mine whether this is a general mechanism.

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Registry No. Chlorpyrifos, 2921-88-2; 3,5,6-trichloro-2-pyridinol, 6515-38-4; *O,O*-diethyl phosphorothioic acid, 2465-65-8.

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Determination of the Phenolic Metabolites of Carbofuran in Plants by Gas Chromatography/Mass Spectrometry

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A gas chromatographic procedure using mass spectrometry in the selected ion mode as the detection system for the determination of residues of the phenolic metabolites of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is described. The method is general, having been applied without change to 12 different crop matrices including those having high-moisture, low-moisture, and high-lipid content, as well as fruit, vegetable, and root crops. Method sensitivities of 0.05–0.10 ppm for each phenol were achieved. Average recoveries of the three phenolic metabolites ranged from 72 to 104%. The method involved release of the conjugated phenols by acid hydrolysis, extraction, ethoxylation, partition of the phenols into base, acidification, extraction, and silica gel Sep-PAK cleanup of the samples.

Investigations by Metcalf et al. (1968) and Knaak et al. (1970a,b) have shown that there are three major phenolic metabolites of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate). These metabolites were identified as 2,3-dihydro-2,2-dimethyl-7-benzofuranol (7-phenol), 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol (3-keto-7-phenol), and 2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol (3-hydroxy-7-phenol). The phenolic residues occur as water-soluble conjugates bound at the 3- and/or 7-position of the benzofuranol system.

Only one residue procedure of any generality has been reported for these phenols (Cook et al., 1977). This method detected the phenols as their dinitrophenyl ether derivatives. Archer et al. (1977) have reported a similar residue method for these compounds in strawberry and strawberry leaves. The general procedure reported by Cook is workable; however, the procedure is a lengthy, complex, multistep procedure. The chromatographic cleanup of the samples is variable from crop matrix to crop matrix and low-level coextractive interferences created quantitation

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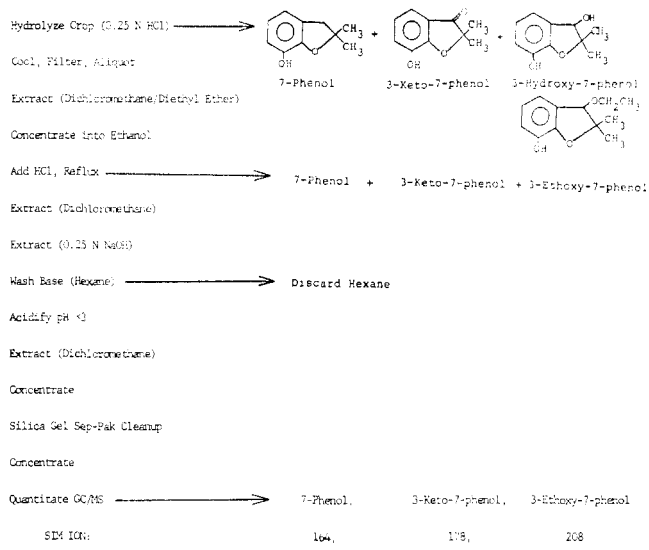

Figure 1. Determination of 7-phenol, 3-keto-7-phenol, and 3-hydroxy-7-phenol residues by gas chromatography/mass spectrometry (GC/MS).

problems in certain crop matrices.

We therefore sought a faster, more general procedure for the residue analysis of the phenolic metabolites of carbofuran. The method reported herein has been applied to 12 plant matrices without change.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Hewlett-Packard 5992B gas chromatograph/mass spectrometer equipped with a Hewlett-Packard 9825A desktop computer, a Hewlett-Packard 9885 flexible disk drive, and a Hewlett-Packard 9866B printer plotter or a Hewlett-Packard 5993 gas chromatograph/mass spectrometer equipped with a Hewlett-Packard 7906 disk drive, Hewlett-Packard 2648A graphics terminal, and a Hewlett-Packard 9876A printer plotter were used for detection. Standard laboratory glassware was used throughout the procedure. Baker Resi-Analyzed solvents (or their equivalent) were employed.

Sample Preparation. Each bulk sample (minimum 500 g) was macerated in a food chopper (Hobart) by using liquid nitrogen as the coolant. If further size reduction was required, a grinder or mill was employed by using liquid nitrogen as the coolant. Since relatively small samples (5 or 10 g) were used for analysis, care was exercised to ensure 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 non-phosphorus detergent in a laboratory glassware washer. Figure 1 diagrams the flow scheme of the analysis.

Hydrolysis. Ten grams of crop was placed in a 1-L round-bottom flask. Two hundred fifty milliliters of 0.25 N hydrochloric acid was added and the mixture refluxed for 1 h. The hot mixture was filtered through glass wool and the filtrate rinsed with 130 mL of 0.25 N hydrochloric acid. The volume of the hydrolyzed material was adjusted to exactly 400 mL. A 200-mL aliquot (5 g of crop equiv) was then removed.

Crops with high lipid content (i.e., sunflower seed, corn grain, peanuts, and soybeans) required a lower sample-to-acid ratio at hydrolysis and a 5-g sample was hydrolyzed as described. A 1.25 g crop equiv aliquot (100 mL/400 mL) was taken. This aliquot was brought to 200 mL with distilled water. This variation resulted in limiting emulsion problems; thus allowing better recovery of the phenols.

Initial Extraction. The 200-mL aliquot was extracted 3 times with 150 mL of dichloromethane (DCM)–diethyl ether (3:1 v/v) after the addition of enough 4% aqueous sodium lauryl sulfate (0–4 mL depending on the crop) to break any emulsion. The organic phase was filtered through approximately 10 g of anhydrous sodium sulfate (prewashed with ethyl acetate and DCM) into a Kuderna-Danish evaporator that was equipped with a 50-mL round-bottom flask. Fifty milliliters of absolute ethanol was added and the mixture was concentrated in a steam bath to approximately 25 mL.

Ethoxylation. To the 25 mL of concentrated ethanol solution in the above round-bottom flask was added 6 drops of concentrated HCl. This mixture was refluxed for 0.5 h. The contents were allowed to cool and then transferred to a 250-mL separatory funnel with a 100-mL rinse of distilled water.

Base Extraction. Following ethoxylation, the aqueous mixture was extracted 3 times with 50 mL of DCM. The 150 mL of combined DCM extract was returned to the separatory funnel and partitioned twice with 50 mL of 0.25 N NaOH. The 100 mL of combined base solution was washed with 100 mL of hexane. The hexane was discarded. The extracted NaOH solution was then acidified with 6 mL of concentrated HCl. This acidic solution was partitioned 3 times with 50 mL of DCM. The 150 mL of DCM solution was dried with anhydrous sodium sulfate (prewashed with ethyl acetate and DCM) and decanted into a Kuderna-Danish evaporator. The DCM mixture was concentrated to ca. 10 mL of a steam bath. The volume was further reduced to 5 mL with the aid of a gentle stream of nitrogen on a N-EVAP evaporator.

Silica Gel Sep-PAK Cleanup. The concentrated sample was given an additional cleanup prior to GC/MS quantiation with a Silica Gel Sep-PAK (Waters Associates) cartridge. The cartridge was prepared for use by washing with 10 mL of DCM followed by 20 mL of DCM–hexane (7:3 v/v). The Sep-PAK was then conditioned for residue elution with 10 mL of methanol–water (1:1 v/v). The excess water and methanol were flushed from the gel with 10 mL of DCM. The 5 mL of sample in DCM was syringed onto the Sep-PAK and the eluant collected. The phenol residues were then eluted by passing sufficient DCM–hexane (7:3 v/v) through the cartridge to yield a final 10-mL volume. Methanol (2 mL) was added to this eluant solution. The eluant was slowly evaporated to 1 mL with a stream of nitrogen. Caution: do not concentrate to dryness or loss of phenolic residue may occur. Samples from high-lipid crops were concentrated to 0.5 mL.

Quantitation. The three phenols were quantitated by GC/MS in the selected ion mode. The molecular ion of each compound, 164 for 7-phenol, 178 for 3-keto-7-phenol, and 208 for 3-ethoxy-7-phenol, was used for quantitation. Use of the molecular ion provided most specific detection and avoided coextractive responses. A 122 cm by 2 mm (i.d.) glass column packed with 5% OV-3 on Chromosorb W-HP (80–100 mesh) supplied by Supelco was used. A column temperature of 154 °C and an inlet temperature of 250 °C were used. The flow rate was 25 mL/min helium. With the GC operating under the listed, conditions, 7-phenol eluted at 1.1 min, 3-keto-7-phenol at 1.8 min, and 3-ethoxy-7-phenol at 2.6 min.

Each day a combination standard (0.5 ng/μL each) of 7-phenol, 3-keto-7-phenol, and 3-ethoxy-7-phenol was prepared in methanol. The response of the ions of interest was optimized before the injection of any sample. Typically no adjustment of the mass spectrometer settings was

Table I. Recovery of 7-Phenol, 3-Keto-7-phenol, and 3-Hydroxy-7-phenol from Fortified Crop Samples

crop	fortification range, ppm, for each phenol	no. of fortified samples	av % recovery (SD)		
			7-phenol	3-keto-7-phenol	3-hydroxy-7-phenol
alfalfa	0.10-2.0	14	77 (12)	99 (8)	87 (10)
alfalfa hay	0.10-3.0	19	76 (9)	95 (8)	81 (9)
grapefruit	0.05-0.20	5	94 (8)	104 (7)	87 (2)
orange	0.05-0.2	4	82 (6)	98 (10)	83 (8)
peanut	0.10-0.40	11	98 (12)	100 (13)	82 (11)
peanut hull	0.10-1.0	9	73 (5)	99 (7)	92 (8)
potato	0.05-0.50	6	78 (8)	89 (11)	90 (9)
soybean	0.10-2.0	17	79 (9)	98 (12)	81 (8)
sunflower	0.05-0.20	9	75 (10)	98 (8)	84 (4)
sweet corn ear (kernel and cob)	0.10-1.0	13	72 (9)	96 (8)	81 (11)
sweet corn fodder	0.10-2.0	13	76 (7)	92 (10)	83 (9)
sweet corn husk	0.10-2.0	12	75 (10)	96 (12)	85 (11)

required from day to day. When adjustment was required, it was a minor adjustment of the amu gain or offset. Samples were injected and a standard (typically 1 ng on the column) was injected after each sample. Areas of the peaks of interest were measured by the GC/MS computer. The resulting five or six standard injection areas were averaged.

Calculations. The following formula was used to calculate ppm values for the samples:

$$\text{ppm} = (\text{area of unknown} \times \text{ng of standard})/(\text{average area of standard} \times \text{mg of crop injected})$$

For calculation of 3-hydroxy-7-phenol, the number obtained is multiplied by 0.865, the molecular weight ratio of 3-hydroxy-7-phenol to 3-ethoxy-7-phenol.

Analytical Limits. Quantitatively reliable measurement of response, i.e., method sensitivity, for each phenol was determined by satisfactory recovery of the compound from artificially fortified control samples. Recognition of response was possible when the peak of interest gave an area count of approximately 5–10% (0.05–0.10 ng on the column) of the standard. This value was defined as the limit of detection (method detectability) and was normally 0.005–0.010 ppm. Peaks giving areas less than this value were defined as nondetectable. For routine work values between the method detectability and the method sensitivity should be considered estimates since their quantitative reliability is untested.

RESULTS AND DISCUSSION

Table I reports recovery values from 12 crop matrices for each phenol. Average recovery of 7-phenol ranged from 72 to 98% and gave a grand average of 78%. Recovery of 3-keto-7-phenol ranged from 89 to 104% with a grand average of 97%. Recovery of 3-hydroxy-7-phenol as its 3-ethoxy derivative ranged from 81 to 92% with a grand average of 84%.

Table I clearly demonstrates the utility and generality of the method. Figures 2 and 3 display typical GC/MS chromatograms derived through the method.

The efficiency of the individual extraction steps, the ethoxylation, and the Sep-PAK cleanup was verified by using radiolabeled compounds as well as analytical standards. No individual step had an efficiency of less than 95%.

The generality of the method hinges on the Sep-PAK cleanup and the use of the mass spectrometer as a routine quantitation tool. Of all the crops studied, only potato samples had no hindering coextractives and, therefore, required no Sep-PAK cleanup. To date the Sep-PAK cleanup has successfully dealt with all interfering coextractives. Very low level interferences at the limit of method detection (0.005–0.01 ppm) have been encountered

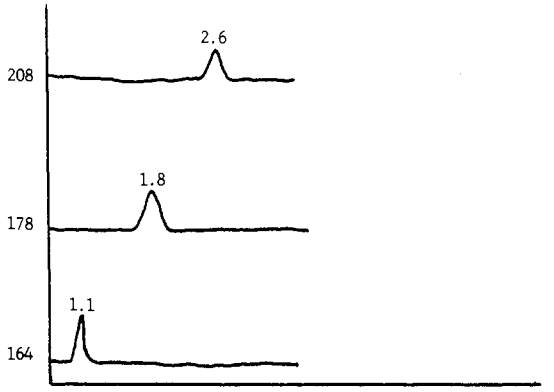

Figure 2. 1 ng injected; 7-phenol (164), 3-keto-7-phenol (178), and 3-ethoxy-7-phenol (208).

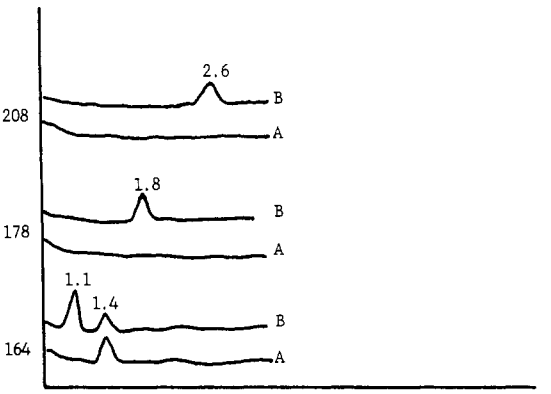

Figure 3. (A) Soybean check sample, 10 mg injected. (B) Soybean check sample fortified at 0.1 ppm (10 mg injected) of 7-phenol (164; 0.087 ppm), 3-keto-7-phenol (178; 0.097 ppm), and 3-ethoxy-7-phenol (208; 0.080 ppm of 3-hydroxy-7-phenol).

in the 7-phenol region in several crops in some control samples. These low-level responses presented no difficulty in quantitation at the 0.1-ppm level. The 3-keto-7-phenol and 3-ethoxy-7-phenol regions have been free of interferences in all crops.

The mass spectrometric detection system was shown to be linear over the range 0.25–2.0 ng injected. An indication of the precision of the GC/MS system is the coefficient of error (standard deviation/mean) observed for the alternating standard injections. The coefficient of error for each of the phenols reported in this work ranged from 0.03 to 0.07. Further, five repetitive injections of a 0.10 ppm fortified sample resulted in calculated values for recovery with a standard deviation of $<\pm 5\%$.

CONCLUSIONS

The method as written provides a reliable, general method for the analysis of carbofuran phenols and demon-

strates the successful use of gas chromatography/mass spectrometry as a routine residue analysis tool.

Application of gas chromatography/mass spectrometry has allowed the quantitation of carbofuran phenols with a minimum amount of sample cleanup. This has reduced the per sample analysis time by half relative to the that of the published derivatization procedure.

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Registry No. Carbofuran, 1563-66-2; 7-phenol, 1563-38-8; 3-keto-7-phenol, 17781-16-7; 3-hydroxy-7-phenol, 17781-15-6.

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Metabolism of Pentachloronitrobenzene by Goats and Sheep

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The metabolic fate of a single oral dose of pentachloronitro[^{14}C]benzene ([^{14}C]PCNB) was examined in four goats and a sheep. Two general pathways of metabolism were observed. One involved the reduction of the nitro group to an amine and formation of secondary metabolites such as the *N*-glucuronide of pentachloroaniline (PCA), the sulfamate of PCA, and tetrachloroaminophenol. This was the principal pathway for animals receiving 2 mg of [^{14}C]PCNB/kg. Only [^{14}C]PCA and small amounts of [^{14}C]PCNB were identified in feces of these animals. The other pathway results in replacement of a nitro group with a sulfur-containing group such as a thiol, a methylthio, an *N*-acetylcysteine, and an *S*-glucuronide or in which both the nitro and a chlorine have been replaced to form compounds such as the *S*-glucuronide of tetrachloro(methylthio)thiophenol, bis(methylthio)tetrachlorobenzene) or *S*-[(methylthio)tetrachlorophenyl]-*N*-acetylcysteine. This type of metabolism was observed with animals receiving 30 mg or more of [^{14}C]PCNB/kg of body weight. In these animals, [^{14}C]PCNB was isolated from feces in amounts equal to or greater than those of [^{14}C]PCA.

Pentachloronitrobenzene (PCNB) is a soil fungicide that is relatively persistent in soil (Beck and Hansen, 1974; Murthy and Kaufman, 1978). Some information is available about the metabolic fate of PCNB in rabbits (Betts et al., 1955), cattle (St. John et al., 1965; Borzelleca et al., 1971), dogs and rats (Kuchar et al., 1969), and sheep (Avrahami and White, 1976). However, these studies did not employ radiotracers, and therefore, analysis was for suspected metabolites and parent compound. More recently, metabolism studies using radiotracer techniques have been reported. Kogel et al. (1979a–c) observed excretion of ^{14}C and the pattern of radiolabeled metabolites after oral administration of [^{14}C]PCNB to three rhesus monkeys (single dose of 2 and 91 mg/kg of body weight; 2 ppm in diet for 71 days). Excretion occurred via both feces and urine with slightly larger amounts in feces. The pattern of metabolites was similar in the excreta from the three monkeys and pentachloroaniline (PCA) was the predominant metabolite in both feces and urine. Other metabolites in excreta were pentachlorobenzene, bis(methylthio)tetrachlorobenzene, pentachlorothiophenol, tetrachloroaniline (TCA), and tetrachloroaminothioanisole. Pentachlorophenol, tetrachlorophenol, tetrachlorothioanisole, and tetrachlorophenyl methyl sulfoxide were found only in urine while PCNB was found only in feces. O'Grodnick et al. (1981) reported *S*-(pentachlorophenyl)-*N*-acetylcysteine as the predominant urinary metabolite from Osbourne-Mendel rats after a single oral dose of 5 mg of [^{14}C]PCNB/kg of body weight. PCA was the predominant metabolite in feces and also a major urinary metabolite. Small amounts of pentachlorothioanisole were recovered from urine and feces. PCNB was found only in feces. PCA was recovered from one unidentified chromatographic fraction (from urine) after acid hydrolysis. Bahig et al. (1981) observed the fate of [^{14}C]PCNB added to water that contained fish. The major products were conjugates of pentachlorophenol and pentachlorothiophenol. We have investigated the metabolic fate of PCNB in goats and a sheep with [^{14}C]PCNB, and the results are presented in this report.

MATERIALS AND METHODS

Chemicals. [^{14}C]PCNB used in this study was synthesized in two batches from uniformly labeled [^{14}C]-nitrobenzene (Mallinckrodt, Amersham); one as described by Kadunce and Lamoureux (1976) and the other by a modification of that procedure and the procedure of Sandrock et al. (1978). Because a high yield of PCNB was not obtained when a Dewar condenser was used as in the method of Kadunce and Lamoureux (1976) or when chlorine was bubbled into the reaction mixture as in the

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