Gas–Liquid Chromatographic Determination of *O*-Ethyl *S*-(4-Chlorophenyl) Ethanephosphonodithioate (Stauffer N-2596) and Its Metabolite in Crops, Soils, Milk, and Tissues of Cattle and Chickens

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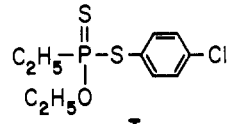
A gas chromatograph equipped with a flame photometric detector in the phosphorus mode provided a highly sensitive method for determining residues of *O*-ethyl *S*-(4-chlorophenyl) ethanephosphonodithioate (Stauffer N-2596) and its metabolite *O*-ethyl *S*-(4-chlorophenyl) ethanephosphonothioate in crops, soils, milk, and tissues of cattle and chickens. After extraction, crops and soils required no clean-up prior to chromatography; tissues, milk, and eggs required solvent partitioning. The detection limit was 0.01 ppm for each compound. Recoveries from crops, soils, eggs, milk, and most tissues were 76–110% for N-2596 and 75–105% for N-2596 metabolite. Recoveries of N-2596 metabolite from kidney, liver, and cardiac muscle were 0–64%.

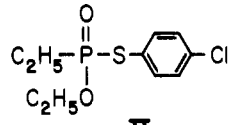
Stauffer Chemical Company's N-2596 (*O*-ethyl *S*-(4-chlorophenyl) ethanephosphonodithioate) is an experimental insecticide whose efficacy has been demonstrated for use against maggots, wireworms, and rootworms on a wide variety of crops including cole crops, corn, and sugar beets. It is readily desulfurized in vivo to its oxygen analogue (*O*-ethyl *S*-(4-chlorophenyl) ethanephosphonothioate). Since the oxygen analogue is nearly 10^4 more effective in inhibiting cholinesterase activity, it is believed that this metabolite is responsible for the insecticidal activity of N-2596 (Miaullis et al, 1977).

A method is described for determining these two compounds at levels of 0.01–5.0 ppm in the above crops as well as in beef and chicken tissues, milk, cream, eggs, and soils. Crops and soils were extracted with benzene; the extract was analyzed by gas chromatography without cleanup or concentration. A Melpar flame photometric detector was sufficiently sensitive and selective to require no processing of the extracts of these materials. Animal tissues, milk, and cream were extracted with hexane, the organophosphates were partitioned into acetonitrile, and the acetonitrile was concentrated prior to chromatography. Eggs were macerated with acetonitrile, which was diluted with water, extracted with benzene, concentrated, and chromatographed.

The structural formulas for N-2596 (I) and N-2596 oxygen analogue (II) are as shown.



I



II

EXPERIMENTAL SECTION

Reagents and Equipment. Exposure to all solvents (Mallinckrodt Nanograde) was minimized by conducting all operations in a fume hood using solvent-impermeable gloves. The hazardous nature of benzene required special care. Standard solutions were prepared from analytical standards (>99% purity, Stauffer Chemical Co.). The rubber gaskets in the Waring Blendor jars (0.5-L capacity) were replaced with Teflon ones.

Gas Chromatography. A Hewlett-Packard Model 5711A gas chromatograph equipped with a flame photometric detector in the phosphorus mode (526-nm filter) and a 1-mV recorder (Linear Instruments Corp.) was used.

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Table I. Retention Times (Relative to Parathion) of I, II, and Potentially Interfering Organophosphorus Pesticides

	Relative retention time	
	OV-17	QF-1 + DC-200
Parathion	1.00[a]	1.00[b]
Malathion	0.94	
Fenitrothion	0.93	
N-2596	0.82	0.56
Paraoxon	0.82	1.19
Methyl parathion	0.80	0.75
N-2596 oxygen analogue	0.64	
Dyfonate	0.47	
Diazinon	0.40	
Dyfonate oxygen analogue	0.37	

[a] Absolute retention time is 3.50 min. [b] Absolute retention time is 4.09 min.

A 190-cm × 2-mm i.d. borosilicate glass column packed with 10% OV-17 on 80/100 Gas-Chrom Q (Applied Science Laboratories) and conditioned overnight using a low nitrogen flow gave excellent resolution. Optimum column temperature was 220 °C. Operating temperature for the injector (glass insert) and detector was 250 °C. The nitrogen carrier, hydrogen, air, and oxygen flows were 50, 200, 50, and 20 mL/min, respectively. At these conditions, the retention times of I and II were 2.8 and 2.2 min, respectively. Retention times of I and II and some potentially interfering organophosphorus pesticides relative to parathion are given in Table I. Note that while I, paraoxon, and methyl parathion are not separated on the OV-17 column, they are well resolved on a 145-cm × 2-mm i.d. column of 15% QF-1 + 10% DC-200 on 80/100 Gas-Chrom Q at 200 °C. Other commonly used organophosphorus pesticides do not interfere (Bowman and Beroza, 1970).

The detector response was linear for 0.05–5.0 ng of I and II when a constant injection volume of 5 μL was used. The detection limit D (2× noise level) was 0.05 ng of I, but varied for II. Typically, during a series of analyses, D_{II} gradually increased from 0.5 D_I to 0.9 D_I as the column became conditioned with crop or tissue extractives. Following an extended column bake-out, D_{II} temporarily dropped to <0.5 D_I.

Residues in the fortified control samples were calculated by direct proportion using peak heights of 5-μL injections each of sample and standard solutions. Soil residues were calculated on a dry-weight basis by correcting the soil weight for its water content. No moisture correction was applied to crops or tissues.

Table II. Recoveries of N-2596 and N-2596 Oxygen Analogue from Fortified Crops, Soils, Tissues, Milk, and Cream

Sample	Fort. level,[a] ppm	% recovery[b,c] N-2596	% recovery[b,c] Analogue
Cole crops			
Broccoli	0.05	85	99
Brussels sprouts[d]	0.05	86	100
Cabbage	0.05	96	100
Cabbage[d]	0.50	91	104
Cabbage[d]	5.0	96	99
Cauliflower[d]	0.05	106	96
Corn			
Ears	0.05	100	102
Ears[d]	0.50	101	92
Ears[d]	5.0	90	98
Stalks	0.05	100	103
Sugar Beets			
Roots	0.05	92	99
Tops[d]	0.05	90	96
Soil			
Sand (0.5% organic)	0.05	102	101
Sandy loam (1.5% organic)	0.05	104	102
Silty clay loam (2.3% organic)	0.05	90	97
Beef			
Fat (omental)	0.05	78	85
Kidney	0.05	87	99,[e] 45, 16[f]
Liver	0.05	99	5[e], 0
Muscle, cardiac	0.05	88	64
Muscle, skeletal	0.05	103	95
Milk, whole	0.01	91	98
Cream	0.01	76	75
Chicken			
Fat	0.03	87	87
Liver	0.03	100	40
Muscle, skeletal	0.03	100	80
Egg, whole	0.03	96	87
Egg, yolk	0.03	110	90
Egg, albumen	0.03	77	105

[a] N-2596 and N-2596 oxygen analogue each fortified at same level. [b] Average of at least two determinations, except as noted. [c] 10-min delay between fortification and extraction except as noted. [d] One determination. [e] No delay between fortification and extraction. [f] 75-min delay between fortification and extraction.

Extraction of Crops. All crops, except dry corn grain, were thoroughly chopped while frozen using a Hobart food chopper. Dry corn grain was ground in a Wiley mill (2-mm screen). For all crops except dry corn stalks, a 50-g representative sample was blended with 200 mL of benzene in a 0.5-L Waring Blendor for 5 min at moderate speed. Dry corn stalks (30 g) were wetted with 50 mL of water, allowed to stand for 30 min, and blended with 300 mL of benzene. Extracts were filtered through a 5-cm plug of anhydrous sodium sulfate contained in a funnel with a coarse glass frit. Excessively wet filtrates, as evidenced by cloudiness, were shaken with Na_2SO_4 until clear. They were stored over sodium sulfate in amber bottles. The recoveries of I and II from extracts of sugar beet tops, sugar beet roots, and cabbage were reduced about 10% in 6 weeks when stored in this manner. Corn grain extracts showed no such degradation. The extracts prepared as described were chromatographed without concentration or cleanup.

Extracts of Soils. Soil samples of 1–2 kg were pulverized and thoroughly mixed prior to weighing a representative 50-g subsample into an 8-oz jar. To this was added 50 mL of deionized water and 100 mL of benzene. The jars were clamped in a horizontal position on an oscillating table shaker and shaken for 30 mim. After separation, the organic layer was passed through a 5-cm plug of anhydrous sodium sulfate as described above and chromatographed without cleanup.

Extraction of Tissues, Milk, and Cream. Samples were carefully trimmed from surrounding tissue and diced into 1-cm pieces. Whole milk was vigorously shaken immediately prior to sampling to disperse the cream. A 50-g sample was blended with 200 mL of hexane at high speed for 5 min and then percolated through sodium sulfate. A 20-mL aliquot was partitioned with 2 × 30 mL of acetonitrile. The combined acetonitrile extracts were backwashed with 2 × 10 mL of hexane, reduced in volume to <1 mL on a rotary evaporator, and diluted to 1.0 mL with acetonitrile prior to chromatography. Extracts of tissue samples were analyzed immediately in order to obviate the possible loss of II; see the Results and Discussion section.

Extraction of Eggs. Whole eggs (shell included) were thoroughly macerated prior to sampling. Twenty grams of egg (yolk, albumen, or whole) were extracted with 80 mL of acetonitrile and 1 mL of glacial acetic acid for 3 min using a Polytron mixer. One-fourth of the resulting mixture was shaken for 1 min with 160 mL of water, 50 mL of saturated sodium chloride solution, and 20 mL of benzene. Following extraction with a second 20-mL portion of benzene, the combined extracts were percolated through sodium sulfate, which was then washed with 2 × 5 mL of benzene. All benzene solutions were combined and concentrated under vacuum to <0.5 mL. The extracts were diluted to 0.50 mL with benzene prior to chromatography.

RESULTS AND DISCUSSION

The efficiency of the procedure was tested by adding known amounts of I and II to control samples immediately prior to extracting them. While such recovery experiments do not reflect the extraction efficiencies of field-weathered

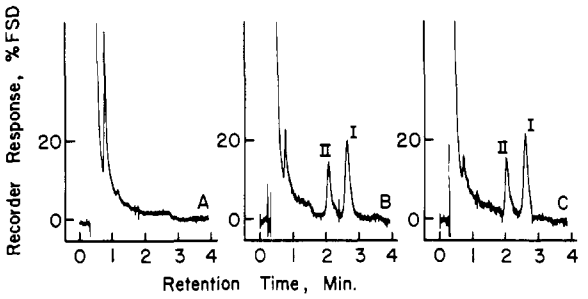

Figure 1. Chromatograms from extracts of whole milk: (A) 25 mg of control milk; (B) 25 mg of control milk fortified (0.01 ppm) with 0.25 ng each of I and II; (C) standard containing 0.25 ng each of I and II.

samples, they are an accurate test of manipulative efficiency. These experiments also demonstrated the absence of interfering co-extractives. Unpublished data (J. B. Miaullis, Stauffer Chemical Co., 1976) on the metabolism of N-2596 in a corn plant indicate that 95% of the N-2596 and 90% of the N-2596 oxygen analogue (both ring-^{14}C labeled) remaining in the plant are removed by benzene extraction. The recovery results from fortifications of 0.01–5.0 ppm are shown in Table II. Each value represents the average of at least two determinations. The analysis precision was ±10%. Figure 1 shows a typical chromatogram for whole milk for the 0.01 ppm fortification level.

All recoveries of I and II were satisfactory for each sample type except beef liver, beef cardiac muscle, and chicken liver. However, the high fat content of cream and beef fat lowered the recoveries from these tissues to near 75%. Using this method, the recoveries of I from field-weathered (1 month) sand and silty clay loam were within 20% of the values obtained upon 24-h Soxhlet extractions of these soils using benzene or acetonitrile.

Table II shows that recovery of II from freshly fortified beef liver was only 5%; a delay of 10 min between fortification and extraction reduced the recovery to zero. Likewise, if there was a delay between fortification and extraction of kidney tissue, recoveries of II were reduced from 99% for tissue extracted immediately after fortification to 45% for a 10-min delay and 16% for a 75-min delay. These results were obtained when the tissue extracts were analyzed immediately after extraction. However, if the kidney extract giving 99% recovery was stored at room temperature for 7 days, the recovery of II was reduced to 67%. The low recoveries of II in these tissues, as well as the low values obtained for beef cardiac muscle and chicken liver may be the result of enzymatic degradation of II. Similar behavior has been reported for ethion dioxon (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorothioate) in liver and kidney tissues of turkey and cattle (Ivey and Mann, 1975). The recoveries of I were unaffected by delay between fortification and extraction for any tissue.

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A Rapid Gas–Liquid Chromatography Method for Determining Benomyl Residues in Foods

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A GLC method for determining residues of benomyl as a composite value of MBC and 2-AB acetates was described. Using a high-resolution glass capillary GLC technique, and nitrogen–phosphorus selective and EC detectors, a sensitivity of 0.00001 ppm benomyl for a 10-g sample was achieved. The method was tested for various vegetables and fruits.

Methyl 1-(butylamino)carbonyl-1*H*-benzimidazol-2-ylcarbamate, generally known as benomyl, is a protective and eradicant fungicide, effective against a wide range of fungi affecting fruits, berries, nuts, vegetables, field crops, turf, and ornamentals.

Because of its relatively low toxicity, the recommended maximum amounts of benomyl and its degradation products in foods may be high as several milligram/kilogram in the case of citrus fruits and wine grapes. On the other hand, the recommended maximum amount of benomyl residues in vegetables may be, depending on legislation, as low as 0.1 ppm (Coduro, 1974).

In acidic conditions, benomyl is easily hydrolyzed to methyl benzimidazol-2-ylcarbamate (MBC) and a further hydrolysis leads to 2-aminobenzimidazole (2-AB). Benomyl is known to degradate in animal systems to 5-hydroxybenzimidazol-2-ylcarbamate (5-HBC) and 4-hydroxybenzimidazol-2-ylcarbamate (4-HBC) (Gardiner et al., 1974).

In foods the residues of benomyl and its degradation products can be determined by several different methods, the first steps of the analysis being similar in each case. Benomyl is quantitatively hydrolyzed to MBC, which is then determined, or MBC may be further hydrolyzed to 2-AB, which in turn is determined. The residues of benomyl, MBC and 2-AB are then measured as a composite value.

Aharonson and Ben-Aziz (1973) measured MBC by a direct fluorometric method. Pease and Gardiner (1969) and Pease and Holt (1971) have described a method where 2-AB is determined by fluorometric measurement, or by colorimetric analysis following bromination. Mestres et

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