Fate of 2-Methyl-2-(methylthio)propionaldehyde *O*-(Methylcarbamoyl) oxime (Temik) in Cotton Plants and Soil

J. R. Coppedge,[1] D. A. Lindquist,[1] D. L. Bull,[1] and H. W. Dorough[2]

In the cotton plant, Temik [10% granular formulation of UC-21149, 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] was rapidly metabolized to its sulfinyl derivative (Temik-sulfoxide); further oxidation to the sulfonyl compound (Temik-sulfone) was slow. No evidence of oxidative *N*-demethylation was found. The sulfinyl derivative was degraded primarily by hydrolysis to a sulfinyl-oxime. Both Temik-sulfoxide and Temik-sulfone were stable in cotton. Relatively large amounts of radioactivity were lost from C^{14}-Temik-treated excised cotton leaves. In the three soil types studied under laboratory conditions, Temik was degraded more slowly than in cotton. However, similar metabolites were recovered from both cotton plants and soil.

Temik [registered trade-mark for a 10% granular formulation of 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, UC-21149; Temik is used interchangeably with UC-21149 in this manuscript] is effective as a systemic insecticide against a wide spectrum of phytophagous insects and mites including several species that attack cotton. Experimental results have shown that Temik is effective for 7 to 9 weeks against the cotton aphid, *Aphis gossypii* Glover, thrips, *Frankliniella* spp., and the cotton fleahopper, *Psallus seriatus* (Reuter) after in-furrow application at time of planting (Davis *et al.*, 1966; Hopkins and Taft, 1965). Side dress applications of Temik were effective for 2 to 3 weeks against cotton fleahoppers (Cowan *et al.*, 1966) and lygus bugs, *Lygus hesperus* Knight (Ridgway *et al.*, 1966) and also show promise for control of the boll weevil, *Anthonomus grandis* Boheman (Hopkins and Taft, 1965), and spider mites, *Tetranychus* spp. (Hopkins and Taft, 1965; Weiden *et al.*, 1965). Temik is not effective against the bollworm, *Heliothis zea* (Boddie), or the cabbage looper, *Trichoplusia ni* (Hübner) (Hopkins and Taft, 1965).

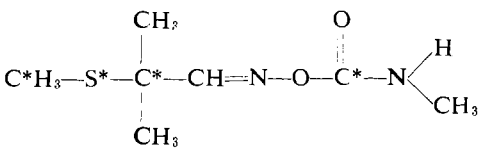
Recent studies on the fate of Temik in the bollworm (Bull *et al.*, 1967) indicate that slow rates of cuticular penetration and rapid metabolism and excretion are some of the reasons for poor bollworm control. Also, these studies indicated the possibility that Temik did not inhibit the cholinesterase of bollworms nearly as efficiently as cholinesterases from other sources. The pathway of Temik metabolism in the housefly (Metcalf *et al.*, 1966) follows the route of Temik → Temik-sulfoxide → oxime-sulfoxide and Temik-sulfone (see Table I for chemical names). In rats, Temik was rapidly oxidized to Temik-sulfoxide, 2-methyl-2 (methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime, and to oxime-sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldoxime (Andrawes *et al.*, 1967; Knaak *et al.*, 1966) and excreted primarily in the urine.

A recent article (Metcalf *et al.*, 1966) describes the metabolism of Temik in cotton using isolated leaves, stem injection, and direct application of the Temik to the stem of cotton plants. Essentially these authors found the major pathway of Temik metabolism in cotton leaves to be Temik → Temik-sulfoxide → oxime-sulfoxide and Temik-sulfone (see Table I for chemical names).

Because of the unusually long effectiveness of Temik in controlling certain cotton pests following soil applications, fate of this toxicant in cotton plants and soil was studied.

EXPERIMENTAL

The separate radiolabels utilized in these studies are indicated below:



Two batches of S^{35}-Temik (initial specific activities of 1.3 and 10.8 mc. per mmole) and one of C^{14}-(S-methyl)Temik (1.2 mc. per mmole) were prepared in this laboratory from a procedure supplied by Union Carbide Corp. (Bartley *et al.*, 1966; Knaak *et al.*, 1966; Payne *et al.*, 1966). C^{14}-(carbonyl)Temik (2.08 mc. per mmole) was synthesized as described by Andrawes *et al.* (1967). The crude Temik from these preparations was purified by using a 60- to 100-mesh Florisil column and eluting with a mixture of *n*-hexane and chloroform. The initial mixture of the solvent was 2 to 1 (v./v.) *n*-hexane and chloroform but was gradually changed to 1 to 2 for final elution. Over-all yields ranged from 30 to 60%. C^{14}-(tertiary)Temik (3.9 mc. per mmole), S^{35}-Temik (55.6 mc. per mmole) and C^{14}-(S-methyl)Temik (4.9 mc. per mmole) were supplied by Union Carbide Corp., as were theoretical metabolites (Table I). All radioactive materials used exceeded 95% purity as shown by thin-layer chromatography (TLC) and autoradiography. Radioactivity was assayed at ambient temperature by liquid scintillation, and necessary corrections were made for quenching and radioactive decay.

Radiolabeled Temik-sulfoxide and Temik-sulfone (see Table I for chemical names) were prepared by oxidizing S^{35}-Temik with 1 and 2 equivalents of *m*-chloroperoxybenzoic acid, respectively, in chloroform. Radiolabeled

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

[2] Texas Agricultural Experiment Station, Texas A&M University, College Station, Tex.

Temik-oxime was prepared by hydrolysis of S^{35}-Temik with dilute sodium hydroxide in 50% ethanol. Purification, when needed, was accomplished by column or preparative thin-layer chromatography.

Cotton plants used were of the Deltapine-Smoothleaf variety grown in the greenhouse in 1-gallon cans. The potting soil consisted of about two parts of Lufkin sandy loam and one part of peat moss. Insects used for bioassay included 6- to 9-day-old adult boll weevils and 4- or 5-day-old adult lygus bugs, *Lygus hesperus* Knight. Both species were from insecticide-susceptible laboratory colonies reared under controlled environmental conditions.

All plant material was frozen immediately after harvesting and usually processed within 2 hours. Processing involved maceration with 1 to 1 (v./v.) acetone and water at 0° C., centrifugation, evaporation under vacuum at 40° C., and chromatography. A measure of the total radioactivity was obtained by radioassaying aliquots of the extracts. Plant residues containing C^{14} were burned in a Parr oxygen bomb (Payne and Dove, 1954), and the evolved $C^{14}O_2$ was trapped in a mixture of 2 to 1 (v./v.) methyl Cellosolve and ethanolamine (Jeffay and Alvarez, 1961). Plant tissues containing S^{35} were digested with nitric acid and hydrogen peroxide. Aliquots from samples obtained by either method were assayed with a liquid scintillator.

Soil samples were extracted for 2 hours with 1 to 1 (v./v.) acetone and 95% ethanol on a mechanical shaker; the extracts then were treated as described for plant extracts.

Temik metabolites were tentatively identified by cochromatography on TLC plates with authentic standards in two or more of the systems listed in Table I. Either silica gel G (Brinkmann Instruments, Inc., Westbury, N.Y.) or microcrystalline cellulose (FMC Corp., Newark, N.J.) 0.25 mm. thick, was used. Two-dimensional chromatography, using two of the systems in Table I, was used for better resolution of certain metabolites. Nonradioactive standards were detected by spraying the plates lightly with 0.2% trichloro-*P*-benzoquinoneimine in ethanol, followed by exposure to ultraviolet light (Smith and Birchenough, 1960). This procedure produced yellow spots with all of the available standards; however, the sulfones were slow in developing. A light spray of 1% $KMnO_4$ in water also was used; this produced yellow spots with all standards. Some of the chromatograms of radiolabeled standards were first exposed to no-screen x-ray film to locate areas of radioactivity, then sprayed with one of the chromogenic agents. In metabolism studies, areas of radioactivity were scraped from the TLC plate directly into scintillation vials and radioassayed.

TEST PROCEDURE, RESULTS, AND DISCUSSION

Chromatography and Preliminary Experiments. The chromatographic behavior of Temik and Temik metabolites in several solvent systems is shown in Table I.

Table I. Chromatographic Behavior of Temik, Related Compounds, and Temik Metabolites in the Presence of Biological Material

Chemical Name	Abbreviated Name	R_f Values in Indicated System[a]					
		A	*B*	*C*	*D*	*E*	*F*
2-Methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime	Temik	0.87	0.84	0.82	0.87	0.92	0.98
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Temik-sulfoxide	0.16	0.10	0.39	0.12	0.71	0.96
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Temik-sulfone	0.52	0.47	0.52	0.29	0.87	1.00
2-Methyl-2-(methylthio)propionaldehyde *O*-carbamoyloxime	*N*-Demethyl-Temik	0.87	0.84	0.63	0.65	0.90	1.00
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-carbamoyloxime	*N*-Demethyl-Temik-sulfoxide	0.13	0.12	0.18	0.04	...	...
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-carbamoyloxime	*N*-Demethyl-Temik-sulfone	0.40	0.45	0.36	0.12	...	...
2-Methyl-2-(methylthio)propionaldoxime	Oxime	0.97	0.96	...	...	0.93	1.00
2-Methyl-2-(methylsulfinyl)propionaldoxime	Oxime-sulfoxide	0.40	0.38	0.44	0.20	0.69	0.84
2-Methyl-2-(methylsulfonyl)propionaldoxime	Oxime-sulfone	0.87	0.84	0.80	0.55	0.85	0.96
2-Methyl-2-(methylthio)propionitrile	Nitrile	[b]	[b]	...	...	0.96	1.00
2-Methyl-2-(methylsulfinyl)propionitrile	Nitrile-sulfoxide	0.59	0.54	0.57	0.51	0.80	0.96
2-Methyl-2-(methylsulfonyl)propionitrile	Nitrile-sulfone	0.87	0.84	0.80	0.79	0.86	1.00
2-Methyl-2-(methylthio)propionic acid	Acid	[c]	[c]	...	...	0.36	0.40
2-Methyl-2-(methylsulfinyl)propionic acid	Acid-sulfoxide	[c]	[c]	...	...	0.17	0.22
2-Methyl-2-(methylsulfonyl)propionic acid	Acid-sulfone	[c]	[c]	...	...	0.93	...
	Unknown(s) 1	0.00	0.00	...	0.00	...	...
	Unknown 3	0.22	0.16	...	...	...	...
	Unknown 5	0.69	0.63	...	...	...	...
	Unknown 6	0.82	0.76	...	...	...	...
	Unknown 7	0.94	0.88	...	...	...	...

[a] *A* = Dioxane-benzene, 1 to 1, silica gel G.
B = Diethyl ether–*n*-hexane–ethanol, 4:1:1, silica gel G.
C = Chloroform–*n*-hexane–ethyl acetate–ethanol, 5:1:1:1, silica gel G.
D = Chloroform–ethyl acetate–*n*-hexane–dioxane, 5:1:1:1, silica gel G.
E = Acetonitrile–water–ammonium hydroxide, 20:9:1, silica gel G.
F = Acetonitrile–water–ammonium hydroxide, 20:9:1, microcrystalline cellulose.
[b] Runs with solvent front.
[c] Compounds streaked badly, no R_f values obtained.

Systems *A*, *B*, and *C* were used predominantly. Unless fresh developing solutions were used, system *A* oxidized Temik to Temik-sulfoxide and Temik-sulfoxide to Temik-sulfone. System *D* or *E* was used with systems *B* or *C* for two-dimensional chromatography.

A digestive procedure using HNO_3 and H_2O_2, similar to the procedure commonly used for P^{32}, worked very well for estimating the S^{35} remaining in extracted materials. Recoveries ranged from 92 to 100% with a mean of 95% when either S^{35}-Temik or S^{35}-Temik-sulfoxide was added to leaf samples.

Temik and Temik-sulfoxide were added to cotton leaves, processed through the procedure described, and chromatographed. From 4 to 8% of the Temik was converted to Temik-sulfoxide; however, no degradation of added Temik-sulfoxide occurred. The relative amounts of Temik or Temik-sulfoxide in extracts that were held at room temperature decreased significantly within a few days. All samples were chromatographed immediately after extraction, and no corrections were made for Temik degradation.

Excised Leaves. The metabolic fate of S^{35}-, C^{14}-(carbonyl), C^{14}-(S-methyl)-, and C^{14}-(tertiary)Temik as well as S^{35}-Temik-sulfoxide, S^{35}-Temik-sulfone, and S^{35}-oxime was studied in young mature excised cotton leaves. The leaves were allowed to take up 0.15 ml. of aqueous solutions containing 100 to 200 μg. of the materials and were then held in distilled water in the laboratory at 25° to 27° C. under continuous light. Sufficient leaves were treated so that duplicate one-leaf samples were available at each harvest date. The leaves were processed as described.

The results of the excised cotton leaf experiment using the four radiolabels (Table II) show that Temik had a half life of slightly more than 24 hours. The predominant metabolite in all extracts was Temik-sulfoxide. Three days after treatment, the concentration of Temik-sulfoxide accounted for about 60% of the applied dose and 14 days after treatment, 35 to 43% in the case of all four labels. No major differences were found among the metabolites in extracts of leaves treated with S^{35}-, C^{14}-(S-methyl), or C^{14}-(tertiary)Temik. Unknowns 3 and 5 were not carbamates as indicated by their absence from extracts of leaves treated with C^{14}-(carbonyl)Temik. Unknown 6, detected in the 1- and 7-day C^{14}-(carbonyl)Temik–treated leaves, but not in the 3- and 14-day samples, appears to be a carbamate. The unidentified metabolites recovered from treated excised leaves did not correspond chromatographically to the oxime-sulfone, nitrile-sulfone, or the *N*-demethyl derivatives of Temik, Temik-sulfoxide, and Temik-sulfone. About 55% of the total radioactivity from C^{14}-(carbonyl)Temik was lost during the 14-day experiment. During a similar period of time, S^{35}-, C^{14}-(tertiary)-, and C^{14}-(S-methyl)Temik–treated leaves lost 34, 30.8, and 23.5%, respectively, of the applied radioactivity. The higher loss of radioactivity from leaves treated with C^{14}-(carbonyl)Temik than from leaves treated with the other radiolabels probably was a result of hydrolysis and subsequent degradation of the carbonyl portion of the molecule to $C^{14}O_2$ and methylamine.

When the fates of S^{35}-Temik, Temik-sulfoxide, Temik-sulfone, and oxime (Table III) were compared, Temik-sulfone was stable in cotton leaves (a half life of approximately 14 days) compared with Temik-sulfoxide (a half life of about 4 days). The metabolism of Temik was similar to that described in the preceding experiment, except that small amounts of oxime-sulfone were detected. The S^{35}-metabolites recovered from Temik-sulfoxide–treated leaves were comparable with those recovered from the Temik-treated leaves except that unknown 3 was recovered in only the 14-day sample of the Temik-sulfoxide–treated leaves. Also, the quantities of most S^{35}-metab-

Table II. Metabolism of Temik in Excised Cotton Leaves[a]

Product	Per Cent of Total Dose at Indicated Days after Treatment			
	1	3	7	14
		S^{35}-Temik[b]		
Temik	59.2	11.4	1.0	0
Temik-sulfoxide	29.3	61.1	51.6	37.0
Temik-sulfone	0.8	5.3	4.0	4.1
Nitrile-sulfoxide	0.2	1.8	5.9	2.0
Oxime-sulfoxide	0.5	0.9	3.6	10.5
Unknown(s) 1	3.3	1.6	3.4	3.1
Unknown 3	0.4	2.0	0	0.7
Unknown 5	0.2	0	0	0
Unknown 6	0.6	0.5	0.6	1.4
Total extracted	94.5	84.6	70.1	58.8
Residue	8.6	11.5	8.6	7.2
Lost	0	3.9	21.3	34.0
		C^{14}-(S-methyl)Temik[c]		
Temik	50.8	...	1.4	0
Temik-sulfoxide	37.4	...	49.9	43.4
Temik-sulfone	0.1	...	3.7	3.4
Nitrile-sulfoxide	0.1	...	5.9	1.8
Oxime-sulfoxide	0.1	...	4.3	11.3
Unknown(s) 1	1.8	...	5.7	4.8
Unknown 3	T		T	0
Unknown 5	0.3	...	0	0
Unknown 6	1.7	...	1.2	1.6
Total extracted	92.3	...	72.1	67.8
Residue	7.6	...	8.6	8.7
Lost	0.1	...	19.3	23.5
		C^{14}-(tertiary)Temik[d]		
Temik	58.3	5.9	0.6	0
Temik-sulfoxide	30.9	59.8	43.1	40.8
Temik-sulfone	0.5	4.8	2.9	3.6
Nitrile-sulfoxide	0.3	1.5	6.1	1.8
Oxime-sulfoxide	0.3	0.8	3.6	10.8
Unknown(s) 1	0.9	1.8	4.0	2.8
Unknown 3	1.1	1.1	0	0.8
Unknown 5	0.1	0	0	0
Unknown 6	1.0	1.1	0.5	0.7
Total extracted	93.4	76.8	60.8	61.3
Residue	6.7	8.2	5.2	7.9
Lost	0	15.0	34.0	30.8
		C^{14}-(carbonyl)Temik[e]		
Temik	51.5	6.7	0.6	0
Temik-sulfoxide	37.6	59.9	44.8	35.0
Temik-sulfone	0	1.8	4.3	4.0
Unknown(s) 1	0.6	3.5	1.8	0.9
Unknown 6	1.7	0	0.2	0
Total extracted	91.4	71.9	51.7	39.9
Residue	8.5	10.0	7.3	4.5
Lost	0.1	18.1	44.0	55.6

[a] 200 μg. Temik per leaf; all data based on radioassay and expressed as average of duplicate chromatograms of duplicate samples.
[b] 2.5×10^6 c.p.m. per leaf.
[c] 1.2×10^6 c.p.m. per leaf.
[d] 1.2×10^6 c.p.m. per leaf.
[e] 0.6×10^6 c.p.m. per leaf.

Table III. Metabolism of S^{35}-Temik, Temik-sulfoxide, Temik-sulfone, and Oxime in Excised Cotton Leaves

Product	Per Cent of Total Dose at Indicated Days after Treatment, Based on Radioassay[a]				
	1	3	7	14	21
			TEMIK[b]		
Temik	49.0	24.4	3.0	0	0
Temik-sulfoxide	36.9	51.6	49.9	35.1	29.1
Temik-sulfone	0.4	0.9	1.9	2.7	3.6
Oxime-sulfoxide	0.7	1.2	1.9	1.2	1.0
Nitrile-sulfoxide	0.2	1.1	3.1	6.5	8.3
Nitrile-sulfone	0	0	0	0.6	1.0
Unknown(s) 1	1.1	2.3	3.8	5.1	11.0
Unknown 3	3.8	1.3	1.2	1.3	0.5
Unknown 5	0	0	0.1	0	0
Unknown 6	1.8	0.6	0.2	0.5	0.2
Unknown 7	0.1	0	0.1	0.1	0
Total extracted	94.0	83.4	65.2	53.1	54.8
Residue	6.0	8.3	6.7	6.6	6.5
Lost	0	8.3	28.1	40.3	38.7
			TEMIK-SULFOXIDE[c]		
Temik-sulfoxide	76.8	54.6	38.4	23.1	25.4
Temik-sulfone	0.7	1.2	2.4	2.6	3.9
Oxime-sulfoxide	3.7	4.3	2.5	1.6	0.9
Nitrile-sulfoxide	2.5	4.1	7.2	8.0	12.3
Nitrile-sulfone	0	0.1	0.5	0.8	1.9
Unknown(s) 1	0.5	1.8	3.6	4.6	9.3
Unknown 5	0	0.1	0	0	0
Unknown 6	0	0.1	0.2	0.3	0.2
Unknown 7	0	0.1	0	0	0
Total extracted	84.6	66.4	54.8	41.2	53.9
Residue	9.6	7.4	6.6	4.9	6.9
Lost	5.8	26.2	38.6	53.9	39.2
			TEMIK-SULFONE[d]		
Temik-sulfone	87.2	84.0	61.0	49.6	48.1
Nitrile-sulfone	1.7	0.6	1.8	2.5	1.1
Unknown(s) 1	0.3	0.5	1.4	4.3	6.5
Unknown 5	0.2	0.4	0.5	0	0
Unknown 6	1.1	2.3	1.4	0	0
Unknown 7	0.3	0.5	0	0	0
Others[e]	1.2	1.2	3.7	4.5	3.7
Total extracted	92.0	89.5	69.8	60.9	59.4
Residue	8.0	9.4	8.8	7.8	6.0
Lost	0	1.1	21.4	31.3	34.6
			OXIME[e]		
Oxime	0.3	0	0	0	...
Oxime-sulfoxide	47.5	17.3	13.1	0.8	...
Nitrile-sulfoxide	3.1	6.2	11.9	15.8	...
Nitrile-sulfone	0	0.2	1.3	1.9	...
Unknown(s) 1	19.1	41.0	55.8	42.3	...
Unknown 3	18.9	18.6	11.8	16.2	...
Unknown 5	0.7	0.7	0.7	0	
Others[f]	5.0	2.1	1.6	9.3	...
Total extracted	94.6	87.9	96.5	86.3	...
Residue	8.3	10.1	8.5	14.0	...
Lost	0	2.0	0	0	...

[a] Average of duplicate chromatograms of duplicate samples.
[b] 171 μg. per leaf, 6.9×10^6 c.p.m. per leaf.
[c] 155 μg. Temik-equivalents per leaf, 6.3×10^6 c.p.m. per leaf.
[d] 123 μg. Temik-equivalents per leaf, 5.0×10^6 c.p.m. per leaf.
[e] 109 μg. Temik-equivalents per leaf, 4.4×10^6 c.p.m. per leaf.
[f] Unidentified metabolites other than those listed in Table I.

olites were similar except that oxime-sulfoxide and nitrile-sulfoxide were present in higher concentrations initially in the Temik-sulfoxide–treated leaves than in the Temik-treated leaves. Radioactivity was lost initially more rapidly from the Temik-sulfoxide–treated leaves than from the Temik-treated leaves. After 20 days, 48.1% of the applied S^{35}-Temik-sulfone was unchanged; 34.6% of the dose had been lost by volatilization, leaving only 17.3% present as metabolites. Included in these were trace amounts of an S^{35}-labeled metabolite whose chromatographic behavior was identical to Temik-sulfoxide. This was also found by Metcalf *et al.* (1966). The remaining metabolites were primarily polar compounds that remained at the baseline of the plates [unknown(s) 1] and other unidentified metabolites, as well as a small amount of oxime-sulfone.

No significant radioactivity was lost from leaves treated with S^{35}-oxime. Only trace amounts of oxime were recovered 1 day after treatment and none thereafter. In another experiment with S^{35}-oxime–treated leaves, less than 10% of the applied radioactivity was recovered as the oxime 1 hour after treatment. The oxime was rapidly metabolized to oxime-sulfoxide which represented nearly 50% of the applied dose 1 day after treatment, and to polar metabolites [unknown(s) 1] which remained at the base line of the chromatogram. These polar compounds were the major metabolites in all but the 1-day sample. Significant quantities of nitrile-sulfoxide were found; after 21 days this metabolite represented more than 15% of the applied dose. Unknown 3 accounted for 15 to 19% of the applied dose in all samples. Only small amounts of oxime-sulfone were recovered, even though relatively large amounts of oxime-sulfoxide were found initially, which suggests that further oxidation of the oxime-sulfoxide was relatively slow.

After the loss of radioactivity from treated excised leaves was found to be as much as 50% in 3 weeks, experiments were designed to determine whether this loss was a result of volatilization of radioactivity from the leaves. Insignificant amounts of radioactivity (less than 0.3% of total dose) were recovered from the water in which the treated leaves were held. Excised cotton leaves, each treated with 200 μg. of Temik, were placed in a glass container, 11.5 cm. in diameter and 23 cm. tall. Air was drawn through this container at a rate of about 15 liters per hour and passed through two or three gas-washing flasks containing 100 ml. of 2 to 1 (v./v.) methyl Cellosolve and ethanolamine for trapping CO_2. The trapping solutions were changed every 24 or 48 hours and aliquots of the trapping solution radioassayed. Figure 1 shows that

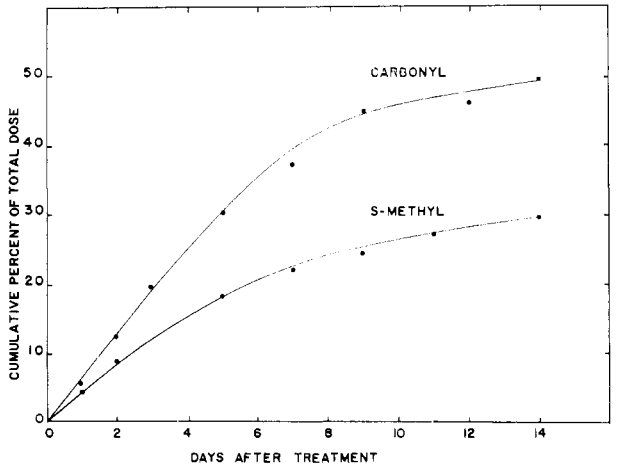


Figure 1. Radioactivity trapped from C^{14}-(carbonyl)- and C^{14}-(S-methyl)Temik–treated excised cotton leaves

through 14 days nearly 50% of the applied radioactivity from C^{14}-(carbonyl)Temik and 30% from C^{14}-(S-methyl)-Temik were recovered in the trapping solution. It is not known whether all trapped C^{14} was $C^{14}O_2$. Experiments with S^{35}-Temik, using 2% glycerol in 0.5*N* NaOH as the trapping solution, resulted in 20 to 25% of the applied radioactivity recovered in the trapping solution after a 14-day experiment. Using the data from Figure 1, nearly 100% of the applied radioactivity can be accounted for in Tables II and III.

Attempts to identify the volatilized compounds were not entirely successful. Air was drawn from a chamber, containing S^{35}-Temik–treated excised cotton leaves, through two U-tubes submerged in a dry ice–acetone bath. Small amounts of Temik-sulfoxide were recovered on each of the 3 days of the experiment; on the third day Temik-sulfone and unknown 3 also were recovered. In a bioassay experiment, two cotton leaves were treated with 200 μg. of Temik and volatilized materials drawn through a flask containing adult lygus bugs. During the first 8 hours after treatment, 100% mortality of the lygus bugs was observed. From 24 through 48 hours after treatment, 50% of a second group of lygus bugs were killed, and no mortality occurred after 48 hours

Petiole Injection. Young mature leaves of greenhouse-grown cotton plants were each treated with 200 μg. of aqueous S^{35}-Temik (1.5×10^6 c.p.m. per leaf) by injection in the leaf petioles with finely pointed capillary tubes. Plants were approximately 4 weeks old at time of treatment. After treatment the plants were held in an environmental chamber programmed for 14 hours of light (>600 foot candles) at 29° C. and for 10 hours of dark at 24° C. per day. At intervals after treatment, leaves were removed from the plants and analyzed.

The data from the petiole injection experiment (Table IV) indicated again that Temik had a very short half life in cotton plants. After 1 day, less than 25% of the applied dose remained in the form of the parent molecule, and after 3 weeks no Temik was recovered. Most of the applied Temik appeared to be oxidized rapidly to its sulfoxide derivative. At all times, Temik-sulfoxide was the major metabolite recovered. This metabolite accounted for 56.4% of the applied dose after 1 day, reached a peak of 71.8% at 3 days, and, at the end of 5 weeks accounted for 5.3% of the applied dose.

Oxidation at the sulfur atom of Temik-sulfoxide to give the sulfone derivative appears to be a somewhat slower process. The maximum quantity of Temik-sulfone was recovered at 3 weeks and accounted for only 10.4% of the applied dose. These data indicate that the major portion of the sulfoxide undergoes chemical changes other than oxidation to the sulfone derivative. It is possible that hydrolysis of Temik-sulfoxide to its oxime derivative is a major degradative route of Temik-sulfoxide. The small amount of oxime-sulfoxide (maximum recovery of only 2.5% of the total dose) is probably due to its unstable nature in plants as demonstrated in previous experiments.

The metabolite tentatively identified as the nitrile-sulfoxide increased in concentration from 2.0% of the total dose at 1 day to 4.8% at 1 week and then declined to 1.9% at the final harvest date (Table IV, 5 weeks). This compound would be formed via dehydration of oxime-sulfoxide. The biological degradation of oximes usually involves hydrolysis to yield aldehydes or ketones and hydroxylamine or reduction to form primary amines (Williams, 1959). The nitrile-sulfoxide has been prepared chemically, however, (Payne *et al.*, 1966) and cochromatographs identically with the product produced in cotton plants in several solvent systems.

During the course of this experiment, four compounds (unknowns 3, 5, 6, and 7) were encountered which did not cochromatograph with available standards. No tentative identifications were made on these compounds. The maximum combined recovery of these compounds was less than 6% of the total dose, and at the final harvest, none of the four was detected.

In addition to the unknowns mentioned above, 4.5 to 13.9% of the applied radioactivity remained at the origin of TLC systems *A*, *B*, or *C* [unknown(s) 1]. Limited studies of this fraction, using TLC system *F*, has shown that 12 or more S^{35}-labeled metabolites are present.

Recovery of applied radioactivity from leaves declined progressively until only 27.8% was measured after 5 weeks. Previous studies with excised leaves indicated that this loss was a result of volatilization of the radioactivity from the treated leaves. Another experiment using S^{35}-Temik demonstrated that certain radioactive compounds were translocated from treated leaves to other parts of the plant (6 to 10% of the dose). The chemical nature of these products was not established.

Soil Studies. To obtain a general understanding of the fate of Temik in soil, a study was conducted using three soil types: virgin Houston clay, Norwood silty clay loam, and Lakeland fine sand. These soils were selected primarily for their different organic matter content (Table V). Before treatment, the moisture level of each soil was adjusted to one moisture equivalent according to a method described by Bouyoucos (1935). Ten-gram samples of soil were placed in 250-ml. wide-mouthed amber bottles equipped with screw caps; then 200 μg. of S^{35}-Temik (1.5×10^7 c.p.m.) in 50 μl. of water were added to each and mixed thoroughly with the soil and the bottles tightly capped. The samples were held in the dark at 25° to 28° C. Frequent weighing of the random samples indicated that

Table IV. Metabolism of Temik in Intact Cotton Leaves[a]

Product	Per Cent of Total Dose at Indicated Days after Treatment					
	1	3	7	21	28	35
Temik	24.5	3.5	2.5	0.5	0	0
Temik-sulfoxide	56.4	71.8	52.1	28.9	19.3	5.3
Temik-sulfone	2.7	3.2	6.3	10.4	4.7	2.6
Oxime-sulfoxide	2.5	1.5	0.8	0.7	0	0
Nitrile-sulfoxide	2.0	2.1	4.8	3.4	0.6	1.9
Unknown(s) 1	4.5	5.0	3.3	13.9	9.1	13.1
Unknown 3	0	1.6	0.6	0	0	0
Unknown 5	2.0	0	0.2	0.1	0	0
Unknown 6	2.6	0.5	0.2	0.3	0.1	0
Unknown 7	0.6	0.3	1.2	0	0	0
Total in extract	98.0	89.5	72.0	58.2	33.8	22.9
Residue	2.0	6.5	2.6	5.7	1.2	4.9
Lost	0	4.0	25.4	36.1	65.0	72.2

[a] 200 μg. S^{35}-Temik per leaf administered by petiole injection; data based on radioassay and expressed as average of duplicate chromatograms of triplicate samples.

Table V. Some Chemical and Physical Properties of Three Soil Types

Soil	Per Cent Organic Matter[a]	pH	Per Cent H_2O at One Moisture Equivalent[b]	Mechanical Analysis, %[c] Sand	Silt	Clay
Houston clay[d]	4.2	8.0	23.5	4.4	40.1	55.5
Norwood silty clay loam	1.0	8.0	21.4	23.9	53.5	22.6
Lakeland fine sand	0.4	6.3	3.4	92.0	6.0	2.0

[a] Determined by method of Peach *et al.* (1947).
[b] Determined by method of Bouyoucos (1935).
[c] Determined by method of Bouyoucos (1936) after organic matter was digested with H_2O_2.
[d] Noncultivated soil.

there was no loss of moisture during the experiment.

At intervals during the 12-week experimental period, three replicates of each soil type were removed from storage and processed. Each sample was handled individually. The soil samples were extracted twice with 50 ml. of 1 to 1 (v./v.) acetone and ethanol on a mechanical shaker for 1 hour. The solvent was decanted from the bottles after extraction and cooled for 30 minutes at 0° C. to facilitate removal of colloidal soil particles by centrifugation. After centrifugation the soil extracts were handled as described for plant material.

Certain chemical and physical properties of the different soils are shown in Table V. Temik decomposed more slowly in soils (Table VI) than in plants (Table IV). The approximate half life was 9, 7, and 12 days for Houston clay, Norwood silty clay loam, and Lakeland fine sand, respectively. Four weeks after treatment, these soils contained 6.1, 0.3, and 27.2%, respectively, of the applied dose of Temik. All soils contained measurable quantities of Temik at the end of the 12-week experiment.

As in plants, the major product recovered from soil during the 12-week experimental period was Temik-sulfoxide. This compound appeared to be stable in all soils tested, particularly in Lakeland fine sand. The apparent slow rate of hydrolysis as indicated by the small amounts of oxime-sulfoxide recovered, possibly contributed to the persistence of Temik-sulfoxide in sand. The Temik-sulfoxide was least persistent in the Houston clay soil. The oxidation of Temik-sulfoxide to the sulfone derivative was slow, as indicated by the low recovery of Temik-sulfone. Oxime and nitrile-sulfoxide were recovered only in trace amounts. Unidentified compounds recovered from soil appeared to be the same as those from plants.

Side-Dress Treatment. Greenhouse cotton plants growing in 1-gallon cans of potting soil were treated with S^{35}-Temik at the rate of 17 mg. of toxicant per 1-gallon can (18.4×10^7 c.p.m. per can) to simulate a side-dress treatment. Two plants each with 6 to 8 true leaves at the time of treatment were growing in each can. The insecticide, dissolved in 4 ml. of water, was divided among four 2-inch deep holes spaced evenly around the two plants. The gallon cans were watered from the top once a week and from the bottom the remainder of the time. This procedure minimized loss of Temik or its metabolites by leaching.

The plant parts from three treated plants were harvested at weekly intervals. Leaves from each plant were handled separately and were separated into mature, young, and new, based on their age at time of treatment. New leaves were those that formed after the plants were treated.

Table VI. Fate of 20 P.P.M. S^{35}-Temik in Three Types of Soils[a]

Product	Per Cent of Total Dose at Indicated Weeks after Treatment 1	2	4	6	9	12
	HOUSTON CLAY (VIRGIN)[b]					
Temik	57.3	31.5	6.1	1.1	0.8	0.7
Temik-sulfoxide	31.5	41.1	35.3	23.3	16.0	9.5
Temik-sulfone	4.3	3.4	3.8	6.2	4.8	4.8
Nitrile-sulfoxide	0	0	0.2	0.2	0	0
Oxime	0.2	0.2	0	0	0	0
Oxime-sulfoxide	3.8	4.3	3.4	3.1	1.5	0.8
Unknown(s) 1	0.8	0.9	0.8	1.9	1.8	2.3
Unknown 3	1.7	2.4	2.1	1.7	0.8	0.6
Unknown 5	0	0	0.2	0.2	0	0
Unknown 6	0	0	0.2	0	0.1	0.1
Unknown 7	0.4	0.1	0	0	0	0
Residue[c]	0	16.1	47.9	62.3	74.2	81.2
	NORWOOD SILTY CLAY LOAM					
Temik	53.1	18.3	0.3	0.5	0.5	0.4
Temik-sulfoxide	41.5	61.6	51.2	37.3	30.0	25.0
Temik-sulfone	1.2	2.1	4.9	6.4	6.8	6.3
Nitrile-sulfoxide	0	0.8	0.1	0.2	0	0
Oxime-sulfoxide	1.9	2.8	2.1	3.2	1.0	1.0
Unknown(s) 1	0.8	0.9	0.3	0.4	0.4	0.5
Unknown 3	1.6	4.1	5.2	7.6	4.7	5.1
Unknown 5	0	0.7	0.1	0.2	0	0
Unknown 6	0	0	0.3	0	0.1	0.1
Unknown 7	0	0	0	0	0.1	0
Residue[c]	0	8.7	35.5	44.2	56.4	61.6
	LAKELAND FINE SAND					
Temik	65.1	54.7	27.2	2.9	6.0	3.6
Temik-sulfoxide	27.5	33.8	40.7	55.5	51.3	49.8
Temik-sulfone	3.9	3.8	5.7	11.6	8.0	13.4
Nitrile-sulfoxide	0	0	0.2	0	0.1	0
Oxime	0.1	0	0	0	0	0
Oxime-sulfoxide	1.5	0	0	0	0	0.6
Unknown(s) 1	0.7	0.9	0.8	0.8	1.2	3.3
Unknown 3	1.0	1.0	1.7	3.1	2.1	5.0
Unknown 5	0	0	0.2	0	0	0
Unknown 6	0	0	0	0	0.1	0.2
Unknown 7	0.2	0.4	0.1	0	0.1	0.1
Residue[c]	0	5.4	23.4	26.1	31.1	24.0

[a] Average of duplicate chromatograms of triplicate samples.
[b] Noncultivated soil.
[c] Nitric acid digestion of extracted soil. Essentially 100% recovery of applied radioactivity was obtained from each sample.

Bracts from the fruit were removed and included with the new leaves. The plant parts were weighed immediately after harvesting and analyzed as described previously.

Four cores of the potting soil (½ inch in diameter and 5 to 6 inches deep with an average combined weight of 60 grams) were taken from each treated pot at each sampling date, extracted on a mechanical shaker, and analyzed.

Bioassay data using adult boll weevils were obtained with plants treated exactly as described above except with nonradiolabeled Temik. Various plant parts were removed from these treated plants and fed to adult boll weevils for 3-day periods. Three replicates of 15 weevils each were used to bioassay each plant part.

All side-dress–treated cotton plants remained in the greenhouse for the duration of the experiments. Lighting was programmed for 14 hours of light and 10 hours of dark per day. The side-dress experiment was conducted twice.

Mature leaves accumulated the highest concentration of S^{35}-Temik equivalents, followed by young leaves and new leaves after side-dress treatments (Table VII). Smaller amounts of radioactivity were found in the terminals and fruit than in the leaves. After 3 weeks, 135 p.p.m. of S^{35}-Temik equivalents were recovered from mature leaves, while 117, 50, 24, and 18 p.p.m. of Temik-equivalents were recovered from the young leaves, new leaves, terminals, and fruit, respectively. The maximum recovery of radioactivity from the leaves present at time of treatment (mature leaves and young leaves) occurred 2 weeks after treatment; the maximum recovery from leaves formed following treatment (new leaves) occurred 5 weeks after treatment. Radioactivity in the terminals increased continuously over the 6-week period; however, the concentration in the fruit was 18 p.p.m. of the Temik-equivalents at 3 weeks (the first harvest at which fruit was present on the treated plants) and decreased to 6 p.p.m. after 6 weeks. The part per million of Temik-equivalents in fruit was undoubtedly affected by an increase in weight over the test period.

Uptake of radioactivity by side-dress-treated cotton plants reached a peak at 2 weeks and remained fairly constant thereafter. These data indicate that no significant uptake occurs after 2 weeks; however, loss by volatilization from the plants or soil was not considered. If radioactivity is lost from the foliage of side-dress–treated cotton plants as rapidly as Temik-treated excised leaves, then continuous uptake during the entire experiment could have gone undetected.

The data in Table VIII indicate that leaves of Temik-treated cotton plants are toxic to boll weevils even though the parent compound is not present. For example, at 1 week after application, 10.4 p.p.m. of Temik were found in the young leaves of treated cotton, and 100% weevil mortality was recorded; however, after 5 weeks, no Temik was found in the same leaves, and weevil mortality was 84%. These studies clearly indicate that Temik-sulfoxide is persistent in foliage of side-dress–treated cotton. In mature leaves, Temik-sulfoxide reached a peak concentration of 104.7 p.p.m. during the second week after treatment and then declined slowly to 41.4 p.p.m. at the final harvest date. The concentration of Temik-sulfone increased steadily throughout the test period until, at 5 weeks in the new leaves and 6 weeks in the young leaves and mature leaves, more Temik-sulfone was extracted than Temik-sulfoxide. These data indicate that insect control on side-dress–treated cotton is closely related to the concentration of Temik-sulfoxide.

No major differences were found in the concentration of Temik and/or its metabolites in the mature leaves and young leaves; however, less Temik and a higher ratio of Temik-sulfone to Temik-sulfoxide was found in the new

Table VII. Uptake of S^{35}-Temik by Side-Dress–Treated Cotton Plants

Plant Part	Temik-Equivalents at Indicated Weeks after Treatment, P.P.M.[a]					
	1	2	3	4	5	6
Terminals	18	22	24	24	27	33
Squares and bolls	...	...	18	15	10	6
New leaves	...	46	50	42	57	51
Young leaves	135	120	117	107	110	118
Mature leaves	133	167	135	130	91	123
Total[b]	106	100	89	72	65	49
Per cent of total dose	4.2	7.1	7.1	6.8	8.4	6.4

[a] All data based on radioassay and expressed as average of duplicate chromatograms of triplicate samples. [b] P.p.m. in total fresh plant weight excluding stems and roots.

Table VIII. Metabolism and Bioassay of S^{35}-Temik in Side-Dress–Treated Cotton Plants

Product	P.P.M. (Fresh Weight) of S^{35}-Temik Equivalents in Various Age Leaves at Indicated Weeks after Treatment and Boll Weevil Mortality, %[a, b]		
	N	*Y*	*M*
		1 Week	
Temik	...	10.4	5.4
Temik-sulfoxide	...	79.6	76.3
Temik-sulfone	...	4.3	5.7
Weevil mortality	...	100	93
		2 Weeks	
Temik	0.3	1.3	1.5
Temik-sulfoxide	20.9	73.7	104.7
Temik-sulfone	5.1	12.2	21.5
Weevil mortality	18	87	83
		3 Weeks	
Temik	0.4	2.1	3.4
Temik-sulfoxide	18.7	57.4	70.6
Temik-sulfone	10.2	19.1	26.2
Weevil mortality	7	84	96
		4 Weeks	
Temik	0.4	0.6	1.2
Temik-sulfoxide	7.7	34.0	51.7
Temik-sulfone	6.8	17.3	26.8
Weevil mortality	13	59	86
		5 Weeks	
Temik	0	0	0
Temik-sulfoxide	16.0	37.2	33.4
Temik-sulfone	19.5	32.9	26.8
Weevil mortality	15	84	86
		6 Weeks	
Temik	0	0	0
Temik-sulfoxide	6.0	26.2	41.4
Temik-sulfone	23.7	28.5	44.4
Weevil mortality	23	74	79

[a] Metabolism data based on radioassay and expressed as average of duplicate chromatograms of triplicate samples. Weevil mortality data are based on three replicates. *N* = new leaves, *Y* = young mature leaves, and *M* = mature leaves.

leaves. Possible explanations of this would be that the smaller amounts of toxicant were metabolized at a faster rate in the new leaves compared with the larger amounts in the older foliage, or the labeled compounds in the new leaves were partially derived from the potting soil where some degradation had taken place. Indications are that the latter may be more important, since the metabolites recovered from the potting soil and from the new leaves were similar (Table IX).

Considerable variation was found between the fate of Temik in potting soil following side-dress application (Table IX) and in the soil study reported in Table V; however, these data are not directly comparable. The potting soil in the side-dress experiment was in open containers subject to leaching, volatilization, absorption by the large amount of organic material (peat moss), and uptake by plants, while the soil study data were unaffected by these factors. Differences in recovery cannot explain all the variation, however, since the degradation of Temik appears to progress somewhat faster in the potting soil from the side-dress treatment. It is possible that the increased temperature and moisture in the greenhouse together with the presence of growing plants afforded a more biologically active medium for the degradation of Temik. Similar metabolites were recovered from both the side-dress and soil study.

DISCUSSION

The initial rapid oxidation of Temik in cotton to Temik-sulfoxide and the relative stability of the latter is strong evidence that most if not all of the insecticidal activity of Temik is a result of Temik-sulfoxide. Temik-sulfone perhaps contributes some insecticidal activity from side-dress treatment, particularly since this compound is present in soil following applications of Temik and probably is taken up by cotton plants. However, bioassay tests indicated Temik-sulfone was essentially nontoxic to the boll weevil. Excised leaves (average weight of 2 grams) containing 50 μg. of Temik, Temik-sulfoxide, or Temik-sulfone were fed to adult boll weevils for 72 hours; the resulting mortalities were 80, 77, and 0%, respectively. Dosages of 200 μg. of Temik-sulfone per excised leaf resulted in less than 20% weevil mortality. Temik-sulfone accumulates to relatively high concentrations in aged residues (Table VIII). However, Temik-sulfoxide is a much more potent cholinesterase inhibitor than Temik-sulfone (Bull *et al.*, 1967; Payne *et al.*, 1966). *N*-Demethyl-Temik was not found in any extracts of Temik-treated cotton or soil.

There is little direct chromatographic evidence for the hydrolysis of Temik to oxime in cotton plants. The oxime, however, is unstable in cotton leaves (Table III). The greater loss of C^{14} from leaves treated with C^{14}-(carbonyl)-Temik than from the other radiolabels (Table II) indicates that hydrolysis does occur. Also, the formation of unknown 3 appears to be associated with the oxime, since large amounts of unknown 3 were found in leaves treated with the oxime (Table III). Unknown 3 also was found in leaves treated with Temik [except the C^{14}-(carbonyl)-Temik] but not in leaves treated with Temik-sulfoxide or Temik-sulfone (Table III). Thus it appears that unknown 3 is formed from the oxime.

Temik-sulfoxide is subject to oxidation of the sulfur atom to form Temik-sulfone; theoretically both Temik-sulfoxide and Temik-sulfone could form *N*-demethyl derivatives via oxidative *N*-demethylation. The authors found no evidence that the latter takes place in cotton or soil, although it has been reported for insects (Bull *et al.*, 1967). This oxidative *N*-demethylation has been reported to occur with organophosphorus compounds such as Bidrin, 3-hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate (Bull and Lindquist, 1964), Azodrin, 3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate (Bull and Lindquist, 1966; Menzer and Casida, 1965), schradan (O'Brien, 1960), and dimefox (O'Brien, 1960). However, Dorough and Casida (1964) did not detect the *N*-demethyl compound in studies with the carbamate carbaryl (1-naphthyl *N*-methylcarbamate) but did recover 1-naphthyl *N*-hydroxymethylcarbamate.

Hydrolysis of Temik-sulfoxide and particularly Temik-sulfone to their respective oximes is apparently slow. However, since the oxime-sulfoxide is unstable (Table III), the hydrolysis of Temik-sulfoxide to oxime-sulfoxide may be more important than the Temik-sulfoxide metabolism data in Table III indicate. Oxime-sulfoxide apparently is converted to nitrile-sulfoxide and unknown(s) 1, both of which accumulated rather slowly in Temik-sulfoxide-treated leaves (Table III), but were the predominant metabolites at 7, 14, and 21 days after treatment.

Of the 5 unknowns reported (1, 3, 5, 6, 7), 3, 5, and 7 are not carbamates, since they were not detected in cotton leaves that had been treated with C^{14}-(carbonyl)Temik. Unknown 7 was found both in plant extracts and in certain radiolabeled samples of Temik. Unknown 6 probably is a carbamate, since it was recovered from leaves treated with all radiolabels (Table III). Unknown 1 is a complex mixture of more polar compounds, some of which are probably carbamates. Unknowns 2 and 4 were not found in plant or soil extracts but have been found in insect extracts (Bull *et al.*, 1967).

A tentative metabolic pathway of Temik in cotton is shown below

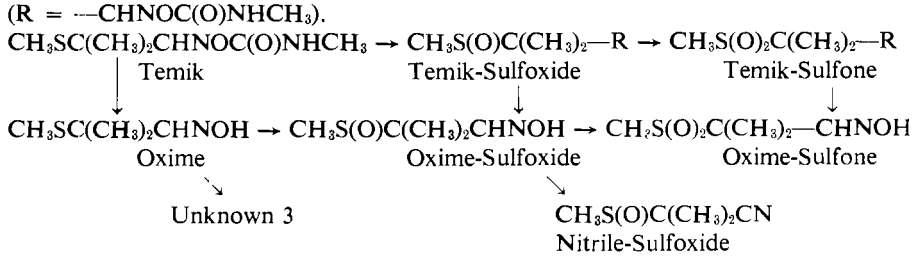


Table IX. Fate of S^{35}-Temik in Potting Soil Following Side-Dress Application to Greenhouse Cotton Plants

Product		Temik-Equivalents Extracted from Soil at Indicated Weeks after Treatment,[a] P.P.M.				
		1	2	3	4	6
Temik	0.49	0.12	0.02	T	0	
Temik-sulfoxide	1.63	0.86	0.22	0.08	0.01	
Temik-sulfone	0.05	0.12	0.08	0.06	0.03	
Nitrile-sulfoxide	0.07	0.08	0	T	0	
Oxime-sulfoxide	0.11	0.25	0.01	0	T	
Unknown(s) 1	0.09	0.05	0.01	0.02	T	
Unknown 6	0.02	0.01	0	T	0	
Unknown 7	0	0	T	0	0	
Total	2.47	1.48	0.33	0.17	0.05	

[a] Dry weight; T = trace amounts. Data based on radioassay of extracts only. No attempts were made to assay residues. All results are expressed as average of duplicate chromatograms of duplicate samples.

The major pathway of Temik metabolism in cotton appears to be Temik → Temik-sulfoxide → oxime-sulfoxide and Temik-sulfone. The Temik-sulfoxide subsequently is converted to the nitrile-sulfoxide. This pathway is similar to that reported by Metcalf *et al.* (1966), except that they did not report the nitrile derivatives. Also, Metcalf *et al.* (1966) did not report as extensive loss of radioactivity from treated leaves or intact plants as the authors found. It is possible that some of the unknown compounds reported by Metcalf *et al.* (1966) are the nitrile derivatives.

Metcalf *et al.* (1966) suggest that their metabolite II, which was recovered from Temik-treated isolated leaves 13 days after treatment and did not contain the $N—C^{14}H_3$ group, was the oxime of Temik. However, the authors found that the oxime had a half life of less than 1 hour in excised cotton leaves. This would indicate that Metcalf's metabolite II is not the oxime. Metcalf *et al.* (1966) suggest that metabolite III is the oxime of Temik-sulfone. The authors did not recover this from any of their Temik-treated plants or soil.

LITERATURE CITED

Andrawes, N. R., Dorough, H. W., Lindquist, D. A., *J. Econ. Entomol.*, to be published.
Bartley, W. J., Heywood, D. L., Steele, T. E. N., Skraba, W. J., J. AGR. FOOD CHEM. **14,** 604 (1966).
Bouyoucos, G. J., *Soil Sci.* **41,** 165 (1935).
Bouyoucos, G. J., *Soil Sci.* **42,** 225 (1936).
Bull, D. L., Lindquist, D. A., J. AGR. FOOD CHEM. **12,** 310 (1964).
Bull, D. L., Lindquist, D. A., J. AGR. FOOD CHEM. **14,** 105 (1966).
Bull, D. L., Lindquist, D. A., Coppedge, J. R., J. AGR. FOOD CHEM. **15,** 610 (1967).
Cowan, C. B., Jr., Ridgway, R. L., Davis, J. W., Walker, J. K., Watkins, W. C., Jr., Dudley, R. F., *J. Econ. Entomol.* **59,** 958 (1966).
Davis, J. W., Watkins, W. C., Jr., Cowan, C. B., Jr., Ridgway, R. L., Lindquist, D. A., *J. Econ. Entomol.* **59,** 159 (1966).
Dorough, H. W., Casida, J. E., J. AGR. FOOD CHEM. **12,** 294 (1964).
Hopkins, A. R., Taft, H. M., *J. Econ. Entomol.* **58,** 746 (1965).
Jeffay, H., Alvarez, J., *Anal. Chem.* **33,** 612 (1961).
Knaak, J. R., Tallant, M. J., Sullivan, L. J., J. AGR. FOOD CHEM. **14,** 573 (1966).
Menzer, R. E., Casida, J. E., J. AGR. FOOD CHEM. **13,** 102 (1965).
Metcalf, R. L., Fukuto, T. R., Collins C., Borck, K., Burk, J., Reynolds, H. T., Osman, M. F., J. AGR. FOOD CHEM. **14,** 579 (1966).
O'Brien, R. D., "Toxic Phosphorus Esters," p. 293, Academic Press, New York, 1960.
Payne, L. K., Stansbury, H. A., Weiden, M. H. J., J. AGR. FOOD CHEM. **14,** 356 (1966).
Payne, P. R., Dove, J., *Nature* **174,** 27 (1954).
Peach, M. L., Alexander, L. T., Dean, L. A., Reed, J. F., *U.S. Dept. Agr., Circ.* No. **757,** 5–11 (1947).
Ridgway, R. L., Jackson, C. G., Patana, R., Lindquist, D. A., Reeves, B. G., Bariola, L. A., *J. Econ. Entomol.* **59,** 1017 (1966).
Smith, I., Birchenough, M., "Imidazoles Chromatographic Techniques," Vol. **I,** p. 217, Interscience, New York, 1960.
Weiden, M. H. J., Moorefield, H. M., Payne, L. K., *J. Econ. Entomol.* **58,** 154 (1965).
Williams, R. T., "Detoxication Mechanisms," p. 160, Wiley, New York, 1959.

Received for review November 14, 1966. Accepted June 16, 1967. The authors thank D. L. Heywood, Union Carbide Corp., for his assistance and cooperation. Research supported in part by U.S. Public Health Service Research Grant ES-00085. Mention of proprietary names does not necessarily imply endorsement by the U.S. Department of Agriculture.