Carbofuran and 3-Hydroxycarbofuran Determination in Lettuce by Alkali-Flame Gas Chromatography

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A gas chromatographic method capable of determining carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) and 3-hydroxycarbofuran (2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) as residues in weathered lettuce is presented. The carbamate pesticide is transesterified with methanol *via* reaction gas chromatography to form methyl *N*-methylcarbamate which is assayed by an alkali-flame ionization detector. Acid hydrolysis converted the water-soluble conjugated carbamate residue forms to organo-extractable aglycones. Methylene chloride extracts of lettuce required only a Nuchar-Attaclay cleanup. Recoveries from lettuce extracts fortified with 0.05 to 1.0 ppm 3-hydroxycarbofuran were quantitative.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) is a broad spectrum insecticide–nematocide effective as a foliar applied contact-short residual toxicant and as a soil applied contact agent for soil insects and nematodes. When applied to the soil, carbofuran is usually absorbed and translocated to some degree by the growing plant. The persistence of carbofuran translocated into cotton plants grown under greenhouse conditions seemed to be controlled by both the organic matter and clay content of the soils investigated by Abdellatif *et al.* (1967). It has been noted by Cook *et al.* (1969) and McCarthy (1970) that 80–90% of the carbamate residues found in weathered corn or alfalfa were either 3-hydroxycarbofuran or 3-hydroxycarbofuran glycoside. A similar metabolic pattern was apparent in plants grown in soil previously treated with carbofuran, or 3 to 4 weeks following a foliar application. It was reported that, following the application of carbofuran to the soil at planting time, the carbamate residue in corn foliage at silage and grain stage consisted almost exclusively of 3-hydroxycarbofuran glycoside.

Kuhr and Casida (1967) demonstrated that the water soluble metabolites found in bean plants resulted in part from hydroxylation of the carbamate followed by conjugation, primarily to glycosides. These glycosides were found to be quite persistent and often exhibited anticholinesterase activity following hydrolysis by β-glucosidase. Cook *et al.* (1969) report the mechanism of carbofuran metabolism in field corn involves oxidation followed by conjugation at the 3 position of the benzofuran ring, and/or hydrolysis followed by conjugation at the 7 position of the benzofuran ring. The metabolism of benzofuranyl-7a-C^{14} and carbonyl-C^{14} carbofuran was investigated, and Knaak *et al.* (1970) identified the major metabolites in alfalfa and beans as the glycosides of 3-hydroxycarbofuran, 2,3-dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran, and 2,3-dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran.

Since a considerable percentage of the carbofuran metabolites in aged plant samples appear to be in the water soluble glycosidic form, an analytical method capable of measuring not only the parent compound but also any carbamate aglycones and glycosides was required. In the procedure reported here, all water soluble glycosides were converted to water-insoluble materials following digestion with dilute hydrochloric acid. The resultant carbamate aglycones, along with carbofuran itself, were extracted quantitatively with a nonpolar solvent.

The method reported here is based on the extraction and clean-up procedure presented by Cook *et al.* (1969) and Cassil *et al.* (1969), with the primary difference being the on-column transesterification of the intact carbamate, and the final detection system employed. Whereas these previous workers used a microcoulometric nitrogen detection system for the determination of the intact methylcarbamate, the described procedure utilizes transesterification of carbofuran with methanol to form methyl *N*-methylcarbamate (Figure 1). This product, as previously described by Moye (1971), was detected by glc in the low nanogram range using a rubidium sulfate alkali-flame ionization detector (AFID-$RbSO_4$). Extracted samples of lettuce, previously treated in the field with carbofuran, have been analyzed by this technique.

EXPERIMENTAL

Field Experiment. This was conducted at the Everglades Experiment Station, Belle Glade, Fla., in muck soil considered 100% organic matter. The soil applications of carbofuran were applied as 10% granules by sifting into the furrow in a 6 in. band along the line where seeding of the head lettuce was made. A seed drill mixed the granules with the soil to a depth of about 3 in. at rates of 2 and 4 lb active ingredient per acre. In a separate set of field plots, seven foliar applications composed of 1 lb of active 75% wettable powder carbofuran were applied to lettuce at weekly intervals during the latter stages of growth. Planting occurred on November 13, 1969, and the foliar applications on January 29, February 4, 11, 17, and 24, and March 3 and 10, 1970. Five mature heads per plot were selected at random on each sampling date, with the normal number of outer leaves being stripped to simulate normal commercial practices. The samples were placed in containers with sufficient dry ice and air-expressed to the laboratory at Gainesville.

Sample Preparation. The lettuce was finely chopped in a Hobart foodcutter, placed in polyethylene bags, and kept frozen until analyzed. Sample analysis was initiated following approximately 2 months of frozen storage.

Extraction. The procedure followed was essentially that of Cook *et al.* (1969), with indicated exceptions. After adding 250 mg of sodium lauryl sulfate to the filtrate, the aqueous phase was extracted with 300 ml of methylene chloride three successive times. The methylene chloride extracts were com-

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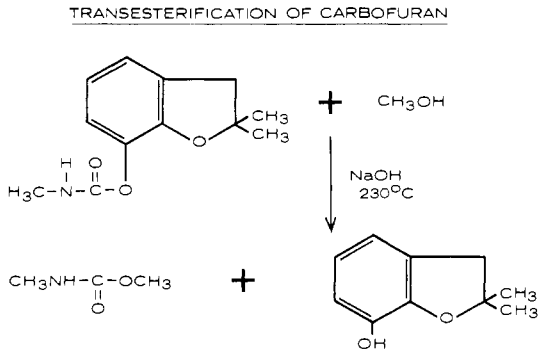

Figure 1. On-column transesterification of carbofuran with methanol to form methyl *N*-methylcarbamate

bined, dried over anhydrous sodium sulfate, and concentrated to approximately 15 ml in a 1-l. Kuderna-Danish evaporative concentrator.

Cleanup. The column cleanup employed was very similar to that presented by Cook *et al.* (1969), except that the use of silica gel was eliminated. Following concentration in the K-D evaporator, the methylene chloride extract was added to the top of the Nuchar-Attaclay column and eluted with successive 100-ml rinses of 30% hexane-ethyl acetate (v/v). Approximately 250 ml of eluent was collected in a graduated 500-ml Erlenmeyer flask and reduced to about 3 ml at 45–50° C using a stream of nitrogen to facilitate evaporation.

Methylation. Following transfer to a graduated concentrator tube, the eluent was concentrated to approximately 1 ml using dry nitrogen and heat. Approximately 3 ml methanol were added, and the solution was concentrated to less than 1 ml. One hundred-fifty microliters of 2 *N* sodium hydroxide were added and the final volume was adjusted to 1.0 ml with methanol. Upon injection into the gas chromatograph, on-column transesterification occurred instantly, catalyzed on the glass beads by the sodium hydroxide.

Conversion Efficiency. Methyl isocyanate was reacted with methanol to furnish a standard quantity of methyl *N*-methylcarbamate. To test the conversion efficiency of the reaction used in the experiment, 38.8 ng carbofuran, 3-hydroxycarbofuran, or several mixtures of the two compounds were methylated on column to form methyl *N*-methylcarbamate. Table I illustrates the consistency of conversion for the various carbamate mixtures with or without the presence of crop extractives. Two levels of carbofuran, 3-hydroxycarbofuran, and ratios of 1:4 and 4:1 parent compound to metabolite were reacted to test the efficiency of transesterification to form methyl *N*-methylcarbamate. The two ratios employed were an attempt to simulate the relative ratios of parent compound and primary carbamate metabolite early and later in the conversion process. For all carbamate mixtures tested, transesterification resulted in a very consistent percentage of conversion (67–71%).

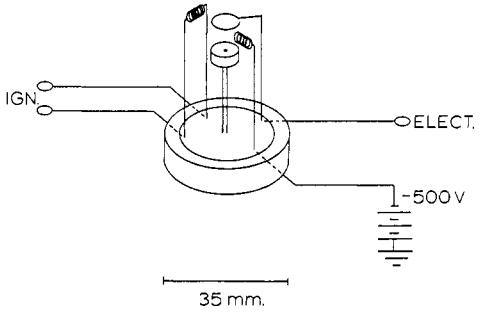

Figure 2. Packard hydrogen-flame ionization detector converted to a rubidium sulfate cup-type AFID. (All parts to scale)

Table I. Percent Conversion Efficiency of Carbofuran/3-OH Carbofuran

Carbamate	Ratio	Ng injected	w/o Crop[a]	w/Crop[a]
Carbofuran	...	50	69	68
3-OH carbofuran	...	50	68	67
C + 3-OH	1:4	50	68	70
C + 3-OH	4:1	50	71	67
Carbofuran	...	200	68	70
3-OH carbofuran	...	200	69	69
C + 3-OH	1:4	200	67	71
C + 3-OH	4:1	200	70	68

[a] W and w/o lettuce extractives.

Percent Recoveries. Fifty grams of untreated lettuce were fortified with 0.05, 0.2, 0.5, and 1.0 ppm 3-hydroxycarbofuran prior to digestion and carried through the entire procedure. Since the majority of the weathered carbamate residues have been found to be either in the 3-hydroxy aglycone or glycoside form, it was decided to use the primary metabolite as a standard in preference to the parent material. Recoveries of 0.05 to 1.0 ppm 3-hydroxycarbofuran added to the crop (50 g) prior to extraction were approximately 100%. The standard curve for 3-hydroxycarbofuran was linear throughout this range.

Gas Chromatography. A Packard 7620 gas chromatograph with a hydrogen-flame ionization detector converted to AFID operation (Figure 2) was used. The conversion was made by removing the standard Packard flame jet and fitting a short length of thin wall $^1/_{16}$ in. stainless steel tubing in place. The Rb_2SO_4 salt cup was formed by placing a $^1/_{16}$ in. stainless steel Swagelok nut at the upper end, supported by a $^1/_{16}$ in. front ferrule that had been swaged onto the tubing. The nut was supported at a height on the tubing so that the end of the tubing was level with the upper end of the nut. Rb_2SO_4, as a slurry in water, was added to the cup thus formed and allowed to slowly air-dry before use. A collector loop was formed from $^1/_{16}$ in. tubing also, with a diameter of 9 mm, and placed at a height of 9 mm above the salt cup. The polarizing coil was formed from 26 gauge stainless steel wire.

A minus 500 V polarizing voltage was supplied by a Smith-Florence 127 power supply, and bucking current was supplied internally by a Sargent MR recroder. The internal bucking current from the MR recorder was required since, by changing the polarizing voltage from positive to negative, the sign of the electrometer current was also reversed. The Packard gc electrometer can control negative current but could not provide the required negative bucking. The column used was a 6 ft by 4 mm i.d. Pyrex spiral packed with Porapak P and topped with approximately a 1 in. layer of micro (80/120 mesh) glass beads. A plug of glass wool was placed at each end of the glass bead layer. Injections were made directly on the glass beads in order to assure instantaneous transesterification. The injection port and column were maintained at approximately 200° C, whereas the detector was kept at about 245° C. Flow rates (ml/min) were: hydrogen, 36; helium, 81; and air, 205.

DISCUSSION AND RESULTS

Previous work has shown that the metabolite pathway of carbofuran in plants involves numerous carbamate and

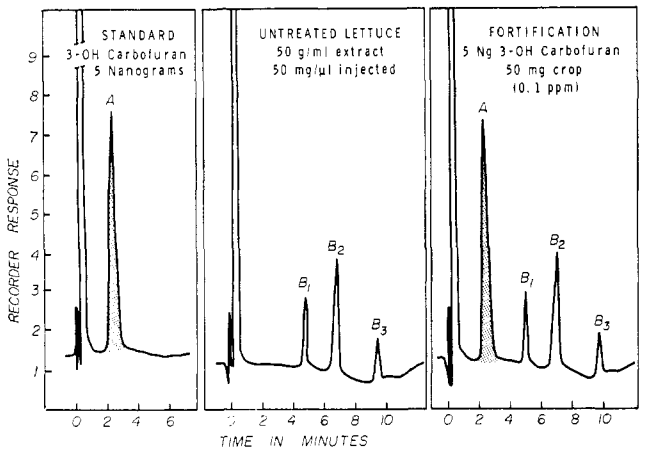

Figure 3. Typical gas chromatographs of 3-hydroxycarbofuran with and without lettuce extract background. A. 3-Hydroxycarbofuran. B. Lettuce extract background

phenolic metabolite residues. However, for toxicological purposes, only the cholinesterase-inhibiting carbamate residues need to be considered. Therefore, an acceptable analytical method for carbofuran residues must be capable of measuring the total carbamate residues remaining on and in a crop following various methods of application and periods of weathering. Accordingly, FDA has established several tolerances for carbofuran on grain forage and hay, based on the combined residues of the parent compound and its primary 3-hydroxy metabolite.

The extraction procedure used removed not only the parent compound and its 3-hydroxy metabolite, but also converts all water-soluble conjugates to organoextractable aglycones. This conversion to aglycones is vital to any valid analytical method, since a considerable percentage of carbofuran metabolites in weathered plants appear to be in the water-soluble glycosidic form.

A primary advantage of the gas phase transesterification is the consistently high conversion of carbofuran or its primary carbamate metabolite to methyl *N*-methylcarbamate in the presence of considerable crop extractives. The reaction is simple and instantaneous on the gc column. It is also much easier to gas chromatograph methyl *N*-methylcarbamate intact, as compared to a larger and more complex molecule such as carbofuran or its 3-hydroxy metabolite.

Figure 3 illustrates typical gas chromatograms of 3-hydroxycarbofuran as a pure standard and in the presence of the normal lettuce extract background following the described cleanup. The chromatograms of the lettuce extract with and without the fortification of 5 ng of 3-hydroxycarbofuran demonstrate that the crop background does not appear to interfere with the quantitative determination of the pesticide.

The total carbofuran residues determined by alkali-flame gas chromatography following methylation of the extracts are shown in Table II. Systemic pesticides applied to soils are influenced by many factors which control their uptake and persistence in plants. Such factors as the plant species (type of root system, maturity, etc.), type of pesticide applied,

Table II. Combined Residues of Carbofuran + 3-Hydroxycarbofuran in Lettuce Resulting from Soil and Foliar Applications (Average of Three Field Replications and Expressed in ppm)

Soil[a] Lb Active/Acre		Foliar[b] Days Since Last Application		
2	4	0	7	14
0.07	0.19	0.46	0.15	0.14

[a] One application (10% granules) at planting time. [b] Seven applications (75% W.P.) at weekly intervals.

soil type, as well as weather conditions, all have a profound effect. Probably a major factor in the very limited uptake of carbofuran or its metabolites by the lettuce plants may be the soil type involved in this experiment. Muck soils, because of their extremely high organic matter content, present large suface areas and high exchange capacities. It has been shown by previous investigators that the organic matter content can play an important role in the reduced absorption of systemic pesticides by plants. In addition, microbial processes are usually rapid in high organic matter soils, resulting in significant degradation of the bound pesticides. Excessive rainfall during the latter part of the growing season can probably help to explain the low concentrations of carbamate residues detected on the foliar-treated lettuce. For example, over 2 in. of rainfall occurred between the second and third foliar application, and over 5 in. was measured during the 2 days immediately preceding the final foliar application.

At present there are no official tolerances established for carbofuran on any vegetables. Several official tolerances ranging from 0.2 to 0.5 ppm have been established on the grain, hay, or forage of corn and rice. An evaluation of the observed total carbofuran residues found in this study indicated that a 0.5 ppm tolerance on lettuce would be adequate to cover any translocated carbofuran previously applied to the soil at rates as high as 4 lb active per acre.

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