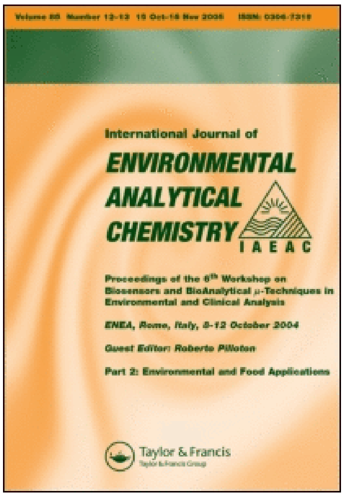

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Determination and Persistence of Bay 68138 and Two of its Metabolities in Turf Grass

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Determination and Persistence of Bay 68138 and Two of its Metabolities in Turf Grass

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Residues of Bay 68138 (Nemacur P®) [ethyl 4-(methylthio)-*m*-tolyl isopropylphosphoramidate] and its sulfoxide and sulfone were determined in turf grass. The extracts were separated by liquid chromatography on silica gel into two fractions which were concentrated and injected into a gas chromatograph equipped with a flame photometric detector sensitive to phosphorus. Recoveries were 95 to 105% for all three compounds at the 0.10-ppm level; sensitivity to each compound was 0.01 ppm or better. Retention times for the three compounds on six liquid phases, *p*-values in seven solvent systems, and conditions for separating the compounds by liquid chromatography were determined. Total residues in turf grass treated with a granular formulation at the rate of 15 lb (active ingredient) per acre, declined from 4930 to 102 ppm (dry basis) during a 49-day period of weathering. Bay 68138 was found to oxidize to its sulfoxide and sulfone.

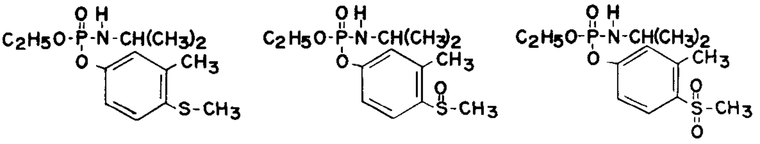
INTRODUCTION

Bay 68138, Nemacur P®, ethyl 4-(methylthio)-*m*-tolyl isopropylphosphoramidate, a product of Chemagro Corporation, is a systemic pesticide effective against insects and nematodes.[1]

The compound contains an alkyl thioether linkage which may be oxidized to form a sulfoxide and sulfone; both metabolites were identified in plants and animals treated with the chemical.[2] Formulas of the three compounds are shown in Figure 1.

Thornton[3] reported a method for determining residues of Bay 68138: the compound and its sulfoxide (II) were oxidized to the sulfone (III) with potassium permanganate. Final detection of the sulfone was accomplished by the phosphorus-sensitive alkali flame detector. Sensitivity of this method is about 0.01 ppm. The individual metabolites were not determined.

A method sensitive to 0.01 ppm or less of each metabolite was therefore devised to determine each of the three compounds. The procedures are similar to those described by Bowman and Beroza[4] for fenthion and five of its metabolites and by Bowman and Hill[5] for Dasanit®, *O*,*O*-diethyl *O*-[*p*-(methylsulfinyl) phenyl] phosphorothioate, and three of its metabolites. After the substrate is extracted, the extract is separated into two fractions by liquid chromatography on a silica-gel column. Aliquots of each fraction are then injected into a gas chromatograph equipped with a flame photometric detector sensitive to phosphorus. Recoveries are 95 to 105% for all three compounds.



I II III

FIGURE 1 Bay 68138 (I) and 2 of its metabolites. I, P=O,S; II, P=O,SO; III, P=O,SO_2.

Also, as part of this study, the persistence of the three compounds in turf grass in the field was determined during the 49 days of weathering that followed application of Bay 68138 as a granular formulation.

EXPERIMENTAL

Apparatus

A Hewlett–Packard Co., Model 5750 gas chromatograph equipped with the Melpar flame photometric detector was operated in the phosphorus mode (526 nm filter).

The liquid chromatographic columns (Kontes No. 42000) were 12 mm i.d. glass. The Soxhlet extractors were Fisher Scientific Co., No. 9-556 B.

Reagents and Solvents

Analytical-grade samples of Nemacur P and the two metabolites and the 15% Nemacur P granular formulation were kindly supplied by the Chemagro Corporation.

The silica gel (J. T. Baker Chemical Co., No. 3405) was found to contain 4.8% water (loss in weight after overnight drying at 110°C). Therefore, for the liquid chromatography, the water content was adjusted to 15% by weight by adding the required amount of distilled water to a 1-kg batch of the adsorbent in a reagent jar and rolling it overnight on a ball mill (with no balls in jar).

The chloroform, benzene, acetone, and absolute methanol used were CP grade solvents; all except methanol were distilled before use. Sodium sulfate was the anhydrous granular reagent-grade chemical.

Sample Preparation and Extraction

Samples of turf grass from the mower (ca. 10-20 mesh) were mixed well. Then 20 g of each sample was transferred to a Soxhlet apparatus containing a plug of glass wool (to prevent insoluble plant material from siphoning over during the solvent exchanges) and extracted under carbon dioxide for 4 hr with 150 ml of 10% methanol in chloroform at a rate of about six solvent exchanges/hr. The extract was allowed to cool and then percolated through a plug of sodium sulfate (ca. 25 mm dia. × 35 mm thick). After the flask and plug were washed with 10 ml of fresh chloroform, the extract was evaporated just to dryness on a 50°C water bath under water pump vacuum (any residues of methanol, chloroform, or water must be removed to avoid difficulty in the subsequent liquid chromatography). The residue was taken up in 10 ml of benzene for liquid chromatography.

The dry matter content, i.e., material not volatile in a 100°C oven overnight, was also determined for each sample.

Liquid Chromatography

A silica-gel column was prepared by adding successively to the glass chromatographic column a plug of glass wool, 2 g of sodium sulfate, 5 g of silica gel (15% water), and 2 g of sodium sulfate. The column was washed with 25 ml of benzene, and the wash liquid was discarded. The grass extract was added to the column and washed into the adsorbent with two 5-ml portions of 2.5% acetone in benzene. The column was then eluted with an additional 20 ml of 2.5% acetone in benzene. This eluate, which contained only extraneous plant material, was discarded. Next, the column was eluted with 45 ml of 25% acetone in benzene; the eluate (fraction A) contained Nemacur P (I) and its sulfone (III). Finally, the column was eluted with 50 ml of acetone; the eluate (fraction B) contained the sulfoxide (II). Each fraction was evaporated just to dryness under water-pump vacuum with a 50°C water bath. The residues were transferred to calibrated tubes and adjusted to exactly 5 ml with acetone (5 mcl equivalent to 20 mg of grass) for gas chromatographic analysis.

The liquid chromatographic operations are summarized in Figure 2.

Gas Chromatographic Analysis

The following conditions were used for gas chromatography:

Column:	Glass, 45 cm × 4 mm i.d. (6 mm o.d.)
Packing:	OV-17, 5% (w/w) on 80 to 100 mesh Gas Chrom Q
Gases:	Nitrogen (carrier) 160 ml/min; oxygen 40 ml/min; hydrogen 200 ml/min.
Temperatures:	Column 230°C; injection port 250°C; detector (external) 250°C.

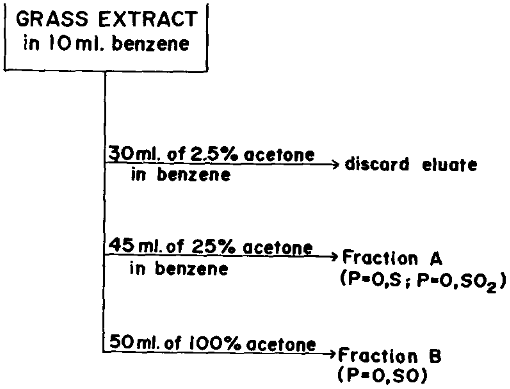


FIGURE 2 Summary of operations in liquid chromatography on silica gel.

The column was conditioned overnight at 250°C and then conditioned further (with the instrument operated as described) by injecting 250-ng amounts of the appropriate pesticide until several 5-ng amounts produced a constant response. Five microliters of each fraction (or a dilution thereof) was injected alternately with standard solutions, and residues in the unknowns were calculated on the basis of peak height, which was shown to be proportional to concentration of compound injected.

Recovery Experiments

Duplicate 20-g samples of turf grass were separately spiked with 5.0 and 0.10 ppm of each compound prior to the Soxhlet extractions; others were spiked with mixtures containing 50, 5.0, and 0.10 ppm of each compound. Unspiked controls were also carried through the procedure.

Field Treatment and Sampling of Turf Grass

Four plots of turf grass (Tifton 328) at Winter Haven, Florida, were treated March 5, 1971, by broadcasting a 15% granular formulation of Bay 68138 at the rate of 15 lb (active ingredient) per acre with a mechanical spreader (Cyclone Seeder Co., Inc.). Analysis of the granular formulation showed that it contained 15.0, 0.0243, and <0.0002% by weight of Bay 68138 and its sulfoxide and sulfone, respectively.

Samples (0.25 lb/plot) were taken with a Toro Greenmaster mower on the day of application and 1, 7, 14, 21, 28, 35, 42, and 49 days later. The samples were frozen immediately after collection. No measurable rainfall occurred during the 49-day test; however, about 0.25 in. of water was applied with overhead sprinklers for about 15 min each night.

RESULTS AND DISCUSSION

The Soxhlet extraction procedure was used to obtain maximum recoveries of the residues from the field-weathered samples.[6] An extraction period of 4 hr was necessary to remove all residues recoverable by the method. The extractions were performed under an atmosphere of carbon dioxide to prevent oxidation of Bay 68138 and its sulfoxide since the atmosphere of nitrogen normally used in our Soxhlet procedures was inadequate because slight oxidation of the residues occurred during the extraction.

Recoveries from samples spiked as described and carried through the entire analytical procedure were 95-105% for all compounds at all levels. The sensitivity to each compound was 0.01 ppm or better (based on twice noise) on a wet-sample basis. No evidence was found that Bay 68138 or its sulfoxide oxidized during analysis. However, when the grass was spiked with a large amount of any one compound, the impurities in that compound were detected. An analysis of each of the standards for the other metabolites showed that P=O,S contained 0.13% of P=O,SO; P=O,SO contained 0.11% of P=O,S and 0.97% of $P{=}O,SO_2$; and $P{=}O,SO_2$ contained 0.02% of P=O,S and 0.34% of P=O,SO.

Gas chromatograms of fractions A and B obtained from the liquid chromatography of turf grass on silica gel are presented in Figure 3. The retention time of Bay 68138 was 1.00 min; those of the sulfoxide and sulfone were both 3.05 min. The peak in fraction A at about 1.6 min is a naturally occurring material found in untreated turf grass taken at both Winter Haven, Florida and Tifton, Georgia: in gas chromatography with a 45-cm column of 10% OV-101 (methyl silicone), it emerged under the sulfone peak; with a column of 5% OV-25 (methyl phenyl silicone, 75% phenyl) of the same length, it emerged under the Bay 68138 peak. Therefore, a 10% OV-17 (methyl phenyl silicone, 50% phenyl) column was selected for the analyses because it shifted the peak of this material to a portion of the chromatogram where it did not interfere.

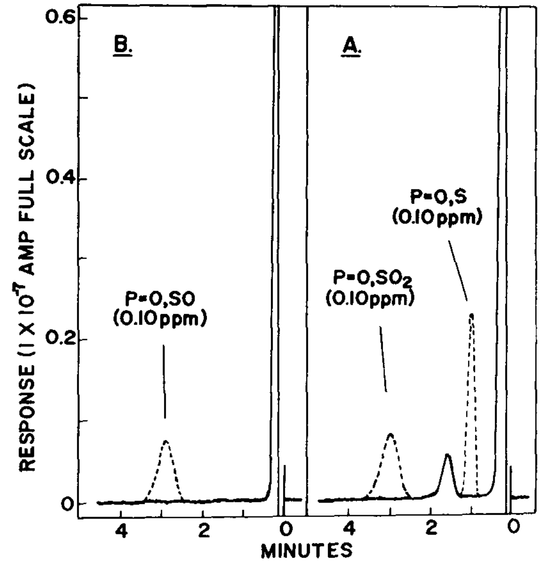


FIGURE 3 Gas chromatograms of Bay 68138 and 2 of its metabolites in fractions A and B obtained by liquid chromatography on silica gel. Solid lines are unfortified controls of turf grass equivalent to 20 mg; broken lines are the same fractions spiked with 2 ng (0.10 ppm) of the 3 compounds.

TABLE I

Retention times of Bay 68138 and two of its metabolites on eight gas chromatographic columns

Gas chromatographic column†	Column temperature (°C)	Retention time (min) for indicated compound		
		P=O,S	P=O,SO	P=O,SO$_2$
5% OV-225	250	1.15	5.60	6.70
5% OV-25	230	1.35	4.60	4.50
5% OV-210	230	0.60	3.50	3.80
5% Dexsil 300	230	1.30	4.30	4.35
10% OV-17	240	2.30	6.75	6.80
10% OV-17 (45 cm)	230	1.00	3.05	3.05
	250	0.50	1.55	1.55
10% OV-101	220	1.70	4.20	3.85
10% OV-101 (45 cm)	200	1.45	3.90	3.65
	220	0.70	1.75	1.60

† All columns are borosilicate glass, 6 mm o.d. (4 mm i.d.) × 100 cm long except as noted. Gas Chrom Q (80–100 mesh) was used to prepare all packings; columns were conditioned overnight at 250°C prior to use.

TABLE II

p-Values of Bay 68138 and two of its metabolites in seven binary solvent systems

Compound	Hexane-water	Hexane-20% MeCN (80% water)	Hexane-40% MeCN (60% water)	Hexane-60% MeCN (40% water)	Hexane-80% MeCN (20% water)	Hexane-MeCN	Benzene-water
Bay 68138 (P=O,S)	0.94	0.65	0.20	0.051	0.023	0.038	0.98
Bay 68138 sulfoxide (P=O,SO)	0.002	0.002	0.002	0.002	0.002	0.004	0.52
Bay 68138 sulfone (P=O,SO_2)	0.009	0.006	0.002	0.002	0.002	0.004	0.93

Retention times of the three compounds on six different column packings are presented in Table I; these may prove useful in confirming the identities of peaks or in circumstances where interferences occur. As a further aid in confirming identities of the compounds at the ng level, *p*-values[7,8] were determined in seven solvent systems; these values are presented in Table II.

Since the sulfoxide and sulfone of Nemacur P were not completely separated on our nonpolar gas chromatographic columns (Table I), we sought a means of separating them prior to analysis. Experiments were conducted with silica-gel columns (5 g), containing 4.8, 10, and 15% moisture and eluted with benzene, acetone, or various mixtures of both solvents. Complete separation of the three compounds was obtained with all columns; however, oxidation of about 10 and 2% of Nemacur P to its sulfoxide was observed on the silica gel containing 4.8 and 10% water, respectively. No oxidation was detected with the silica gel containing 15% water; therefore, it was employed in the analytical method.

The separation of 100 mcg of each of the three compounds contained in 1 ml of benzene and introduced on a column prepared from 5 g of silica gel containing 15% water is illustrated in Figure 4. These results may be useful

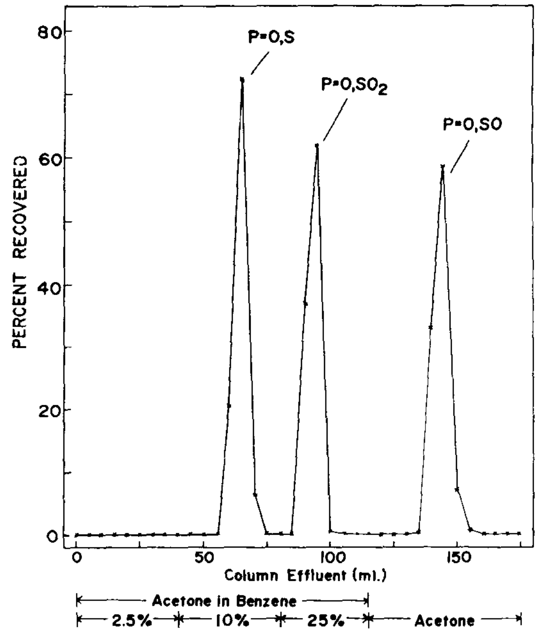


FIGURE 4 Separation of Bay 68138 and 2 of its metabolites by liquid chromatography on silica gel.

TABLE III

Residues of Bay 68138 and two of its metabolites in turf grass after treatment with 15% granular formulation at the rate of 15 lb (active ingredient) per acre. Winter Haven, Florida 1971.†

Days after application	Dry matter (%) ($\bar{x}\pm$S.E.M.)	Residues of indicated compound (ppm, $\bar{x}\pm$S.E.M., dry basis)			
		P=O,S	P=O,SO	P=O,SO$_2$	Total
0	21.0±0.2	4540 ±410	224 ±17	170 ±20	4930±450
1	17.2±0.4	4100 ±160	583 ±16	208 ±10	4890±180
7	36.0±0.4	110 ± 3.0	769 ±28	935 ±27	1810± 40
14	35.4±0.4	79.2 ± 6.5	678 ±47	772 ±19	1530± 40
21	22.0±0.2	16.8 ± 0.8	485 ±13	218 ±14	720± 13
28	21.0±0.9	13.5 ± 2.5	304 ±17	150 ±11	468± 20
35	17.2±0.3	5.62± 0.13	165 ± 4.0	63.1± 1.1	234± 11
42	19.1±0.4	4.13± 0.30	111 ± 5.0	45.3± 2.6	160± 8
49	13.8±0.8	1.60± 0.04	70.8± 2.4	29.3± 1.2	102± 4

† Each value is the mean of four replicates.

in adapting the method to other substrates or to other analyses (e.g., metabolic studies).

Results of the field persistence tests with Bay 68138 applied to turf grass are presented in Table III. Because of the wide variation in moisture content of the samples, residues were calculated on a dry basis to provide a meaningful comparison of data for the various intervals. Residues may be converted to their original basis (wet) by using the values for dry-matter content presented in the table. Total residues declined from 4930 to 102 ppm (dry basis) during the 49 days of weathering. Nemacur P was found to oxidize to its sulfoxide and sulfone. Relative persistences were: P=O,S < P=O,SO$_2$ < P=O,SO.

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