diphenadione and did not represent a metabolite already present in the tissues.

Secondary hazards were evaluated by feeding the livers of treated cattle to laboratory rats because this species is highly susceptible to the anticoagulant effects of diphenadione (Saunders et al., 1955; Bentley and Larthe, 1959). The liver–oats formulation was highly palatable and all animals consumed their daily ration soon after it was offered. None of the test animals died or exhibited signs of chronic toxicity during the test or the following 14-day observation period; all appeared vigorous and showed weight gains comparable with those of the controls. After 14 days of feeding, prothrombin clotting times (means ± standard deviation) were similar in all groups: 17.7 ± 0.4 s for 10 rats fed liver from cattle killed at 30 days posttreatment, 18.6 ± 0.6 s for 10 fed liver from cattle killed at 60 days, 17.4 ± 0.5 s for 10 fed liver from cattle killed at 90 days, and 18.7 ± 0.5 s for 10 fed untreated liver. Blood and liver samples from these rats contained no detectable diphenadione. These results indicated that the minute diphenadione residues in the tissues of treated cattle caused no observable adverse effects in secondary consumers.

Diphenadione has been used as a prothrombinopenic anticoagulant in human therapy. "Remington's Pharmaceutical Sciences" (1970) lists the range of human daily dosages as 2.5 to 30 mg for anticoagulant therapy.

Therefore, if the liver of treated cattle contained the highest residue level found, 0.15 ppm, a 70-kg human would have to eat about 17 kg of it per day to receive the minimum dosage used in therapy.

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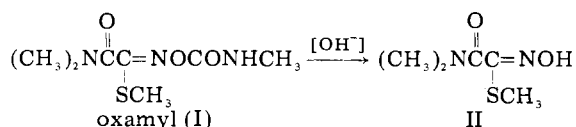
Determination of Oxamyl Residues Using Flame Photometric Gas Chromatography

Richard F. Holt and Harlan L. Pease*

Oxamyl (methyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate) residues in plant and animal tissues and in soil are determined by initial extraction with ethyl acetate, alkaline hydrolysis to the more volatile oximino fragment, and final determination by gas chromatography with sulfur-sensitive flame photometric detection. Method sensitivity is 0.02 ppm based on 25-g samples. Recoveries of added material average about 90% in the 0.02–10 ppm range.

Oxamyl is the approved common name for methyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate. This material was formerly known as DPX-1410 and is the active ingredient in Du Pont's "Vydate" Oxamyl Insecticide/Nematicide.

The residue method for oxamyl (I) is based on the gas chromatographic measurement of the corresponding oximino fragment II, methyl N',N'-dimethyl-N-hydroxy-1-thiooxamimidate, after extraction of oxamyl from the substrate with ethyl acetate and subsequent alkaline hydrolysis to produce the more volatile, but stable derivative:

$$(CH_3)_2N\overset{\overset{O}{\|}}{C}C=NOCONHCH_3 \xrightarrow{[OH^-]} (CH_3)_2N\overset{\overset{O}{\|}}{C}C=NOH$$

$$\underset{\text{oxamyl (I)}}{\underset{|}{SCH_3}} \qquad \underset{\text{II}}{\underset{|}{SCH_3}}$$

Biochemicals Department, Experimental Station, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.

EXPERIMENTAL SECTION

Apparatus and Reagents. The Perkin-Elmer Model 3920 gas chromatograph (Perkin-Elmer, Norwalk, Conn.) equipped with a flame photometric detector with interference filter for spectral isolation of sulfur emission at 394 mμ was used. The chromatographic column was 10% SP-1200/1% H_3PO_4 on 80–100 mesh Chromosorb W AW (Supelco, Inc., Bellefonte, Pa.), 3 ft glass, 0.25 in. o.d., $1/16$ in. i.d.

Homogenization and extractions were conducted using a blender–centrifuge bottle and adapter base as shown in Figures 1 and 2. These items were designed in this laboratory. It is not necessary to construct this specialized equipment unless desired. Conventional blender bottles and centrifuge tubes may be used but are somewhat more time consuming. Centrifugation was carried out with an International Size 1, Type SB Centrifuge capable of accommodating the 250-ml bottle shown in Figure 1.

The reference standards of I and II were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The solvents used were distilled-in-glass,
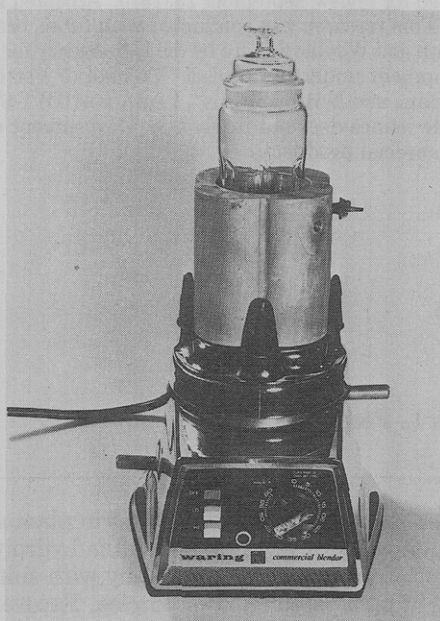
Figure 1. Blender–centrifuge bottle.

Figure 2. Combined extraction unit: Waring Blendor base, adapter base, and blender–centrifuge bottle. Figures 1 and 2 (Pease and Holt, 1971) reprinted by permission of copyright owners.

purchased from Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

Isolation. Weigh 25 g of a representative solid crop or tissue sample into the blender–centrifuge bottle, add 100 ml of ethyl acetate, cover, place in the adapter base, and blend at high speed for about 5 min. Centrifuge at 1500 rpm for 10–15 min and carefully decant the ethyl acetate through a 1.5-in. bed of anhydrous sodium sulfate contained in a 45° funnel and collect in a 500-ml round-bottomed flask. Repeat the extraction two more times each time using 100 ml of ethyl acetate. Add 50 ml of water to the combined extracts and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

When analyzing soil, weigh 25 g into a 250-ml glass-stoppered Erlenmeyer flask, add 100 ml of ethyl acetate and 25 ml of water, stopper, and shake on a wrist-action shaker for 15 min. Allow to settle, and filter the extract through cotton into a 500-ml round-bottomed flask. Repeat the extraction two more times each time using 100 ml of ethyl acetate. Add 50 ml of water to the combined

extract and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

For analysis of milk or other liquids, transfer a 50-g sample into a 250-ml separatory funnel, add 100 ml of n-hexane, and shake gently for 2 min. Allow the phases to separate (centrifuge if necessary to obtain clean separation). Discard the hexane wash. Extract the aqueous phase with three 100-ml portions of ethyl acetate using 2-min shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate through a 1.5-in. bed of anhydrous sodium sulfate contained in a 45° funnel into a 500-ml round-bottomed flask. Add 50 ml of water to the combined extracts and evaporate the ethyl acetate in a vacuum rotary evaporator at 60°C.

For all samples, transfer the remaining water ($\sim$40 ml) from the 500-ml flask to a 250-ml separatory funnel using several small volumes of water as wash. Dilute to approximately 50 ml with water. Add 50 ml of n-hexane to the separatory funnel, shake gently for about 1 min, and allow the phases to separate. Centrifuge, if necessary, to obtain a clean separation. Discard the hexane layer. Repeat the hexane wash two more times using additional 50-ml portions of solvent. Discard the hexane after each wash.

Adjust the pH to about 12 by adding 3 ml of 1 N NaOH (check with pHydrion paper; add more if necessary) and add 50 ml of chloroform. Shake gently for about 1 min and allow the phases to separate. Discard the chloroform layer. Repeat the chloroform wash one more time using 50 ml of chloroform. Discard the chloroform.

Heat the aqueous phase on a steam bath with occasional stirring to remove the residual chloroform. Cover and continue to heat on the steam bath for an additional 15 min to convert I to II.

Cool and quantitatively transfer to a 250-ml separatory funnel, using several small water washes. Add 50 ml of chloroform, shake gently for about 1 min, and allow the phases to separate. Discard the chloroform layer. Repeat the wash using a second 50-ml portion of chloroform. Discard the chloroform.

Saturate the aqueous phase by adding 15 g of NaCl (add more if needed) and extract with four 50-ml portions of ethyl acetate–methanol (90:10, v/v) using 2-min shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate–methanol phase through a 1.5-in. bed of anhydrous sodium sulfate into a 250-ml round-bottomed flask.

Concentrate the combined ethyl acetate–methanol extracts to about 10 ml in a vacuum rotary evaporator at 60°C. Quantitatively transfer the concentrated extract to a 30-ml beaker, using ethyl acetate as wash (if traces of water are present, filter through sodium sulfate). Continue to concentrate the solution to about 0.5 ml by evaporation at room temperature in a well-ventilated hood. Transfer the concentrated extract to a 1-ml volumetric flask using a dropper with a fine tip and several small washes of ethyl acetate. Dilute to volume with ethyl acetate and mix thoroughly.

Gas Chromatographic Calibration. Equilibrate the chromatograph under the following conditions: inlet temperature, 230°C; detector temperature, 200°C; column temperature, 180°C; helium carrier gas flow, 70 cm^3/min; oxygen flow, 20 cm^3/min; air flow, 40 cm^3/min; hydrogen flow, 180 cm^3/min. After conditioning the chromatographic column by maintaining the temperature at 180°C with carrier gas flowing for at least 24 hr, set the initial column temperature at 100°C. Inject aliquots (1 to 3 μl) of a standard solution of II prepared in ethyl acetate to

Table I. Oxamyl Recovery Data

Crop	Residue, ppm	No. of detns.	Recovery, % Av	Range
Peanut, nut	0.02-0.80	6	103	73-120
Peanut, hull	0.10-5.0	8	99	82-120
Peanut, foliage	0.05-10	12	93	70-113
Tobacco	0.02-5.0	19	93	72-110
Apples	0.04-0.40	3	98	94-100
Turf grass	0.02-4.0	6	94	76-105
Peaches	0.04-2.0	4	76	54-105
Lettuce	0.04-1.0	4	92	81-99
Oranges	0.04-2.0	11	83	70-90
Cottonseed	0.02-0.20	3	95	84-102
Grapefruit	0.08-0.40	9	79	72-85
Coffee beans	0.02-2.0	2	98	96-100
Grapes	0.02-2.0	3	85	70-94
Potatoes	0.02-4.0	9	91	76-120
Tomatoes	0.02-2.0	11	83	71-109
Celery	0.04-2.0	12	84	70-109
Peppers	0.02-1.0	3	91	84-100
Carrots	0.02-0.20	6	88	79-96
Soil	0.04-6.6	21	94	74-120
Urine	0.40-4.0	4	100	75-130
Feces	0.20-2.0	4	83	72-100
Liver	0.04-0.40	6	90	75-102
Kidney	0.04-0.40	6	89	82-100
Lean meat	0.04-0.40	6	107	85-114
Fat	0.04-0.40	6	98	80-130
Milk[a]	0.02-0.20	6	83	70-110

[a] Used 50-g sample.

contain 0.5, 1, 2, 3, 5, and 10 μg/ml so that the peak will not exceed full scale deflection. Program the column temperature at 16°C per minute to a maximum of 200°C. Hold the column at this temperature for about 8 min. The retention time for II is about 7 min. Operate the flame photometric detector according to instructions furnished by the manufacturer. Construct calibration curves for the different attenuations by plotting micrograms of II injected vs. peak height. Use log–log paper to obtain a straight line. Chromatograph one or more calibration solutions daily to ensure that the calibration curve remains accurate.

Gas Chromatographic Analyses. Equilibrate the instrument and chromatograph aliquots of the residue extracts as described under Gas Chromatographic Calibration. Measure the peak height of II and determine the micrograms of this material in the aliquot, using the calibration curve previously prepared. Calculate the amount of oxamyl in parts per million by dividing the micrograms of II found, corrected for the molecular weight conversion (1.35), aliquot, and recovery factors, by the sample weight in grams.

RESULTS AND DISCUSSION

The gas chromatographic method described is sensitive to about 0.5 μg of oxamyl or 0.02 ppm based on a 25-g sample. Since oxamyl is difficult to chromatograph at the levels required for this sensitivity, the analytical procedure is based on the gas chromatographic measurement of the characteristic oximino fragment, methyl N^1,N^1-dimethyl-N-hydroxy-1-thiooxamimidate (II). This derivative is easily formed by simple alkaline hydrolysis in aqueous solution.

The applicability of the method for determining oxamyl residues has been demonstrated on a variety of substrates. Recovery of this compound added to untreated control samples is essentially quantitative as is shown in Table I. The recoveries were conducted by adding known amounts of oxamyl to the samples contained in the Blender–Centrifuge Bottle. After evaporation of the solvent, analyses were then initiated by addition of the first portion of ethyl acetate.
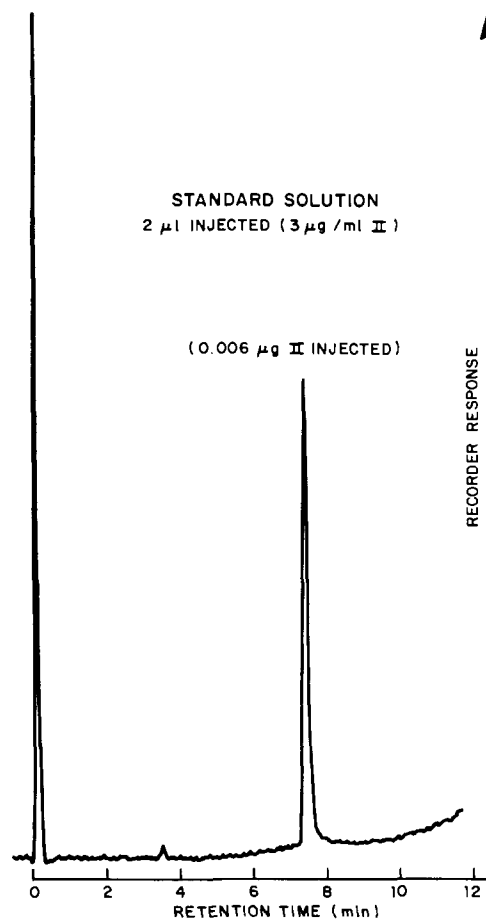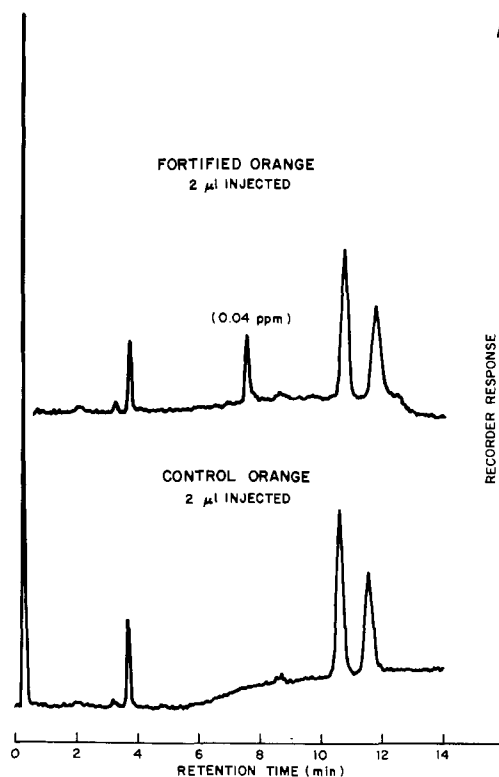

Figure 3. Standard solution of II.

Figure 4. Extracts of oranges.

During the analysis of highly acidic substrates, i.e. oranges, grapefruit, and peaches, somewhat lower recoveries were noted. This was attributed to the detrimental effect of heating oxamyl and the oximino fragment in the acidic media which results from the initial extractions of fruit

crops. Somewhat higher recoveries can be obtained by concentrating these extracts over 1 N NaOH instead of distilled water (see section on Isolation, paragraph 1) thereby neutralizing the extract.

The use of the sulfur-sensitive flame photometric detector provides for a highly selective measurement of the desired compound and no interference was encountered in the majority of the untreated controls analyzed. For illustration, Figures 3 and 4 show typical chromatograms. Figure 3 shows a standard chromatogram for the oximino fragment (II). Figure 4 shows chromatograms obtained on extracts of oranges, the upper curve obtained on a sample fortified with 0.04 ppm of oxamyl, the lower curve representing a control orange extract.

Some of the initial chromatography for this method was conducted using a column packed with 30% OV-101 on Gas-Chrom Q. However, the 10% SP 1200/1% H_3PO_4 column has proven to be more precise and selective. The column life is equivalent. In addition, the new column requires less conditioning than the earlier OV-101 column.

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Trifluoroacetylation of Mesurol [4-Methylthio-3,5-xylyl-N-methylcarbamate], Its Sulfoxide, Sulfone, and Phenol Analogs for Analysis by Gas Chromatography

Roy Greenhalgh,*[1] William D. Marshall,[1] and Russell R. King[2]

Reaction conditions are established for the trifluoroacetylation of the carbamate insecticide Mesurol and its metabolites, Mesurol sulfoxide and Mesurol sulfone, together with their respective phenol analogs. The reactions with Mesurol sulfoxide and its phenol are anomalous in that they yield di rather than mono TFA derivatives. This is due to initial reaction taking place at the sulfoxide moiety. The carbamate trifluoroacetyl sulfoxide undergoes rearrangement to form an alkyl trifluoroacetoxy derivative, whereas the phenol analog gives an aryl trifluoroacetoxy derivative. The reaction of trifluoroacetic anhydride proceeds more slowly with the carbamate and phenolic moieties. Mass spectra, nuclear magnetic resonance, and infrared spectral data are used to deduce the structures of the compounds. All the trifluoroacetyl derivatives of Mesurol and its metabolites can be chromatographed by gas chromatography and detected by a flame photometric detector in S mode. Thus, trifluoroacetylation can be used for the quantitation of Mesurol and its metabolites or for confirmatory purposes.

Mesurol (I) in common with other thioethers is readily converted to a sulfoxide (II) and sulfone (III) by aerial oxidation, microsomal oxidases, and living organisms. These metabolites have been found in plants (Abdel-Wahab et al., 1966) and are known to be cholinesterase inhibitors (Metcalf et al., 1967). It is desirable that residues of these two metabolites are determined in any analytical method in addition to those of Mesurol. Of the two current methods available for Mesurol (Thornton and Drager, 1973; Bowman and Beroza, 1969), only the latter is capable of determining all three compounds individually, but it is rather involved and a simpler procedure would be an advantage.

Mesurol can be determined directly by gas chromatography (GC) (Lorah and Hemphill, 1974); however, II and III, like most N-methylcarbamates, have poor GC characteristics and must be derivatized for analysis. Perfluoroacylation has been employed for this purpose in the case of carbaryl (Khalifa and Mumma, 1972) and carbofuran (Wong and Fisher, 1975). Seiber (1972) has shown that I forms a trifluoroacetyl (TFA) derivative which is suitable for GC analysis. This paper reports on further studies with the reaction of trifluoroacetic anhydride (TFAA) on I, II, and III, together with their phenol analogs, Mesurol phenol IV, Mesurol sulfoxide phenol V,

and Mesurol sulfone phenol VI. The TFA derivatives formed are characterized by mass spectrometry (MS), nuclear magnetic resonance (NMR), and infrared (ir) spectroscopy. In addition, the feasibility of the reaction for the GC analysis of Mesurol and its metabolites is demonstrated.

EXPERIMENTAL SECTION

Chemicals. Trifluoroacetic anhydride (TFAA) was obtained from Aldrich Chemical Company and was used as received. Analytical samples of I, II, and III were kindly provided by Chemagro, Kansas City, Kansas.

Equipment. Ir spectra were determined as films or Nujol mulls using a Beckman IR-20A spectrophotometer. NMR spectra were obtained in $CDCl_3$ solution with Me_4Si as an internal standard on a Varian T-60 NMR spectrometer. The MS were determined by a Finnigan 3100 GC-MS coupled to a D 6000 data acquisition system.

Gas Chromatography. A Pye gas chromatograph, Model 104, fitted with a Bendix Sulphur Phosphorus Emission Detector (flame photometric detector), was operated in the S mode (394 nm). A glass column, 2 ft × 0.25 in. o.d., was packed with acid-washed 80–100 mesh, Chromosorb W coated with 5% DC-200. The column flow was 50 ml/min of nitrogen, and the air and hydrogen flow to the detector selected for optimum response. With a column temperature of 170°C, the retention times of Mesurol TFA (VII), Mesurol sulfoxide di-TFA (VIII), and Mesurol sulfone TFA (IX) were 2.0, 3.2, and 6.3 min, respectively, while those for Mesurol phenol TFA (X), Mesurol sulfoxide phenol di-TFA (XI), and Mesurol

[1]Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

[2]Fredericton Research Station, Agriculture Canada, Fredericton, New Brunswick, Canada.