Table II. Products Formed from DDT by Isolates from Amended Sewage

Products formed	Number of isolates		
	Sewage	Sewage + glucose	Sewage + diphenylmethane
DDD, DDE, DBP	29[a]	17	31
DDD, DDE	31	35	34
DDD, DBP	2	11	3
DBP	1	0	1
DDD	2	8	0
None	75	69	71

[a] Number represents total number of isolates forming these products from DDT.

of the existence of microorganisms which, in culture at least, can effect its extensive biodegradation, it has been postulated that the insecticide is metabolized by few microorganisms, none of which is capable of utilizing the compound as a carbon or energy source (Pfaender and Alexander, 1972). On the basis of the present data, it seems more likely that DDT persists owing to the fact that cells which can cometabolize the chemical, although sometimes numerous, do not express a high activity. The activity of some of the organisms may not be expressed at all. Furthermore, inasmuch as a species particularly active in cometabolism has no necessary selective advantage in nature, its population density would not increase in response to DDT application.

ACKNOWLEDGMENT

The authors express their thanks to Janice Zelaska and Theresa Ho for able technical assistance.

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Received for review October 20, 1972. Accepted January 8, 1973. This investigation was supported by Public Health Service Training Grant ES-00098 from the Division of Environmental Health Sciences.

Determination of Carbofuran and 3-Hydroxycarbofuran Residues in Small Fruits

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A method is described for determining residues of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and its 3-hydroxy metabolite in strawberries, raspberries, blueberries, and cranberries. The extraction method of Cook *et al.* (1969) was modified to include a dichloromethane extraction of the filtered solids. Cleanup was on a silica gel–alumina column with mixtures of dichloromethane–ether and acetone–methanol as eluents. Determination was by gas chromatography (gc) with nitrogen detection using a Coulson conductivity detector. Recoveries of both carbofuran and 3-hydroxycarbofuran from fortified samples averaged greater than 86% over the range 0.2 to 5.0 ppm.

Recent field experiments have demonstrated the efficacy of spray applications of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) for controlling root weevils and a moth larva in small fruits. These include: the strawberry root weevil, *Brachyrhinus ovatus* (L.), and the bush weevil, *Nemocestes incauptus* (Horn), in strawberries; the bud weevil, *B. singularis* (L.) in raspberries; the black vine weevil, *B. sulcatus* (Fab.), in blueberries; and the black-headed fireworm, *Rhopobota naevana* (Hub.), in cranberries. For further evaluation of this insecticide for use on these crops, analysis of the fruit for residues of both the parent compound and its toxic 3-hydroxy metabolite (2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate) was required. This paper describes the method developed and recoveries obtained from fortified samples of strawberries, raspberries, blueberries, and cranberries.

Several methods for the determination of carbofuran residues have been published and various crops have been analyzed (Bowman and Beroza, 1967; Butler and McDonough, 1971; Cassil *et al.*, 1969; Cook *et al.*, 1969; Van Middelem *et al.*, 1971). Apparently there has been no previous study of carbofuran in small fruits. Most of the published methods follow similar techniques for extraction and cleanup based on the original method of Cook *et al.* (1969) for corn. Acid hydrolysis is used to release any conjugated 3-hydroxycarbofuran to the aglycone form. This is followed by extraction of the aqueous phase with dichloromethane and column cleanup to remove possible interferences. Final determination is by gc, either directly with nitrogen detection or after conversion to compounds sensitive to electron capture or flame photometric detection. The method to be described employs direct determination with conductivity detection of nitrogen.

For small fruits some modifications were required in the basic method of Cook *et al.* (1969) and some points of interest arose which should have a bearing on methodology for other crops.

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EXPERIMENTAL SECTION

Apparatus. A boiling flask and condenser, as described by Cook *et al.* (1969), were used. The filtration apparatus

was a 150-ml glass Büchner funnel with a coarse fritted disk, modified by the addition of a 29/42 ⅀ joint and a sidearm. This was attached to a 250-ml graduated cylinder with a 27 ⅀ opening. A 300 × 20 mm i.d. liquid chromatographic column with Teflon stopcock was used. The gas chromatograph was a Micro-Tek Model MT-220 equipped with a heated transfer tube of Teflon-lined ¼-in. o.d. aluminum (Analabs Inc., North Haven, Conn.) and attached to a Dohrmann Model S-200 pyrolysis furnace fitted with a Cassil-design quartz pyrolysis tube (Cassil *et al.,* 1969). The outlet of the pyrolysis tube was attached to a Coulson conductivity detector by a short length of 2 mm i.d. glass capillary tubing fitted with 12/5 semi-ball joints and lined with 2-mm o.d. Teflon tubing. Gc conditions were as follows. The column was a 6-ft × ¼-in. o.d. glass U tube packed with a mixture of 6% OV210 and 4% OV101 on 60–80 mesh Gas Chrom Q. The packing was prepared by dissolving both stationary phases in sufficient chloroform to saturate the required amount of support, and then evaporating the solvent on a rotary vacuum evaporator. Temperatures were 160° for the column, 225° for the inlet, and 225° for the transfer tube. Flow of hydrogen used as a carrier gas was 125 ml/min. Pyrolysis conditions were as described by Cassil *et al.* (1969).

Reagents. Analytical grade carbofuran and 3-hydroxycarbofuran were supplied by Niagara Chemical Division, FMC Corp., Middleport, N. Y. Solvents were reagent grade, redistilled. Silica gel, Davidson, Grade 923 (Davidson Chemical, Baltimore, Md.), was used as received. Loss on heating overnight at 110° was 1.3%. Florisil was used as received. Norit A neutral activated charcoal (Fisher Scientific) was acid treated by the procedure of Storherr *et al.* (1971) before use. Woelm W 400 acid alumina (Waters Associates), partially deactivated by the addition of 4% water, was used. Keeper solution was a 1% solution of OV 101 in ethyl acetate. A 4% aqueous solution of sodium lauryl sulfate was used to break emulsions. Two eluting solutions were used: solution A, a 1:4 (v/v) mixture of ethyl ether and dichloromethane; and solution B, a 12:88 (v/v) mixture of methanol and acetone. Both were dried for 1 hr with 13X molecular sieve (10% w/v) before use.

Table I. Recovery of Carbofuran and 3-Hydroxycarbofuran from Strawberries, Raspberries, Blueberries and Cranberries, Fortified with Both Compounds before Extraction and Cleanup

	Fortification, ppm		Recovery, %	
Crop	Carbofuran	3-Hydroxy-carbofuran	Carbofuran	3-Hydroxy-carbofuran
Strawberries	0.2	0.2	101.0	101.0
			98.1	105.8
	1.0	1.0	102.3	81.0
	5.0	5.0	82.4	82.5
Raspberries	0.2	0.2	86.2	95.0
			85.9	103.2
	1.0	1.0	83.3	91.5
			79.7	92.0
	5.0	5.0	73.1	71.1
			71.8	81.7
Blueberries	0.2	0.2	98.9	105.7
			85.0	77.4
	1.0	1.0	80.4	75.8
			92.1	84.0
	5.0	5.0	77.9	76.3
			77.7	72.4
Cranberries	0.2	0.2	98.0	73.8
			84.8	71.6
	1.0	1.0	91.3	80.5
			80.0	77.8
	5.0	5.0	85.2	72.0
			83.8	81.2

Procedure. *Extraction.* Transfer a representative 50-g sample of berries to the boiling flask, add 180 ml of distilled water, 10 ml of 6 *N* hydrochloric acid, and 10 ml of a saturated aqueous solution of mercuric chloride. Reflux the mixture for 1 hr with occasional swirling. Allow the extract to cool, then, with the aid of suction, filter through a glass fiber filter in the Büchner funnel, receiving the filtrate in the 250-ml graduate. Wash the residual solids on the filter with small portions of distilled water, then continue suction until as much as possible of the water has been removed. Make up the filtrate to exactly 250 ml. Extract the residual solids on the filter with two 50-ml portions of dichloromethane, using suction and collecting the filtrate in a graduate. Record the volume obtained.

Cool the aqueous extract to room temperature, mix thoroughly, then transfer a 100-ml aliquot (representing 20 g of fruit) to a 500-ml separatory funnel, add 6 drops of sodium lauryl sulfate solution, and extract with three 50-ml portions of dichloromethane. Collect the combined extracts in a 250-ml glass-stoppered Erlenmeyer flask and add ⅖ of the dichloromethane extract (representing 20 g of fruit) derived from the residual solids. Add an excess of anhydrous sodium sulfate and 1 g of acid-washed charcoal. Thoroughly shake the mixture and allow it to settle for 30 min.

Cleanup. To the chromatographic column add, in order from the bottom, a small plug of glass wool, a 0.5-cm layer of anhydrous sodium sulfate, 15 g of alumina, 0.5 cm of anhydrous sodium sulfate, 10 g of silica gel, and 1.0 cm of anhydrous sodium sulfate. Prewash the column with 50 ml of dichloromethane dried with 13X molecular sieve. Transfer the dichloromethane extract to the column, decanting it from the charcoal to avoid slowing the column flow. Collect the eluate in a 600-ml beaker to which has been added 1 ml of keeper solution. As the last of the solution enters the column, add 100 ml of eluting solution A, and when this has eluted, add 100 ml of eluting solution B.

Concentration. Evaporate the combined eluates on a 70° water bath in a moderate current of air, taking care that the beaker does not become warm in the final stages of evaporation. When the organic solvents have evaporated and only condensed water remains (generally about 1–2 ml), transfer the water to a 50-ml beaker, measure its volume approximately, then add sufficient sodium chloride to make a 10% solution. Wash down the large beaker with 3 ml of ethyl acetate using a Pasteur pipette. Transfer the washings to the smaller beaker, mix thoroughly with the aqueous phase, and then decant the ethyl acetate layer into a 10-ml Kuderna-Danish concentrating tube. Repeat this operation twice more to ensure that no residue remains in the large beaker and that extraction from the aqueous phase is complete. Evaporate the combined extracts to exactly 1 ml on a 40° block heater in a gentle current of air. The sample is now ready for gc analysis.

Additional Cleanup. In samples showing a considerable amount of waxy residue in the final concentrate, additional cleanup may be desirable. This is readily accomplished by transferring the concentrate to a micro-cleanup column, consisting of a 3-cm layer of Florisil in a Pasteur pipette, and then washing it through with three 1-ml portions of ethyl acetate. After reconcentrating to 1 ml, the solution is ready for gc analysis.

RESULTS AND DISCUSSION

Recovery of carbofuran and 3-hydroxycarbofuran from berries fortified at various levels is given in Table I. In fortifying these berries, an appropriate volume of benzene solution containing 500 μg/ml of carbofuran and 3-hydrox-

ycarbofuran was added to the boiling flask containing the berries prior to refluxing.

Recoveries of both compounds, while somewhat lower than desirable, were reasonably consistent. Recovery from strawberries averaged 94.3% total carbofuran and 3-hydroxycarbofuran, from raspberries 84.5%, from blueberries 83.6%, and from cranberries 81.7%. The lower recoveries appear to be associated with berries having greater amounts of wax on their surface.

Use of the additional Florisil cleanup step in the procedure caused no additional loss of either carbofuran or 3-hydroxycarbofuran.

The cleanup used in the method described proved satisfactory, with the only interference in the gc step being an increase in the size of the initial peak as compared with that of the standard. No peaks with retention times corresponding to either carbofuran or 3-hydroxycarbofuran were observed in check samples of any of the berries. Typical chromatograms are shown in Figure 1.

A gradual buildup of nonvolatile impurities does occur at the inlet end of the gc column but, by having a small plug of silanized glass wool on top of the packing and replacing it whenever recorder response to standard samples decreases, this potential source of trouble is readily eliminated.

Low recovery was one of the main problems encountered in developing methodology for berries. The waxy layer on some was found to hold up significant amounts of both carbofuran and its 3-hydroxy derivative. This necessitated the extra extraction of the filtered solids described in the procedure.

Another problem encountered was the condensation of a small amount of water during the concentration step. The evaporation of a highly volatile solvent such as dichloromethane, if carried out with reasonable rapidity, causes sufficient cooling for condensation of some atmospheric moisture to occur. Evaporating this to dryness results in loss of both carbofuran and its 3-hydroxy derivative. Prolonged heating may also cause some carbofuran to be converted to 3-hydroxycarbofuran. Since both compounds are appreciably soluble in water, the choice of an organic solvent for extracting them from the aqueous phase becomes important. Originally benzene was employed, but by substituting ethyl acetate, in which both carbofuran and 3-hydroxycarbofuran are considerably more soluble, and by adding sodium chloride to the aqueous phase, quantitative extraction was achieved.

In atmospheres of relatively low humidity, where condensation of moisture is not a problem, direct dilution and transferral of the concentrated extract with ethyl acetate or benzene may be used.

Other methods of concentration, such as Kuderna-Danish or vacuum evaporation, may be preferred in some laboratories. Having tried both of these we still tend to favor the method described.

Butler and McDonough (1971) advocate the use of mineral oil as a keeper to minimize loss during the concentration step. While this has a beneficial effect, the use of the silicone oil OV101 appears preferable and the addition of small amounts of this compound to the column is in no way detrimental since it replaces any lost by bleed.

The use of mercuric chloride in the aqueous extract requires explaining. Initially it was added to eliminate mercaptans when crucifer crops were being analyzed for carbofuran. It proved so effective in reducing emulsion formation during the subsequent dichloromethane extraction that it was retained for all crops.

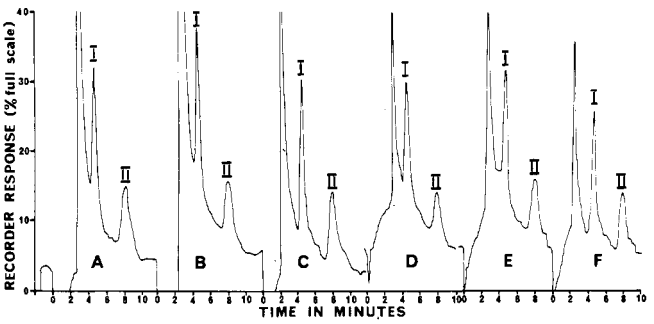

Figure 1. Typical gas chromatograms of berries fortified with 0.2 ppm of carbofuran (I) and 3-hydroxycarbofuran (II). A, strawberries; B, cranberries; C, corresponding standard representing 20 ng each of I and II; D, raspberries; E, blueberries; F, corresponding standard representing 20 ng each of I and II.

The use of molecular sieve as a drying agent for the eluents used in the cleanup step may not always be necessary, but it has been our experience that its use generally leads to more consistent results. While a considerable amount is used, it is readily regenerated by heating to 300° overnight and then cooling in an atmosphere of dry nitrogen.

In the method of Butler and McDonough (1971), carbofuran and 3-hydroxycarbofuran were removed from the cleanup column in two separate fractions. This is not possible in the method described here, since some carbofuran elutes with 3-hydroxycarbofuran in the first 50 ml of eluting solution B. However, by using an extra 50 ml of eluting solution A and changing the collecting beaker after this has reached the top of the column, the two compounds may be collected separately if this is desirable.

The Teflon-lined aluminum transfer tube between the gc and the pyrolysis furnace may require conditioning before installation due to the apparent presence of a nitrogen-containing compound associated with the Teflon coating. Conditioning is best accomplished by heating the tube to 200° under vacuum for a period of at least 24 hr.

Recent work has demonstrated that the method described may be used equally well for the analysis of potatoes, carrots, and cauliflower.

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Received for review November 30, 1972. Accepted January 30, 1973.