Determination of Carbofuran and 3-Hydroxycarbofuran Residues in Plant Tissue by Nitrogen Selective Gas Chromatography

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A general method for determining residues of carbofuran and its major carbamate plant metabolite, 3-hydroxycarbofuran, is reported. A method sensitivity of 0.1 ng/mg (ppm) for both compounds was obtained. Recovery of carbofuran from 16 different crops averaged 82%. Recovery of 3-hydroxycarbofuran from the same crops averaged 77%. Carbofuran and 3-hydroxycarbofuran were analyzed individually. The carbofuran analysis involves blending with 0.25 N hydrochloric acid, hexane partition, and Florisil column cleanup. The 3-hydroxycarbofuran analysis involves hydrolysis, methylene chloride partition, ethoxylation, and Florisil column cleanup. Quantitation of intact carbofuran and 3-hydroxycarbofuran (as its ethyl ether) is accomplished by gas chromatography with nitrogen selective detection.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is an effective broad spectrum insecticide–nematicide (Thomson, 1973). It is used on a wide variety of crops; therefore, a need for a broad spectrum residue analysis procedure was recognized. Several electron-capture (EC) gas chromatographic methods have appeared in the literature (Butler and McDonough, 1971; Coburn et al., 1976; Wong and Fisher, 1975). All these methods require a derivatization step. None of the EC methods for carbofuran have been shown to be broad spectrum, probably due to the nonselective nature of the EC system.

A method based on gas chromatography and nitrogen specific detection (Hall or microcoulometric) has been reported (Cook, 1973). In practice, the method reported by Cook is broad spectrum. However, crop related interferences have required modification of the method on an individual crop basis, particularly in the areas of extraction and cleanup. The detection of 3-hydroxycarbofuran has been troublesome due to crop matrix related variability in the chromatography of this relatively polar compound.

The method reported herein involves separate analysis of carbofuran and 3-hydroxycarbofuran (as its ethyl ether). The method has been applied successfully without change to 16 crops including those having high moisture, low moisture, and high lipid content, as well as fruit, vegetable, grain, and root crops.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Hewlett-Packard 5730 gas chromatograph equipped with a nitrogen–phosphorus thermionic detector (NPD) was used. Standard laboratory glassware was used throughout the procedure. Baker Resianalysed solvents (or their equivalent) were employed.

Sample Preparation. Each sample was macerated in a food chopper. If further size reduction was required, a grinder or blender was employed. Since relatively small samples (10 g) were used, ample care was exercised to insure good mixing of the bulk sample to provide a representative subsample. Once ground, the samples were packaged in plastic lined cloth bags, frozen, and stored at −10 °C until analyzed.

Procedure. All glassware was thoroughly washed with a nonphosphorus detergent, water rinse, Chemsolve (Mallinckrodt) or caustic soak, water rinse, and final methanol rinse. Glassware contamination could be a major problem if great care is not exercised in cleaning.

A. Carbofuran. Figure 1 diagrams the flow scheme for the analysis of carbofuran. Specifically, 10 g of crop is blended at high speed with 300 mL of 0.25 N hydrochloric acid for 5 min (Waring blender). The sample is filtered through a glass Buchner funnel with a coarse sintered glass frit. Sea sand (Union Camp) may be used as a filter aid where required. Other filter aids have caused contamination problems (filter paper, glasswool, cheesecloth). A 30-mL (1 g) aliquot is removed and extracted four times with 60 mL of hexane. One to eight drops of 4% aqueous sodium lauryl sulfate may be required to break emulsions. The hexane is decanted carefully from the separatory funnel to minimize aqueous carry-over. Diethyl ether (anhydrous, 25 mL) is added to the combined hexane extracts. A minimum (1–2 g) amount of anhydrous sodium sulfate which has been rinsed with ethyl acetate and air-dried is used to dry the solution. The dried extract is concentrated to 5 mL in a Kuderna-Danish concentrator. Further concentration to 1 mL is accomplished using a gentle stream of nitrogen. The samples must not be allowed to go to dryness.

Florisil Column Cleanup. A plug of glasswool which has been rinsed with ethyl acetate and approximately 1 cm of sea sand are placed in a column (20 cm × 19 mm) which is equipped with a Teflon stopcock and a 250-mL bulb reservoir. Ten grams of 100–200 M Florisil (Floridan Co.) deactivated to a moisture content of 2.5 ± 0.3% weight percent is added and topped with 0.5 cm of sea sand. The column bed should be approximately 11 cm. The column is prewet with 20–30 mL of hexane. The sample is quantitatively transferred to the column with several 1-mL rinses of 1:9 ethyl acetate/hexane (v/v). Elute with 100 mL of 1:9 ethyl acetate/hexane (v/v) as a wash and discard. Elute with 60 mL of 3:7 ethyl acetate/hexane (v/v) and collect in a Kuderna-Danish concentrator. Two milliliters of toluene was added to the eluant in the concentrator. The toluene serves as a keeper and injection solvent. The eluant is then concentrated to 2–3 mL on a steam bath. The residue is transferred to a graduated centrifuge tube and reduced to exactly 1 mL under a gentle stream of nitrogen for injection. The sample is now ready for GC/NPD quantitation.

B. 3-Hydroxycarbofuran. Figure 2 diagrams the flow scheme for the analysis of 3-hydroxycarbofuran as 3-ethoxycarbofuran.

Hydrolysis and Extractions. Ten grams of crop is placed in a 1-L, round-bottom flask. Two hundred mil-

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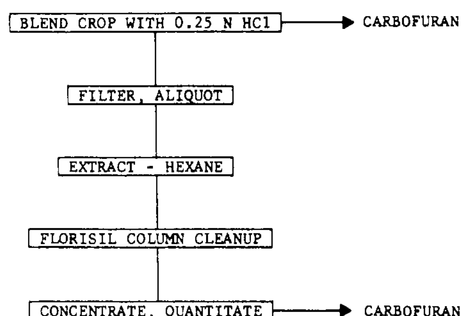

Figure 1. Carbofuran analysis.

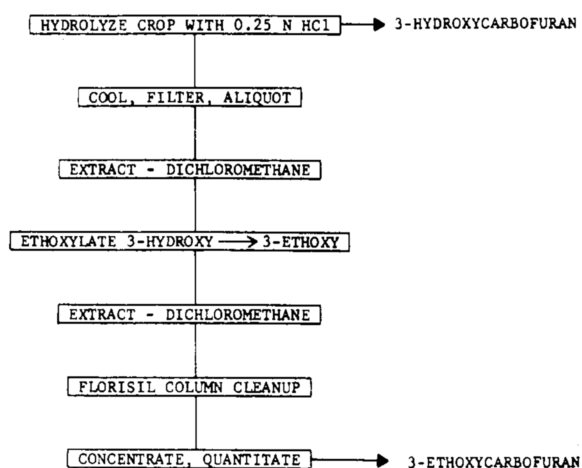

Figure 2. 3-Hydroxycarbofuran analysis.

liliters of 0.25 N hydrochloric acid is added. Hard crops (rice, dry corn kernels, dry beans etc.) are first blended with 150 mL of 0.25 N hydrochloric acid and transferred to the hydrolysis flask with a 50-mL 0.25 N hydrochloric acid rinse. The mixture is refluxed 1 h and cooled. The sample is filtered through a coarse sintered glass Büchner funnel. Sea sand may be used as a filter aid if required. The filtered solids are rinsed with enough 0.25 N hydrochloric acid to bring the volume to exactly 300 mL. A 30 mL (1 g) aliquot is removed and extracted three times with 30 mL of dichloromethane. One to eight drops of 4% sodium lauryl sulfate may be required to break emulsions. The dichloromethane is dried with a minimum (1–2 g) amount of sodium sulfate which has been prerinsed with ethyl acetate. The dried extract is decanted into a Kuderna-Danish concentrator attached to a 50-mL, round-bottom flask.

Ethoxylation. Fifty milliliters of absolute ethanol is added to the concentrator and the volume reduced to approximately 25 mL on a steam bath. Six drops of concentrated hydrochloric acid is added to the flask and the mixture refluxed 0.5 h. The cooled ethanolic extract is placed in a separatory funnel. One-hundred-fifty milliliters of 0.25 N hydrochloric acid is added to the funnel. The aqueous ethanol is extracted with two 175-mL portions of dichloromethane. The extract is dried over a minimum amount of prerinsed sodium sulfate. The extract is concentrated in a Kuderna-Danish concentrator to approximately 5 mL on a steam bath. Further reduction of the volume to 1 mL is accomplished under a gentle stream of nitrogen. The sample must not be allowed to go to dryness.

Florisil Column Cleanup. The cleanup of this extract was accomplished by a column and elution identical with that used for the carbofuran extracts.

Analysis. Detection of both carbofuran and 3-hydroxycarbofuran (as 3-ethoxycarbofuran) was accomplished using a Hewlett-Packard 5730 gas chromatograph equipped with a NPD detector. The operating parameters were as follows. Detector parameters: hydrogen (ultrapure) flow rate, 3.2 mL/min; air (ultrapure) flow rate, 40 mL/min; temperature, 300 °C; voltage applied to detector, 16–20 V (as needed); attenuation (carbofuran), 16; attenuation (3-ethoxycarbofuran), 8. Column parameters: composition, silanized glass; length, 122 cm; diameter, 2 mm; packing, 5% OV-3 on Chromosorb WHP (80–100 mesh); injector temperature, 200 °C; column temperature, 175 °C; carrier (helium, ultrapure) flow rate, 60 mL/min. With the instrument operating under the conditions listed above, a 40–60-mm response for 1 ng of carbofuran (160 mm equals 1 mv FSD) should be observed at 2.1 min. Similarly, a response of 25–40 mm should be observed at 4.5 min for 3-ethoxycarbofuran.

The detector exhibited linearity for both compounds over a concentration range of ten decades by both peak height and area measurement. A standard deviation of less than 5% was obtained by either method. In general, injection standards were 0.5 ng/μL in concentration.

Quantitation. Uncorrected ppm values for carbofuran were calculated using the following formula:

$$\text{ppm} = \frac{\text{response unknown} \times \text{ng standard injection}}{\text{response standard} \times \text{mg crop injected}}$$

Uncorrected ppm values for 3-hydroxycarbofuran were calculated using the same formula and then multiplied by 0.893, the molecular weight conversion factor for 3-ethoxycarbofuran. Corrected residues were calculated by dividing the uncorrected ppm values by the experimentally established average method recovery value.

Analytical Limits. Quantitatively reliable measurement of response, i.e., method sensitivity, for each compound was determined to be 0.10 ppm. Method sensitivity was validated by satisfactory recovery from fortified samples. Visual recognition of detector response as a peak, defined as method detectability, was possible when a response began to exceed 1 mm (i.e., 0.01 ppm for carbofuran and 0.02 ppm for 3-hydroxycarbofuran). For routine work, observed values lower than method detectability would be considered nondetectable. Values observed between the method detectability and method sensitivity would be reported as estimated values, since their quantitative reliability would be questionable for routine residue work.

RESULTS AND DISCUSSION

Sixteen different crops were subjected to the analytical method, without change. After initial development efforts, no attempt was made to adjust the method to maximize recoveries for individual crops. Recovery of carbofuran averaged 81.5 ± 7.5%. Recovery of 3-hydroxycarbofuran averaged 76.6 ± 6.9%. A background response in the carbofuran region averaging 0.02 ± 0.01 ppm was observed in all analyses of carbofuran. Similarly, reagent blank analyses with no crop present resulted in a background response in the carbofuran region averaging 0.02 ± 0.01 ppm. This indicated that this background response was reagent and/or environment related. Recovery values for individual crops are reported in Table I. Representative chromatograms are displayed in Figures 3 and 4.

The generality of this method was established by separating the analysis for the compounds of interest. This separation allowed the extraction of carbofuran by a blend with 0.25 N hydrochloric acid, followed by a partition with a nonpolar solvent (hexane). This minimized troublesome coextractives, which may be created by an acid hydrolysis and/or partitioned out with a more polar solvent (dichloromethane). Since it was known that carbofuran has not been found to any appreciable extent as a conjugated

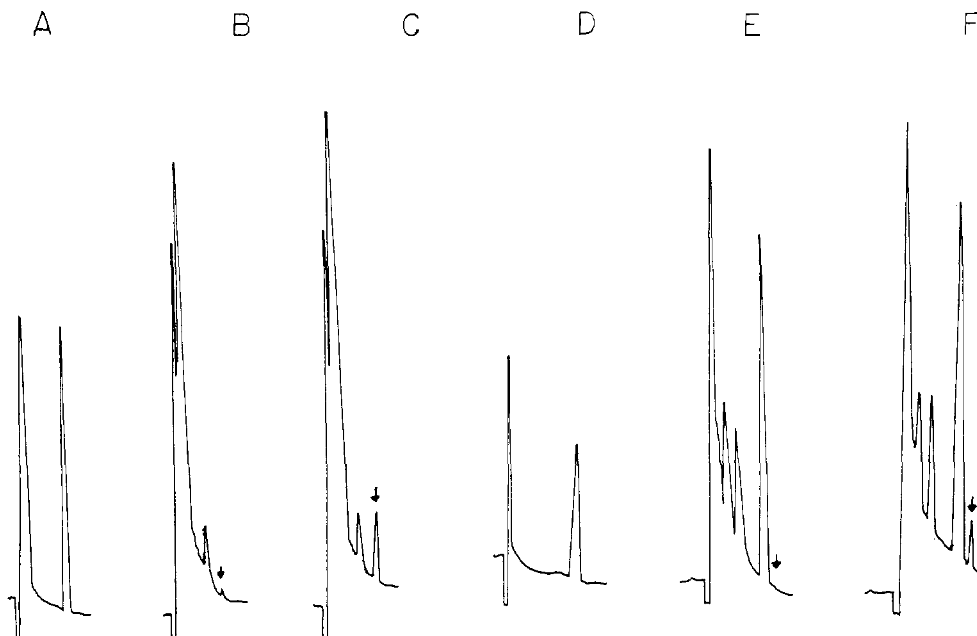

Figure 3. Typical chromatograms for the analysis of carbofuran and 3-hydroxycarbofuran in wheat grain: (A) 1 ng of carbofuran; (B) wheat grain check sample, 3 mg injected (0.01 ppm carbofuran); (C) wheat grain check sample fortified with 0.1 ppm carbofuran (0.076 ppm carbofuran), 3 mg injected; (D) 1 ng of 3-ethoxycarbofuran; (E) wheat grain check sample, 3 mg injected; (F) wheat grain check sample fortified with 0.1 ppm 3-hydroxycarbofuran (equivalent to 0.082 ppm 3-hydroxycarbofuran), 3 mg injected.

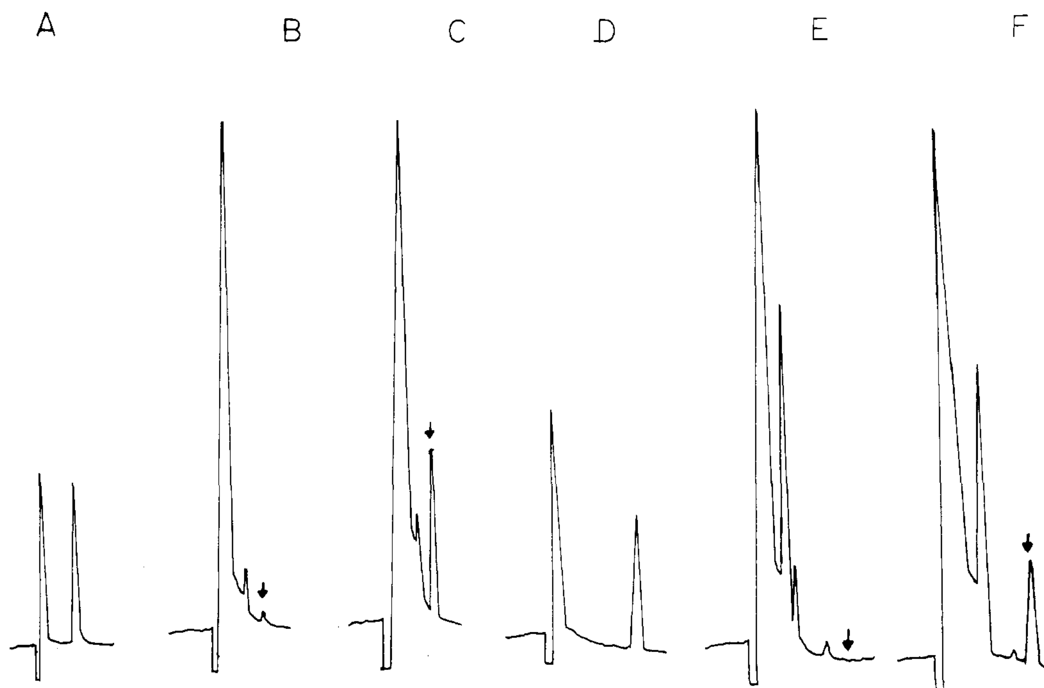

Figure 4. Typical chromatograms for the analysis of carbofuran and 3-hydroxycarbofuran in potatoes: (A) 1 ng of carbofuran; (B) potato check sample, 3 mg injected (0.028 ppm carbofuran); (C) potato check sample fortified with 0.3 ppm carbofuran, 3 mg injected (0.241 ppm carbofuran); (D) 1 ng of 3-ethoxycarbofuran; (E) potato check sample, 3 mg injected; and (F) potato check sample, fortified with 0.3 ppm 3-hydroxycarbofuran, 3 mg injected (equivalent to 0.249 ppm 3-hydroxycarbofuran).

residue, this appeared to be an attractive procedure. In order to prove the viability of the method (blend), samples of corn silage and potatoes known to contain residues of carbofuran were subjected to this method and the method of Cook (hydrolysis). Table II illustrates the comparison. Further verification was provided by analyzing an alfalfa hay sample obtained from alfalfa which had been grown in soil treated with ring-^{14}C-labeled carbofuran. The blending procedure was run six times. Residues ranging from 0.17 to 0.24 ppm averaging 0.20 ± 0.02 ppm were found. The hydrolysis procedure was run eight times. Residues ranging from 0.17 to 0.24 ppm averaging 0.20 ± 0.02 ppm were found. In addition, the blending samples showed 2272 ± 70 dpm attributable to the ^{14}C-labeled carbofuran, while the hydrolysis samples showed 2695 ± 328 dpm attributable to the ^{14}C-labeled carbofuran. Since these numbers were experimentally identical, it was concluded that there was no difference in the amount of carbofuran available to extraction by either method.

Since 3-hydroxycarbofuran is found in a conjugated form, an acid hydrolysis was required to free the compound from its conjugate and render it extractable by organic

Table I. Recovery of Carbofuran and 3-Hydroxycarbofuran from Various Crops

crop	fortification level, ppm	recov[a] carbofuran, %	3-hydroxy-carbofuran	crop	fortification level, ppm	recov[a] carbofuran, %	3-hydroxy-carbofuran
alfalfa	0.0	0.02[b]	0.03[b]	potato	0.0	0.01[b]	ND
	0.0	0.02[b]	0.03[b]		0.0	0.03[b]	ND
	0.1	83.0	73.0		0.3	85.0	81.1
	0.3	76.3	82.3		0.5	81.0	85.8
	0.5	70.0	72.2		av	83.0	84.4
	1.0	83.4	72.6	pumpkin flesh	0.0	0.02[b]	ND
	av	78.1 ± 6.4	75.0 ± 4.9		0.1	94.0	74.4
apple	0.0	0.03[b]	ND[c]		0.3	91.7	66.7
	0.1	89.5	88.2		0.5	95.0	73.6
	0.3	93.7	87.5		1.0	81.8	-
	av	91.6	87.8		av	90.6 ± 6.0	71.6 ± 4.2
banana	0.0	0.03[b]	ND	rice grain	0.0	0.03[b]	ND
	0.1	83.0	74.0		0.0	0.02[b]	ND
	0.3	84.2	76.7		0.1	94.0	67.7
	av	83.6	75.3		0.3	106.0	80.0
broccoli	0.0	0.03[b]	ND		0.5	82.4	67.0
	0.5	84.4	82.2		1.0	87.3	77.6
corn grain	0.0	0.02[b]	ND		av	92.4 ± 10.2	73.1 ± 6.7
	0.0	0.02[b]	ND	tomato	0.0	0.03[b]	ND
	0.1	74.5	87.8		0.0	0.03[b]	ND
	0.3	70.9	77.9		0.1	77.0	68.5
	0.5	69.5	84.8		0.3	72.7	82.0
	1.0	71.9	81.2		0.5	86.6	77.0
	av	71.7 ± 2.2	82.9 ± 4.3		1.0	87.3	66.6
corn silage	0.0	0.03[b]	0.02[b]		av	80.9 ± 7.2	73.5 ± 7.2
	0.0	0.02[b]	0.01[b]	wheat grain	0.0	0.01[b]	ND
	0.1	85.0	72.0		0.0	0.01[b]	ND
	0.3	84.3	68.0		0.1	66.0	81.9
	0.5	76.6	69.0		0.3	78.3	73.0
	1.0	82.9	75.0		0.5	71.6	72.1
	av	82.2 ± 3.8	69.7 ± 2.1		1.0	74.0	68.7
dry bean	0.0	0.03[b]	ND		av	72.5 ± 5.1	73.9 ±
(northwestern)	0.5	90.0	89.2	wheat straw	0.0	0.03[b]	ND
green bean	0.0	0.03[b]	ND		0.0	0.03[b]	ND
	0.5	74.6	85.8		0.1	101.0	99.2
green pea	0.0	0.02[b]	ND		0.3	84.3	69.8
	0.5	82.4	84.8		0.5	82.4	72.8
kidney bean	0.0	0.02[b]	ND		1.0	75.1	74.4
	0.5	86.4	95.3		av	85.7 ± 10.9	79.1 ± 13.6

grand average
carbofuran: 82.6 ± 8.7% (16 crops, 43 samples)
3-hydroxycarbofuran: 77.6 ± 8.0 (16 crops, 42 samples)
grand average adjusted for values deviating from average by >2 standard deviation
carbofuran: 81.5 ± 7.5% (16 crops, 42 samples)
3-hydroxycarbofuran: 76.6 ± 6.9% (16 crops, 40 samples)

[a] Corrected for background response if observed. [b] Crop or environment related background response. [c] ND = none detected.

Table II. Comparative Study of Hydrolysis and Blend Methods

crop	carbofuran[a] found in hydrolysis method, ppm	carbofuran[a] found in blend method, ppm
corn silage	0.20	0.25[b]
corn silage	0.50	0.49[b]
corn silage	0.68	0.63[b]
corn silage	1.35	1.64
potato	0.22	0.26
potato	0.10	0.10
potato	0.07	0.09

[a] Corrected for experimental recovery factor. [b] Average of two values.

solvents. The compound is relatively polar due to its hydroxy moiety and required a relatively polar partition medium (dichloromethane) to insure quantitative recovery. Conversion of 3-hydroxycarbofuran to 3-ethoxycarbofuran allowed the use of a Florisil column for cleanup. This in turn allowed the omission of any liquid partition for "oil" removal from crops having a high lipid content. The Florisil columns employed for both carbofuran and 3-ethoxycarbofuran were identical. The elution patterns were also identical and, as far as could be determined, totally crop independent. Florisil is a universal, standard absorbent and provided adequate cleanup for this analysis. It was shown that the moisture content of the Florisil was an important variable. A moisture content of 2.5 ± 0.3 weight percent proved to be optimum. If the moisture content was lower, loss of compound, particularly carbofuran, was observed. Wheat and corn grain were analyzed by this procedure, but Florisil which was 1.9 weight percent moisture was substituted for the normal 2.5%. Inspection of Table I shows that the average recovery of carbofuran from these samples was 10% lower than the average recovery of the rest of the samples.

Quantitation of the samples for both carbofuran and 3-hydroxycarbofuran (as 3-ethoxycarbofuran) was accomplished using 5% OV-3 on 80/100 M Chromosorb WHP. Apeizon L, OV-1, and OV-17 as well as Ultrabond columns were tested. The OV-3 column proved to be superior to

all these liquid phases for both sensitivity and selectivity. Interfering peaks, particularly in the 3-ethoxy region, were observed for some crops (rice, tomatoes, wheat grain) on all columns except the 5% OV-3. The chromatography of 3-ethoxycarbofuran was shown to be excellent with no crop related variation.

While the split procedure appears to be the most general, it is possible that analysis of both carbofuran and 3-hydroxycarbofuran could be done for certain crops by the 3-hydroxy procedure alone.

Practitioners of this method should be aware of possible difficulties which were discovered during the development of this method. The NPD system is very sensitive and is a selective but not a specific detector. Interfering peaks which were artifacts of the method and its reagents were observed frequently during the development of the method. Specifically, responses were found to come from the following: (1) contaminated glassware, (2) phosphorus detergents, (3) filter aids (filter paper, glasswool, cheesecloth), (4) sodium sulfate, (5) butyl rubber, (6) deterioration of the detector bead (sensitivity is achievable when selectivity is not, detectors exhibiting wide solvent regions should be considered suspect). Once these problems were identified, isolated, and dealt with, they did not recur.

The quantitative nature of all partition steps and the ethoxylation step were verified by radiotracer experiments as well as recovery of standard compounds.

Two areas where significant loss of compound occurred were noted. First, the samples must not be allowed to go to dryness during concentration. Significant loss will occur if the solvent is totally removed. Second, carbofuran was shown to be absorbed from hexane onto sodium sulfate. Radiotracer experiments showed that the loss was irreversible. This phenomenon was sodium sulfate batch related. The loss was easily avoided by adding diethyl ether (10%, v/v) to the hexane extracts before the addition of a minimum amount of sodium sulfate. Magnesium sulfate and barium oxide also resulted in significant loss of carbofuran if used as drying agents.

CONCLUSIONS

The method, as written, was designed to be as general as possible. It was applied without change to the 16 crops in Table I. Variations of the method are possible with individual crops. Some of these variations include (1) analysis of the carbofuran fraction with no cleanup column (peas, potatoes, tomatoes), (2) analysis of both carbofuran and 3-hydroxycarbofuran by the 3-hydroxycarbofuran procedure alone, (3) alteration of the Florisil column elution pattern, and (4) analysis using a different GC column. These variations may work for an individual crop and shorten the procedure. However, given a crop of unknown characteristics, the method practiced exactly as written has the best chance of success.

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Hydrolysis of Fenitrothion in Model and Natural Aquatic Systems

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The hydrolysis of fenitrothion [*O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] was studied in the dark in buffered distilled water, natural lake water, and buffered lake water. Above pH 8, specific base catalysis was the predominant degradative reaction resulting in the formation of 3-methyl-4-nitrophenol. Below pH 7, a second reaction involving dealkylation also took place to form demethylfenitrothion. The extent of this reaction was temperature dependent. Amino fenitrothion was also detected as a reaction product, but only in natural lake water systems. The $t_{1/2}$ for the disappearance of fenitrothion at 23 °C and pH 7.5 in natural lake water in the dark and in the field were 49.5 and 1.5–2 days, respectively. This difference suggests that photolysis and microbiol processes are the main degradative route of fenitrothion in natural aquatic systems.

Fenitrothion [*O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] (**1**) has been used extensively in Canadian forests for the control of lepidopterous defoliators. Application is made by aerial spray, which can result in the inadvertent contamination of aquatic systems, either directly or indirectly from surface run-off following rainfall. Levels of fenitrothion in river water following spraying have been reported ranging from 6 ppb to 64 ppm (Symons, 1977; Flannagan, 1973; Edit and Sundaram, 1975; Moody et al., 1978). Information about the persistence and modes of degradation of this insecticide in fresh water is therefore important in assessing its impact on aquatic flora and fauna.

Like other organophosphorus insecticides, fenitrothion is known to be degraded in water by both photolysis

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