Determination of Carbofuran and 3-Hydroxycarbofuran Residues in Peppermint Hay and Peppermint Oil

A general method for determining residues of carbofuran and its major carbamate plant metabolite, 3-hydroxycarbofuran, was modified for the analysis of peppermint hay and oil. The hay was refluxed with acid and carbofuran residues were partitioned into 25% ethyl acetate in hexane followed by Florisil cleanup. The metabolite analysis involved extraction of the hydrolysis mixture with dichloromethane, charcoal–silica gel chromatography, ethoxylation, and Florisil cleanup. The oil samples were diluted with hexane and extracted with water, and the residues were partitioned into dichloromethane followed by ethoxylation and Florisil cleanup. Quantitation was by nitrogen-specific gas chromatography. These methods are sensitive to 0.05 ppm in hay and 0.1 ppm in oil for the parent compound and to 0.1 ppm for the metabolite in both substrates. Recoveries averaged 85%.

The insecticide carbofuran (Furadan, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) is effective for the control of strawberry root weevil (*Fumibotys fumalis*) larvae and mint root borer (*Otiorhynchus ovatus*) larvae in peppermint (Berry, 1971). An analytical method for determining this insecticide and its principal metabolite, 3-hydroxycarbofuran (2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate), in mint hay and in oil distilled from the hay was needed. A general method for determining these residues (Nelsen and Cook, 1980) in 16 different crops was applied to peppermint hay and oil and was found to be suitable for these substrates with the modifications described below. Analysis of hay samples for residues of 3-hydroxycarbofuran required an additional column cleanup step to remove interferences.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Hewlett-Packard 5710A gas chromatograph equipped with dual nitrogen–phosphorus thermionic detectors was used. Analytical-grade standards of carbofuran and 3-hydrocarbofuran were supplied by FMC Corp., Middleport, NY. All solvents were distilled in glass.

Procedure. Carbofuran and 3-Hydroxycarbofuran in Hay. Extraction of both residues from chopped and throughly mixed hay was accomplished by first blending a 20-g representative sample with 250 mL of water in an Omnimixer followed by refluxing with 10 mL of concentrated hydrochloric acid for 1 h. After filtration, separate aliquots of the acid extract equivalent to 5 g of the sample were taken for each determination.

The carbofuran residue was partitioned into 25% ethyl acetate in hexane (v/v) by using four 50-mL portions of the solvent, the extract was dried and concentrated just to dryness, and the residue was redissolved in 1 mL of benzene and 0.5 mL of ethyl acetate. Five milliliters of hexane was added and the residue applied to a cleanup column containing 10 g of 5% water deactivated Florisil (100–200 mesh, Floridin Co., Berkeley, WV). The column was first eluted with 50 mL of 10% ethyl acetate in hexane (v/v), which was discarded, and then with 125 mL of 15% ethyl acetate (v/v), which was collected and concentrated for gas chromatography. The gas chromatograph was equipped with a 75 × 0.2 cm i.d. glass column packed with 10% OV-3 on 120–140-mesh Chromosorb WHP and operated at 210 °C with 30 mL/min helium carrier gas.

Residues of 3-hydroxycarbofuran were extracted from the acid solution with four 50-mL portions of dichloromethane, dried, and concentrated for chromatography on a mixed sorbent column (Maitlen and Powell, 1982). The residue was transferred to a 1.9 cm i.d. glass column containing 8 g of mixed charcoal–silica gel adsorbent (4 g of charcoal, Norite A decolorizing carbon, Fisher Scientific Co., Fairlawn, NJ, mixed with 50 g of 2% water deactivated silica gel, Grade 950, 60–200 mesh, Davidson Chemical Division, W. R. Grace and Co., Baltimore, MD). The absorbent bed was first washed with 50 mL of 5% methanol in dichloromethane (v/v) and then with 50 mL of dichloromethane. After the sample residue was transferred to the column it was eluted with 50 mL of dichloromethane which was discarded. The column was then eluted with 100 mL of 5% methanol in dichloromethane, discarding the first 20 mL and collecting the last 80 mL of the eluate. The residue was ethoxylated as described by Nelsen and Cook (1980) and subjected to a final cleanup on a 10-g 5% water deactivated Florisil column using 75 mL of a 25% ethyl acetate in hexane (v/v) solvent system, discarding the first 25 mL and collecting the last 50 mL. The final eluate was concentrated for gas chromatography on a 168 × 0.2 cm i.d. glass column packed with 6% DC-200 on 100–200-mesh Chromosorb 750 and operated at 190 °C with 30 mL/min helium carrier gas.

Table I. Residues of Carbofuran and 3-Hydroxycarbofuran in Mint Hay and Oil from Junction City, OR, after Foliar Application[a]

days after application	residues, ppm, from application of carbofuran[b]			
	2.2 kg of AI/ha		4.5 kg of AI/ha	
	carbofuran	metabolite[c]	carbofuran	metabolite[c]
0	309	<1[g]	315	1[g]
7	1.8	5.7	6.5	13.0
14	0.77	2.9	5.0	13.3
28	0.95	3.0	4.5	11.3
96	0.06	0.47	0.21	1.15
181	<0.05	0.1	0.06	0.35
345[d]	<0.05	0.4	0.14	0.1
345[e]	0.1	0.11	0.1	0.1
345[f]	<0.1	<0.1	<0.1	<0.1

[a] One application of Furadan 4F, 0.48 kg/L, by ground sprayer. Applied Sept 16, 1977; harvested Aug 27, 1978. [b] Not corrected for recoveries. [c] 3-Hydroxycarbofuran. [d] Mint hay at harvest. [e] Mint hay after distillation. [f] Mint oil distilled from hay. [g] Estimated value. Accurate determination of 3-hydroxycarbofuran was difficult because of the large quantity of carbofuran present.

Carbofuran and 3-Hydroxycarbofuran in Peppermint Oil. Both carbofuran and 3-hydroxycarbofuran residues were extracted from 5-g samples of peppermint oil by first dissolving the oil in 100 mL of hexane and then partitioning 3 times with 100-mL portions of doubly distilled water. The combined aqueous phases were acidified with 6.5 mL of concentrated hydrochloric acid, 6 g of sodium chloride was added, and the aqueous phase was extracted with four 50-mL portions of dichloromethane. This

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Table II. Recovery of Added Carbofuran and 3-Hydroxycarbofuran from Untreated Crops

crop	no. of recoveries	fortification levels, ppm	average[a] recovery, %	SD
		Carbofuran		
fresh mint hay	10	0.5-1.0	76	±14.4
spent mint hay	3	1.0	73	±10.0
mint oil	9	0.1-2.0	83	±16.0
		3-Hydroxycarbofuran		
fresh mint hay	10	0.5-1.0	95	±11.2
spent mint hay	3	1.0	101	±30.4
mint oil	9	0.12-4.0	85	±14.0
grand average				
carbofuran			78	±15.1
3-hydroxycarbofuran			92	±17.2

[a] Corrected for background response if observed.

extract was dried, ethoxylated, concentrated to near dryness, and solvent exchanged into benzene. The residue was chromatographed on a 10-g 5% water deactivated Florisil column with 75 mL of 10% ethyl acetate in hexane (v/v), which was discarded, and then with 85 mL of 30% ethyl acetate in hexane (v/v), which was concentrated for gas chromatography. A 75 × 0.2 cm i.d. glass column packed with 10% OV-3 on 120–140-mesh Chromosorb WHP and operated at 210 °C with 30 mL/min helium carrier gas served for quantitation of both residues.

Method Sensitivity. The sensitivity limits of the analytical method were estimated on the basis of the gas chromatographic response to analytical standards and the sample size. The method sensitivity for mint hay was estimated to be 0.05 ppm for carbofuran and 0.1 ppm for 3-hydroxycarbofuran with a 5-g sample and for mint oil 0.1 ppm for both compounds with a 5-g sample. The retention time on the OV-3 column for carbofuran was about 2.4 min and for 3-hydroxycarbofuran 4.7 min at 210 °C. The retention time of 3-hydroxycarbofuran on the DC-200 column was about 5.3 min at 190 °C.

RESULTS AND DISCUSSION

Several modifications of the general method developed by Nelsen and Cook (1980) were required to obtain satisfactory recovery of added carbofuran and 3-hydroxycarbofuran and to remove interferences when this method was applied to peppermint hay and oil. Extracting both residues after hydrolysis of the hay samples was found to be advantageous, even though a more polar solvent, 25% ethyl acetate in hexane, was required for satisfactory carbofuran recovery. Separation of the carbofuran from coextractives, however, required a separate Florisil elution scheme.

Interferences were observed in the analysis of the hay samples for 3-hydroxycarbofuran on several different gas chromatography columns, even after liquid chromatography of the ethoxylated residue on a variety of adsorbents. Chromatography of the underivatized 3-hydroxycarbofuran on the mixed bed column, followed by ethoxylation and chromatography on Florisil, was found to be adequate if the gas chromatographic analysis was performed on the longer, more heavily loaded nonpolar column. Hay samples taken early in the season, before the synthesis of the oils within the plant had taken place, could be successfully analyzed without the mixed bed column. The extracts of peppermint oil were made suitable for analysis of both residues after ethoxylation and cleanup on a Florisil column.

This method has been successfully used for the determination of residues of carbofuran and 3-hydroxycarbofuran in mint hay and oil. Typical residue data are presented in Table I. The fall application was made after harvest and before much of the regrowth had started; therefore, the initial residues of carbofuran found on hay were high. The nearly identifical initial residues of carbofuran resulting from high and low treatment rates were probably due to the difficulty of obtaining a representative sample of plants which were very small at this time. In addition, it is also likely that some of the plants were contaminated by the treated soil. Carbofuran residues persisted under Oregon winter conditions of cool temperatures and high rainfall for more than 6 months, and traces of carbofuran were present even at harvest at the higher rate of application. The principal metabolite of carbofuran, 3-hydroxycarbofuran, also persisted throughout the growing season. However, residues of both compounds were absent in the product used for human consumption, peppermint oil.

The reliability of the analytical method was tested by adding known amounts of carbofuran and its metabolite to fresh and spent mint hay and to mint oil, followed by extraction and analysis. The range of fortifications and recoveries are shown in Table II.

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Registry No. Carbofuran, 1563-66-2; 3-hydroxycarbofuran, 16655-82-6.

Supplementary Material Available: Expanded recovery data of those in Table II (2 pages). Ordering information is given on any current masthead page.

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