The previous discussion has been based on the assumption that the following hydrolytic intermediates are very unstable under biological conditions: CH_3-NHCOOH, $(CH_3)_2NCOOH$, and their *N*-hydroxymethyl derivatives, if present. The fate of these intermediates was not independently examined by directly administering them to rats. Further study will be necessary to establish whether the rate-limiting step in formation of $C^{14}O_2$ from the carbonyl- and methyl-labeled carbamates is the hydrolysis of the carbamate grouping or the subsequent degradation of the intermediates.

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INSECT METABOLISM

Metabolism of 3-Hydroxy-*N*-methyl-*cis*-crotonamide Dimethyl Phosphate (Azodrin) by Insects and Rats

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AZODRIN (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate) is a substituted vinyl-phosphate insecticide that has shown unusual effectiveness against certain lepidopterous insect pests in field trials with cotton plants (*3*). A water-soluble compound, Azodrin can function either as a contact or systemic insecticide.

Recent investigations (*1*, *6*) demonstrated that, in certain biological systems, significant quantities of a closely related compound, Bidrin (3-hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate), were converted to Azodrin by oxidative demethylation via a relatively stable *N*-methylol intermediate. Evidence indicated that further oxidation changed Azodrin to its *N*-methylol derivative, which in turn was converted in minor quantities to the unsubstituted amide derivative (*6*). Metabolic detoxification of both Bidrin and Azodrin was primarily by hydrolysis of vinyl-phosphate and methyl-phosphate bonds, and both insecticides were converted in plants to apparent conjugates that were poten-

Oxidative conversion of Azodrin (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate) to its *N*-methylol derivative occurred in five species of insects and white rats. However, formation of the toxic unsubstituted amide derivative of Azodrin by complete oxidative *N*-demethylation apparently was a minor reaction. Similar metabolites of Azodrin were formed in the different test animals, but at widely different rates.

tially toxic (*1*, *5*). Since widespread use of Azodrin for insect control is anticipated, a comprehensive study was made of its metabolism and system activity in plants (*5*, *8*) and, as reported here, of its metabolism by several insect species and rats.

Experimental

Chemicals. The radiolabeled cis isomer of Azodrin and theoretical metabolites were supplied by Shell Development Co., Modesto, Calif., or synthesized in our laboratory (*1*, *5*, *7*). Different batches of radioactive Azodrin had the following specific activities: Azodrin-*O*-methyl-C^{14}, 1.0 mc. per gram; Azodrin-*N*-methyl-C^{14}, 9.1 mc. per gram; and Azodrin-P^{32}, 4.1 and 10 mc. per gram. Radiolabeled materials were purified (>98%) by chromatography on Celite columns (*5*), and their radiochemical purity was determined by analysis with the chromatographic systems described below.

Treatment of Insects and Rats. All insects were from insecticide-susceptible colonies reared under continuous light at 27° C. Insect species used included adult boll weevils (*Anthonomus grandis* Boheman) of mixed sexes (average weight, 16 mg.), females of adult houseflies (*Musca domestica* Linnaeus) (20 mg.), adult American cockroaches [*Periplaneta americana* (Linnaeus)] of mixed sexes (1.5 grams), and fifth larval instar bollworms [*Heliothis zea* (Boddie)] and tobacco budworms [*Heliothis virescens* (Fabricius)] of mixed sexes (1.0 and 0.75 gram, respectively). A calibrated, micrometer-driven syringe was used for all insect treatments. Houseflies and boll weevils were each treated on the ventral abdominal surface with 1 μl. of a solution of acetone containing 0.05 μg. of Azodrin. Lepidopterous larvae and cockroaches were each treated by a ventral injection into the abdomen of 1 μl. of a solution of water containing 2 μg. of Azodrin. An oral dose of Azodrin was administered to lepidopterous larvae with a blunt-tip 27-gage hypodermic needle. By very careful manipulation, the needle was inserted into the digestive tract and an aqueous solution of Azodrin (2 μg. in 1 μl.) delivered directly into the midgut. Larvae tended to "swallow" the needle, and the operation was accomplished without apparent damage to the alimentary canal. In addition, lepidopterous larvae were allowed to ingest Azodrin and its metabolites in cotton seedlings that had absorbed an aqueous solution of Azodrin through the roots.

Male white rats of the Wistar strain (average weight, 300 grams) were each treated by intraperitoneal injection (5 mg. per kg.) of an aqueous solution of Azodrin.

Analytical Procedures. Detailed procedures for the handling of insects and rats, preparation of extracts of treated materials, and the general techniques for paper chromatography and location of metabolites have been described in detail (*1*, *2*). Use of two ascending paper chromatography systems provided good resolution of the phosphorus-containing metabolites of Azodrin. System A included a mobile phase of acetonitrile–water–ammonium hydroxide (40:9:1 v./v.) and a stationary phase of uncoated Whatman No. 3 paper. System B included a mobile phase of chloroform and a stationary phase of Whatman No. 3 paper impregnated with ethylene glycol (15% in acetone). A thin-layer system, including a stationary phase of silica gel and a mobile phase of chloroform–diethyl ether–methanol (10:1:1 v./v.), was used occasionally for qualitative information.

Aliquots of different extracts and radioactive spots from chromatograms were radioassayed either with a thin-window, gas-flow Geiger counter or with an ambient-temperature liquid scintillation counter. When necessary, appropriate corrections were made for radioactive decay, self absorption, or quenching.

Various procedures were used to identify metabolites. First, radioactive extracts of animals treated with different radiolabels of Azodrin were cochromatographed with authentic standards, whose positions were located colorimetrically and compared with spots on radioautograms. Second, biological materials were treated with equal mixtures (w./w.) of Azodrin-P^{32} with either Azodrin-*O*-methyl-C^{14} or Azodrin-*N*-methyl-C^{14}, and the ensuing metabolites from each treatment were separated by paper chromatography. Metabolites were eluted and counted in a liquid scintillator to determine the relative content of C^{14} and P^{32}. Third, certain metabolites from Azodrin-*N*-methyl-C^{14} treatments were tested for formaldehyde-C^{14} production by formation of the dicoumarol-C^{14} derivative via acid hydrolysis and reaction with 4-hydroxycoumarin (*4*, *9*).

Test Procedure, Results, and Discussion

Chromatography and Identification of Metabolites. Chemical names and R_f values of the phosphorus-containing metabolites of Azodrin that were detected are listed in Table I. System A provided good resolution of most compounds but did not completely separate Azodrin from its unsubstituted amide derivative (*N*-demethyl Azodrin). System B and thin-layer chromatography both allowed good separation of Azodrin, *N*-demethyl Azodrin, and hydroxymethyl Azodrin metabolites; however, these two systems did not resolve the hydrolytic products. All samples were chromatographed in systems A and B and the data combined. Since our results confirmed a previous report (*6*) that *N*-demethyl Azodrin and hydroxymethyl Azodrin partitioned poorly from water into chloroform (or

Table I. Chromatographic Behavior of Azodrin and Its Phosphorus-Containing Metabolites in the Presence of Biological Materials

Abbreviated Name	Chemical Name	R_f Value, System[a] A	B	TLC
Phosphoric acid	Phosphoric acid	0.00	0.00	
Monomethyl phosphate	Methyl dihydrogen phosphate	0.04	0.00	
Unknown A		0.08	0.00	
Dimethyl phosphate	Dimethyl hydrogen phosphate	0.19	0.00	
O-Demethyl Azodrin	3-Hydroxy-*N*-methyl-*cis*-crotonamide methyl hydrogen phosphate	0.24	0.00	
Azodrin acid	3-Hydroxy-*cis*-crotonic acid dimethyl phosphate	0.37	0.54	
Unknown B		0.42	0.00	
Hydroxymethyl Azodrin	3-Hydroxy-*N*-hydroxymethyl-*cis*-crotonamide dimethyl phosphate	0.72	0.26	0.34
N-Demethyl Azodrin	3-Hydroxy-*cis*-crotonamide dimethyl phosphate	0.80	0.43	0.41
Azodrin	3-Hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate	0.85	0.75	0.58

[a] A = acetonitrile–water–ammonium hydroxide (40:9:1 v./v.) with uncoated Whatman No. 3 paper. B = $CHCl_3$ with Whatman No. 3 paper impregnated with ethylene glycol (15% in acetone). TLC = Chloroform–diethyl ether–methanol (10:1:1 v./v.) with silica gel.

other organic solvents), that procedure was used only sparingly. In most cases, the complete water extract was chromatographed.

The product designated as phosphoric acid may have included small amounts of other polar compounds formed by incorporation of inorganic phosphate into natural constituents. Very small concentrations of monomethyl phosphate and unknown A were detected. Unknown A was a phosphorus-containing metabolite that was detected only in excreta of certain insects. Dimethyl phosphate, *O*-demethyl Azodrin, and Azodrin acid were identified initially by cochromatography with known standards. Only trace amounts of Azodrin acid were detected in certain extracts, an indication that hydrolytic degradation of Azodrin by amidase action probably was minimal in the animals tested. Dimethyl phosphate and *O*-demethyl Azodrin, formed by hydrolysis of vinyl-phosphate and methyl-phosphate bonds, respectively, were the major degradation products of Azodrin and its toxic derivatives. The identities of these two metabolites were established by tests with double-labeled Azodrin and with the different single radiolabels of Azodrin. After treatment with an equal mixture of Azodrin-*N*-methyl C^{14} and Azodrin-P^{32}, the dimethyl phosphate spot included only the P^{32} radiolabel and the *O*-demethyl Azodrin spot included both C^{14} and P^{32} in the same proportions as in the original dose. After treatment with an equal mixture of Azodrin-*O*-methyl-C^{14} and Azodrin-P^{32}, the C^{14}-to-P^{32} ratio in the dimethyl phosphate spot was the same as in the original dose (1 to 1) and in *O*-demethyl Azodrin was 1 to 2, which would be expected from a random distribution of C^{14} in the methyl-phosphate groups. In addition, extracts from animals treated with Azodrin-*N*-methyl-C^{14} contained radiolabeled *O*-demethyl Azodrin but not dimethyl phosphate.

Unknown B was a minor metabolite in insects and absent from rat excreta, but is extremely important in plants. The nature of this metabolite will be described in detail in a later publication on the metabolism of Azodrin in plants. Briefly, unknown B seems closely related to a major metabolite formed in plants and insects after treatment with Bidrin (*1*). The intact skeletal structure of the parent molecule is incorporated into the unknown product and acidification results in the release of a toxic material that apparently is hydroxymethyl Azodrin.

Several bits of information supported the identification of hydroxymethyl Azodrin. This metabolite occurred in similar extracts after treatments with each of the three different radiolabels of Azodrin. That it was a toxic compound was demonstrated by in vitro studies of cholinesterase inhibition. Bovine erythrocyte acetylcholinesterase activity was inhibited 35% by 7.5×10^{-6} *M* hydroxymethyl Azodrin (Azodrin-P^3 equivalents), which was isolated from urine of treated rats. The same concentration of pure Azodrin caused 40% inhibition. Acid degradation and subsequent formation of dicoumarol indicated that the metabolite designated as hydroxymethyl Azodrin yielded the theoretical concentration of formaldehyde-C^{14}. Similar tests with pure Azodrin-*N*-methyl-C^{14} were negative. The chromatographic mobility of hydroxymethyl Azodrin, relative to the parent compound and *N*-demethyl Azodrin, as well as cochromatography information, tended to support the identification of this logical oxidative intermediate. In addition, a comparable metabolite of Azodrin in animals was identified and reported by other investigators (*6*).

Table II. Relative Concentrations of Azodrin and Its Metabolites in Boll Weevils and Houseflies Following Topical Treatment with 0.05 μg. of Azodrin-P^{32}

Distribution of Radioactivity	Per Cent of Applied Dose as Azodrin-P^{32} Equivalents at Indicated Hours after Treatment: Boll Weevil, 4	Boll Weevil, 8	Housefly, 4	Housefly, 8
INTERNAL				
Dimethyl phosphate	0.2	0.8	6.6	15.1
O-Demethyl Azodrin	1.2	2.4	3.3	6.7
Hydroxymethyl Azodrin	0.0	0.0	5.3	5.4
Azodrin	19.1	24.1	16.1	15.5
EXCRETED[a]				
Chloroform phase	10.8	14.8	12.2	11.1
Water phase	1.0	1.9	10.7	18.9
External rinse	64.6	48.3	40.4	25.2
Unextracted	3.1	7.7	5.4	2.1

[a] Excreta were partitioned between chloroform and water, but metabolites were not characterized.

Only trace amounts of the metabolite corresponding in chromatographic behavior to *N*-demethyl Azodrin were observed, and these were in extracts of very high specific activity. This evidence suggested that the next major metabolic step after formation of hydroxymethyl Azodrin was degradation, rather than loss of formaldehyde to form the toxic *N*-demethyl Azodrin. The possibility that *N*-demethyl Azodrin was highly unstable and decomposed readily after formation was contraindicated by different tests with pure *N*-demethyl Azodrin. Therefore, it was concluded that demethylation of Azodrin was a minor reaction in animals.

Metabolism

Boll Weevils and Houseflies. The metabolism of Azodrin-P^{32} in boll weevils and houseflies was compared (Table II). Because of the low dose (0.05 μg. per insect), 50 of each species were collected and analyzed at each time to obtain sufficient radioactivity for analysis; each test was replicated three times. Only data obtained 4 and 8 hours after treatment were considered because at these times internal accumulations of radioactivity were maximal. The data clearly indicated that absorbed Azodrin was metabolized much faster in houseflies than in weevils. Relatively large quantities of hydroxymethyl Azodrin were detected in houseflies at 4 and 8 hours after treatment, but none was found in weevils until after 24 hours. Dimethyl phosphate was the dominant hydrolysis product in houseflies while *O*-demethyl Azodrin predominated in weevils.

Results of toxicity tests with the synergist sesamex supported previous reports of the importance of biological oxidation in the detoxification of Azodrin and related compounds in insects. With houseflies, sesamex was shown to synergize several vinyl-phosphate compounds that contained amido groups. Synergism was attributed to inhibition of certain biological oxidative reactions that influenced degradation of the compounds (*11*). This theory was supported by metabolism studies that demonstrated that sesamex caused a definite reduction in the in vivo oxidation of Bidrin and Azodrin in houseflies (*6*). In tests with boll weevils, combinations of sesamex (10 parts synergist to 1 part toxicant) increased the toxicity of Bidrin six times, of Azodrin four times, and of *N*-demethyl Azodrin three and one-half times. The observed increases were not due to changes in rates of penetration because synergist and insecticide were applied separately to different parts of the insect body and were not in contact. Comparable studies with houseflies demonstrated that sesamex increased the toxicity of Bidrin and Azodrin, but not *N*-demethyl Azodrin (*6*). However, in other tests with houseflies, a twofold increase in the toxicity of *N*-demethyl Azodrin by sesamex was reported (*10*) and confirmed in our laboratory. These results sug-

gested that in insects the unsubstituted amide is subject to further degradation as a result of oxidation. Another possibility is that the synergism of this class of compounds by sesamex may involve other supplementary factors.

Azodrin was absorbed and excreted more rapidly by houseflies than by weevils (Table II). Even though internal concentrations of toxic products in the two species were similar at comparable times, Azodrin was about 30 times more toxic to flies than to weevils. After 24 hours, the topical LD_{50} values were 0.065 μg. per fly and 1.9 μg. per weevil. This greater susceptibility among houseflies may result from a more rapid accumulation of toxicant at the actual site of action. Another possibility is that the physiological target in houseflies may be more accessible to the toxicant or more sensitive to inhibition than in boll weevils.

Bollworms, Tobacco Budworms, and American Cockroaches. Representative data from comparisons of the metabolism of Azodrin-P^{32} in these three species are shown in Tables III, IV, and V. At each time after treatments, duplicate samples of three to four insects each were collected and analyzed; tests were replicated six times. No more than four of these insects were used for a given extract because of interference with chromatography by lipids.

Table III shows the relative internal concentrations of Azodrin and six of its metabolites that were detected in the two lepidopterous species and roaches at 2 and 4 hours after oral treatment or injection of each with 2.0 μg. of Azodrin-P^{32}. Extensive oxidative conversion of Azodrin to hydroxymethyl Azodrin occurred in all three species; however, no *N*-demethyl Azodrin was detected. Principal detoxification products were dimethyl phosphate and *O*-demethyl Azodrin. The latter metabolite was more prominent in cockroaches than in either of the lepidopterous species. Tobacco budworms and cockroaches metabolized Azodrin at approximately equivalent rates; however, the metabolic rate in both of these species apparently was considerably faster than that in bollworms. Also, orally administered radioactivity apparently was excreted faster by tobacco budworms than by bollworms. Metabolism data appeared to be closely correlated with toxicity information. For example, at 72 hours after injection with Azodrin, LD_{50} values were 0.75 μg. per bollworm, 3.5 μg. per tobacco budworm, and 3.0 μg. per cockroach.

Excreta were collected from both lepidopterous species after oral treatment or injection of each with 2.0 μg. of Azodrin-P^{32}. Results of analyses indicated that Azodrin and its toxic methylol derivative, as well as six other metabolites, were excreted by both species after both methods of treatment (Table IV). Tobacco budworms appeared to excrete the injected dose of radioactivity slightly faster than the oral dose. After 24 hours, 75% or more of the administered doses were excreted by both species.

The metabolism of naturally ingested doses of Azodrin was compared in bollworms and tobacco budworms. Cotton seedlings were held 24 hours in an aqueous solution containing 25 p.p.m. Azodrin-P^{32}, rinsed thoroughly, and held in nutrient solution. At 1 and 4 days after treatment of seedlings, bollworms or tobacco budworms were placed in individual containers and allowed to feed on treated plants for 8 hours. At the end of the treatment period, seedlings, larvae, and excreta were analyzed separately as described. Results (Table V) indicated the degradation of Azodrin in seedlings was very slow and apparently no hydroxymethyl Azodrin accumulated during the experimental period. Again it was clearly demonstrated that Azodrin was metabolized faster in tobacco budworms than in bollworms. Particularly evident was the more extensive oxidative conversion of Azodrin in tobacco budworms. The only measurable quantities of *N*-demethyl Azodrin were found in the excreta of tobacco budworms.

White Rats. After male rats were treated by intraperitoneal injection with Azodrin-P^{32} (5 mg. per kg.), urine was collected at 2-hour intervals and analyzed. Table VI shows the combined data from tests with six animals and the rate of excretion of Azodrin and its metabolites.

Excretion of radioactive materials by rats was very rapid, particularly during the first 6 hours after treatment (approximately 45% of the dose). Of this, 20% was in the form of the methylol derivative and 46% was hydrolysis products. After 24 hours, more than 60% of the administered dose was excreted in urine (58.4%) and feces (5.1%). Dimethyl phosphate, the principal degradative metabolite formed, exceeded the pro-

Table III. Relative Concentrations of Azodrin and Its Metabolites in Bollworms, Tobacco Budworms, and American Cockroaches Treated Orally or by Injection with 2 μg. of Azodrin-P^{32}

	Per Cent of Applied Dose as Azodrin-P^{32} Equivalents in Internal Extracts at Indicated Hours after Treatment[a]							
	2				4			
	Oral		Injection		Oral		Injection	
Product	Z	V	V	R	Z	V	V	R
H_3PO_4	0.5	1.0	0.5	1.5	0.5	1.0	1.0	2.5
Monomethyl phosphate	0.5	2.0	0.5	0.0	0.0	0.0	1.0	0.0
Dimethyl phosphate	3.5	17.0	16.5	18.0	8.0	31.0	17.0	11.0
O-Demethyl Azodrin	3.5	6.0	3.5	15.5	6.0	1.0	2.5	7.5
Unknown B	0.0	1.5	1.5	0.0	0.0	0.0	1.5	0.0
Hydroxymethyl Azodrin	15.0	18.5	23.5	21.0	13.0	9.0	8.5	12.5
Azodrin	54.0	15.5	13.0	11.0	24.5	2.0	4.5	2.0
Excreted (accumulative)	15.5	30.5	31.0	20.0	40.0	48.0	54.5	59.5
Unextracted	7.5	8.0	10.0	13.0	8.0	8.0	9.5	5.0

[a] Z = *H. zea*, V = *H. virescens*, R = *P. americana*.

Table IV. Relative Concentrations of Azodrin and Its Metabolites in Excreta of Fifth-Instar Bollworms and Tobacco Budworms Treated with 2.0 μg. of Azodrin-P^{32}

	Per Cent of Applied Dose as Azodrin-P^{32} Equivalents at Indicated Hours after Treatment[a]											
	0–2			2–4			4–8			8–24		
Product	ZO	VO	VI	ZO	VO	VI	ZO	VO	VI	ZO	VO	VI
Phosphoric acid	0.1	0.3	0.2	0.2	0.5	0.1	0.2	0.4	0.3	0.3	0.1	0.2
Monomethyl phosphate	0.1	0.7	0.2	0.3	0.9	0.0	0.3	0.6	0.2	0.4	0.2	0.3
Unknown A	0.3	1.0	0.0	0.5	1.0	0.0	0.6	1.0	0.0	0.3	0.8	1.5
Dimethyl phosphate	1.2	2.7	3.3	3.5	3.2	5.9	3.5	4.2	5.9	7.3	4.4	14.2
O-Demethyl Azodrin	1.0	3.3	3.6	5.4	2.8	2.7	3.8	2.8	1.4	4.6	2.6	2.8
Unknown B	0.1	0.8	1.2	0.2	0.8	0.5	0.2	0.4	0.1	0.3	0.1	0.0
Hydroxymethyl Azodrin	2.3	12.0	13.6	3.1	5.2	12.8	2.4	2.9	3.7	2.5	2.5	1.0
Azodrin	10.4	9.7	8.9	11.3	3.1	1.5	6.5	2.8	1.0	1.9	1.3	0.0
Internal radioactivity remaining	84.5	69.5	69.0	60.0	52.0	45.5	42.5	36.9	32.9	24.9	24.9	12.9

[a] ZO = *H. zea*, oral treatment; VO = *H. virescens*, oral treatment; VI = *H. virescens*, injection.

Table V. Relative Concentrations of Azodrin and Its Metabolites in Cotton Seedlings Treated with Azodrin-P[32] and in Lepidopterous Larvae Fed Treated Seedlings

	Per Cent of Recovered Radioactivity as Azodrin-P[32] Equivalents in Indicated Extract[a]									
	Seedling		Internal				Excreta			
Product	1	4	Z-1	V-1	Z-4	V-4	Z-1	V-1	Z-4	V-4
Phosphoric acid	1.0	3.4	2.5	3.2	8.5	8.7	1.3	1.7	4.0	3.6
Monomethyl phosphate	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0
Dimethyl phosphate	1.3	2.3	32.7	31.9	32.9	40.0	7.8	12.2	5.9	9.9
O-Demethyl Azodrin	4.7	5.3	13.3	10.9	11.3	8.5	9.3	11.7	11.0	7.8
Unknown B	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Hydroxymethyl Azodrin	0.0	0.0	14.9	28.5	7.1	28.1	18.0	39.1	11.8	53.2
N-Demethyl Azodrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	3.2
Azodrin	93.0	88.5	36.6	25.5	40.2	12.7	63.6	34.1	67.3	18.3
Azodrin-P[32] µg.-equivalents:										
Extracted	17.0	12.3	4.0	3.5	4.2	4.0	9.7	9.4	6.2	8.0
Unextracted	0.8	1.8	0.7	0.5	0.7	0.7	0.2	0.6	0.4	0.5

[a] Z = *H. zea;* V = *H. virescens;* 1, 4 = number of days after seedlings were treated. Insects were allowed to feed on treated seedlings for 8 hours.

Table VI. Relative Concentrations of Azodrin and Its Metabolites in Excreta of Male Rats Following an Intraperitoneal Injection with 5 Mg./Kg. of Azodrin-P[32]

	µg. Azodrin-P[32] Equivalents of Indicated Products					
Hours after Treatment	H_3PO_4	Dimethyl phosphate	O-Demethyl Azodrin	Hydroxymethyl Azodrin	Azodrin	Per Cent of Dose Excreted
			URINE			
0–2	3.5	89.2	37.7	62.6	152.9	23.1
2–4	2.8	49.4	15.2	38.5	48.8	10.3
4–6	6.0	88.9	14.7	33.9	27.7	11.4
6–8	2.5	39.1	7.1	11.4	11.3	4.8
8–10	1.2	20.6	3.3	5.4	4.8	2.4
10–12	0.7	10.3	1.7	1.9	2.2	1.1
12–24	4.7	46.0	8.7	10.3	9.3	5.3
24–48	7.1	19.5	2.4	3.7	3.8	2.4
			FECES			
0–24	2.6	16.9	15.2	10.1	31.3	5.1
24–48	0.4	6.5	0.9	1.3	1.2	0.7

duction of *O*-demethyl Azodrin by an approximate 4 to 1 ratio. Only trace amounts of *N*-demethyl Azodrin were observed during the experimental period.

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HERBICIDE METABOLISM

Absorption, Translocation, and Metabolism of Diphenamid-1-C[14] by Tomato Seedlings

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Diphenamid, 2,2-diphenyl-*N,N*-dimethylacetamide, absorbed and translocated by tomato seedlings from a nutrient solution, was dealkylated to give 2,2-diphenyl-*N*-methylacetamide and diphenylacetamide in the leaf tissue. Tomatoes grown in diphenamid-treated soil contained up to 11.8 p.p.m. of the desmethyl metabolite in the leaves. None was detected in ripe or green fruit.

DIPHENAMID is commercially useful for the pre-emergence control of weeds in tomato, pepper, potato, strawberry, tobacco, peanut, and other crops. This study was initiated to discover whether diphenamid was absorbed and translocated by a nonsusceptible plant and if translocation occurred, to discover the fate of the material.

Materials and Methods

Radioactive samples were counted in solution using a Packard Tri-Carb scintillation spectrometer, Model 314 EX-2. Paper and thin-layer chromatograms were counted using Vanguard Papergram Scanners (*7*).

Radioactive Diphenamid and Related Compounds. Diphenamid labeled with carbon-14 at the carboxyl carbon position was synthesized: