Metabolism of Carbofuran in Tobacco

Ronald J. Ashworth[1] and Thomas J. Sheets*

Carbofuran, which had been absorbed into tobacco plants through the roots or through the petioles of isolated leaves, was metabolized, with a half-life of about 4 days, by progressive oxidation to 3-hydroxycarbofuran and 3-ketocarbofuran. All three carbamates were hydrolyzed to their corresponding phenols; the phenols and 3-hydroxycarbofuran were then conjugated as glycosides. 3-Hydroxycarbofuran and its glycoside were the major metabolites. Carbofuran applied topically was more persistent because it did not readily penetrate the cuticle of the leaves; the major portion of the applied carbofuran was, therefore, not subject to plant metabolism.

Carbofuran [2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate], is effective against a broad spectrum of insect pests, including some of those which are of particular concern in tobacco (*Nicotiana tobacum* L.) cultivation (Mistric and Smith, 1969, 1970). The metabolism of carbofuran has been studied in beans (*Phaseolus vulgarus* L.), cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.), and animals by Metcalf *et al.* (1968), Dorough (1968a,b), and Stanovich and Burt (1967). Unconjugated metabolites commonly found in plant material by the above investigations are listed in Table I.

The research reported in this paper was carried out to investigate the metabolism and determine the half-life of carbofuran, and was part of a larger study on uptake, translocation, and metabolism of carbofuran by tobacco plants deemed to be of particular relevance in view of the position of carbofuran as a candidate replacement for DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane]. Such information is a prerequisite to the establishment of recommendations for safe and effective use of insecticides and for development of meaningful analytical methods for determination of possible toxic residues.

MATERIALS AND METHODS

The metabolism of carbofuran was followed by single time-sequence studies in young tobacco plants (variety "Hicks"). One-month-old plants growing in solution culture (Crafts and Yamaguchi, 1964) were exposed to ^{14}C-labeled carbofuran by dissolving the tracer in nutrient solutions (Hoagland and Arnon, 1950) in which the roots were suspended and by applying a tracer solution to one of the leaves. In an attempt to introduce the tracer into the plant as rapidly as possible so as to reduce contributory metabolism by microbial contaminants to a minimum, leaves isolated from larger 2-month-old plants were induced to absorb a solution of the tracer through the cut ends of their petioles.

Chemicals. Ring-labeled (position 7a) ^{14}C-carbofuran, specific activity 0.146 mCi/mmol, was supplied by the Niagara Chemical Division, FMC Corp. A stock solution of this tracer in dry benzene, stored under refrigeration for a period of 9 months, showed no chemical modification, as determined by autoradiography of thin-layer chromatograms developed in three different solvent systems. Nonradioactive samples of carbofuran, carbofuran phenol, 3-hydroxycarbofuran, and 3-ketocarbofuran were also supplied by the Niagara Chemical Division. 3-Hydroxycarbofuran phenol and 3-ketocarbofuran phenol were prepared by alkaline hydrolysis of the corresponding carbamates.

Culture of Plants. Plants were grown from seed sown in sand and transferred to solution culture at the fifth-leaf stage, as described for uptake and translocation studies by Ashworth and Sheets (1970).

Root-Uptake Studies. Two experiments were carried out; one without and one with antibiotics in the nutrient solutions containing the ^{14}C tracer. Uniform plants, at the seventh-leaf stage, were selected for treatment after they had been grown in solution culture for 6 to 7 days. The roots were rinsed with distilled water and dipped into 40 ml of distilled water, and the plants were placed in culture jars containing 0.76 mg (5.1 ppm) of the labeled carbofuran dissolved in 150 ml of freshly-boiled nutrient solution. The 40 ml of distilled water, in which the roots had been dipped, were equally divided between two additional culture jars containing similar solutions of the tracer. These jars were set up without plants and served, in some measure, as controls for the chemical and microbial breakdown of the tracer.

The difference between the two root-uptake studies was that the nutrient solutions in the second experiment were fortified by 40 ppm each of penicillin G and streptomycin sulfate. In this experiment the distilled water, used daily to maintain the level of solution in the culture jars, contained half this concentration of antibiotics. Precautions taken to minimize losses of the tracer, to reduce initial microbial contamination, and to prevent algal development in the nutrient solutions were previously described by Ashworth and Sheets (1970).

Plants were removed at intervals of 1, 2, 4, 8, and 12 days; controls were terminated at 6 and 12 days. The roots were rinsed back into the culture jars, excess water was blotted off and the roots were severed from the plant tops. Plant parts were stored at $-18°C$ for 24 hr prior to extraction. Paper tissues used to blot the roots were retained with the culture-jar cork assemblies for subsequent extraction with the nutrient solutions.

Foliar-Uptake Studies. Solutions, consisting of 0.76 mg of ^{14}C-carbofuran in equal volumes of water and ethanol containing 0.1% Triton X-100, were applied to lanolin rings formed near the base of the fifth leaf-blade of plants at the eighth-leaf stage growing in solution culture. Plants were removed at intervals of 2, 4, 8, and 12 days and prepared for extraction as previously described, except that each plant was divided into roots, treated leaf, and remainder of the plant tops.

Studies on Isolated Leaves. Leaves about 25 cm long, taken from the eighth and ninth-leaf positions of plants grown in solution culture, were induced to absorb 0.76 mg of ^{14}C-carbofuran dissolved in an aqueous acetone solution through

Pesticide Residue Research Laboratory, North Carolina State University, Raleigh, North Carolina 27607.

[1] Present address: Research and Development Group, W. D. and H. O. Wills, Bristol, England.

Table I. Chemical Nomenclature for Carbofuran and Unconjugated Metabolites in Plant Material

Common name	Chemical name
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Carbofuran phenol[a]	2,3-Dihydro-7-hydroxy-2,2-dimethylbenzofuran
3-Hydroxycarbofuran	2,3-Dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate
3-Hydroxycarbofuran phenol[a]	2,3-Dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran
3-Ketocarbofuran	2,3-Dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl methylcarbamate
3-Ketocarbofuran phenol[a]	2,3-Dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran

[a] It is considered improper to employ the common name carbofuran in naming derivatives in which the basic molecule is not essentially intact; however, since these hydrolytic products have been so designated in the literature, the use of these common names is continued here.

Table II. R_f Values of Carbofuran and Metabolites on Thin-Layer Chromatography

Compound	Solvent system[a] I	II	III	IV	V
Carbofuran phenol[b]	0.84	0.82	0.81	>0.95	>0.95
3-Ketocarbofuran phenol[b]	0.74	0.65	0.61	>0.95	>0.95
Carbofuran[b]	0.69	0.53	0.58	>0.95	>0.95
3-Ketocarbofuran[b]	0.62	0.47	0.45	>0.95	>0.95
3-Hydroxycarbofuran phenol[b]	0.53	0.52	0.50	>0.95	>0.95
3-Hydroxycarbofuran[b]	0.27	0.20	0.22	>0.95	>0.95
Unknown W[c]	0.00	0.00	0.00	0.69	0.72
Unknown X[c]	0.00	0.00	0.00	0.43	0.58
Unknown Y[c]	0.00	0.00	0.00	0.28	0.33
Unknown Z[c]	0.00	0.00	0.00	0.12	0.15

[a] System I. Benzene:diethyl ether (1:1, v/v) used as supplied. System II. Hexane:diethyl ether (1:3, v/v) saturated with water. System III. Hexane:ethyl acetate (1:1, v/v) saturated with water. System IV. Ethyl acetate:*n*-propanol:water (5:3:2, v/v/v). System V. *n*-Butanol:ethanol:water (10:2:3, v/v/v). [b] Carbofuran and unconjugated metabolites in chloroform-soluble extracts. [c] Glycosidic conjugates in methanol-soluble extracts.

the cut ends of the petioles (Ashworth and Sheets, 1970). When absorption was complete, the leaves were transferred to fresh vials containing 15 ml of nutrient solution without the tracer. Leaves were removed at intervals of 1, 2, 4, 6, and 8 days from the initiation of the treatment and frozen for 24 hr prior to extraction. The nutrient solutions remaining in the vials were examined for ^{14}C compounds secreted by the leaves.

Extraction Procedures. Plant parts were cut into small pieces and extracted three times, in sequence, with 150 ml of acetone. Extraction was accomplished by heating at 55°C for 10 min, while the slurry was stirred and the larger pieces of tissue were crushed with a glass rod. Filtered extracts were combined, reduced to 20 ml on a rotary evaporator, and transferred to a 500-ml separatory funnel containing 25 ml of water. The aqueous acetone was extracted seven times with 50-ml portions of chloroform which were then dried by passage through a 10-cm column of anhydrous sodium sulfate; the volume of the combined eluate was reduced to 20 ml and made up to 100 ml with chloroform in a volumetric flask.

The acetone-extracted plant material was further extracted three times, as above, with 150-ml volumes of hot methanol. The filtered extracts were combined with the aqueous phase from the aqueous:chloroform partition; volumes were reduced and made up to 100 ml with methanol.

Nutrient solutions were extracted once with 100 ml and seven times with 50 ml of chloroform. The initial portions of chloroform were used to rinse the culture-jar assemblies and the tissues (used to dry the roots) before being used to extract the nutrient solutions. The combined chloroform extracts were dried, reduced in volume to 40 ml, and made to 100 ml with chloroform. The extracted aqueous phase was made to 250 ml with water.

Duplicate 5-ml aliquots of the chloroform and methanol-soluble fractions and 4-ml aliquants of the water-soluble fractions were taken for scintillation counting to determine total ^{14}C. Insoluble plant residues were dried, weighed, ground to 20 mesh, and 50-mg portions combusted in a Thomas–Ogg apparatus for scintillation counting.

Thin-Layer Chromatography (tlc). Aliquots from the chloroform-soluble, methanol-soluble, and water-soluble extracts were fortified with 20 μg of each of the six nonradioactive reference compounds, carefully evaporated to 0.05 ml, and spotted on thin-layer plates coated with 0.25 mm of silica gel G and air-dried for 24 hr. Chloroform-soluble extracts were chromatographed in solvent systems I, II, and III (Table II), while methanol-soluble and water-soluble extracts were chromatographed in systems I, IV, and V.

After development in the appropriate solvent system, plates were air-dried and examined under an ultraviolet lamp to locate the two 3-keto compounds which fluoresced strongly; their locations were marked. Plates were then sprayed with 5% potassium hydroxide to prevent volatilization of carbofuran phenol and to hydrolyze the carbamates to their corresponding phenols. When dry, the plates were covered with Saran Wrap and exposed to No-Screen X-ray film for 4 weeks. The plates were then sprayed with freshly prepared 0.1% *p*-nitrobenzene–diazoniumfluoroborate in methanol. The phenols, visualized as reddish spots, were compared to the darkened spots on the autoradiographs to identify those ^{14}C metabolites corresponding to reference compounds in all three solvent systems.

Estimations of quantities of each metabolite were obtained by sectioning the thin-layer plates and removing layer sections into vials for scintillation counting. In addition to scintillation counting of the ^{14}C at positions corresponding to the six reference compounds, other sections were also removed and examined from plates developed in systems I, II, and III. These were material remaining at the origin, material between the origin and 3-hydroxycarbofuran (zone A), material between 3-hydroxycarbofuran and 3-hydroxycarbofuran phenol (zone B), and material above carbofuran phenol (zone C).

Confirmation of Unconjugated Metabolites. Unconjugated, chloroform-soluble metabolites were subjected to alkaline hydrolysis, and the products were reexamined by tlc in solvent systems II and III. By this procedure, carbamates were hydrolyzed to the corresponding phenols while phenols present originally remained unchanged.

Hydrolysis of Conjugates. Methanol-soluble, water-soluble, and insoluble plant fractions were hydrolyzed for 2 hr in 0.5 *N* hydrochloric acid at 100°C in order to liberate the aglycones from their glycosidic conjugates. Aglycones were extracted with chloroform and examined by tlc.

Aliquots of the methanol-soluble extracts suspected of containing glycosidic conjugates were evaporated to dryness and incubated for 21 hr at room temperature with 2% β-glucosidase in 0.0667 *M* phosphate buffer. Chloroform extracts were examined by tlc for the presence of glycosidic aglycones.

Table III. Percent Recoveries of ^{14}C-Carbofuran and Metabolites for Nutrient Solutions from Root-Uptake Studies

Fraction	Compound	Exp. No.[a]	1 Day	2 Days	4 Days	8 Days	12 Days	6-Day control	12-Day control
Chloroform-soluble	Carbofuran phenol	1	0.4	0.4	0.0	0.0	0.0	1.3	5.4
		2	0.2	0.3	0.2	0.3	0.0	0.7	2.0
	3-Ketocarbofuran phenol	1	0.2	0.2	0.0	0.0	0.0	0.2	0.2
		2	0.2	0.3	0.4	0.1	0.0	0.1	0.3
	Carbofuran	1	76.8	55.2	1.5	0.1	0.0	96.0	91.1
		2	87.9	72.8	40.0	13.4	1.0	97.1	94.2
	3-Ketocarbofuran	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		2	0.2	0.3	0.2	0.0	0.0	0.1	0.1
	3-Hydroxycarbofuran phenol	1	0.5	0.4	0.0	0.0	0.0	0.2	0.2
		2	0.1	0.3	0.2	0.0	0.0	0.1	0.2
	Unknown in zone B[b]	1	0.4	0.3	0.0	0.0	0.0	0.0	0.0
		2	0.2	0.2	0.1	0.0	0.0	0.1	0.1
	3-Hydroxycarbofuran	1	0.8	0.9	0.4	0.0	0.0	0.2	0.3
		2	0.2	0.6	0.8	0.4	0.0	0.1	0.2
	Unknown in zone A[b]	1	0.3	0.3	0.2	0.0	0.0	0.0	0.0
		2	0.1	0.3	0.5	0.5	0.2	0.0	0.0
	Unknown at origin[b]	1	0.4	1.2	0.8	0.1	0.2	0.3	0.1
		2	0.1	0.2	0.2	0.2	0.2	0.2	0.1
	All compounds	1	79.8	58.9	2.9	0.2	0.2	98.2	97.3
		2	89.2	75.3	42.6	14.9	1.4	98.5	97.2
Water-soluble		1	5.7	10.3	19.8	8.2	6.7	0.3	0.7
		2	0.6	3.6	7.2	8.7	5.5	0.2	0.5
All fractions		1	85.5	69.2	22.7	8.4	6.9	98.5	98.0
		2	89.8	78.9	49.8	23.6	6.9	98.7	97.7

[a] Experiment 1 without and experiment 2 with antibiotics. [b] Refers to positions on thin-layer chromatograms in systems I, II, and III.

RESULTS AND DISCUSSION

Root-Uptake Studies. The antibiotics were, presumably, responsible for the extended life of carbofuran and its reduced conversion to polar, water-soluble products in the nutrient solutions of the second experiment (Table III). However, the production of polar products was delayed, rather than inhibited, by the antibiotics. This is indicative of an induction period required for the multiplication of organisms with resistance to the antibiotics. The small extent to which carbofuran was converted to water-soluble products in the control solutions certainly eliminates any major contribution by nonbiochemical mechanisms. However, the role of microorganisms is not necessarily excluded because these, if present, may have been rhizosphere or symbiotic species unable to develop in the control solutions without living plants. The role of the plant roots in the production of these water-soluble products was not resolved. The small quantities of 3-hydroxycarbofuran, 3-ketocarbofuran, and the three hydrolytic phenols consistently found in both treated and control solutions may well have been formed nonbiochemically. Water-soluble radioactivity from the nutrient solutions of both experiments streaked and failed to form discrete spots, when chromatographed in tlc solvent systems IV and V; moreover, chloroform extracts of the acid hydrolysates did not contain activity capable of migrating from the origin in systems I and II. There was, therefore, no evidence of glycosidic conjugates in these fractions.

In consequence of the protection to carbofuran afforded by the antibiotics, there was a reduction in the accumulation of methanol-soluble and insoluble activity in the plant roots (Table IV) and an enhanced accumulation of carbofuran in the plant tops of the second experiment.

Carbofuran did not accumulate in the plant roots and tended to disappear altogether as carbofuran in the solutions became exhausted (Table IV). Unconjugated metabolites (unconjugated metabolites always occurred in the chloroform-soluble extracts) were not found in the roots. Methanol-soluble metabolites did not migrate from the origin in tlc system I; in systems IV and V activity streaked nearly the length of the plates without forming discrete spots to be expected from glycosidic conjugates. Only trace quantities of glycosidic algycones, mainly 3-hydroxycarbofuran, were extracted into chloroform after acid hydrolysis of the methanol-soluble root extracts. The identified aglycones accounted for only 17 and 15% of the methanol-soluble root activity from the respective experiments. Some unidentified activity was also found in zones A and B (tlc systems I and II) of the chloroform extracts of the acid hydrolysates. Carbofuran accumulated rapidly in the plant tops (Table IV) and, after reaching a maximum, decreased with a half-life of about 4 days. In the chloroform-soluble fractions, 3-hydroxycarbofuran was the only unconjugated metabolite found in appreciable quantity, although traces of 3-ketocarbofuran and 3-ketocarbofuran phenol were found consistently. Traces of carbofuran phenol and 3-hydroxycarbofuran phenol were found only occasionally in the second experiment, together with unidentified activity in zones A and B and at the origin of tlc plates developed in systems I to III.

Some activity, which remained at the origin of thin-layer chromatograms developed in systems I, II, and III, was found in the chloroform-soluble extracts from all experiments. Investigation revealed this material to be essentially the same as the polar activity occurring in the methanol-soluble or water-soluble extracts and resulted from the exhaustive extraction, with chloroform, of the nutrient solutions and aqueous acetone plant extracts necessary for the complete recovery of 3-hydroxycarbofuran phenol in the chloroform-soluble fraction.

Methanol-soluble radioactivity increased in plant tops throughout the 12 days (Table IV). Tlc systems IV and V resolved four distinct ^{14}C components, indicating the presence of glycosidic conjugates. Examination of chloroform extracts of the acid hydrolysates by tlc revealed 3-hydroxycarbofuran to be the major aglycone; lesser quantities of the three phenols were also found in these hydrolysate extracts. The four aglycones liberated by acid hydrolysis accounted for 50

Table IV. Percent Recoveries of ^{14}C-Carbofuran and Metabolites for Plant Roots and Tops from Root-Uptake Studies

Fraction	Compound	Exp no.[a]	1 Day	2 Days	4 Days	8 Days	12 Days
		Roots					
Chloroform-soluble	Carbofuran	1	0.4	0.6	0.1	0.0	0.0
		2	0.4	0.4	0.4	0.2	0.1
	Unknown at origin[b]	1	0.0	0.0	0.2	0.3	0.4
		2	0.0	0.0	0.0	0.1	0.1
	All compounds	1	0.4	0.6	0.3	0.3	0.4
		2	0.4	0.4	0.4	0.3	0.2
Methanol-soluble		1	0.1	0.4	1.9	3.4	3.7
		2	0.0	0.1	0.4	0.9	1.3
Insoluble residue		1	2.0	5.8	22.1	21.9	21.1
		2	0.4	1.5	6.2	15.1	15.8
All fractions		1	2.5	6.8	24.3	25.6	25.2
		2	0.8	2.0	7.0	16.3	17.3
		Tops					
Chloroform-soluble	Carbofuran phenol	1	0.0	0.0	0.0	0.0	0.0
		2	0.0	0.0	0.1	0.1	0.2
	3-Ketocarbofuran phenol	1	0.0	0.1	0.1	0.1	0.1
		2	0.0	0.1	0.2	0.2	0.2
	Carbofuran	1	8.7	15.6	20.8	10.5	4.6
		2	6.8	13.1	29.0	29.9	17.0
	3-Ketocarbofuran	1	0.0	0.1	0.2	0.1	0.0
		2	0.2	0.3	1.0	0.9	0.4
	3-Hydroxycarbofuran phenol	1	0.0	0.0	0.0	0.0	0.0
		2	0.0	0.0	0.0	0.0	0.1
	Unknown in zone B[b]	1	0.0	0.0	0.0	0.0	0.0
		2	0.0	0.1	0.2	0.2	0.2
	3-Hydroxycarbofuran	1	0.2	0.8	2.3	3.2	2.1
		2	0.2	0.5	2.5	5.3	6.3
	Unknown in zone A[b]	1	0.0	0.0	0.0	0.0	0.0
		2	0.0	0.0	0.0	0.1	0.2
	Unknown at origin[b]	1	0.0	0.2	0.7	1.2	1.4
		2	0.1	0.2	0.6	1.3	2.4
Chloroform-soluble	All compounds	1	8.9	16.8	24.1	15.1	8.2
		2	7.3	14.3	33.6	38.0	27.0
Methanol-soluble	Unknown W[c]	1	0.2	0.9	3.3	9.5	11.3
		2	0.1	0.5	2.1	5.8	15.8
	Unknown X[c]	1	0.0	0.0	0.0	0.0	0.0
		2	0.0	0.0	0.2	0.8	1.7
	Unknown Y[c]	1	0.0	0.2	0.6	2.7	3.6
		2	0.0	0.1	0.5	2.8	10.7
	Unknown Z[c]	1	0.0	0.0	0.2	0.7	1.4
		2	0.0	0.0	0.0	0.2	0.7
	All compounds	1	0.2	1.1	4.1	12.9	16.3
		2	0.1	0.6	2.8	9.6	28.9
Insoluble residue		1	0.3	0.4	1.3	3.3	3.2
		2	0.1	0.3	0.9	2.6	6.0
All fractions		1	9.4	18.3	29.5	31.3	27.7
		2	7.5	15.2	37.3	50.2	61.9

[a] Experiment 1 without and experiment 2 with antibiotics. [b] Refers to positions on thin-layer chromatograms in systems I, II, and III. [c] Separated in tlc systems IV and V (Table II).

and 59% of the methanol-soluble activity in the respective experiments. Unidentified activity was also present in zones A and B of tlc plates developed in solvent systems I and II.

Isolated-Leaf Studies. During the course of this experiment the leaves wilted progressively until the sixth or seventh day, when wilting was practically permanent. Despite this, there was no significant (<0.1%) secretion of ^{14}C activity from the petioles into the solutions in which they were immersed. During the initial period (up to 6 days) carbofuran decreased with a half-life of about 4 days (Table V); however, no loss occurred between 6 and 8 days. This result was, presumably, a reflection of the state of dehydration of the plant during the final 2 days of treatment. As in the root-uptake studies, 3-hydroxycarbofuran was the major unconjugated metabolite with traces of carbofuran phenol, 3-ketocarbofuran, 3-ketocarbofuran phenol, and unidentified radioactivity in zones A and B.

Three distinct areas containing activity were resolved from the methanol-soluble fractions by tlc systems IV and V; these ^{14}C components appeared to correspond to three of the four found in the root-uptake studies. Chloroform extracts of the acid hydrolysates of these methanol-soluble fractions contained all four hydroxy aglycones with 3-hydroxycarbofuran predominating. These identified aglycones accounted for 54% of the methanol-soluble metabolites extracted from the leaves. Several unidentified compounds in zones A and B were also found in the hydrolysate extracts.

Foilar-Uptake Studies. No ^{14}C activity was found in plant parts other than the treated leaves. Differences were apparent between this and the root-uptake and isolated leaf studies.

Table V. Percent Recoveries of ^{14}C-Carbofuran and Metabolites in Leaves from Studies on Isolated Leaves

Fraction	Compound	1 Day	2 Days	4 Days	6 Days	8 Days
Chloroform-soluble	Carbofuran phenol	0.3	0.1	0.2	0.2	0.3
	3-Ketocarbofuran phenol	0.2	0.2	0.2	0.2	0.3
	Carbofuran	72.4	55.0	38.4	25.0	25.4
	3-Ketocarbofuran	0.8	0.8	0.5	0.3	0.4
	Unknown in zone B[a]	0.2	0.1	0.2	0.0	0.1
	3-Hydroxycarbofuran	7.4	11.5	13.1	6.2	5.0
	Unknown in zone A[a]	0.1	0.1	0.1	0.0	0.1
	Unknown at origin[a]	1.0	1.9	3.3	2.2	2.9
	All compounds	82.4	69.7	56.0	34.1	34.5
Methanol-soluble	Unknown W[b]	3.5	10.8	22.9	32.6	28.2
	Unknown Y[b]	0.6	2.1	5.8	10.9	10.2
	Unknown Z[b]	0.3	0.6	1.6	1.9	2.4
	All compounds	4.4	13.5	30.3	45.4	40.8
Insoluble residue		1.9	4.6	4.4	6.2	11.2
All fractions		88.7	87.8	90.7	85.7	86.5

[a] Refers to positions on thin-layer chromatograms in systems I, II, and III. [b] Separated in tlc systems IV and V (Table II).

Table VI. Percent Recovery of ^{14}C-Carbofuran and Metabolites for Treated Leaves from Foliar-Uptake Studies

Fraction	Compound	2 Days	4 Days	8 Days	12 Days
Chloroform-soluble	Carbofuran phenol	5.7	0.8	0.6	1.3
	3-Ketocarbofuran phenol	0.4	0.1	0.2	0.3
	Carbofuran	78.7	93.6	82.7	72.3
	3-Ketocarbofuran	0.2	0.1	0.3	0.5
	3-Hydroxycarbofuran phenol	0.2	0.0	0.3	0.4
	Unknown in zone B[a]	0.2	0.2	0.4	0.6
	3-Hydroxycarbofuran	0.3	0.2	0.5	1.0
	Unknown in zone A[a]	0.3	0.2	0.3	0.6
	Unknown at origin[a]	0.9	0.4	0.7	2.1
	All compounds	86.9	95.6	86.0	79.1
Methanol-soluble	Unknown W[b]	0.3	0.2	0.5	1.3
	Unknown X[b]	0.2	0.1	0.2	0.5
	Unknown Y[b]	0.2	0.0	0.3	1.2
	Unknown Z[b]	0.1	0.0	0.0	0.4
	All compounds	0.8	0.3	1.0	3.4
Insoluble residue		1.6	0.4	0.9	2.3
All fractions		89.3	96.3	87.9	84.8

[a] Refers to positions on thin-layer chromatograms in systems I, II, and III. [b] Separated in tlc systems IV and V (Table II).

First, carbofuran was considerably more persistent (Table VI); and second, the hydrolysis product carbofuran phenol was the major unconjugated metabolite, whereas in previous studies the oxidation product 3-hydroxycarbofuran had predominated. These facts strongly support a previous report (Ashworth and Sheets, 1970) that the major portion of the parent compound does not penetrate the plant cuticle and is, therefore, not subject to plant metabolism. Methanol-soluble activity was similar in composition to that found in plant tops from studies reported above.

Insoluble Plant Residues. Acid hydrolysis of samples of insoluble plant residue resulted in only traces of activity being rendered chloroform-soluble, less than half of which migrated from the origin in solvent systems I and II. This activity may represent incomplete removal of methanol-soluble conjugates during extraction. Even so, it was apparent that the insoluble residues did not contain more than traces of glycosidic conjugates derived from ^{14}C-labeled hydroxy metabolites of carbofuran.

Enzymatic Confirmation for the Presence of Glycosidic Conjugates in the Methanol-Soluble Extracts. Methanol-soluble extracts were subjected to both acid and enzymatic hydrolysis to compare the quantities of activity rendered chloroform soluble. In all cases the yield from the enzymatic action was somewhat lower than from the acid. However, this is not surprising in view of the vastly different techniques employed. Tlc of the chloroform extracts showed identical aglycones produced by both methods of hydrolysis. In the plant top and leaf extracts, the major aglycone was 3-hydroxycarbofuran, with smaller quantities of the three phenols. However, the unidentified products (zones A and B) produced (and reported above) by acid hydrolysis were not present after enzymatic hydrolysis, and some doubt must exist as to whether these were artifacts of the acid hydrolysis or genuine carbofuran metabolites bound as some acid-labile nonglycosidic entity.

CONCLUSIONS

Carbofuran was metabolized in the leaves of tobacco plants with a half-life of approximately 4 days undergoing progressive oxidation to 3-hydroxycarbofuran, the major unconjugated metabolite, and to 3-ketocarbofuran. All three carbamates were hydrolyzed to their corresponding phenols, and the four hydroxy compounds subsequently were conjugated as glycosides. 3-Hydroxycarbofuran was the major glycosidic aglycone.

In the roots of tobacco plants, the metabolic picture was confused by absorption of unidentified water-soluble products

of carbofuran from the nutrient solutions. Unconjugated metabolites were absent, and only a minor portion of the polar activity was identified as being glycosidic.

The activity in the insoluble plant residues was not identified except for possible traces of glycosidic conjugates.

The half-life of carbofuran, applied topically to the leaves of tobacco plants, was very much greater than 4 days. This, and a somewhat different pattern of metabolism, indicates that foliar-applied carbofuran does not readily penetrate the cuticle.

LITERATURE CITED

Ashworth, R. J., Sheets, T. J., *J. Econ. Entomol.* **63,** 1301 (1970).
Crafts, A. S., Yamaguchi, S., Manual 35, California Agricultural Experiment Station Extension Service (1964).
Dorough, H. W., *Bull. Environ. Contam. Toxicol.* **3,** 164 (1968a).
Dorough, H. W., J. AGR. FOOD CHEM. **16,** 319 (1968b).
Hoagland, D. R., Arnon, D. I., Circular 347, California Agricultural Experiment Station Extension Service (1950).
Metcalf, R. L., Fukuto, T. R., Collins, C., Borck, K., El-Aziz, S. A., Munoz, R., Cassil, C. C., J. AGR. FOOD CHEM. **16,** 300 (1968).
Mistric, W. J., Smith, F. D., *J. Econ. Entomol.* **62,** 712 (1969).
Mistric, W. J., Smith, F. D., *J. Econ. Entomol.* **63,** 509 (1970).
Stanovich, R. P., Burt, J., Niagara Chemical Division, FMC Corporation, Middleport, New York, unpublished report (1967).

Received for review June 1, 1971. Accepted November 1, 1971. Contribution from the North Carolina State University Agriculture Experiment Station. Published with the approval of the Director of Research as paper No. 3492 of the Journal Series. This research was partially supported by Niagara Chemical Division, FMC Corp., Middleport, New York. The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Experiment Station of the products named nor criticism of similar ones not mentioned.

Bux Insecticide Soil Metabolism

Beverly V. Tucker* and Donald E. Pack

Bux Insecticide [3 to 1 mixture of *m*-(1-methylbutyl)-phenyl methylcarbamate and *m*-(1-ethylpropyl)-phenyl methylcarbamate] is readily metabolized by soil organisms by attack at the carbamate ester linkage. Soils treated separately with *m*-(1-methylbutyl)phenyl methylcarbamate-*carbonyl*-^{14}C and *m*-(1-ethylpropyl)phenyl methylcarbamate-*carbonyl*-^{14}C evolved $^{14}CO_2$ as the parent carbamate content decreased. Each parent carbamate formed only one ^{14}C metabolite in the soil and it was present only in trace amounts. The main emphasis was placed on the major isomer of Bux Insecticide and its metabolite was identified as *m*-(1-hydroxy-1-methylbutyl)phenyl methylcarbamate. This metabolite is not persistent in soil but is degraded at about the same rate as the parent carbamate (50% loss in 1 to 2 weeks under laboratory conditions at ambient temperature). Six hydroxylated derivatives of the major isomer of Bux Insecticide were synthesized to aid metabolite identification.

Bux Insecticide is a 3 to 1 mixture of *m*-(1-methylbutyl)phenyl methylcarbamate and *m*-(1-ethylpropyl)-phenyl methylcarbamate which is used to control corn rootworm. It is presently available as a 10% granule and applied to the soil in a band at the time of planting. Soil samples from six test locations in Iowa and Nebraska contained less than 0.02 to 0.24 ppm of Bux Insecticide at corn harvest after a 2 lb/acre application at planting. The samples contained less than 0.02 ppm (limit of detection) of the phenolic hydrolysis product of Bux Insecticide. The average Bux Insecticide residue found was 0.08 ppm, which shows there is a minimum of 90% loss of Bux Insecticide from corn planting (treatment) to corn harvest (5 to 6 month interval). These tests were performed at twice the maximum anticipated commercial rate of 1 lb/acre. Bux Insecticide residues were determined by total *in vitro* acetylcholinesterase inhibition with thin-layer chromatography confirmation of residues. The phenolic hydrolysis product of Bux Insecticide was determined by thin-layer chromatography.

The phenolic hydrolysis product of Bux Insecticide is degraded rapidly in soils (50% loss in 24 hr). In laboratory tests the maximum amount of the phenol found in soils treated with Bux Insecticide was 2% of the carbamate added and this was several days after treatment. Due to the nonpersistence and low toxicity of the phenol, the fate of Bux Insecticide after the carbamate functional group is lost is of minor importance.

There are few reports in the literature concerning the soil metabolism of methylcarbamates. Kaufman (1967) reviewed the available knowledge on carbamate degradation in soil and postulated degradation mechanisms based on analogies with metabolic pathway of carbamates in other media. He postulates that methylcarbamates would be degraded by initial attack at the ester linkage, giving methylamine, carbon dioxide, and the phenol.

This paper is concerned with the metabolism of Bux Insecticide in soil. Carbonyl-^{14}C-labeled carbamate was used so that the fate of the carbamate functional group could be followed. Radiotracer techniques were used in the studies and the fate of the molecule after the carbamate group was lost was not followed. Model compounds that were considered potential metabolites were synthesized to develop extraction and chromatography procedures for identifying actual metabolites. The model compounds were *m*-(1-methylbutyl)phenyl *N*-hydroxy-*N*-methylcarbamate (II), *m*-(1-hydroxy-1-methylbutyl)phenyl methylcarbamate (III), *p*-hydroxy-*m*-(1-methylbutyl)phenyl methylcarbamate (IV), *m*-(1-methylbutyl)phenyl *N*-hydroxymethylcarbamate (V), *m*-

Ortho Division, Chevron Chemical Co., Richmond, California 94804.