Metabolism of 2-Methyl-2-(methylthio)propionaldehyde *O*-(Methylcarbamoyl)oxime (Temik, UC-21149) in Insects

D. L. BULL, D. A. LINDQUIST, AND J. R. COPPEDGE

The fate of radiolabeled 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime (Temik, UC-21149) in insects was examined. Topically applied Temik was absorbed rapidly by adult boll weevils, *Anthonomus grandis* Boheman, but very slowly by third instar tobacco budworms, *Heliothis virescens* (F.). Oxidation at the sulfur atom was the predominant reaction in both species of insects, yielding primarily the sulfoxide derivative of Temik and to a lesser extent the sulfone. Traces of *N*-demethyl derivatives in excreta of the tobacco budworm indicated some oxidation at the *N*-methyl position. The principal products of hydrolysis were the sulfoxide and sulfone derivatives of 2-methyl-2-(methylthio)propionaldehyde oxime.

Temik [trademark for a 10% granular formulation of 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime (UC-21149), used in this publication to represent the technical product] is the first carbamate insecticide to demonstrate good systemic activity in cotton plants, and it also appears promising for control of certain major arthropod pests (Davis *et al.*, 1966; Hopkins and Taft, 1965; Ridgway and Gorzycki, 1965). The chemistry and biological activity of Temik and related *O*-(methylcarbamoyl)oximes (Bartley *et al.*, 1966; Payne *et al.*, 1966; Weiden *et al.*, 1965) and the metabolism of Temik in cotton plants (Metcalf *et al.*, 1966; Coppedge *et al.*, 1967), houseflies, *Musca domestica* L., (Metcalf *et al.*, 1966), and mammals (Knaak *et al.*, 1966; Andrawes *et al.*) have been reported. This report deals with the fate of Temik in certain insect pests of cotton.

Materials and Methods

Chemicals. Difference samples of Temik radiolabeled either with C^{14} at the *S*-methyl (specific activity 4.9 mc. per mmole), tertiary (3.9 mc. per mmole), and carbonyl (2.5 mc. per mmole) positions, or with S^{35} (initial specific activity of 10.8 and 55.6 mc. per mmole) were supplied by Union Carbide Co., Clayton, N.C., or synthesized by their procedures (Bartley *et al.*, 1966). All radiolabeled compounds administered to insects had a chromatographic purity of 98% or greater.

Also supplied by Union Carbide Co. were pure samples of the following potential metabolites of Temik: Temik sulfoxide, 2-methyl-2-(methylsulfinyl)-propionaldehyde *O*-(methylcarbamoyl)oxime; Temik sulfone, 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime; *N*-demethyl Temik, 2-methyl 2-(methylthio)propionaldehyde *O*-carbamoyloxime; oxime, 2-methyl-2-(methylthio)propionaldehyde oxime; oxime sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldehyde oxime; oxime sulfone, 2-methyl-2-(methylsulfonyl)propionaldehyde oxime; nitrile, 2-methyl-2 (methylthio)propionitrile; nitrile sulfoxide, 2-methyl-2-(methylsulfinyl)propionitrile; nitrile sulfone, 2-methyl-2-(methylsulfonyl)propionitrile; acid, 2-methyl-2-(methylthio)propionic acid; acid sulfoxide, 2-methyl-2-(methylsulfinyl)propionic acid; acid sulfone, 2-methyl-2-(methylsulfonyl)propionic acid.

N-demethyl Temik sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-carbamoyl oxime, and *N*-demethyl Temik sulfone, 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-carbamoyl oxime, were prepared by the chemical oxidation of *N*-demethyl Temik with one and two equivalents, respectively, of *m*-chloroperoxybenzoic acid in a chloroform solution, and isolated by thin-layer chromatography.

Insects and Their Treatment. Adult boll weevils, *Anthonomus grandis* Boheman, and houseflies, and third or fifth instar bollworms, *Heliothis zea* (Boddie), and tobacco budworms, *Heliothis virescens* (F.), were selected at random from insecticide-susceptible laboratory colonies for use in different experiments. All insects were reared and tested under continuous light at 27° C.

Before treatment, the insects were anesthetized lightly with CO_2. Solutions of insecticide for the studies of both toxicity and metabolism were administered with a calibrated, micrometer-driven syringe. For topical treatments of adult boll weevils, 0.25 μl. of a solution of acetone containing the insecticide was placed dorsally on the intersegmental membrane between the thorax and abdomen. On third instar lepidopterous larvae, topical doses (1 μl.) were placed on the dorsal abdominal surface. Fifth instar larvae were treated by a ventral injection of 1 μl. of an aqueous insecticide solution into the abdominal cavity. For metabolism studies, duplicate samples of 25 boll weevils or two lepidopterous larvae, each, were prepared per time interval and all experiments were repeated at least three times. Insects used for toxicity tests were allowed to feed during test periods; those used for studies of absorption and metabolism were confined without food in individual glass containers.

Preparation and Analysis of Extracts. If the insects were treated topically, unabsorbed radioactivity was recovered by rinsing them with five successive 10- to 15-ml. portions of acetone. Internal extracts were prepared by homogenizing treated insects in 10 ml. of distilled water at 4° C. Solids that precipitated after addition of 30 ml. of acetone were removed by centrifugation and then re-extracted three times with

Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, College Station, Tex. 77841.

Table I. R_f Values of Temik and Certain of Its Metabolites in Different Thin-Layer Chromatography Systems

Compound (Abbreviated Name)	System					
	A	*B*	*C*	*D*	*E*	*F*
	R_f Value					
Temik	0.87	0.84	0.82	0.84	0.92	0.98
Temik sulfoxide	0.16	0.10	0.39	0.10	0.71	0.96
Temik sulfone	0.52	0.29	0.52	0.47	0.87	1.00
N-demethyl Temik	0.87	0.65	0.63	0.84	0.90	1.00
N-demethyl Temik sulfoxide	0.13	0.04	0.18	0.12	...	...
N-demethyl Temik sulfone	0.40	0.12	0.36	0.45	...	...
Oxime	0.97	0.85	...	0.96	0.93	1.00
Oxime sulfoxide	0.40	0.20	0.44	0.38	0.69	0.84
Oxime sulfone	0.87	0.55	0.80	0.84	0.85	0.96
Nitrile sulfoxide	0.59	0.51	0.57	0.54	0.80	0.96
Nitrile sulfone	0.87	0.79	0.80	0.84	0.86	1.00
Acid sulfoxide[a]	...	...	...	...	0.17	0.22
Acid sulfone[a]	...	...	...	...	0.93	0.98
Unknown(s) 1	0.00	0.00	0.00	0.00	...	...
Unknown 2	0.05	0.00	0.15	0.00	...	...
Unknown 3	0.22	0.10	...	0.10	...	...
Unknown 4	0.40	0.10	0.46	...	...	...

[a] These compounds streaked badly in all systems except *E* and *F*.

10-ml. portions of acetone. Acetone was removed under vacuum from combined acetone–water extracts. With lepidopterous larvae, the remaining aqueous volume was reduced to about 5 ml., diluted 1 to 4 (v./v.) with acetonitrile, and then extracted twice with petroleum ether. Petroleum ether removed much of the interfering lipoid materials but only traces of radioactivity. Excreta of treated insects, recovered from glass containers by scrubbing first with water and then with acetone, were processed similarly. After radioassay, extracts were concentrated under vacuum or in a stream of dry nitrogen and chromatographed.

Samples of tissue containing unextractable S^{35} were digested in boiling nitric acid. After removal of the color with hydrogen peroxide, aliquots of the solutions were radioassayed by liquid scintillation. Tissues containing C^{14} were dried, pulverized, and radioassayed in planchets with a gas-flow, Geiger-Mueller detector.

Chromatography. Thin-layer chromatography (TLC) was employed using silica gel G on glass (0.25 mm. thick) with solvent mixtures (v./v.) as follows: system *A*, 1 to 1 benzene and dioxane; system *B*, 5:1:1:1 chloroform, *n*-hexane, ethyl acetate, and dioxane; system *C*, 5:1:1:1 chloroform, *n*-hexane, ethyl acetate, and ethanol; system *D*, 4:1:1 diethyl ether, *n*-hexane, and ethanol; system *E*, 4 to 1 acetonitrile and water, and microcrystalline cellulose on glass (0.25 mm. thick) with 4 to 1 acetonitrile and water (system *F*). R_f values of Temik and certain of its metabolites are listed in Table I.

After chromatographic development, radioactive areas were located by autoradiography; authentic compounds were located colorimetrically with potassium permanganate (1% in water) or *N*,2,6-trichloro-*p*-benzoquinoneimine (0.2% in ethanol).

Identifications were based primarily on coincidence of the radioactive products with authentic compounds after two-dimensional development in several combinations of different solvent systems. Additional information supporting tentative identifications was obtained by use of the different radiolabels of Temik, and by observing products formed after chemical alteration of metabolites.

Anticholinesterase Studies. Stock solutions of Temik and its sulfoxide and sulfone derivatives were prepared first in ethanol and then diluted to the desired concentrations with sodium bicarbonate buffer (Moorefield and Tefft, 1958) immediately before each analysis. The final concentrations of ethanol never exceeded 1% and did not interfere with enzyme activity. The anticholinesterase activity of these inhibitors was assessed manometrically with a differential respirometer by using the general procedures of Moorefield and Tefft (1958). Sources of enzyme included homogenates of housefly heads (final concentration of 1 per ml.), whole boll weevils (2 per ml.), whole fifth instar lepidopterous larvae (0.15 per ml.), and bovine erythrocyte acetylcholinesterase (2.5 units per ml., purchased from Sigma Chemical Co., St. Louis, Mo.).

Results

Toxicity Tests. Results of topical toxicity tests with adult boll weevils are shown in Table II. After 72

Table II. Influence of Synergists on the Toxicity of Temik and Temik Sulfoxide Applied Topically to Adult Boll Weevils[a]

Toxicant, μg. per Insect	Synergist	72-Hour Mortality at Indicated Ratio of Toxicant-Synergist, %						
		1:0	1:1	1:5	1:10	1:20	1:50	1:100
Temik (0.1)	Sesamex	13	36	52	77	..	91	..
Temik (0.1)	Piperonyl butoxide	13	16	26	39	..	51	77
Temik sulfoxide (0.2)	Sesamex	20	..	..	96	..	..	..
Temik sulfoxide (0.1)	Sesamex	0	..	..	..	50	..	..

[a] LD_{50} concentrations after 72 hours: Temik 0.22 μg. per weevil and Temik sulfoxide 0.42 μg. per weevil.

hours, the LD_{50} concentration of Temik was 0.22 μg. per insect, that of Temik sulfoxide was 0.42 μg. per insect, and that of Temik sulfone was greater than 25 μg. per insect. Combination with the synergistic compounds sesamex and piperonyl butoxide increased the toxicity of Temik to adult weevils. For example, at a toxicant to synergist ratio of 1 to 50, sesamex caused a sevenfold increase and piperonyl butoxide a fourfold increase in the toxicity of Temik. Use of sesamex also increased the toxicity of Temik sulfoxide.

Toxicity tests with lepidopterous larvae were somewhat erratic because of the apparent high tolerance of these insects to Temik. The approximate 72-hour LD_{50} dose of Temik applied topically to third instar tobacco budworm larvae (30- to 40-mg. weight range) was 20 μg. per insect. When fifth instar larvae were injected with solutions of Temik in a mixture of water, propylene glycol, and ethanol (1:1:1 v./v.), the approximate 72-hour LD_{50} concentrations were 30 μg. per bollworm and 50 μg. per tobacco budworm. In combination with sesamex (1 to 10), the LD_{50} concentrations of Temik were reduced to 10 μg. per bollworm and 30 μg. per tobacco budworm.

Anticholinesterase Studies. Results were in general agreement with those of Payne *et al.* (1966). In all cases where inhibition was observed, Temik sulfoxide was a more active anticholinesterase agent than Temik or Temik sulfone (Table III). Compared, for example, with Temik and Temik sulfone, respectively, Temik sulfoxide was 47 and 25 times more effective in inhibiting fly-head activity, 23 and 60 times more effective against pure bovine erythrocyte acetylcholinesterase, and about twice as effective with weevil homogenates. Even at very high concentrations, none of the compounds inhibited the hydrolysis of acetylcholine by homogenates of lepidopterous larvae. However, final assessment of the importance of the latter result to the great tolerance of these insects to injected doses of Temik must await more critical studies with an at least partially purified cholinesterase. The poor in vitro inhibition of the activity of the larval homogenates could not be attributed to extensive degradation of Temik. Control studies with S^{35}-Temik under identical assay conditions demonstrated that metabolism in vitro was negligible; only very small concentrations of Temik sulfoxide were formed.

Absorption. Studies of the absorption of Temik by adult boll weevils (Figure 1) demonstrated a triphasic

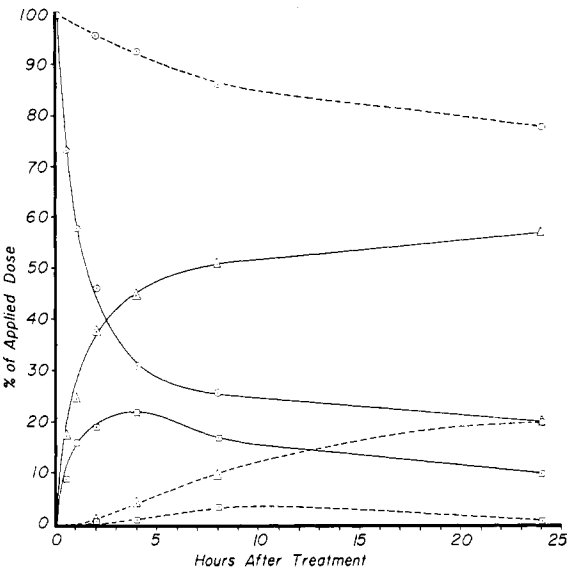

Figure 1. Absorption of S^{35}-Temik by adult boll weevils (solid lines) and third instar tobacco budworm larvae (broken lines)

⊙ = external, ◻ = internal, △ = excreta

penetration pattern that was somewhat similar to that reported for comparable tests with other animals (Buerger and O'Brien, 1966). During the first hour after topical treatment of individual weevils with 0.1 μg. in 0.25 μl. of acetone, S^{35}-Temik penetrated the cuticle at a rapid rate (42% of the dose). Through the next 3 hours, the rate of absorption was slower (27% of the dose), and it was much slower from 4 through 24 hours. The rate at which absorbed radioactivity was excreted was particularly striking. After only 1 hour, 25% of the applied dose was recovered from containers. (Special tests were made to demonstrate that externally applied insecticide was actually absorbed and not lost by contact of the treated insects with the container walls; the treated insects were either suspended individually from threads, or the dose was placed under elytra, thus eliminating any chance of rub-off. These tests confirmed the rapid disappearance of external radioactivity and also the rapid rate of excretion.) The maximum internal accumulation of radioactivity (22%) was reached at 4 hours after treatment, a time coincident with severe symptoms of cholinesterase inhibition.

Table III. Anticholinesterase Activity of Temik and Its Sulfoxide and Sulfone Derivatives

Enzyme Source[a]	I_{50}, Molar		
	Temik	Temik sulfoxide	Temik sulfone
Housefly heads	4.7×10^{-5}	1.0×10^{-6}	2.5×10^{-5}
Whole boll weevils	1.0×10^{-4}	5.8×10^{-5}	1.1×10^{-4}
Bovine erythrocyte AChE	1.8×10^{-5}	8.1×10^{-7}	4.9×10^{-5}
Whole fifth instar bollworms[b]	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$
Whole fifth instar tobacco budworms[b]	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$

[a] Substrate was acetylcholine bromide at a final molar concentration of 0.01 for housefly heads and 0.005 for all others.
[b] No inhibition was obtained at indicated concentrations.

Comparable tests demonstrated that S^{35}-labeled Temik sulfoxide was absorbed at a slower rate by weevils than was Temik. For example at 1, 4, and 24 hours after treatment, unabsorbed radioactivity accounted for 70, 61, and 34% of the applied dose, respectively. Temik sulfone was absorbed by weevils at an even slower rate; after 1, 4, and 24 hours, unabsorbed radioactivity accounted for 97, 91, and 84% of the dose, respectively.

S^{35}-Temik was absorbed very slowly by third intsar tobacco budworm larvae (Figure 1). Even after 24 hours, 78% of the applied dose was recovered in the external rinse, and after 48 hours, 60% still remained. Of particular significance were the very low levels of internal radioactivity at all sample times; the maximum internal concentration measured was only 3.5% at 8 hours after treatment.

Metabolism in Boll Weevils. The fate of S^{35}-Temik after topical treatment of adult boll weevils (0.1 μg. per insect) is shown in Table IV. Substantial amounts of Temik were oxidized to Temik sulfoxide on the external surfaces of treated insects, but little hydrolysis occurred. Temik and at least five metabolites were detected and measured in excreta and internal extracts. The major metabolite at all times was Temik sulfoxide. Also recovered were the sulfone derivative of Temik and the hydrolytic products, oxime sulfoxide and oxime sulfone. The relatively large concentrations of parent compound excreted and the preponderance of toxic metabolites in internal extracts, even after 24 hours, were of particular interest. Over-all concentrations of degradative products were low. Also, a certain amount of radioactivity [unknown(s) 1] remained at the origin of TLC system *B*. As many as 12 unidentified metabolites could be resolved from unknown(s) 1 in system *F*.

Metabolism in Lepidopterous Larvae. Injection studies were made with both lepidopterous species. Except for a slightly slower metabolic rate in bollworms, results were very similar and only those obtained in experiments with tobacco budworms are presented.

Data summarized in Tables V to VII compare the metabolism of similar injected doses of Temik, Temik sulfoxide, and Temik sulfone in fifth instar tobacco budworms. The oxidative derivatives of radiolabeled Temik were prepared by chemical oxidation with *m*-chloroperoxybenzoic acid and isolated with TLC system *B*.

Table IV. Relative Concentration of S^{35}-Temik and Its Metabolites after Topical Treatment of Adult Boll Weevils (0.1 μg. per Insect)[a]

Product	Percentage of Applied Dose at Indicated Hours after Treatment					
	0	1	2	4	8	24
	External					
Temik sulfoxide	0.8	11.4	24.1	17.5	13.8	13.3
Oxime sulfoxide	0.0	0.0	0.0	0.0	0.0	4.3
Temik	99.2	46.6	21.4	13.5	12.2	2.5
	Internal					
Unknown(s) 1	...	0.2	1.0	0.5	0.5	1.0
Temik sulfoxide	...	3.4	6.5	9.0	5.6	3.7
Oxime sulfoxide	...	0.4	0.8	1.3	1.2	0.8
Temik sulfone	...	0.3	0.7	1.8	1.4	1.4
Oxime sulfone	...	0.3	1.3	1.0	0.8	0.8
Temik	...	11.4	8.8	8.4	7.4	2.2
	Excreta (cumulative)					
Unknown(s) 1	...	0.3	0.5	0.6	0.7	0.9
Temik sulfoxide	...	9.8	12.6	14.2	15.6	17.0
Oxime sulfoxide	..	1.1	1.5	1.6	1.8	2.0
Temik sulfone	...	0.0	0.0	0.2	0.3	0.5
Oxime sulfone	...	0.8	1.2	1.3	1.4	1.6
Temik	...	13.1	20.3	27.2	31.3	35.0

[a] Analyses done in system *B*.

Table V. Relative Concentrations of Temik and Its Metabolites in Fifth Instar Tobacco Budworm Larvae after Individual Injections with 5 μg.[a]

Product	Percentage of Applied Dose at Indicated Hours after Treatment					
	0	1	2	4	8	24
	Internal					
Unknown(s) 1	0.0	1.0	1.0	1.2	1.3	1.0
Unknown 2	0.0	0.7	0.0	0.0	0.0	0.0
Temik sulfoxide	5.8	61.0	62.1	54.7	31.5	5.9
Oxime sulfoxide	0.0	2.0	2.8	3.0	6.9	3.6
Temik sulfone	0.0	1.6	1.3	1.6	1.8	0.3
Nitrile sulfoxide	0.0	1.3	1.5	1.3	1.2	0.5
Oxime sulfone	0.0	1.2	2.4	1.4	1.0	0.5
Nitrile sulfone	0.0	0.4	0.0	0.0	0.4	0.0
Temik	94.2	17.8	6.9	2.8	0.9	1.2
	Excreta (cumulative)					
Unknown(s) 1	...	0.6	1.0	1.4	2.3	4.7
Unknown 2	...	0.1	0.2	0.3	0.4	0.6
Temik sulfoxide	...	7.5	15.7	25.1	37.9	51.6
Unknown 3	...	0.1	0.2	0.3	0.5	0.7
Oxime sulfoxide	...	0.3	0.5	0.9	2.2	6.8
Temik sulfone	...	0.1	0.2	0.4	0.8	1.7
Nitrile sulfoxide	...	0.1	0.2	0.3	0.5	1.1
Oxime sulfone	...	0.1	0.2	0.4	0.7	1.3
Nitrile sulfone	...	0.1	0.2	0.3	0.4	0.6
Temik	...	3.0	3.4	4.0	4.1	4.2

[a] Data obtained by combining results of tests with C^{14}-(*S*-methyl)- and S^{35}-labeled Temik, and analyses in systems *A*, *B*, and *D*.

Temik was metabolized at an extremely rapid rate by budworms, primarily by oxidative conversion to the sulfoxide (Table V). After 1 hour, only 17.8% of the dose remained in internal extracts as unchanged Temik, and only minor amounts were excreted. Temik sulfoxide accumulated to a peak internal concentration after 2 hours (62.1%) and then declined as it was excreted or converted to other products. Other tentatively identified metabolites, formed in lesser concentrations, included Temik sulfone, the sulfoxide and sulfone derivatives of the oxime, nitrile sulfoxide, and nitrile sulfone. Also three unidentified products were detected: Unknown(s) 1 was formed from all radiolabels of Temik and was comparable with that reported for boll weevils as it included several products, at least three of which retained the carbonyl group. Unknowns 2 and 3 were formed from all radiolabels of Temik except carbonyl-C^{14} but were not comparable with any of the available theoretical metabolites including the acid or its oxidative derivatives. At no time was any *N*-demethyl Temik detected; however, trace amounts of products that cochromatographed with its sulfoxide and sulfone derivatives were detected in samples of high activity.

The general procedure of Jeffay and Alvarez (1961) was used to trap $C^{14}O_2$ expired by fifth instar tobacco budworms that were individually injected with 5 μg. of either C^{14}-(*S*-methyl)- or C^{14}-(carbonyl)-Temik. Insects were processed in groups of 10 each, and tests were duplicated. Larvae treated with C^{14}-(*S*-methyl)-Temik expired no $C^{14}O_2$ through 24 hours; those treated with C^{14}-(carbonyl)-Temik expired 25% of the dose as $C^{14}O_2$ during the same period of time. When larvae were treated with a combination of sesamex and C^{14}-(carbonyl)-Temik (10 to 1, w./w.), the evolution of $C^{14}O_2$ was reduced to 15.6% of the dose through 24 hours. A study of metabolism indicated that the most prominent change caused by the inclusion of sesamex was a reduction in the rate of the initial oxidation of Temik. For example, after 1 and 4 hours, respectively, internal extracts of larvae treated with Temik contained 15.3 and 3.3% of the injected dose as the unchanged parent compound. At the same times, in larvae injected with sesamex and Temik, 50.7 and 20.0% of the dose remained in the parent form.

The metabolites detected in tobacco budworm larvae injected with S^{35}-Temik sulfoxide (Table VI) were similar to those reported for Temik. Of particular interest was the very slow rate at which the compound was metabolized. After 24 hours, the combined amount of unchanged Temik sulfoxide in excreta and internal extracts was 57.4% of the dose.

The larvae metabolized S^{35}-Temik sulfone at a more rapid rate than the sulfoxide, but at a somewhat slower rate than Temik (Table VII). Relatively large concentrations of unknown(s) 1 were detected in internal extracts and in excreta, and another metabolite (unknown 4) was detected that did not correspond to any theoretical metabolites available.

The oxime was not detected in any extracts of insects treated with Temik. This compound is somewhat volatile and may have been lost from the chromatoplates during analysis. Other possibilities were that oxidation of Temik always preceded hydrolysis or that the

Table VI. Relative Concentrations of S^{35}-Temik Sulfoxide and Metabolites in Tobacco Budworms after Individual Injection with 5 μg.[a]

Product	Percentage of Applied Dose at Indicated Hours after Treatment					
	0	1	2	4	8	24
			Internal			
Unknown(s) 1	0.0	0.0	0.7	1.2	3.3	1.0
Temik sulfoxide	94.4	70.3	56.4	40.5	21.8	9.0
Oxime sulfoxide	1.3	5.5	6.8	7.1	7.8	2.1
Temik sulfone	3.8	1.9	3.1	3.1	1.6	0.4
Nitrile sulfoxide	0.5	5.8	5.5	4.6	2.9	5.4
Oxime sulfone	0.0	0.0	0.0	2.5	3.7	1.7
			Excreta (cumulative)			
Unknown(s) 1	...	0.1	0.2	1.6	3.5	4.0
Unknown 2	...	0.0	0.0	1.1	1.8	5.0
Temik sulfoxide	...	12.6	20.1	26.5	37.3	48.4
Unknown 3	...	0.1	0.2	1.7	1.8	2.4
Oxime sulfoxide	...	0.8	1.3	2.0	3.5	8.4
Temik sulfone	...	0.3	0.6	1.2	2.1	2.7
Nitrile sulfoxide	...	0.6	0.6	1.0	1.6	2.1
Oxime sulfone	...	0.0	0.0	0.0	0.3	0.4

[a] Analyses done in systems *A* and *C*.

Table VII. Relative Concentrations of S^{35}-Temik Sulfone and Metabolites in Tobacco Budworms after Individual Injections with 5μg.[a]

Product	Percentage of Applied Dose at Indicated Hours after Treatment					
	0	1	2	4	8	24
			Internal			
Unknown(s) 1	0.4	1.9	10.3	18.4	27.1	21.3
Unknown 2	0.0	0.0	0.6	0.6	0.8	0.5
Unknown 4	0.0	2.5	2.0	1.9	4.3	0.2
Temik sulfone	97.9	83.2	57.4	36.9	9.3	3.0
Oxime sulfone	1.6	6.5	13.7	10.1	6.5	0.3
Nitrile sulfone	0.1	1.0	1.4	1.7	2.3	5.7
			Excreta (cumulative)			
Unknown(s) 1	0.0	0.0	0.2	1.3	4.9	11.8
Unknown 2	0.0	0.0	0.0	0.2	0.7	1.4
Unknown 4	0.0	0.1	0.3	1.5	5.6	9.4
Temik sulfone	0.0	4.5	11.8	18.8	25.6	29.8
Oxime sulfone	0.0	0.2	0.5	0.7	2.3	3.7
Nitrile sulfone	0.0	0.0	0.1	0.2	0.3	0.3

[a] Analyses done in systems *A* and *B*.

oxime was oxidized to the sulfoxide as rapidly as it was formed, thus precluding isolation of unchanged oxime.

The metabolism of C^{14}-(*S*-methyl)oxime in tobacco budworms was studied by using an injected dose of 42.8 μg. per larva. Results (Table VIII) indicated the oxime was oxidized very rapidly to the sulfoxide; no parent material was recovered, even in samples prepared immediately after treatment. Percentage recoveries of the injected radioactivity ruled out excessive loss of oxime through volatilization. Substantial portions of radioactivity remained at the origin of system *A* chromatoplates; in system *F* this radioactivity separated into 11 metabolites, none of which was identified. Relatively small amounts of nitrile sulfoxide and oxime sulfone also were detected.

To support the tentative identifications, radiolabeled Temik and its metabolites were subjected to certain chemical reactions, and ensuing products were identified by two-dimensional cochromatography with authentic standards. As expected, oxidation with *m*-chloroperoxybenzoic acid converted Temik (the only sulfide recovered) to Temik sulfoxide and all sulfoxide derivatives were converted to the corresponding sulfones. No oxidative alteration of the *N*-methyl group of different carbamates was detected. Hydrolysis with alcoholic potassium hydroxide (0.2%) resulted in degradation of all carbamates to their respective oximes; however, oximes and nitriles were not changed. When benzene solutions of different metabolites were reacted with an excess of methyl isocyanate in a sealed ampoule for 12 hours at 37° C., oxime derivatives were converted to their respective carbamates, but nitriles and carbamates were not changed.

Table VIII. Relative Concentrations of C^{14}-(*S*-Methyl)-oxime in Fifth Instar Tobacco Budworm Larvae after Individual Injections with 42.8 μg.[a]

Compound	Percentage of Applied Dose at Indicated Hours after Treatment					
	0	1	2	4	8	24
	Internal					
Unknown(s) remaining at base line	17.2	39.3	18.9	6.7	3.1	3.1
Oxime sulfoxide	78.9	29.6	26.4	22.9	11.1	5.5
Nitrile sulfoxide	3.9	5.2	7.0	2.1	2.0	0.5
Oxime sulfone	0.0	2.6	1.6	1.3	1.1	1.3
	Excreta (cumulative)					
Unknown(s) remaining at base line	...	12.9	31.5	51.1	61.3	67.0
Oxime sulfoxide	...	2.1	4.5	5.1	9.7	11.4
Nitrile sulfoxide	...	0.6	1.0	2.3	2.9	3.3

[a] Analyses done in system *A*.

Discussion and Conclusions

In field experiments, Temik has been very effective against boll weevils and certain other cotton pests; however, its use often leads to substantial increases in populations of tobacco budworms and bollworms (Hopkins and Taft, 1965; Ridgway *et al.*, 1967). One apparent factor contributing to these increases is the reduction in numbers of beneficial insects that normally help keep the lepidopterous species in check (Ridgway *et al.*, 1967). A second factor, demonstrated by toxicity tests, is the tolerance of tobacco budworm and bollworm larvae to relatively large concentrations of Temik.

The experimental evidence provided a good explanation of the susceptibility of adult boll weevils to topically applied Temik. The compound was absorbed rapidly and was not extensively degraded. Although excreted freely, substantial concentrations of Temik and its toxic oxidative derivatives tended to accumulate internally.

Physiological mechanisms that protect lepidopterous larvae from the toxic effects of Temik are less clear. Certainly topical treatments are ineffective because Temik is absorbed very slowly through the larval cuticle and the toxicants do not accumulate internally. The low effectiveness of injected doses is puzzling because, though a certain amount of hydrolytic degradation occurs, the predominant metabolic products are toxic compounds—particularly the highly toxic sulfoxide derivative of Temik. Temik sulfoxide was formed very rapidly from Temik and was surprisingly stable to enzymatic attack. Toxic compounds were excreted readily by larvae, but relatively large concentrations persisted internally during the critical first 8 hours after treatment. According to Heywood (1965), Temik sulfoxide is strongly basic and highly soluble in water (>33%). These properties might limit the penetration of the compound into the nerve cords of larvae.

In vitro studies of the anticholinesterase activity of Temik and its sulfoxide and sulfone derivatives may have provided another possible clue to the high tolerance of the lepidopterous larvae. The toxic compounds, particularly Temik sulfoxide, were relatively effective as inhibitors of boll weevil cholinesterase activity but did not influence the activity of lepidopterous larval homogenates, even at high concentrations. The cholinesterase of boll weevils has properties very similar to those reported for acetylcholinesterase from various animals, but the enzyme in bollworm and tobacco budworm homogenates that hydrolyzes choline esters appears to be somewhat different. Preliminary studies have shown the enzyme in whole-body or head homogenates is not inhibited by excess acetylcholine and that the rate of hydrolysis of choline esters increases with increasing carbon atoms in the acidic moiety of the molecule. Therefore, since Temik was specifically designed to resemble acetylcholine structurally (Payne *et al.*, 1966), its geometric configuration may not permit an optimum interaction with the active site of the lepidopteran cholinesterase. These considerations are still under investigation.

The general pathway for the metabolism of Temik in insects appears similar to that reported in other biological systems, except for the expected differences in reaction rates. The sulfur atom of the Temik molecule is oxidized readily, a reaction that occurs both nonenzymatically and by enzyme action. Experimental evidence suggests that this reaction precedes all others. Further oxidation of the sulfoxide to the sulfone definitely occurs in insects but at a much slower rate. Once formed, the sulfoxide and sulfone derivatives of Temik are surprisingly stable to degradative attack. Also, at least some *N*-methyl oxidation occurred, as indicated by the apparent recovery of traces of the sulfoxide and sulfone derivatives of *N*-demethyl Temik in excreta of tobacco budworms that had been treated with Temik. Perhaps the intermediates of this oxidative *N*-dealkylation were accounted for by the carbamates that remained at the base lines of the chromatograms. The toxic carbamates apparently were detoxified primarily by hydrolysis, which resulted in the formation of oxime derivatives. Small amounts of nitrile derivatives were formed during the metabolism of different carbamates; however, their significance cannot be assessed until the exact mechanism of their formation is established. Metabolites remaining at the base lines of chromatograms may have included conjugates or natural products that had incorporated radioactive fragments.

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