indicating polar breakdown products, since Colep is not water-soluble. The same was shown for the activity remaining in the intestine. This would indicate that some disruption occurred in the intestine also. Williams (*20*), in discussing the metabolic fate of phenol in mammals, gives pertinent references showing phenyl glucuronoside, phenyl sulfuric acid, and conjugated catechol and quinol to be the urinary metabolites. Phenol conjugation was shown in both liver and intestinal tissue, and more recently in the kidney as well (*17*). Since the radioactivity in Colep is incorporated in the phenol moiety, we would anticipate activity to occur in polar materials if it is degraded in the animal. The concentration of radioactivity in the urine of both animals indicates a disruption of Colep and release of phenol.

There was no measurable activity incorporated in the abdominal fat layer nor released as CO_2. Apparently Colep is not found in fat where the benzene- and $CHCl_3$-soluble and water-insoluble Colep might be expected to be transported. Mammals are not known to be able to oxidize phenol to CO_2 and water so the absence of respiratory $C^{14}O_2$ is not surprising.

Thin layer chromatography in solvent *E* of the urine resulted in a large broad band of radioactivity from R_f 0 to 0.6, peaking at R_f 0.25 to 0.35, suggesting a mixture of unresolved highly polar materials. No activity was found in the region associated with Colep.

To determine the ionic character of the excretory products, samples of urine were passed through ion exchange columns. The results are shown in Table X. While most of the activity passed through the cationic column, 25% was retained and could be partly recovered by NaOH elution. This suggests a basic radioactive component. The radioactivity in the effluent from the cation exchange column was completely retained by the anionic column resisting even 1*N* HCl for elution. Either such a small amount of material is involved as to be irreversibly adsorbed, or the component is strongly acidic and difficult to elute. A strongly acidic metabolite could be phenyl sulfuric acid. Williams (*20*) indicates that, in rabbits, the predominant phenolic excretion conjugate for low levels of phenol (as is present in this situation) is phenyl sulfuric acid.

Acid hydrolysis of the urine samples showed the release of phenol as in plant extracts. Further identification of the metabolic products was not attempted. The remainder of the animals was not analyzed, nor was the carcass washed for possible urine adhering to the legs and fur. This could account for some of the losses in recovery noted in Table IX.

These initial data indicate an effective degradation of Colep and rapid excretion of breakdown products within a 24-hour period, with little deposition of radioactivity in some of the key tissues of rats. The radioactivity excreted is in the form of polar molecules which liberate phenol when subjected to acid hydrolysis.

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INSECTICIDE METABOLISM

Metabolism of 3-Hydroxy-*N,N*-dimethylcrotonamide Dimethyl Phosphate by Cotton Plants, Insects, and Rats

D. L. BULL and D. A. LINDQUIST

Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, College Station, Tex.

BIDRIN [(registered trademark, Shell Oil Co.), defined as the *cis* isomer only] (3-hydroxy-*N*, *N*-dimethylcrotonamide dimethyl phosphate), formerly known as compound SD-3562 (Shell Development Co., Modesto, Calif.), has shown promise as a short-residual, systemic insecticide that is toxic to a wide spectrum of phytophagous insects and mites. Foliar treatments with Bidrin are particularly effective for control of early-season insect pests of cotton (*2, 5*). This substituted-vinyl phosphate compound is soluble in water, of good stability (at 38° C. the half life is 1200 and 2400 hours at pH 9.1 and 1.1, respectively), and has a rat oral LD_{50} of 25 mg. per kg. (*14*). The technical product consists of a mixture of *cis* (85%) and *trans* (8%) isomers, of which the *cis* form is the more insecticidally active. Basic research on certain substituted-vinyl phosphate and other compounds structurally related to Bidrin has been reviewed in considerable detail in recent publications (*9, 11*). However, there have been no published reports of detailed investigations of the systemic action and metabolic behavior of Bidrin in plants and animals. This report presents the results of investigations of the rates of absorption, in vivo metabolism, and excretion of C^{14}- or

The nature and rate of the in vivo metabolism of the experimental systemic insecticide Bidrin were compared in cotton plants, two species of cotton insects, and white rats, through the use of radiometric techniques. Oxidative demethylation of the toxicant to its equally toxic *N*-methyl derivative occurred in all biological materials, but all toxic products decomposed rapidly. Of nine phosphorus-containing metabolites detected, six hydrolytic and two oxidative products were identified tentatively. A major metabolite that occurred in treated plants was not completely identified, but was shown to contain almost all the original Bidrin molecule.

P^{32}-labeled *cis*-Bidrin by cotton plants, insects, and white rats.

Materials and Methods

Biological Material. Cotton plants of the Deltapine-15 variety were grown in complete nutrient solution (*7*) and in soil or sand in 1-gallon metal containers. The insects used [5- to 7-day old adult boll weevils (*Anthonomus grandis* Boheman) and fifth-instar bollworm larvae (*Heliothis zea* (Boddie)] were from insecticide-susceptible laboratory colonies that were reared under controlled environmental conditions. Treatment of insects and handling procedures have been described in detail elsewhere (*3*). The white rats used were adult males weighing 290 to 310 grams, each.

Chemicals. Radioactive and nonradioactive Bidrin and most of the analytical standard chemicals were provided by the Shell Development Co. Dimethyl 1-methylvinyl phosphate was synthesized by reacting trimethyl phosphite with chloroacetone (*12*). Desmethyl Bidrin acid was prepared by refluxing Bidrin acid with sodium iodide in acetone (*16*). The *cis*-Bidrin was labeled either with C^{14} in the methoxy groups (specific activity 2.5 mc. per gram), or at the dimethylamino group (specific activity 4.0 mc. per gram), or with P^{32} (two batches with initial specific activities of 12 and 20 mc. per gram, respectively). When received, the P^{32}-labeled Bidrin shipments were mixtures of *cis* (70 to 85%) and *trans* (5 to 10%) isomers with small quantities of impurities. The *cis* and *trans* isomers were separated from each other and from impurities by partition chromatography for which benzene and crushed firebrick impregnated with ethylene glycol or water (1:1 w./w.) (*14*) were used. Radiolabeled Bidrin used for the experiments was determined to be 98 to 100% *cis* isomer by analyses in two paper chromatographic systems.

Analytical Techniques. Treated plant or insect materials were homogenized in water at 0° C., centrifuged immediately and decanted, then centrifuged again after acetone precipitation of proteins in the supernatant. Liquid volumes were reduced under vacuum, and samples were held in a deep freeze for analysis. Sample preparation procedures, either in the presence or absence of biological materials, did not cause degradation of pure Bidrin standards.

In the ascending paper chromatographic techniques (*3*) used to separate Bidrin and its metabolites, two different solvent systems were employed: System *A*—acetonitrile:water:ammonium hydroxide (80:18:2 v./v.) with uncoated Whatman No. 1 filter paper; and System *B*—benzene with Whatman No. 1 filter paper impregnated with ethylene glycol (8% in acetone). After the total water extract was analyzed by System *A*, it was partitioned with chloroform, and the chloroform layer analyzed by System B. Locations of different radioactive products on developed chromatograms were determined by autoradiography with x-ray film and identified tentatively by cochromatography with authentic standards, for which locations were demonstrated colorimetrically (*1, 8*). Spots of radioactivity were marked, excised, and radioassayed individually to provide quantitative measurements.

Radioassays of whole biological tissues, aliquots of tissue extracts, or spots from chromatograms were made with an ultrathin-window, gas-flow, Geiger-Mueller detector used in conjunction with an automatic sample changer. When necessary, data were corrected for self-absorption and radioactive decay.

Test Procedures, Results, and Discussion

Chromatographic Systems. The two paper chromatography systems used provided adequate resolution of Bidrin and its metabolites (Table I). System *A* gave good separation of all products except Bidrin and its two oxidative metabolites. Only Bidrin and its oxidative metabolites partitioned into chloroform (>95%), and these products were resolved quite well by System *B*. Phosphoric acid remained at the origin in both systems. Other phosphorus-containing compounds such as phosphoprotein might also be expected to exhibit a similar chromatographic behavior. These phosphorus-containing compounds could become radioactive by synthetic incorporation of phosphoric acid-P^{32} that was liberated by the metabolism of Bidrin-P^{32}. Therefore, spots of radioactivity identified as phosphoric acid may also have included small quantities of other phosphorus-containing products.

Metabolism in Plants. The metabolism of Bidrin-P^{32} was compared in excised, mature cotton leaves and in seedling cotton plants. Excised leaves were treated by allowing each to absorb 0.25 ml. of aqueous Bidrin-P^{32} solution from a 1-ml. cell or by spreading a uniform film of aqueous insecticide solution over the upper surface of each leaf. Some of the seedling plants were grown from treated seeds; others were treated by application of Bidrin-P^{32} on the upper surfaces of cotyledons. Plants from treated seeds were held in a greenhouse (80° to 85° F.); all other plant materials were held in the laboratory (78° to 82° F.).

The metabolism of Bidrin-P^{32} in plants was quite complex, involving simultaneous oxidative and hydrolytic attacks on the molecule as well as extensive incorporation of inorganic P^{32} into different normal constituents. In excised leaves that each absorbed 100 μg. of the toxicant through the stem, Bidrin and nine phosphorus-containing metabolites were detected (Table II).

Analyses of the water extracts of treated leaves by System *A* allowed detection of a rapid in vivo decomposition of Bidrin (and metabolites of similar R_f) during the first 2 weeks after treatment. The initial detoxification of the Bidrin molecule included hydrolysis of the vinyl-phosphate bond to form dimethyl phosphate and *N,N*-dimethylacetoacetamide, cleavage of a methylphosphate bond to form desmethyl Bidrin and methanol, and hydrolysis of the amide bond to form Bidrin acid and dimethylamine. Though *N,N*-dimethylacetoacetamide, methanol, and dimethylamine were not identified specifically, they are logical products of these reactions.

Concomitant with the initial hydrolytic reactions, the Bidrin molecule also was subjected to oxidative attack at the dimethylamino group. The oxidation reaction resembled those reported for schradan and dimefox (*9, 11*) and that postulated (*11*) from results of studies of amiton (*13*). An important dissimilarity exists, however, because Bidrin is a direct inhibitor that does not require oxidative demethylation to become toxic

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

Abbreviated Name	Chemical Name	R_f Value, System A[a]	R_f Value, System B[b]
Phosphoric acid	Phosphoric acid	0.00	0.00
Monomethyl phosphate	Methyl dihydrogen phosphate	0.04	0.00
Desmethyl Bidrin acid	3-Hydroxy-*cis*-crotonic acid methyl hydrogen phosphate	0.10	0.00
Dimethyl phosphate	Dimethyl hydrogen phosphate	0.19	0.00
Desmethyl *N*-methyl Bidrin	3-Hydroxy-*N*-methylcrotonamide methyl hydrogen phosphate	0.24	0.00
Desmethyl Bidrin	3-Hydroxy-*N*,*N*-dimethylcrotonamide methyl hydrogen phosphate	0.34	0.00
Bidrin acid	3-Hydroxycrotonic acid dimethyl phosphate	0.37	0.05
Unknown A		0.46	0.08
Unknown B (presumably hydroxymethyl Bidrin)	3-Hydroxy-*N*-(hydroxymethyl)-*N*-methylcrotonamide dimethyl phosphate	0.73	0.04
N-Methyl Bidrin	3-Hydroxy-*N*-methylcrotonamide dimethyl phosphate	0.79	0.12
trans-Bidrin	3-Hydroxy-*N*,*N*-dimethyl-*trans*-crotonamide dimethyl phosphate	0.81	0.18
cis-Bidrin	3-Hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate	0.84	0.44

[a] Acetonitrile:water:ammonium hydroxide (80:18:2 v./v.); with uncoated Whatman No. 1 filter paper.
[b] Benzene; with Whatman No. 1 filter paper impregnated with 8% ethylene glycol (in acetone v./v.).

Table II. Relative Concentrations of Bidrin and Its Degradative Products in Internal Extracts of Excised Cotton Leaves Following Uptake of 100 μg. of Bidrin-P^{32} per Leaf[a]

Product	Per Cent of Applied Dose as Bidrin-P^{32} Equivalents at Indicated Days after Treatment: 1	3	7	14	28
Phosphoric acid	1.5	5.4	5.8	4.3	2.6
Monomethyl phosphate	0.0	0.2	0.0	0.1	0.0
Desmethyl Bidrin acid	0.1	1.7	1.4	1.4	0.4
Dimethyl phosphate	6.3	10.9	10.1	9.0	2.8
Desmethyl Bidrin	5.1	5.1	3.6	2.1	1.5
Bidrin acid	0.1	2.2	1.9	1.0	0.0
Unknown A	1.8	12.0	10.8	11.3	4.9
Hydroxymethyl Bidrin	6.0	5.0	0.8	0.1	
N-Methyl Bidrin	0.0	2.8	1.6	1.4	0.8
Bidrin	65.1	37.7	16.0	3.3	
Unextractable radioactivity	14.0	17.0	48.0	66.0	87.0

[a] Data combined from analyses by both chromatographic systems employed.

as is the case with schradan and dimefox. Further, the oxidative reaction was not a direct degradation as suggested for the oxidative deethylation of amiton. Oxidation of Bidrin yielded two products, one of which was tentatively identified as *N*-methyl Bidrin. By analogy with comparable reactions reported for other dialkylamino compounds, Bidrin probably was oxidized first to the hydroxymethyl amide then converted to *N*-methyl Bidrin. The toxicities of acetone solutions of Bidrin and *N*-methyl Bidrin to the insects used were similar. For adult boll weevils, the topical LD_{50} doses of Bidrin and *N*-methyl Bidrin were 0.8 and 0.6 μg. per insect, respectively, and for third-instar bollworm larvae, 1.6 and 1.5 μg. per insect, respectively. Studies of the in vitro inhibition of adult boll weevil acetylcholinesterase by Bidrin and *N*-methyl Bidrin indicated the compounds had I_{50} values of 8×10^{-6} *M*.

The above suggested sequence of events for the oxidative metabolism of Bidrin was supported by experimental data. As noted previously, of all the phosphorus-containing products recovered in water extracts of plant (or animal) tissues, only Bidrin and its two oxidative derivatives partitioned from water into chloroform (>95%). Experiments with Bidrin labeled with C^{14} in the methyl-phosphate or dialkylamino groups of the molecule indicated that these three chloroform-partitioning compounds each contained at least one intact C—O—P group, and at least one carbon atom linked to the nitrogen atom. Hydroxymethyl Bidrin, prepared by reacting *N*-methyl Bidrin (37.7% w.) in 37% aqueous formaldehyde, was chromatographed in both systems and was found to have an R_f value identical to that of the more polar oxidative metabolite (unknown B). Similarly, pure *N*-methyl Bidrin had an R_f value identical to that of the oxidative metabolite of intermediate polarity. The relative polarities of the three materials (Table I) and the agreement in chromatographic behavior with authentic standards tended to support the tentative identifications. The more polar of the two oxidative metabolites (hydroxymethyl Bidrin) was formed first in plants at 1 day after treatment then diminished at subsequent times as it was converted to a compound of intermediate polarity (*N*-methyl Bidrin) or hydrolyzed. Bidrin and both oxidation products were detoxified at a rapid rate. Apparently the degradation of the oxidative metabolites was accomplished primarily by hydrolysis of vinyl-phosphate bonds; however, the data from experiments with plants were insufficient to allow identification of the exact sequence of events.

Products of the initial hydrolyses of Bidrin and its oxidative metabolites also were subjected to further hydrolytic degradation (Table II). Only small concentrations of Bidrin acid were detected. This compound has the same structure as Phosdrin acid. In pea plants, Phosdrin acid was metabolized primarily to dimethyl phosphate and to a lesser extent to desmethyl Phosdrin acid (*15*). It could be expected that Bidrin acid encountered a similar metabolic fate in cotton plants. The nature and rate of the subsequent metabolism of desmethyl Bidrin were demonstrated by treating excised cotton leaves with desmethyl Bidrin-P^{32} that was prepared by reacting Bidrin-P^{32} with sodium iodide (*16*). Leaves that each absorbed 100 μg. of desmethyl Bidrin-P^{32} through the stem were analyzed at different times during an 8-day period. After 2 days, extracts of treated leaves contained desmethyl Bidrin (17%), desmethyl Bidrin acid (5%), monomethyl phosphate (1%), and phosphoric acid (77%). After 8 days, the desmethyl Bidrin was completely degraded to desmethyl Bidrin acid (10%) and phosphoric acid (90%).

The decarboxylation derivatives of Bidrin acid (dimethyl 1-methylvinyl phosphate) and desmethyl Bidrin acid (monomethyl 1-methylvinyl phosphate) were not detected in any of the extracts. Though desmethyl *N*-methyl Bidrin is a logical detoxification product of *N*-methyl Bidrin, it was not detected in any of the extracts of treated materials.

The most interesting of the metabolic events involving Bidrin in cotton plants was the formation of unknown metabolite A. Since unknown A was the major component of aged plant residues, its identification is essential. The following experimental evidence was sufficient for a partial characterization of the unknown product.

When partitioned between water and chloroform or benzene, all of unknown A

remained in the water layer. In the chromatographic solvent systems used, the unknown was less polar than any of the hydrolytic metabolites of Bidrin but was considerably more polar than Bidrin and the oxidative metabolites of Bidrin.

Qualitative information from experiments with Bidrin labeled with C^{14} in the methyl phosphate or the dimethylamino groups indicated the unknown molecule contained at least one intact C—O—P group and at least one carbon atom linked to the nitrogen atom. Since it was not detected in cotton leaves that were treated with desmethyl Bidrin-P^{32}, formation of the unknown probably requires the presence of both methoxy groups. Since no unknown A was detected in leaves treated with *N*-methyl Bidrin-C^{14}, its formation probably requires the presence of an intact—N(C)(C) group.

Unknown A was very stable in different biological systems. When bollworm larvae were injected with 20 μg. of P^{32}-labeled unknown A, each, 80% of the dose was excreted after 2 hours. Of this excreted radioactivity, 87% was unknown A, 7.6% was dimethyl phosphate and 5.4% was a mixture of what appeared to be hydroxymethyl Bidrin and *N*-methyl Bidrin. After 24 hours, radioactive products in the excreta of treated larvae included unknown A (90%) and dimethyl phosphate (10%). After 4 hours' incubation of 100 μg. of the unknown with rat liver slices in Krebs-Ringer phosphate buffer (*17*), the mixture contained unknown A (93.4%), dimethyl phosphate (4.8%), and hydroxymethyl Bidrin and *N*-methyl Bidrin (1.8%). After 8 days, extracts of excised leaves that were each treated with 100 μg. of P^{32}-labeled unknown A contained the unknown (79%), dimethyl phosphate (7.4), Bidrin acid (2.4%), phosphoric acid (2.9%), desmethyl Bidrin acid (4.8%), and a mixture of hydroxymethyl Bidrin and *N*-methyl Bidrin (3.5%). Thus the very slow degradation of the unknown molecule was achieved primarily by cleavage of the vinyl-phosphate bond. The evidence was insufficient to determine if the hydrolytic reactions occurred directly with the unknown molecule or with the toxic products that formed from the unknown. Of great importance from an insecticidal residue standpoint was the conversion of small quantities of the unknown back to toxic products. Also important, the unknown itself is a weak acetylcholinesterase inhibitor (approximate I_{50} with weevil AChE is 1×10^{-4} *M* Bidrin equivalents).

Changes in the pH of aqueous solutions of unknown A caused a drastic change in its chromatographic behavior. When the pH was adjusted from pH 5 to < pH 1 with hydrochloric acid, the unknown disappeared from its normal chromatographic position in System *A* and all the radioactivity shifted to R_f position 0.75. Adjustment of the same solution back to pH 5 with sodium hydroxide or potassium hydroxide resulted in a return of almost all the radioactivity to the original R_f position of the unknown. If the < pH 1 solution was partitioned with chloroform, most of the radioactivity was recovered in the organic layer. Analysis of this chloroform layer by both chromatographic systems indicated the radioactivity consisted primarily of hydroxymethyl Bidrin (>98%) or another product with a similar R_f value. The product was shown to be a potent acetylcholinesterase inhibitor. When adult boll weevil acetylcholinesterase was used, the I_{50} concentration of the chloroform extractable product was 8.4×10^{-6} *M*. The chromatographic behavior of unknown A was unchanged over a pH range of 5 to 11, but substantial degradation occurred under highly alkaline conditions.

The evidence suggests that either Bidrin or hydroxymethyl Bidrin is

Table III. Relative Concentrations of Bidrin and Its Metabolites Recovered from Excised Cotton Leaves and Seedling Cotyledons Following Topical Treatment with 50 μg. and 20 μg. of Bidrin-P^{32} [a]

Product	Per Cent of Applied Dose as Bidrin-P^{32} Equivalents at Indicated Days after Treatment					
	1	3	7	14	21	28
A	Excised Leaves—External Rinses					
Phosphoric acid	0.6	0.6	0.8	0.4	1.0	
Dimethyl phosphate	5.2	5.8	2.4	1.6	1.2	
Desmethyl Bidrin	13.5	15.0	10.8	6.6	3.4	
Hydroxymethyl Bidrin	0.0	0.0	0.2	0.0		
N-Methyl Bidrin	0.0	1.0	0.4	0.2	0.4	
Bidrin	23.6	12.2	2.8	0.8		
	Excised Leaves—Internal Extracts					
Phosphoric acid	1.4	6.8	4.0	11.6	11.4	
Desmethyl Bidrin acid	0.0	0.8	1.0	0.8	0.8	
Dimethyl phosphate	1.8	2.4	4.0	5.0	5.8	
Desmethyl Bidrin	1.4	3.4	2.8	3.4	4.0	
Bidrin acid	0.0	0.0	1.6	0.0	0.0	
Unknown A	0.0	5.4	8.0	10.0	9.4	
Hydroxymethyl Bidrin	2.0	3.4	1.6			
N-Methyl Bidrin	0.0	0.8	1.6	10.2	1.8	
Bidrin	37.2	42.4	22.4			
	Excised Leaves—Radioactivity Lost					
Unextractable radioactivity	5.8	7.8	19.0	19.4	24.8	
Radioactivity lost by volatilization	7.5	4.8	16.6	30.0	36.0	
B	Cotyledons—External Rinses					
Phosphoric acid	0.0	0.0	0.0	0.0	0.2	0.2
Dimethyl phosphate	0.8	0.8	0.8	0.4	0.4	0.3
Desmethyl Bidrin	2.4	1.6	2.8	2.0	2.0	2.4
Hydroxymethyl Bidrin	32.0	14.8	10.8	6.0	2.4	2.0
N-Methyl Bidrin						
Bidrin						
	Cotyledons—Internal Extracts					
Phosphoric acid	0.6	1.6	10.8	7.6	6.4	11.2
Desmethyl Bidrin acid	0.0	0.0	0.0	0.0	0.8	0.0
Dimethyl phosphate	0.8	0.8	1.6	2.0	2.8	3.2
Desmethyl Bidrin	4.6	1.4	0.8	2.4	2.8	2.0
Unknown A	0.4	0.6	0.8	4.0	5.6	4.8
Hydroxymethyl Bidrin	0.8	1.2	3.2	1.2		
N-Methyl Bidrin	0.0	2.4	0.8	1.6	12.8	8.0
Bidrin	42.8	40.4	30.8	15.6		
	Cotyledons—Radioactivity Lost					
Unextractable radioactivity	3.6	5.6	7.6	14.8	20.2	22.0
Radioactivity lost by volatilization	11.2	28.8	29.2	42.4	43.6	43.9

[a] Data combined from analyses by both chromatographic systems employed.

Table IV. Relative Concentration of Bidrin and Its Metabolites in Cotton Seeds and Seedling Plants Following Treatment with 0.4-Mg. Bidrin-P^{32} per Seed[a]

	Per Cent of Recovered Radioactivity as Bidrin-P^{32} Equivalents at Indicated Days after Treatment[b]						
	1 Seed[c]		2 Seed		3 Seed		4 Seedling
Product	Ext	Int	Ext	Int	Ext	Int	Cotyl
Phosphoric acid	0.0	6.4	0.0	9.7	0.0	12.2	23.0
Desmethyl Bidrin acid	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Dimethyl phosphate	0.9	2.2	2.1	3.2	2.1	3.5	6.6
Desmethyl Bidrin	0.0	8.8	0.0	3.3	0.0	4.6	4.6
Unknown A	0.0	0.0	0.0	0.0	0.0	2.1	8.2
Bidrin[d]	99.1	82.6	97.9	83.8	97.9	77.6	57.1
Bidrin-P^{32} μg.-equivalents recovered		16.7		22.3		37.5	14.7

	7 Seedling	14 Seedling			21 Seedling		
	Cotyl	Whole	Cotyl	Leaves	Whole	Cotyl	Leaves
Phosphoric acid	42.4	43.5	37.6	66.4	39.7	20.2	74.3
Desmethyl Bidrin acid	1.2	1.4	1.1	0.0	1.9	3.2	0.0
Dimethyl phosphate	11.1	12.8	8.5	24.0	8.0	17.0	25.7
Desmethyl Bidrin	7.9	6.3	6.6	0.0	15.3	6.6	0.0
Unknown A	13.9	24.2	28.4	0.0	18.7	38.2	0.0
Bidrin[d]	23.5	11.8	17.8	9.6	16.4	14.8	0.0
Bidrin-P^{32} μg.-equivalents recovered	22.9	23.3	16.0	5.0	16.8	10.3	4.7

[a] Chromatographic System *A*.
[b] Seeds planted in sand immediately after treatment.
[c] Ext = external rinse of seedcoat; Int = internal extract of seedmeat.
[d] Bidrin plus oxidative metabolites.

changed in plants to form a comparatively stable product. This change might involve either a structural modification or a combination of one of the materials with a normal plant constituent. Experiments necessary for final identification of unknown A are currently in progress.

In excised leaves that were treated topically with 50 μg. of Bidrin-P^{32}, each, the metabolism of absorbed toxicant was somewhat similar to that described above (Table III*A*). The reactions of unabsorbed Bidrin (removed by rinsing each leaf in water for 3 minutes), however, differed considerably from those occurring within the leaf. Only three hydrolytic products were detected on leaf surfaces—desmethyl Bidrin, dimethyl phosphate, and inorganic phosphate. Bidrin also was oxidized on leaf surfaces, with resulting formation of the two derivatives described above. Hydrolytic and oxidative reactions on biological and nonbiological surfaces are not uncommon (*11*); similar reactions occurred with unabsorbed dimethoate on corn, pea, potato, and cotton leaf surfaces (*6*). The latter authors assumed the reactions occurring on leaf surfaces were nonenzymatic since they could be duplicated on nonbiological (glass) surfaces. When Bidrin was applied to a glass surface, however, oxidation was negligible, and hydrolytic reactions were very slow during a 2-week experimental period. For example, of products recovered from treated glass surfaces after 14 days, 91.3% was Bidrin and minute quantities of its oxidative products, 6.7% was desmethyl Bidrin and 2.0% was dimethyl phosphate. Therefore, the relatively large concentrations of Bidrin metabolites that were detected in external rinses of treated leaves might have been formed by reactions catalyzed by components of the leaf surface.

Cotyledons of cotton seedlings were treated topically with 20 μg. of Bidrin-P^{32}, each, and the plants were forced to remain in the cotyledonous stage by removal of true-leaf growth. The metabolic pathways of absorbed and unabsorbed Bidrin were similar to those discussed above for excised leaves; however, the apparent reaction rates were somewhat slower in cotyledons (Table III*B*). The rate of oxidation of absorbed Bidrin was comparable to that shown for excised leaves, but only trace amounts of oxidative metabolites were detected on the surfaces of cotyledons. The differences in reaction rates that were observed are a further indication that surfaces of cotton foliage are not inert but may have components of natural origin that are associated with metabolism of unabsorbed insecticides.

Cotton seeds treated externally with 0.4 mg. of aqueous Bidrin-P^{32}, each, were planted in sand and a certain number collected daily until seedlings emerged. After removal from sand, the seeds were split, the seedmeats removed, and separate extracts made of seedcoats and seedmeats. After emergence, seedlings were transferred to nutrient solution and held for harvest at weekly intervals; separate extracts were made of whole plants, cotyledons, and true leaves. Experimental results indicated there was no oxidation and only negligible hydrolysis (Table IV) of unabsorbed Bidrin on seedcoats. Extracts of seedmeats contained only small concentrations of oxidation products (3% of recovered radioactivity after 3 days), but hydrolysis of absorbed Bidrin was rather extensive, and after 3 days 22.4% of the recovered radioactivity was in the form of hydrolysis products. Pathways of Bidrin metabolism during seed germination were similar to those discussed previously. Oxidation of Bidrin occurred in cotyledons and leaves of seedlings grown from treated seeds, but the extent of the reactions was not determined. Considerably more inorganic phosphate was recovered in extracts of cotton seedlings than in those from excised leaves, possibly because reincorporation of the inorganic phosphate, formed by Bidrin degradation, was less extensive in seedlings than in mature leaves. Major concentrations of unknown A were formed in cotyledons of seedlings, but the unknown was not detected in true leaves. Minute quantities of Bidrin and its oxidative metabolites were detected in true leaves 10 days after transfer to nutrient solution, but none were detected at later times. Radioactivity in true leaves was supplied chiefly by two hydrolytic metabolites, dimethyl phosphate and inorganic phosphate. These results confirmed bioassay data from experiments with Bidrin (*10*) and other systemic insecticides (*4*) which indicated a failure of effective concentrations of toxic materials to retranslocate from cotyledons to new true leaves. Apparently only the more water-soluble hydrolytic derivatives of Bidrin have any freedom of movement within the cotton plant.

An experiment was designed to determine whether Bidrin or any of its metabolites within treated cotton leaves was excreted on leaf surfaces. Each of several excised leaves was allowed to absorb 100 μg. of Bidrin-P^{32} through the stem, then held in distilled water. Excreted materials were allowed to accumulate for different periods of time on some leaves prior to removal for analysis; on other leaves, the materials excreted during each time interval were determined. Interpretation of the results of the experiment was complicated because different compounds may be excreted, metabolized, and absorbed simultaneously. The results (Table V) indicated a tendency to preferential excretion of less polar materials (Bidrin and oxidation products) because, even after the extensive degradation that is known to occur on leaf surfaces, the relative Bidrin concentrations, per interval (Table V*B*), on the surfaces of leaves were always greater at comparable times

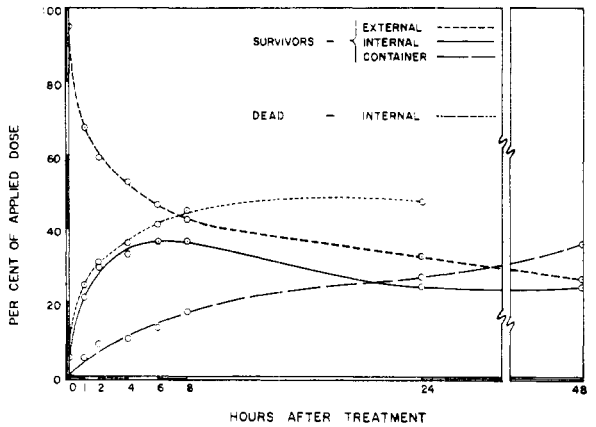

Figure 1. Absorption of Bidrin-C^{14} by adult boll weevils

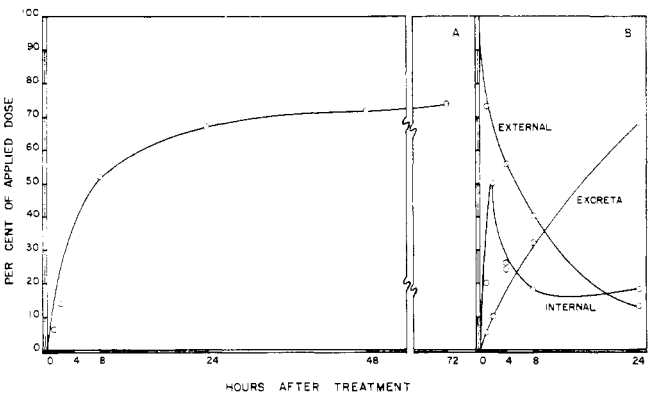

Figure 2. (*A*) Cumulative rate of excretion of injected Bidrin-P^{32} by fifth-instar bollworm larvae
(*B*) Absorption of Bidrin-P^{32} by fifth-instar bollworm larvae

Table V. Excretion of Bidrin and Its Metabolites from Excised Cotton Leaves Following Uptake through Stem of 100 μg. of Bidrin-P^{32} per Leaf[a]

	Per Cent of Recovered Radioactivity as Bidrin-P^{32} Equivalents at Indicated Days after Treatment							
	Accumulated[b] *(A)*				*Per Interval*[c] *(B)*			
Product	*1*	*3*	*7*	*14*	*1*	*3*	*7*	*14*
Dimethyl phosphate	13.1	23.5	37.9	27.1	16.6	18.0	20.4	17.2
Desmethyl Bidrin	10.5	27.3	38.3	58.4	14.7	19.4	25.7	38.4
Bidrin[d]	76.4	49.2	23.8	14.5	68.7	62.6	53.9	44.4
Bidrin-P^{32} μg.-equivalents excreted per leaf	1.8	2.9	3.8	4.2	1.8	1.1	0.9	0.4

[a] Chromatographic System *A*.
[b] The excreted materials were allowed to accumulate from date of treatment to indicated harvest date.
[c] The same leaves were used during the entire experimental period and were rinsed at each indicated time interval.
[d] Bidrin plus oxidative metabolites.

than those in internal extracts except during the first day. Though the quantities of radioactivity excreted were small, the amounts excreted per interval were approximately proportional to the internal, less polar metabolite content (Table V*B*). The results indicated that the two hydrolytic products (dimethyl phosphate and desmethyl Bidrin) were formed on the surface of the leaf and were not extensively absorbed since they tended to accumulate rapidly (Table V*A*) and soon were the major products among recovered radioactive materials. The latter observations were, in part, confirmed experimentally. Excised cotton leaves were treated topically with P^{32}-labeled dimethyl phosphate (25 μg. per leaf) and analyzed for internal and external radioactivity at regular intervals during a 48-hour experimental period. Even after 48 hours, only trace amounts of radioactivity were detected in internal extracts of leaves. Therefore, it appeared probable that only less polar compounds were excreted by cotton leaves and that polar metabolites recovered from leaf surfaces were formed by surface degradation of excreted materials and were not excreted or reabsorbed in significant quantities.

In all treated plant materials, there was a rapid increase of radioactivity in residues of plant extracts that could not be recovered, even by repeated extractions. This evidence strongly suggests that the phosphoric acid formed during the metabolic degradation of Bidrin was incorporated extensively into normal phosphorus-containing constituents of the plants.

Absorption and Excretion by Insects. Adult boll weevils each were treated topically with 0.5 μg. of Bidrin-C^{14} or -P^{32} in 1 μl. of acetone and held in individual glass vials. For absorption studies, fifth-instar bollworm larvae were treated topically with 5.0 μg. of Bidrin-P^{32} in 1 μl. of acetone. For excretion studies, bollworm larvae were injected with 5.0 μg. of Bidrin-P^{32} in 1 μl. of water. Sample preparation and analytical procedures have been described in detail (*3*).

The absorption of Bidrin-C^{14} by adult boll weevils is shown in Figure 1. Contact loss of unabsorbed insecticide was minimized by holding the insects in individual glass vials; thus, radioactivity from containers was supplied primarily by excretion. Excreted radioactivity accumulated at nearly a consistent rate to 36% of the applied dose after 48 hours. The influence of excretion on internal radioactivity was demonstrated further by measurements of the rate of accumulation of internal radioactivity in dead weevils that were treated at the same dose-rate with Bidrin-C^{14}. Insects killed by freezing were treated immediately after confirmation of death and analyzed at the same time intervals as were surviving weevils. Despite possible changes in cuticle permeability, data from tests with dead weevils were useful for evaluation of the accumulation of internal radioactivity in the absence of elimination processes. During the first 4 hours after treatment, the toxicant penetrated the cuticle of dead insects at a rate only slightly faster than was observed in living insects. Internal accumulations of radioactivity were significantly higher in dead insects at succeeding time intervals, however, and the absorption curve apparently reached a constant level after 8 hours. Examination of the composite data from tests with living and dead insects suggested the possibility that externally applied Bidrin tended to "partition" rapidly through the cuticle into the body until a certain saturation level was attained either internally or in the cuticle. These data indicate that once the saturation level was reached, further penetration was dependent on removal by excretion of internal accumulations of Bidrin or its metabolites.

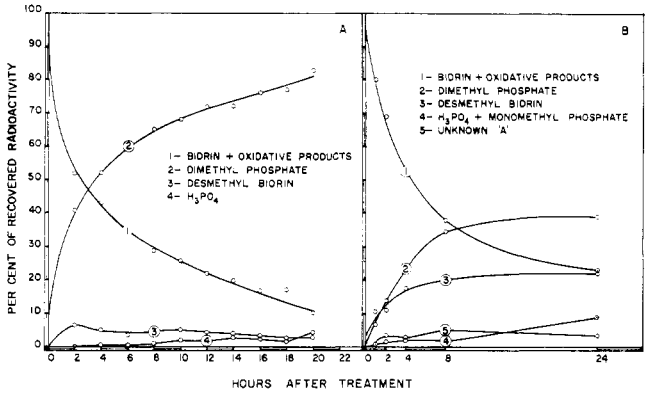

Figure 3. Relative concentrations of Bidrin and its phosphorus-containing metabolites in (*A*) urine of white rats and (*B*) internal extracts of fifth-instar bollworm larvae at different times after injection with Bidrin-P^{32}

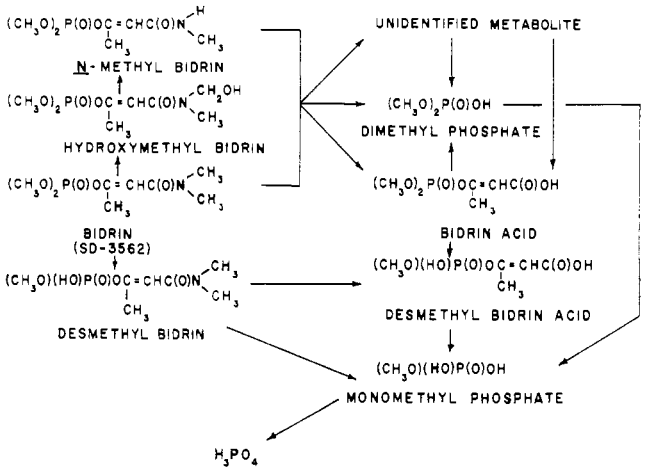

Figure 4. Tentative pathway for the formation of phosphorus-containing metabolites of Bidrin

Table VI. Concentrations of Bidrin and Its Metabolites in Urine of Rats Treated Subcutaneously with Bidrin-P^{32} (3 Mg. per Rat)[a,b]

Product	Bidrin-P^{32} μg.-Equivalents at Indicated Hours after Treatment				
	0–2	4–6	10–12	14–18	18–20
Hydroxymethyl Bidrin	209.3	104.3	12.8	9.8	2.6
N-Methyl Bidrin	88.5	27.6	5.2	5.1	2.0
Bidrin	10.2	6.1	1.0	1.1	0.4
Hydrolytic products	378.0	281.0	71.0	72.0	45.0

[a] Chromatographic System *B*.
[b] Total Bidrin-P^{32} μg.-equivalents excreted after 24 hours: urine, 627 μg. ($CHCl_3$-soluble), 1788 μg. (H_2O-soluble); feces, 51 μg.

Table VII. Relative Concentrations of Bidrin and Its Metabolites Recovered from Adult Boll Weevils Following Topical Treatment with 0.7 μg. of Bidrin-P^{32}[a]

Product	Per Cent of Recovered Radioactivity as Bidrin-P^{32} Equivalents at Indicated Hours after Treatment					
	2	4	8	24	48	72
			INTERNAL EXTRACTS			
Phosphoric acid	1.6	1.4	1.9	5.1	6.0	7.1
Monomethyl phosphate	0.0	0.0	0.0	0.0	0.1	0.9
Dimethyl phosphate	8.0	7.8	8.2	8.9	9.5	17.7
Desmethyl Bidrin	3.1	7.1	7.7	12.2	25.4	26.0
Bidrin[b]	87.3	83.7	82.2	73.8	59.0	48.3
			EXTERNAL RINSES			
Dimethyl phosphate	2.5	3.0	2.9	3.0	8.4	5.6
Desmethyl Bidrin	2.5	3.3	4.2	2.4	14.6	12.1
Bidrin[b]	95.0	93.7	92.9	94.6	77.0	82.3
			FECAL EXTRACTS			
Phosphoric acid	...	...	...	4.1	4.2	7.6
Monomethyl phosphate	...	...	...	3.6	5.7	1.3
Dimethyl phosphate	...	...	...	15.8	17.4	27.8
Desmethyl Bidrin	...	...	...	14.9	11.8	7.6
Bidrin[b]	...	...	...	61.6	60.9	55.7

[a] Chromatographic System *A*. [b] Bidrin plus oxidative metabolites.

Fifth-instar bollworm larvae absorbed topically applied Bidrin-P^{32} at an extremely rapid rate (Figure 2*B*). Figure 2*A* demonstrates the cumulative rate of excretion of injected Bidrin-P^{32} and its metabolites by fifth-instar bollworm larvae. After 96 hours, 75% of the dose was excreted, 15% was recovered from internal extracts of survivors in the form of dimethyl phosphate and inorganic phosphate, and 3% could not be extracted from the tissues by the procedures used. Despite rapid penetration, the highly efficient excretory mechanisms coupled with rapid metabolic degradation of the toxicant enabled the two insect species to tolerate relatively large doses of Bidrin.

Metabolism in Insects and Rats. Fifth-instar bollworm larvae were treated by injection or by feeding on treated cotton seedlings, and adult boll weevils were treated topically at the same doses used for absorption and excretion studies described above. Surviving insects were sacrificed at the designated times, homogenized (unabsorbed insecticide first was removed from weevils (*3*) and analyzed separately) in water, and the extracts prepared for chromatographic analysis as described above for plants. Feces of both insect species were collected periodically and extracted similarly. Male white rats were each injected subcutaneously with a sublethal 10 mg. per kg. dose of aqueous Bidrin-P^{32} and placed in a metabolism cage that was suspended over an automatic fraction collector where urine samples were collected at 2-hour intervals for 24 hours.

The results of studies of the degradation of Bidrin-P^{32} by rats and fifth-instar bollworm larvae are shown in Figure 3. Hydrolysis of the vinyl-phosphate bond of Bidrin (or its oxidative metabolites), to produce dimethyl phosphate was the predominate initial degradative step in rats (Figure 3*A*). Desmethyl Bidrin and inorganic phosphate also were detected in urine in minor concentrations. The detoxification of injected Bidrin was extremely rapid; after 20 hours 83% of the recovered radioactivity was in the form of dimethyl phosphate and after 24 hours the urine contained only traces of radioactivity. Bidrin and its metabolites were excreted rapidly by rats. After 6 hours, 65% of the injected dose was excreted and after 24 hours 83% was excreted. Table VI shows representative data on the actual quantities of chloroform and water-soluble products excreted during different time intervals. In bollworm larvae, hydrolytic attack at vinyl-phosphate and methyl-phosphate bonds of the Bidrin molecule resulted in the formation of dimethyl phosphate and desmethyl Bidrin at an approximate

able VIII. Relative Concentration of Bidrin and Its Degradative Products in :otton Seedlings Treated with Bidrin-P^{32} and in Feces of Fifth-Instar Bollworm Larvae Fed with Treated Seedlings[a]

Product	Per Cent of Recovered Radioactivity as P^{32}-Bidrin Equivalents at Indicated Days after Treatment of Seedlings					
	1		7		14	
	Cotyl[b]	Feces	Cotyl	Feces	Cotyl	Feces
hosphoric acid	2.2	2.0	10.3	15.8	18.1	19.6
lonomethyl phosphate	0.0	0.5	0.0	2.1	0.3	0.2
)esmethyl Bidrin acid	0.2	1.7	2.0	0.1	0.9	1.2
)imethyl phosphate	3.2	14.3	9.8	18.4	10.8	13.1
)esmethyl Bidrin	6.5	9.2	11.6	13.3	7.3	9.0
idrin acid	0.0	0.0	0.0	0.0	0.7	0.7
'nknown A	1.3	1.1	12.6	6.1	15.3	15.9
idrin[c]	86.6	71.2	53.7	44.2	46.6	40.3

[a] Chromatographic System *A*. [b] Cotyl = cotyledons of cotton seedlings. [c] Bidrin plus xidative metabolites.

)ncentration ratio of 2:1, respectively Figure 3*B*). Monomethyl phosphate, ıorganic phosphate, and unknown A also ere formed in bollworms in minor con-ntrations. The decomposition of in-cted Bidrin in bollworms was a rapid rocess; after 24 hours, only 21% of covered radioactivity was Bidrin or s oxidation products.

In white rats there was extensive oxi-ative conversion of Bidrin (Table VI). fter only 2 hours, the major chloroform ctractable materials in the urine were ıe two oxidative metabolites of Bidrin. he peak concentration (209.3 μg.) hydroxymethyl Bidrin was reached ter 2 hours then diminished rapidly succeeding time intervals as the metab-ite was converted to *N*-methyl Bidrin hydrolyzed. The data tend to indi-te that the extensive conversion of drin to its hydroxymethyl derivative ay be an important factor in accelerat-g hydrolytic degradation. Hydroxy-ethyl Bidrin might be expected to be ss stable than Bidrin or *N*-methyl drin. Since its formation was so pid in rats, and since the over-all oduction of hydrolysis products pro-eded at such a fast rate, then hydroxy-ethyl Bidrin could be the major target hydrolytic degradation. *N*-Methyl drin also was subject to hydrolytic eakdown because it did not tend to cumulate in urine and was degraded an insignificant concentration after 24 urs. The sequence of metabolic events ıpeared to be somewhat similar to at proposed for the oxidation and hy-olysis of schradan (*11*); however, a ore critical evaluation is needed before finite conclusions may be reached.

The metabolism of Bidrin by adult boll weevils was similar to that demonstrated in bollworms (Table VII). The phosphorus-containing metabolites of the absorbed insecticide were primarily dimethyl phosphate and desmethyl Bidrin. Desmethyl Bidrin accumulated in internal extracts, but dimethyl phosphate tended to accumulate in feces, an indication that the later metabolite may be preferentially excreted by treated weevils. Unabsorbed insecticide was degraded slowly by the same processes that were described above for leaf and glass surfaces. In both insect species, Bidrin was oxidized to form the same products shown for plants and rats; but at the time of analyses, extracts contained insufficient activity for quantitative determinations.

The degradation of Bidrin and its metabolites in the digestive tract of fifth-instar bollworm larvae was investigated. Larvae were fed for 8 hours on cotton seedlings that had been treated with Bidrin-P^{32}. The relative concentrations of Bidrin and its phosphorus-containing metabolites, detected in larval feces and in treated seedlings at the time of feeding, are shown in Table VIII. Only moderate alteration of ingested products occurred in the digestive tracts of bollworms. There was no apparent deviation from metabolic pathways described for injection treatments and no more than about 15% of the toxic materials (Bidrin and oxidation products) was decomposed.

Based on information from experiments with plants and animals, a tentative, composite pathway for the metabolism of Bidrin is proposed (Figure IV). The metabolic routes discussed above for animals were relatively simple and straightforward; however, in plants the most important detoxification route cannot be defined completely until the unknown metabolite is identified.

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