A Rapid High-Performance Liquid Chromatographic Method for the Simultaneous Determination of Oxamyl and Its Oximino Metabolite

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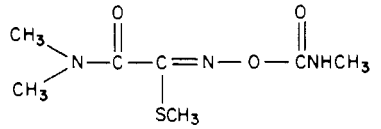
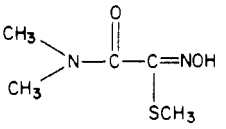
A rapid method for the determination of the insecticide/nematocide oxamyl and its oximino metabolite in water and soil by reverse-phase high-performance liquid chromatography is presented. The sample is extracted from the matrix, cleaned up by passage through a silica Sep-PAK and chromatographed on a Zorbax ODS column. Detection limits of 1 ppb for oxamyl and 5 ppb for the oximato metabolite are obtained.

Oxamyl, methyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate is an insecticide and nematocide sold by Du Pont under the trade name Vydate L Insecticide/Nematocide. It is widely used for the control of various insects, mites, and nematodes in ornamentals, fruits, and vegetable crops both as a foliar spray and a soil drench.

The recommended method for the determination residues of oxamyl in crops (Holt and Pease, 1976) involves the base hydrolysis of oxamyl (I) to its oximino metabolite



I, oxamyl



II, oximino metabolite

II followed by gas–liquid chromatography using a flame photometric detector. This method has been demonstrated to be applicable for the determination of oxamyl residues down to 0.02 ppm. Chapman and Harris (1979), on the other hand, use a trimethylsilica derivative of the oxime with a detector limit of 0.5 ppm. Other methods have also been reported in the literature for the determination of oxamyl residues using different methods of detection. Singhal et al. (1977) have reported both a spectroscopic and a titrimetric (Singhal et al., 1978) method. However, as is the case with the GC method of Holt and Pease, these methods determine the total of both the active ingredient oxamyl and its relatively nontoxic (Oral ALD 11 000 mg/Kg) (Fretz, 1968) oximino metabolite II only.

More recently four high-performance liquid chromatographic methods for oxamyl have appeared in the literature. The method of Harvey and Han (1978) was developed to follow the metabolic fate of oxamyl in the environment and therefore depends on the detection of ^{14}C-labeled oxamyl by a scintillation flow monitor. The method of Thean et al. (1978) determines the intact oxamyl with a lower detection limit of 1 ppm, while the paper of Chiba et al. (1983), which determines both the oxamyl and its oximino metabolite, has detection limits of 0.1 and 0.5 ppm for the oxamyl and oximino metabolite, respectively, while Davis et al. (1978) reports the detection of the parent oxamyl only by UV detection at 212 nm.

Recently, considerable interest (Cohen, 1983) has developed in the persistence of pesticides used in soil application and their ability to leach into the groundwater. As a result, the need has arisen for methodology that can be used to determine oxamyl residues down to as low as 1 ppb and that can distinguish between the active ingredient and its nontoxic metabolite. None of the previously published methods, however, are capable of obtaining these levels of detection and selectivity. A method is therefore reported here that allows the simultaneous determination of both oxamyl and its oximino metabolite down to 1 and 5 ppb, respectively.

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MATERIALS AND METHODS

Apparatus and Reagents. A Hewlett-Packard Model 1084B liquid chromatograph equipped with a 254-nm detector and a 4.6 mm × 25 cm Zorbax ODS column (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898) was used. (b) Rotary Evaporator with a water bath set at 50 °C. Silica Sep-PAK was from Waters Associates, Inc. For the reference oxamyl and oximino metabolite standards (Agrichemicals Department, Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898), separate concentrated standards were prepared at 1 mg/mL by using 100 mg of each compound in 100 mL of acetonitrile and working standards by diluting this to 0.5, 1.0, 2.0, 5.0, and 10.0 μg/mL with acetonitrile. Methylene chloride and acetonitrile were from Burdick and Jackson, distilled-in-glass grade.

Sample Extraction and Cleanup. *Water.* One hundred grams of the water sample was added to a 250-mL separatory funnel. The water was saturated with approximately 20 g of sodium chloride. Fifty milliliters of methylene chloride was added to the separatory funnel and shaken for 2 min. The layers were allowed to separate, and the methylene chloride was transferred to a 250-mL round-bottom flask. The sample was extracted more times with 50 mL of methylene chloride each time. The methylene chloride layers were combined and evaporated by using the rotary evaporator to 2 mL. This was transferred to a 10-mL Kimble tube with a small washing of methylene chloride, and the solvent was evaporated to dryness under nitrogen. The residue was made to 1-mL volume with 15% acetonitrile in distilled, deionized water.

Soil. Fifty milliliters of methylene chloride was added to a 50-g representative sample weighed into a 250-mL polypropylene centrifuge bottle. The mixture was shaken for 10 min, and then the supernatant liquid was decanted through filter paper into a 250-mL round-bottom flask. The soil remaining was extracted in the bottle by again using 50 mL of methylene chloride. The liquid was combined with the first extraction. The combined solutions were evaporated to ca. 5 mL by using a rotary evaporator and a bath temperature of 50 °C. The solution was transferred to a 10-mL Kimble tube with small washing of methylene chloride, and the solvent was evaporated under nitrogen to 5 mL.

A silica Sep-PAK was conditioned by flushing it with 5 mL of methylene chloride. A 10-mL syringe was connected to the Sep-PAK, and the sample was added to the

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Table I. Recovery of Oxamyl and Its Oximino Metabolite from Water

concentration added, ppb		% recovery	
oxamyl	oximino metabolite	oxamyl	oximino metabolite
1		100	
1		99	
1		106	
1	5	105	100
2		96	
2		89	
5	5	100	86
5	5	100	101
5		82	
10	10	100	101
10		90	
10	10	105	72
50	50	90	86
100	100	99	98
100		107	
		av: 98	92
		SD: 7	11

Table II. Recovery of Oxamyl and Its Oximino Metabolite from Soil

concentration added, ppb		% recovery	
oxamyl	oximino metabolite	oxamyl	oximino metabolite
1	5	108	112
10	10	100	92
10	10	98	104
20	20	89	91
100	100	84	88
100	100	76	78
200	200	100	95
500	500	84	84
500	500	86	90
500	500	92	90
1000	1000	98	84
1000	1000	90	94
1000	1000	88	88
		av: 92	92
		SD: 9	9

syringe. The sample was slowly pushed through the syringe followed by 2 mL of methylene chloride, and the methylene chloride was discarded. Four milliliters of a 10/90 solution of methanol/methylene chloride was added to the syringe, and this was pushed through the Sep-PAK. This fraction was collected in a 10-mL Kimble tube. The solvent was evaporated to dryness under nitrogen, and the residue was made to 1 mL with 15% acetonitrile in distilled, deionized water. The sample was ultrasonicated for 5 min to ensure all the residue was dissolved.

Liquid Chromatography. The mobile phase was 15% acetonitrile in distilled deionized water, the flow rate 0.50 mL/min, and the column temperature 35 °C. Under these conditions, the oximino metabolite elutes at about 8.2 min and the oxamyl at about 12.3 min.

Standards. Fifty microliters of each of the standard solutions was injected into the HPLC, and the peak heights were measured. A calibration curve was constructed for each compound by plotting the peak height vs. the concentration for each standard solution.

Samples. Fifty microliters of each sample was injected into the HPLC, and the peak heights were measured for each compound. The peak heights were compared to the calibration curve to obtain the concentration in μg/mL, and then the residues were calculated in ppb by

$$\text{ppb} = (\mu\text{g/mL} \times 1000)/\text{wt of sample (g)}$$

RESULTS AND DISCUSSION

This method has been successfully demonstrated on both water and soil samples received from the field. Av-

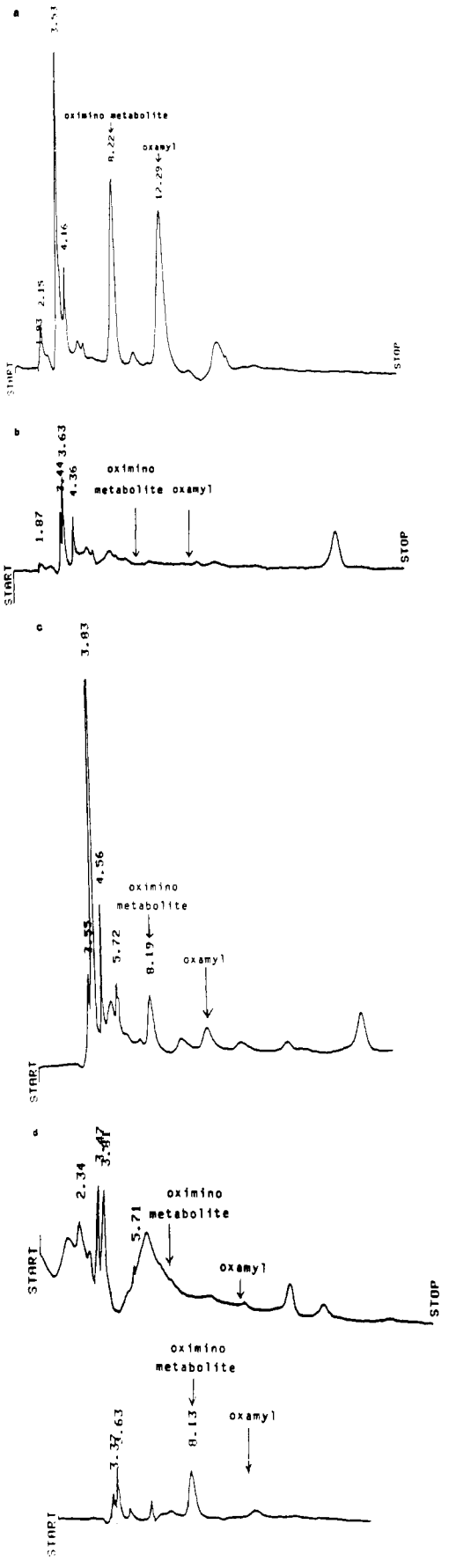

Figure 1. HPLC chromatograms of (a) standard oxamyl and oximino metabolite at 1 μg/mL, (b) well water containing no oxamyl or oximino metabolite, (c) well water fortified with 1 ppb of oxamyl and 5 ppb of oximino metabolite, (d) soil containing no oxamyl or oximino metabolite, and (e) soil fortified with 1 ppb of oxamyl and 5 ppb of oximino metabolite.

erage recoveries for the water samples, listed in Table I, were 98 ± 7% and 92 ± 11% for the oxamyl and the oximino metabolite, respectively, while they were 92 ± 9% for both in soil as listed in Table II. Chromatography of extracts from either soil or water as shown in Figure 1 demonstrates the selectivity and sensitivity of the method. No interference is noted in the control samples and levels of 1 ppb of oxamyl and 5 ppb of the oximino metabolite give more than adequate peak height.

The soil samples used in this study were typical of those used in areas where oxamyl is normally used for nematode control. While the soil was not characterized, we feel that the method should be applicable to any soil type typical of oxamyl nematode use areas.

Registry No. I, 23135-22-0; II, 30558-43-1.

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A Two-Year Field Study To Determine the Fate of Oxamyl in Soil during Flood Irrigation

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A 2-year study was conducted to determine the depth of penetration and degradation of residues of oxamyl in light-textured soils where heavy use of irrigation water was necessary to maintain crop vigor. Monitoring was conducted in sandy desert soils in a southern California lemon grove where a total of 64 acre in. of irrigation water was used during the study period. Oxamyl was applied to flood irrigation basins at a normal required rate for nematode control of 1.0 lb of active ingredient $acre^{-1}$ $month^{-1}$ by metering Du Pont Vydate L Insecticide/Nematicide into the water flow. A total of 17 lb of active ingredient was applied during the 2-year test period. In addition, a single treatment using 10 lb/acre, 10 times the normal monthly use rate, was also made for comparison. Analysis of soil samples showed that under these treatment conditions, oxamyl did not penetrate the soil to depths greater than 60 in. The half-life for oxamyl was 1–4 days, consistent with results from a previous field study using [^{14}C]oxamyl. This information indicates that under these conditions, oxamyl is not likely to accumulate in soil.

DBCP (1,2-dibromo-3-chloropropane) was widely used in California until 1977 as a soil fumigant for nematode control. Agricultural use of DBCP in California was suspended in 1977 by the Department of Food and Agriculture after incidence of male sterility was discovered in workers associated with the manufacture of the product.

In 1979, the California Department of Food and Agriculture detected DBCP contamination of wells in many parts of the state where DBCP had been used, including some municipal water supplies. The Department of Health Services confirmed widespread contamination of groundwater underlying major DBCP use areas.

In a search to find replacement nematicides for DBCP, which would be useful in California agriculture but would not contribute to groundwater contamination, a study was prepared to determine the fate of oxamyl in soil during irrigation.

Members of the Department of Health Services were contacted for help in selecting a test site with a high potential for leaching. Dr. Walter J. Farmer, Associate Professor of Soil Science at the University of California, Riverside, was consulted on requirements to ensure adequate sampling and oxamyl detection. The test site selected was a lemon grove in Thermal, CA, where typical desert soils are deep sands or loamy sands, low in organic matter, and where heavy volumes of flood irrigation water must be applied frequently to maintain the crop.

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EXPERIMENTAL PROCEDURES

In April 1981, the test site was laid out consisting of $^1/_4$-acre blocks, replicated 5 times. Treatments of oxamyl (as Vydate L), at the rate of 1 lb of active ingredient/acre, were metered into flood irrigation water as it flowed from risers into the flood basin between the tree rows. "Water only" check areas were also designated.

Oxamyl treatments were repeated monthly throughout 1981 until Jan 11, 1982 (a total of 10 lb). During that same time interval, a total of 35 acre in. of irrigation water was applied to the site, with the highest volumes required during June, July, and August.

Soil samples were taken on Jan 25, 1982 (14 days after the last treatment), and again Feb 15, 1982 (35 days after the last treatment). The field was irrigated without oxamyl addition between the two soil sampling dates.

Each replicate plot was sampled by using a Giddings drilling rig. Soil samples for analysis of oxamyl residues, water content, and particle size determination were taken at depths of 0–4 in., 4–8 in., 8–12 in., 1–2 ft, 2–3 ft, 3–4

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