

A High-Pressure Liquid Chromatographic Method for the Determination of Carbofuran Residues in Soil and Water

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A method for residue analysis of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) capable of quantitation of residues at the 0.10 ppm level in soil and water is presented. The method involves a methanol/water extraction, base hydrolysis of carbofuran to its phenolic moiety, and quantitation of the phenol which was accomplished by high-pressure liquid chromatography using a UV absorbance detector at 280 nm. Method recovery averaged $82.4 \pm 8.3\%$ for soil and $82.2 \pm 9.6\%$ for water.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a broad spectrum insecticide-nematicide (Thomson, 1977). A variety of analytical methods employing gas chromatography (GC) have been developed for the determination of carbofuran residues. A method employing nitrogen specific detection of the intact carbamate has been reported (Cook, 1973). Several residue procedures involving electron-capture (EC) quantitation have also been reported. One method for plant tissue involves derivatization of carbofuran through its phenol, followed by EC measurement (Butler and McDonough, 1971). Residues in animal tissue have been determined by derivatization of the carbofuran carbamate moiety and EC quantitation (Wong and Fisher, 1975). A residue method for soil and water by derivatization and EC detection has also been reported (Coburn et al., 1976).

Lawrence has recently reported the analysis of carbofuran residue in several food crops by high-pressure liquid chromatography (LC) (Lawrence and Leduc, 1977; Lawrence, 1977). These methods involved direct analysis of carbofuran by UV absorption detection. A LC method for the analysis of carbofuran in food crops employing a fluorescence detector has also been reported (Lawrence and Leduc, 1978).

Since direct soil application of carbofuran (formulated as granules) is a major mode of application, we desired an alternate method that would be rapid and reliable for the routine quantitation of carbofuran residue levels in soil. All the GC and LC methods reported to date involved open column cleanup of extracts and/or the addition of a detectable species to the parent molecule. Several of the methods also employed specialized detection systems. We wish to report a method which includes neither an open column cleanup nor a derivatization. The method presented here employs a high-pressure liquid chromatograph using an ultraviolet (UV) absorbance detection system operating at a standard wavelength (280 nm).

EXPERIMENTAL SECTION

Apparatus. A centrifuge and standard laboratory glassware were necessary. A high-pressure liquid chromatograph (Waters Associates, ALC 202) capable of monitoring a UV absorbance at 280 nm was used for quantitation.

Reagents. Methanol, chloroform, and acetonitrile were Burdick and Jackson distilled in glass. Methylene chloride was laboratory distilled in an all-glass apparatus prior to use. Diethylene glycol and hydrochloric acid were obtained

from Fisher Scientific and used as received. Anhydrous sodium sulfate and sodium hydroxide were used as received from MCB.

Sample Preparation. Bulk soil samples (1–2 kg) were air-dried to constant weight (2–6% water) and ground. The ground soil was sieved through a 2-mm screen.

Extraction. One-hundred-gram subsamples of dried, ground, and sieved soil were blended with 225 mL of methanol-water (2:1, v/v) for 5 min. The blender contents were transferred to a centrifuge bottle and centrifuged at 6000 rpm for 0.5 h.

The liquid was decanted with care. The volume recovered (approximately 170 mL) was recorded for later conversion to grams of sample recovered. The methanol water solutions were filtered through a 1- μ m millipore filter (Nuclepore, Inc.) and extracted twice with 150 mL of methylene chloride. One-hundred-gram water samples, adjusted to pH 3 by the addition of hydrochloric acid, were extracted twice with 100 mL of methylene chloride.

Conversion of Carbofuran to 2,3-Dihydro-2,2-dimethyl-7-hydroxybenzofuran (7-Phenol). The methylene chloride extract from the soil or water sample was washed twice with 150 mL of cold 0.5 N sodium hydroxide. The aqueous phase was discarded. Two drops of diethylene glycol were added as a keeper, and the methylene chloride was reduced in volume to less than 10 mL on a rotary evaporator. The remaining solvent was removed under a gentle stream of nitrogen. Twenty-five milliliters of 0.5 N sodium hydroxide was added to the flask and the flask swirled. The contents of the flask were stirred for 0.5 h at room temperature. The basic hydrolysis mixture was extracted with 50 mL of chloroform and the chloroform discarded. The pH of the hydrolysis solution was adjusted to less than 3 by the addition of concentrated hydrochloric acid. This solution was extracted twice with 25 mL of chloroform, and the combined extracts were dried by filtration through anhydrous sodium sulfate. The filter pad was rinsed with an additional 25 mL of chloroform, and the combined extracts were reduced to less than 10 mL on a rotary evaporator. This extract was transferred to a graduated centrifuge tube, and two drops of diethylene glycol was added as a keeper. The remaining chloroform was removed under a gentle stream of nitrogen at room temperature. The residue was made up to volume (typically 1.5 mL) with acetonitrile.

Quantitation. Fifteen microliters of the final acetonitrile was injected into the liquid chromatograph operating under the following conditions: column, 30 cm C-18 μ Bondapak; temperature, ambient; mobile phase, 42% acetonitrile/58% water (v/v); flow rate, 2 mL/min; wavelength, 280 nm; sensitivity, 0.02 aufs; recorder, 2 mV. With the instrument equilibrated under those conditions 7-phenol had a retention volume of 10.8 mL. The linearity

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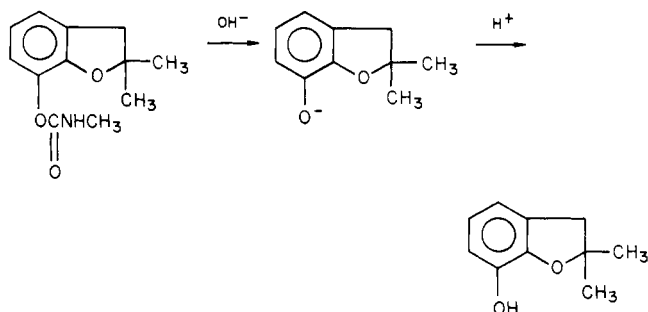


Figure 1. Conversion of carbofuran to 7-phenol.

of the detection system was established for concentrations of 7-phenol ranging from 10 to 30 ng/ μ L. Residues of carbofuran were calculated using the formula:

$$\text{carbofuran (ppm)} = (HCM)/G$$

where H = sample peak height (cm), C = calibration factor from standard ($\mu\text{g cm}^{-1}$), M = molecular weight ratio (carbofuran/7-phenol) = 1.35, G = sample size injected (g).

RESULTS AND DISCUSSION

The method's cleanup and applicability depend on the equation in Figure 1. Control experiments demonstrated that the conversion of carbofuran to 7-phenol was quantitative under the conditions described in this method. It was also possible to prove that the anion of 7-phenol was not extractable from the basic hydrolysis mixture. We demonstrated that base wash of the initial methylene chloride extract did not hydrolyze or extract carbofuran, while it did extract up to 30 ppm 7-phenol. The initial base wash could serve to remove any phenols which might be present in the extract. Therefore, the initial base wash removed acidic coextractives, while the chloroform wash of the hydrolysis mixture removed the neutral and basic coextractives. The advantage of this acid/base clean-up procedure was demonstrated by comparison of chromatograms obtained from this method with chromatograms of soil extracts which were not treated in this manner. The acid base clean-up system removed most polar materials. The relatively nonpolar carbofuran was then converted to the relatively polar phenol. This conversion allowed the use of a reversed-phase column for analysis. Since carbofuran is detectable by UV and is chromatographable (Lawrence, 1977), it is, in theory, possible to simply extract carbofuran from soil and quantitate it without conversion to 7-phenol. The result of all such attempts gave chromatograms which were totally unacceptable for quantitation due to a large number of UV absorbing coextractives.

Individual partitioning efficiencies were shown to be nearly quantitative in all cases by employing radiolabeled standards, as well as nonlabeled materials.

Two sandy loam soils from New York and loam soils from Kansas and Nebraska were subjected to the method. Forty fortified soil samples were analyzed for recovery at levels from 0.1 to 30 ppm. The soil samples were fortified by addition of carbofuran ($1 \mu\text{g}/\mu\text{L}$ in methanol) directly on the soil. The solvent was allowed to evaporate 10 min prior to the addition of the extraction solvent. Results of all fortification experiments are shown in Table I. Recoveries ranged from 69.0 to 102.0% and averaged $82.4 \pm 8.3\%$.

There was no significant difference in recovery due to soil type or spiking level. The recovery data generated compared well with data generated by the method of Cook (1973). Seven fortified water samples were analyzed by the method. Two samples were distilled water and the

Table I. Recovery of Carbofuran from Soil (Loam)

soil type	carbofuran added		% recov.
	μg	ppm	
loam	10	0.1	75.3
loam	10	0.1	76.2
loam	10	0.1	80.8
loam	10	0.1	84.3
loam	10	0.1	85.5
loam	10	0.1	97.2
loam	20	0.2	79.1
loam	20	0.2	82.4
loam	20	0.2	83.4
loam	50	0.5	81.0
loam	50	0.5	85.7
loam	100	1.0	69.0
loam	100	1.0	74.9
loam	100	1.0	76.2
loam	100	1.0	81.8
loam	1000	10.0	69.7
loam	1000	10.0	90.4
loam	2000	20.0	86.7
loam	3000	30.0	69.3
loam	3000	30.0	98.0
silt loam	10	0.1	75.0
silt loam	10	0.1	79.1
silt loam	10	0.1	82.1
silt loam	10	0.1	102.0
silt loam	20	0.2	96.4
silt loam	50	0.5	75.2
silt loam	50	0.5	78.4
silt loam	50	0.5	80.5
silt loam	50	0.5	80.5
silt loam	50	0.5	82.6
silt loam	50	0.5	82.6
silt loam	50	0.5	92.7
silt loam	100	1.0	69.0
silt loam	100	1.0	76.1
silt loam	100	1.0	79.0
silt loam	100	1.0	84.2
silt loam	100	1.0	84.7
silt loam	2000	20.0	80.1
silt loam	2000	20.0	92.7
silt loam	2000	20.0	96.4
av recov loam soil		$81.3 \pm 8.1\%$ (20 values)	
av recov silt loam soil		$83.5 \pm 8.4\%$ (20 values)	
av recov all soils		$82.4 \pm 8.3\%$ (40 values)	

Table II. Recovery of Carbofuran from Water

water type	carbofuran added		% recov.
	μg	ppm	
leachate	10	0.1	76.4
leachate	10	0.1	90.2
leachate	10	0.1	90.5
leachate	20	0.2	72.5
leachate	20	0.2	97.2
leachate	50	0.5	70.3
distilled	50	0.5	83.2
distilled	100	1.0	77.6
av recov		82.2 ± 9.6 (8 values)	

remainder distilled water, which had been allowed to leach through the above mentioned soils. These samples were spiked at levels ranging from 0.1 to 1.0 ppm. Recovery data are reported in Table II. Recoveries ranged from 70.3 to 97.2% and averaged $82.2 \pm 9.6\%$.

The limit of detection (quantitation of a signal two times the noise level) was determined to be 0.05 ppm. No troublesome interferences were encountered in any samples. Figure 2 depicts chromatograms obtained from the method.

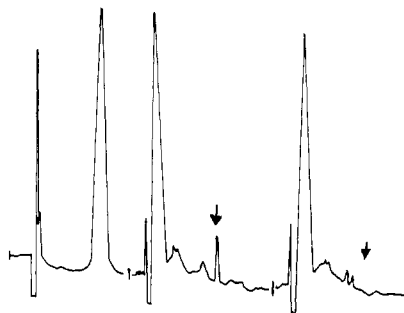


Figure 2. Typical chromatograms for the analysis of carbofuran as 7-phenol in soil: (A) 300 ng of 7-phenol; (B) check soil fortified with 0.1 ppm carbofuran, 782 mg injected (equivalent to 0.081 ppm carbofuran); (C) check soil, 653 mg injected.

One cautionary statement should be noted. The final acetonitrile extracts of soil samples are apparently unstable at room temperature. A sample quantitated directly after completion of workup and requantitated 1 week after storage at -10°C in the dark gave comparable values. However, if the extract was allowed to stand at room temperature in a lighted environment for longer than 24 h, diminished recovery was noted. Therefore, the final acetonitrile extract should be quantitated immediately after workup or be stored at -10°C in the dark if im-

mediate analysis is impossible.

CONCLUSION

The method as presented is a relatively simple and efficient method for determining carbofuran in soil and water. Investigations toward extending the scope of this method are currently in progress.

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Residues of Mancozeb, 2-Imidazoline, and Ethyleneurea in Tomato and Potato Crops after Field Treatment with Mancozeb

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Mancozeb, 2-imidazoline, and ethyleneurea residues were determined in tomato and potato crops at various intervals for 28 days after field treatment with mancozeb. On tomato fruit mancozeb persisted for 28 days while no residues were found in potato tubers. Insignificant residues of 2-imidazoline were found in either crop, and low levels (<0.04 ppm) of ethyleneurea were present in tomatoes up to 28 days after spraying. Ethyleneurea was not detectable in potato tubers.

Several compounds have been identified as a result of studies to determine the fate of ethylenebis(dithiocarbamate) (EBDC) fungicides on plants. Lyman (1971) reported that ethyleneurea, ethylenediamine, and 2-imidazoline were major decomposition products occurring on leafy plants 2 weeks after the application of [^3H]-mancozeb outdoors. Lesser amounts of ethylenebis(isothiocyanate) sulfide (EBIS) and ethylenethiourea (ETU) were also found. Relatively large amounts of ethyleneurea, accompanied by several unknown metabolites, have been found in soybeans grown on soil treated with [^{14}C]maneb (Nash, 1976). In greenhouse experiments with [^{14}C]zineb on lettuce, Vonk (1976) found that while zineb and its degradation products ETU and EBIS decreased with time, ethyleneurea and 2-imidazoline were formed gradually and

persisted for at least 3 weeks.

The dynamics of various EBDC's and some of their decomposition products have been examined in a field study with tomatoes (Newsome, 1976), where it was observed that ETU, EBIS, and ethylenebis(isothiocyanate) declined with time after spraying and that none of the residues constituted more than 0.5% of the parent EBDC. Since it remained to be determined what levels of the desulfurated residues ethyleneurea and 2-imidazoline could occur on food crops treated in the field with EBDC's, the present experiment was initiated. Tomatoes and potatoes were selected as examples of crops which receive widespread application of EBDC's in Canada.

EXPERIMENTAL SECTION

Field Experiment. Studies were conducted during the summer of 1978 at the Ottawa Research Station, Canada Department of Agriculture. Three plots of tomatoes (Ottawa 78 variety) were grown, one of which served as a control, while the others were treated at rates of 1.5 and 3.0 lb of mancozeb formulation (80% active ingredi-

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