of residues of Nemacur [ethyl 4-(methylthio)-*m*-tolyl isopropylphosphoramidate], a promising new nematicide, and its metabolites in plant and animal tissues. Following initial extraction, the extract is oxidized with potassium permanganate to convert Nemacur and its sulfoxide to the sulfone. Final detection of the sulfone is by the phosphorus-sensitive alkali-flame detector, thereby allowing little interference from tissue extractives. Recovery data from experiments run on a large variety of tissues by adding known amounts of Nemacur or metabolites at the blending step were generally in the 75 to 110% range. The method is specific in the presence of other possible organophosphorus chemicals and is sensitive to the 0.01-ppm level.

Nemacur, ethyl 4-(methylthio)-*m*-tolyl isopropylphosphoramidate, also known as Bay 68138, is a low volatile nematicide being developed for use on a wide variety of field, vegetable, and fruit crops. Extensive initial field tests have been made with peanuts, pineapples, and tobacco.

Nemacur has shown high nematicidal activity when applied by a variety of methods including broadcast, row, band, drench, and irrigation applications before or at planting time or to established plantings (Johnson *et al.*, 1969; Jorgenson, 1969; Miller and Taylor, 1970; Stokes and Laughlin, 1970; Taylor and O'Bannon, 1968). Nematicidal activity has been reported against endoparasitic, ectoparasitic, cyst-forming, and root-knotting species. Plant tolerance to the chemical has been excellent. Vegetables, field crops, and fruits have not been injured by applications at several times the effective nematicidal rate.

Metabolism studies (Waggoner, 1969) with both plants and animals have identified the major metabolite as the sulfoxide. Lesser amounts of the sulfone were also formed. The structural formula for the parent compound is



The primary concern in the development of a suitable residue method was to account for the parent compound and its oxidative metabolites with adequate sensitivity. Preliminary work showed that all three compounds could be separated on a gas chromatograph. However, a multicomponent analysis has the disadvantage of increased possibility of interference from crop extractives or other pesticides. Conversion of all three compounds to a single component, the sulfone, would simplify the analysis while still accounting for all three compounds as well as enhance the sensitivity because all three compounds would be concentrated in a single peak.

This paper describes a residue procedure based on conversion of Nemacur and its sulfoxide metabolite to the sulfone using potassium permanganate as the oxidant. The sulfone is then suitably cleaned up and measured gas chromatographically employing an alkali-flame detector. Initial extractions and cleanup schemes are included for several crops as well as animal tissues, since portions of these plants other than the fruit are often used as cattle feed.

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ANALYTICAL METHOD

Apparatus. A Hewlett-Packard Model 5750 gas chromatograph equipped with a flame ionization detector modified for alkali-flame operation as previously described by Thornton and Anderson (1968) was used. Explosion-proof blender motors were used to minimize the fire hazard from volatile organic solvents.

Reagents. Florisil (PR grade, 60–100 mesh) was heated in an oven at 130° C for 24 hr to remove moisture. It was then deactivated by adding 7% water (7 ml of H_2O + 93 g of dried Florisil) and allowed to equilibrate for 24 hr in a tightly stoppered bottle before use. Methanol:sulfuric acid was prepared by diluting 60 volumes of methanol to 100 with 0.05 *N* sulfuric acid. The slightly acidic solvent minimized emulsions in the presence of crop extracts. All solvent partitions between acetonitrile and Skellysolve B are carried out using solvents which have been presaturated each with the other.

Sample Preparation. Grind wet crops and animal tissues in a Hobart food cutter in the presence of Dry Ice and place the samples in frozen storage overnight to allow the Dry Ice to sublime. Grind dry samples in a Wiley mill to pass a No. 3 screen.

Sample Extraction. EXTRACTION OF CITRUS PEEL, CITRUS PULP, AND PINEAPPLE FRUIT. Place 100 g of sample into a Waring Blendor jar marked at the 300-ml level. Add 180 ml of acetone and blend for 2 min at high speed. Dilute to the 300-ml mark with water and blend for one additional minute. Filter through 32-cm Whatman No. 2V fluted filter paper and collect 150 ml of filtrate in a graduated cylinder. Transfer the filtrate to a 500-ml separatory funnel and extract successively with 150-, 75-, and 75-ml portions of chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator at 40° C. Proceed to oxidation.

EXTRACTION OF CURED TOBACCO, PEANUT HULLS, PEANUT VINES, PINEAPPLE BRAN, AND PINEAPPLE FORAGE. Weigh a 25-g portion of sample into a blender jar. Add 300 ml of 60:40 methanol:H_2SO_4 (0.05 *N*) and blend for 3 min at high speed. Filter with vacuum through Whatman No. 541 filter paper, covered with a 0.25-in. layer of Hyflo Super-Cel. Rinse the blender with 100 ml of extraction solvent mixture and use this to wash the filter cake. Transfer the filtrate to a 1-l. separatory funnel. Rinse the filter flask with 200 ml of chloroform and add this to the separatory funnel. Shake the funnel for 30 sec, allow the phases to separate, and drain the lower phase into a 1000-ml round-bottomed flask. Repeat the chloroform extraction steps twice more with 200- and 100-ml portions of fresh chloroform. Evaporate the combined chloroform extracts to near dryness on a rotary vacuum evap-

orator. About 2 to 3 ml of water will remain. Add 200 ml of chloroform to the residue in the flask and transfer to a 500-ml separatory funnel. Rinse the flask with 200 ml of 0.05 *N* H_2SO_4 and add this to the separatory funnel. Shake the funnel for 30 sec, allow the phases to separate, and drain the lower chloroform phase into a 500-ml round-bottomed flask. Repeat the extraction using 100 ml of fresh chloroform. Evaporate the combined chloroform extracts just to dryness on a rotary vacuum evaporator at 40° C. Proceed to oxidation.

EXTRACTION OF PEANUT MEAT AND ANIMAL TISSUES (OTHER THAN FAT). Place 50 g of chopped sample into a blender jar (use 25 g for peanuts). Add 10 g of Hyflo Super-Cel and 200 ml of acetone, and blend for 3 min at high speed. Filter with vacuum through Whatman No. 42 filter paper. Reblend the filter cake with 200 ml of chloroform and filter as before. Rinse the blender with 100 ml of fresh chloroform and use this to wash the filter cake. Transfer the combined filtrate to a separatory funnel. Shake the separatory funnel for 30 sec. Allow the phases to separate and drain the lower phase through 32-cm Whatman No. 2V fluted filter paper containing 1 tsp of Hyflo Super-Cel. Collect the filtrate in a 1000-ml round-bottomed flask. Rinse the filter paper with a fresh 25-ml portion of chloroform. Evaporate the filtrate just to dryness on a rotary vacuum evaporator at 40° C. Transfer the residue to a 500-ml separatory funnel using 250 ml of Skellysolve B. Rinse the flask with 150 ml of acetonitrile and add to the separatory funnel. Shake the funnel for 30 sec, allow the phases to separate, and drain the lower phase into a second 500-ml separatory funnel containing 100 ml of Skellysolve B. Shake the second separatory funnel for 30 sec, allow the phases to separate, and drain the lower phase into a 500-ml round-bottomed flask. Repeat the above two-stage extraction, using a fresh 100-ml portion of acetonitrile. Evaporate the combined acetonitrile extracts just to dryness on a rotary vacuum evaporator at 40° C. Proceed to oxidation.

EXTRACTION OF FAT SAMPLE. Weigh 25 g of chopped sample into a 1-qt blender jar. Add 250 ml of Skellysolve B and 15 g of Hyflo Super-Cel and blend at high speed for 3 min. Filter with vacuum through Whatman No. 42 filter paper. Rinse the blender with 150 ml of acetonitrile and use this to wash the filter cake. Continue with the Skellysolve B/acetonitrile partition steps described under EXTRACTION OF ANIMAL TISSUES above.

Oxidation. Place 5 μg of Nemacur standard in a 100-ml round-bottomed flask in 2 ml of acetone solution and carry through the oxidation procedure. Do not pass the standard through the Florisil column.

Dissolve the sample residue from the previous steps in 2 ml of acetone. Add 5 ml of 20% $MgSO_4$ solution and 20 ml of 0.1 *M* $KNnO_4$ solution, washing down the sides of the flask during the additions. Mix and let stand for 15 min at room temperature with occasional swirling. Transfer to a 125-ml separatory funnel using 20 ml of chloroform to complete the transfer. Shake the separatory funnel for 30 sec, allow the phases to separate (centrifuge if necessary), and drain the lower phase through 15 to 20 g of powdered sodium sulfate retained in a powder funnel with a loose plug of glass wool. Collect the filtrate in a 300-ml round-bottomed flask. Repeat the chloroform extraction two additional times with fresh 20-ml portions of chloroform. Rinse the sodium sulfate with 10 ml of chloroform. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator at 40° C.

Florisil Column (Tobacco, Peanut Hulls, and Animal Tissues Only). Tamp a plug of glass wool into the bottom of a 20 × 400 mm chromatographic column with integral reservoir. Fill the column up to the reservoir with 20% acetone in chloroform. Slowly sprinkle in 7 g of 7% water deactivated Florisil and allow it to settle. Top the column with about 1 in. of granular sodium sulfate. Dissolve the residue from the oxidation steps in 5 ml of 20% acetone in chloroform and transfer to the column. Rinse the flask with three 2-ml portions of 20% acetone in chloroform and add each to the column just as the previous rinse has drained into the sodium sulfate layer. Finally, elute the column with 90 ml of 20% acetone in chloroform at a rate of 2 to 3 drops/sec and collect the total eluate in a 250-ml round-bottomed flask. Evaporate the eluate just to dryness on a rotary vacuum evaporator at 40° C.

Gas Chromatographic Analysis. Dissolve the standard and sample residue from the previous steps in 4 ml of acetone and inject an appropriate aliquot into the alkali-flame modified gas chromatograph maintained at the following conditions: column—1 ft × 4 mm i.d. borosilicate glass column, packed with 6% QF-1, solution coated on 80–100 mesh Gas Chrom Q; gas flows—helium carrier gas, 100 ml/min; hydrogen—adjust hydrogen flow after other gases are set so that approximately a one-half scale peak results from a 5-ng standard injected; temperatures—column, 230° C, injection port, 230° C, detector, 240° C.

Identify the Nemacur sulfone peak by its retention time and measure the area or peak height produced on the recorder strip chart. At the gas chromatographic conditions employed, Nemacur sulfone has a retention time of 4.5 min.

Calculate parts per million of residue in a sample by comparing the response obtained for the unknown to the response obtained for a known amount of Nemacur standard started at the oxidation steps, including appropriate factors for sample size, aliquots, and dilutions. An appropriate dilution of Nemacur sulfone, if available, may be directly injected into the gas chromatograph for use as a standard. Oxidation of Nemacur itself is recommended because the pure sulfone is not readily available to all workers.

DISCUSSION

The method described in this report measures not only Nemacur residues but also its possible metabolites, the sulfoxide and sulfone. Although all three of these compounds give a peak when injected into the gas chromatograph, considerations of simplicity and sensitivity make it more desirable to oxidize the parent compound and the sulfoxide to the sulfone with subsequent gas chromatographic analysis of only one peak. Room temperature oxidation is quantitative using 0.1 *M* potassium permanganate (Tietz and Frehse, 1960) for 15 min. Oxidation also converts most tissue extractives and pigments to a water soluble form, making them easy to remove.

A Florisil column cleanup step is included for animal tissues and crops such as tobacco which require additional cleanup after oxidation, prior to gas chromatographic analysis. Samples other than these may be carried through the column steps if necessary for further cleanup. In some cases the extract may still appear brown-colored even after the Florisil column. This does not appear to cause any difficulty with the alkali-flame detector.

With some oily samples such as certain types of peanuts, emulsions are likely to occur at the Skellysolve B–acetonitrile partition steps after initial extraction. To minimize these

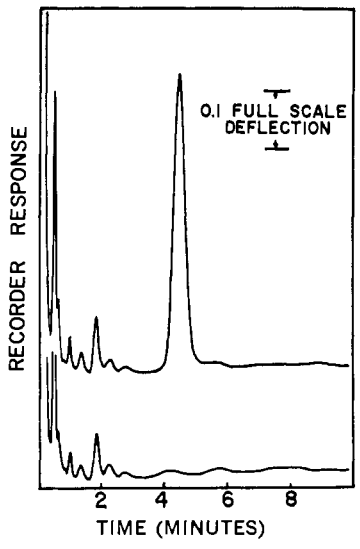

Figure 1. Gas chromatograms of peanut meat control (lower curve) and peanut meat fortified with 0.1 ppm of Nemacur (upper curve)

Table I. Recovery of Nemacur and Metabolites from Representative Samples

Sample type	Compound added[a]	ppm added	Recovery, %[b]
Milk	Nemacur	0.005	114
Milk	Nemacur sulfoxide	0.005	84
Milk	Nemacur sulfone	0.005	104
Bovine liver	Nemacur	0.1	76, 85
Bovine liver	Nemacur sulfoxide	0.1	100, 83
Bovine liver	Nemacur sulfone	0.1	94, 80
Orange peel	Nemacur	0.1	95, 89
Orange peel	Nemacur sulfoxide	0.1	93, 125
Orange peel	Nemacur sulfone	0.1	89, 110
Peanut meat	Nemacur	0.1	96, 78
Peanut meat	Nemacur sulfoxide	0.1	96, 89
Peanut meat	Nemacur sulfone	0.1	79, 72
Tobacco (cured)	Nemacur	0.5	78, 81
Tobacco (cured)	Nemacur sulfoxide	0.5	88, 97
Tobacco (cured)	Nemacur sulfone	0.5	88, 88
Clay loam soil	Nemacur	1.0	97, 100
Clay loam soil	Nemacur sulfoxide	1.0	95, 102
Clay loam soil	Nemacur sulfone	1.0	96, 99
Peanut vines	Nemacur	1.0	99
Peanut vines	Nemacur sulfoxide	1.0	94
Peanut vines	Nemacur sulfone	1.0	92

[a] Controls were <0.01 ppm in all cases (<0.002 for milk). [b] Recovery percents are from separate determinations, fortified in blender.

emulsions, the volumes of solvents may be doubled with no loss in recovery of Nemacur or metabolites.

Recovery experiments were run on a number of crops and animal tissues by adding known amounts of Nemacur and metabolites at the initial blending step. Recoveries were generally determined at the 0.1-ppm level, but a few additional values were obtained at levels ranging from 0.002 ppm in milk to 1.0 ppm in soil. Where samples were fortified with metabolites of Nemacur, an appropriate amount of that compound was oxidized for use as a standard to eliminate corrections for molecular weight and percent purity. A few representative results are shown in Table I. Many other crops not listed in the table have been checked and found to yield good recovery and negligible control values. These include bovine brain, fat, heart, kidney, steak and milk, and green tobacco, orange pulp, peanut hulls, pineapple bran, pineapple fruit, snap beans, and various types of soils. Recovery values at three fortification levels for several different samples were averaged and standard deviations calculated to show the precision of the method. These results are shown in Table II. Chromatograms from controls were very clean in almost every case with only minor extraneous peaks near the solvent peak. Representative chromatograms are shown for a control and a fortified sample of peanuts in Figure 1.

An experiment was conducted to check the efficiency of the initial extraction step in removing residue quantities of Nemacur from crops. Dry tobacco was selected because it is considered a difficult crop to clean up for residue analysis. Also, a dry crop tends to adsorb polar molecules more than a wet crop. Separate, 500-g quantities of dry, cured tobacco were fortified with Nemacur and metabolites at the 0.5-ppm level. These compounds were added to the tobacco in acetone solution and the samples were thoroughly mixed while allowing all traces of solvent to evaporate. The fortified samples were allowed to stand at room temperature for 24 hr and were then analyzed by the normal procedure. Recovery of all three compounds averaged 84%, the lowest of which was 78% for the parent compound, indicating the extraction procedure to be adequate.

The solution coating technique (Applied Science Laboratories, 1967), used to prepare the gas chromatographic column packing, was necessary to produce a column without tailing or adsorption for the Nemacur sulfone peak. After packing, the column was "no flow" conditioned at 250° C for 2 hr, followed by flow conditioning at operating conditions at least 4 hr before use. Several columns have been prepared with identical results, indicating the procedure to be reproducible.

A standard curve was prepared to determine linearity of

Table II. Recovery of Nemacur and Metabolites from Various Sample Types after Fortification at Three Levels

	Recovery in ppm, $\bar{x} \pm S\bar{x}$[a]		
	Nemacur	Nemacur sulfoxide	Nemacur sulfone
Recovery at 0.1 ppm level from various samples including: animal tissues, oranges, peanuts, pineapples, and tobacco	0.080 ± 0.013 (n = 23)	0.094 ± 0.012 (n = 21)	0.094 ± 0.011 (n = 23)
Recovery at 0.5 ppm level from various samples including: peanuts, soils, and tobacco	0.47 ± 0.067 (n = 5)	0.48 ± 0.026 (n = 5)	0.49 ± 0.028 (n = 4)
Recovery at 1.0 ppm level from various samples including: peanuts, soils, and tobacco	1.00 ± 0.034 (n = 4)	0.96 ± 0.095 (n = 6)	1.01 ± 0.090 (n = 5)

[a] All recovery samples were fortified in the blender.

Table III. Compounds Tested for Interference with the Nemacur Residue Analysis Method

Compound	ppm Level tested[a]
Delnav	2.8
Diazinon	1.0
Di-Syston	1.0
Dyfonate	1.0
EPN	3.0
Ethion	2.0
Ethyl parathion	1.0
Guthion	1.0
Malathion	8.0
Methyl parathion	1.0
Naled	3.0
Phorate	0.1
Phosdrin	0.25
Phosphamidon	0.75
Systox	0.75
Trichlorfon	1.0
Trithion	2.0

[a] No interference was noted from any of the chemicals tested.

response in the gas chromatograph for the sulfone. Response was linear over a 50-fold range up to at least 25 ng. Any samples containing residues in excess of this concentration should be diluted and reinjected to ensure that response falls along the linear portion of the curve.

If 0.1 in.^{2} is considered the smallest area which can be accurately measured with a polar planimeter, the level of sensitivity is determined by the amount of Nemacur sulfone necessary to produce this area. In general, 0.1 ppm (5 ng) of standard produced a peak of 1 in.^{2} or better, indicating the sensitivity of the method to be approximately 0.01 ppm. If the criteria of 2× the noise level is selected as the limiting factor, sensitivity would be somewhat better.

To determine the specificity of the method for Nemacur in the presence of other pest control chemicals, an interference study was conducted. Because of the relative specificity of the alkali-flame detector, only organophosphorus chemicals were tested as possible interferences. All of the organophosphorus compounds registered for use on citrus, peanuts, and pineapples were run at the highest level as listed in the N.A.C. News and Pesticide Review (1969). All phosphorus-containing pesticides listed in Handbook No. 331 (1968) for use on tobacco were run at the 1.0-ppm level. A list of these chemicals and the level at which they were tested is shown in Table III. No interferences were noted from any of these chemicals.

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