Metabolism of 2-Methyl-2-(methylthio)-propionaldehyde O-(Methylcarbamoyl)-oxime in Plant and Insect

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The metabolism of the systemic insecticide Temik has been investigated in the cotton plant and in the housefly using C^{14}-labeled radiotracers and column and thin layer chromatography. Temik is readily and completely oxidized to its sulfoxide within 4 to 9 days in cotton leaves at moderate temperatures. The sulfoxide which is more active as a cholinesterase inhibitor is the active metabolite and its long term persistence and relatively slow oxidation to Temik sulfone is responsible for the persistent systemic activity of the compound. Temik sulfoxide is hydrolyzed to the oxime which is the principal degradation product in the cotton plant. Metabolism in the housefly followed a completely similar pattern.

Temik, (registered trademark for Union Carbide 21149) is a novel carbamate insecticide, 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, with a structure resembling acetyl choline. Its synthesis and properties have been described by Payne, Stansbury, and Weiden (*8*). Temik has a high degree of contact toxicity to a variety of insects and is of especial interest because of its remarkable systemic properties. In standardized laboratory tests, 400 mg. of a 5% granular Temik formulation, applied uniformly around the base of cotton plants about 75 cm. high in 6-inch pots, produced 80 to 100% mortality of the following species caged on the upper leaves for the indicated number of weeks after treatment. The parenthetical figures represent the comparable performance of the standard O-P systemic phorate or *O,O*-diethyl *S*-(2-ethylthiomethyl) phosphorodithionate on cotton mite *Tetranychus cinnabarinus* 9 (11), cotton aphid *Aphis gossypii* 13 (14), and cotton leaf perforator *Bucculatrix thurberiella* 16 (13). In field tests applied to cotton as a 10% granular at 3 pounds per acre in early July, Temik gave virtually seasonal protection from attacks by spider mites, leafhoppers, lygus bug nymphs, and other noxious species and resulted in yield increases of from a few hundred to over 900 pounds of seed cotton per acre.

The practical usage of Temik as a cotton insecticide depends upon a knowledge of its metabolic fate in the plant, and this paper describes preliminary studies of this nature together with comparable information on its metabolism in the housefly *Musca domestica*. A very brief account of the systemic behavior and metabolism of Temik has been presented by Reynolds, Metcalf, and Fukuto (*9*).

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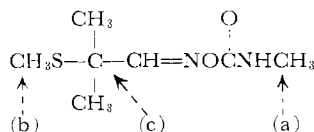
Experimental

The C^{14}-labeled radiotracer preparations of Temik have been described by Bartley *et al.* (*1*). Three preparations were available labeled at (a) in N-$C^{14}H_3$-1.725 μc. per mg., (b) in $C^{14}H_3S$-1.509 μc. per mg., and at (c) in *tert*-C^{14}-1.464 μc. per mg. All three preparations had a chromatographic purity of 98.5 to 99%.



The C^{14}-labeled Temik sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime was prepared as described by Bartley *et al.* (*1*) by adding dropwise 1 equivalent of peracetic acid to the *tert*-C^{14} Temik. The recrystallized product (1.34 μc. per mg.) had a radiochemical purity of 99% by thin layer chromatography and was stored in the refrigerator to prevent decomposition.

The C^{14}-labeled Temik sulfone, 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime, was prepared from both Temik N-$C^{14}H_3$ and Temik *tert*-C^{14} by adding two equivalents of peracetic acid (*1*). The recrystallized N-C^{14} product (1.46 μc. per mg.) had a radiochemical purity of 99+% and the *tert*-C^{14} product (1.25 μc. per mg.) had a radiochemical purity of 98+%.

Samples of the oxime hydrolysis products of the carbamate esters—Temik oxime, 2-methyl-2-(methylthio)propionaldehyde oxime, b.p. 55–62° C./0.5 mm.; Temik oxime sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldehyde oxime, m.p. 107–8° C.; and Temik oxime sulfone, 2-methyl-2-(methylsulfonyl) propionaldehyde oxime, m.p. 131–2° C.—were also provided by Bartley and Heywood. The chromatographic behavior of these compounds is described in Table I.

Cotton plants used were variety Deltapine Smoothleaf and were grown at 80° F. in the glass house. The plants were treated with the radiolabeled Temik preparations in several ways: (a) the petioles of mature leaves

Table I. Chromatographic Properties of Temik and Metabolites

Compound	M.P., °C.	Partition $HCCl_3/H_2O$	Column Elution in	R_f Thin Layer Chromatography, Silicic Acid: SBE	AB with 1% H_2O	EB	Detection: Quinone-imine	Ninhydrin
Temik	100–1	25.8	Ether-hexane	0.65	0.75	0.36	+	+
Temik-SO	108–10	0.95	Methanol	0.15	0.41	0.06	+	+
Temik-SO_2	132–3	3.45	Ethyl acetate	0.36	0.68	0.12	+	+
Temik-oxime	b55–62/ 0.5 mm.	1.66	Ether-hexane	0.69	0.74	0.75	+	−
Temik-SO-oxime	107–8	0.15	Methanol	0.34	0.48	0.07	+	−
Temik-SO_2-oxime	131–2	0.22	Ethyl acetate	0.55	0.73	0.45	+	−

were placed, immediately after cutting, in a small test tube with 2 ml. of water containing 0.5 to 1.0 mg. of the radiotracer, in an environmental plant growth chamber at 65° F. and 40% relative humidity, with 14 hours of daylight. The solution was characteristically imbibed within a few hours, and the leaves were transferred to pure water and held in the cabinet during the sampling period; (b) cotton plants, about 75 cm. high, were treated with 2 mg. of radiotracer as a saturated solution in acetone applied under a cellophane tape band; and (c) cotton plants, about 75 cm. high with developing squares and bolls, were injected with 5 mg. of radiotracer in 1 ml. of 50% ethanol applied through a capillary "funnel" cemented to a small hole in the base of the stem. Plants from treatments (b) and (c) were held in the growth chamber for the duration of the experiments, as described under (a).

The basic separations of the radiolabeled metabolites were carried out in a manner similar to that described by Dorough and Casida (*3*) using chromatographic columns 2 × 30 cm. packed with 60- to 100-mesh silicic acid (Florosil) from a slurry in hexane. Leaf tissues were homogenized in five times their weight of 50% ethanol and the supernatant, after concentration to about 0.5 ml. in vacuo, was added directly to the top of the column. The chromatograms were developed by passing the following sequence of solvents through the column and collecting individual 22-ml. fractions: ether-hexane (3:1), 330 ml.; chloroform, 220 ml.; ethyl acetate, 330 ml.; methanol, 440 ml.; and water, 150 ml. All solvents were reagent grade or were distilled before use.

The chloroform/water partition coefficients in Table I were determined by shaking microgram quantities of C^{14}-labeled compounds for exactly one minute in equal volumes of the solvents and determining the concentrations in aliquots of each layer, except for the values for Temik oxime sulfoxide and Temik oxime sulfone, which were measured by ultraviolet absorption spectrometry.

Thin layer chromatography was carried out in the usual manner using silicic acid (Absorbosil-1) 0.15 mm. thick and the chromatograms were developed in one of the following mixtures: (a) Skellysolve B–benzene–ethanol, 2:2:1 (SBE); (b) acetone–2-butanone, 1:1 with 1% water (AB); (c) ether–benzene, 3:1 (EB). The chromatograms were evaluated by spraying the plates with (a) 0.5% *N* - 2,6 - trichloro - *p* - benzoquinoneimine in cyclohexane and developing at 100° C. for 10 minutes to form orange spots with the thioether group, or with (b) 1% ninhydrin in pyridine and developing at 100° C. for 30 minutes to form red spots with amines produced from the carbamyl group (*3*). Radioautographs were made on Polaroid 3000 film.

Quantitative radioactive measurements were made by liquid scintillation counting using 5- or 10-ml. volumes of standard mixtures of PPO 2,5-diphenyloxazole, POPOP 1,4-bis-2-(5-phenyloxazolyl)-benzene, and toluene for nonpolar solvents, or of PPO, POPOP, and naphthalene in dioxane for polar solvents (*7*), with 0.5 ml. of the eluate from the column or with appropriate solvent extract of sequential 1-cm. sections of the thin layer plates.

The recoveries of radioactivity from the column consistently averaged from 90 to 100% of the radioactivity present in the original homogenates, and the total recovery of radioactivity from that added to the plant ranged from 73 to 90%.

Discussion of Results

The activities of Temik, its sulfoxide, and sulfone, and Temik oxime as anticholinesterases, and as toxicants to *Musca domestica* (S_{NAIDM} females) and to fourth instar larval *Culex pipiens quinquefasciatus* are shown in Table II. It will be noted that both Temik sulfoxide (76x) and Temik sulfone (17x) are more active than Temik as anticholinesterases suggesting that the rapidly formed oxidation products, especially the sulfoxide, are the primary systemic toxicants and are responsible for the long lasting systemic qualities of the compound. However, the more polar sulfoxide is somewhat less toxic by contact to the housefly than Temik although it is better synergized by piperonyl butoxide. These data are in agreement with those of Payne, Stansbury, and Weiden (*8*).

Oxidation and Hydrolysis of Temik-C^{14}. In order to understand the nature of simple changes which might occur in plant and insect with C^{14}-labeled Temik, experiments were carried out using *tert*-C^{14} Temik at room temperature in acetone solution, treated with hydrogen peroxide or sodium hydroxide, followed by column and thin layer chromatography of the C^{14}-labeled products.

Oxidation for 5 minutes with an excess of hydrogen peroxide produced approximately 20% of C^{14} eluting from the column in methanol which was tentatively identified as Temik sulfoxide by its thin-layer behavior (R_f 0.14 SBE, 0.35 BA) and its ninhydrin positive reaction. Longer oxidation produced a higher percentage of sulfoxide and, in addition, relatively small amounts of C^{14} eluting from the column in ethyl acetate and identified as Temik sulfone by its thin-layer behavior (R_f 0.4 SBE) and ninhydrin positive reaction.

Hydrolysis in 0.1% sodium hydroxide solution for 1 hour resulted in 79% of the C^{14} eluting from the column in ether-hexane. Its thin layer behavior (R_f 0.74 SBE) and lack of ninhydrin test indicated Temik oxime. After 24 hours, oxime formation was 95%.

Hydrolysis of Temik sulfoxide in 0.1% aqueous sodium hydroxide for 16 hours resulted in 53% of the C^{14} eluting from the column in methanol. Thin layer chromatography indicated 84% of this to be Temik oxime sulfoxide (R_f 0.34 SBE) and the remainder Temik sulfoxide (R_f 0.17 SBE).

These results are in accord with the known chemical behavior of Temik which is oxidized to sulfoxide and sulfone derivatives (*7*, *8*) and is hydrolyzed by sodium hydroxide to form, principally, the oxime. They suggest that similar simple reactions will occur from the action of oxidases and esterases in plants and animals.

Oxidation of Temik in Cotton. The presence of the methylthio group in Temik suggests a facile oxidation in the

Table II. Biological Properties of Temik and Metabolites

Compound	$I_{50}M$ Fly ChE	*Musca domestica* LD_{50}, μg./G. A (alone)	*Musca domestica* LD_{50}, μg./G. B (1:5 P.B.)[a]	*Culex pipiens 5-fasciatus* LC_{50}, P.P.M.
Temik	8.4×10^{-5}	5.5	3.35	0.160
Temik-SO	1.1×10^{-6}	20	2.4	0.168
Temik-SO_2	5.0×10^{-6}	290	9.0	0.55
Temik-oxime	$>1.0 \times 10^{-3}$	>500		>10
Temik-SO-oxime	$>1.0 \times 10^{-3}$	>500		>10
Temik-SO_2-oxime	$>1.0 \times 10^{-3}$	>500		>10

[a] Piperonyl butoxide synergist.

Table III. Temik Metabolism in Cotton Plants Treated Topically on Stem

Days after Treatment	C^{14} Label	P.P.M. in Leaf	Partition $HCCl_3/H_2O$	% $HCCl_3$ Fraction as Temik	Temik SO	Temik SO_2
2	*N*-CH_3	11.0	1.82	21	79[a]	
	tert-C	14.5	2.08	41	59	
	CH_3S	11.7	1.91	36	64	
4	*N*-CH_3	10.0	1.57	4	96	
	tert-C	13.9	1.53	7	78	15
	CH_3S	11.4	1.74	6	42	52
9	*N*-CH_3	6.8	1.65	0	82	18
	tert-C	6.6	1.26	0	77	23
	CH_3S	5.8	1.26	0	71	29
16	*N*-CH_3	6.5	1.45	0	87	12
	tert-C	12.3	1.09	0	62	38
	CH_3S	12.8	1.14	0	66	36

[a] This fraction was not separated chromatographically.

plant to the corresponding sulfoxide (SO) and sulfone (SO_2) derivatives in analogy with the comparable oxidative metabolism of demeton (Systox), disulfoton (Di-Syston), and phorate (Thimet) which occurs rapidly in cotton (*5*). The chromatographic behavior of the expected sulfoxide and sulfone oxidation products was established as shown in Table I. Cotton plants were treated topically at the base of the stem with 2 mg. each of the three labeled Temik preparations, *N*-$C^{14}H_3$ (a), $C^{14}H_3S$ (b), and *tert*-C^{14} (c) from saturated acetone solutions, and the upper leaves were homogenized in water and the radioactivity partitioned with equal volumes of chloroform at intervals as shown in Table III, and the chloroform layer was subjected to thin layer chromatography using solvent EB. This method separated intact Temik (R_f 0.36) from Temik sulfoxide (R_f 0.06) and sulfone (R_f 0.12). The combined spots for the two oxidation products were then removed from the plate, eluted in acetone, and rechromatographed with solvent SBE to separate Temik sulfoxide and Temik sulfone. Thus, as shown in Table III, the extracts of all three labeled plants behaved almost identically in the slow decrease in the $HCCl_3/H_2O$ partition ratios indicating the formation of more polar metabolites (Table I) and in the rate of disappearance of Temik, which was present only in small amounts after 4 days and could not be detected after 9 days. The rate of oxidation of Temik to the sulfoxide and of the latter to sulfone is considerably slower than previously found for phorate and disulfoton in cotton leaves (*5*). This doubtless is due to the steric hindrance of the thioether resulting from the two methyl groups of the *tert*-carbon.

Plant Metabolism of Temik

The above preliminary results, showing the oxidative metabolism of Temik, suggested that the plant metabolism was likely to be complex and could be evaluated better if a large amount of radiolabeled insecticide were placed in the interior of the cotton plant. Therefore, large isolated cotton leaves were treated with the *N*-C^{14} (a), C^{14}-H_3S (b), and *tert*-C^{14} (c) Temiks by allowing them to transpire about 2 ml. of water containing the radiotracers. Sections of the leaves were homogenized after 4 and 13 days at 65° F. and the C^{14} metabolic products subjected to elution chromatography as shown in Figure 1. These elution patterns for the three C^{14} Temiks are remarkable in their similarities, showing only slight differences at 4 days and only four areas of appreciable difference (II, III, V, VII) at 13 days. The identities of most of the C^{14} fractions can be established, tentatively, from the pattern of elution of known and possible degradation products, from R_f values on three systems of thin layer chromatography, and from behavior with spray reagents (Table I). Temik (I) disappeared rapidly and represented a small amount (A-5%, B-13%, C-18%) of the total activity after 4 days. The Temik sulfoxide (VI) was clearly the major metabolite

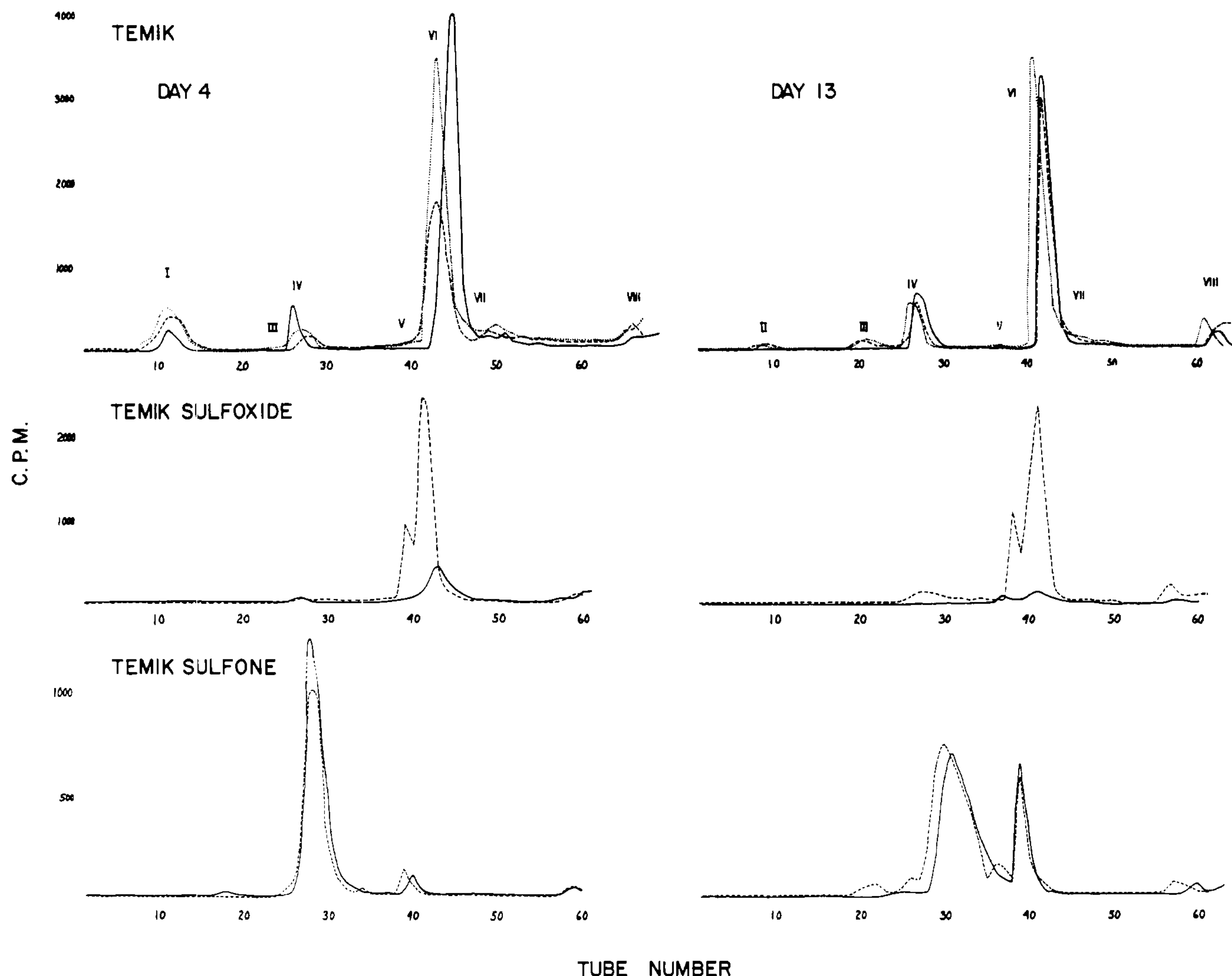


Figure 1. Elution chromatography of C^{14} Temik metabolites from isolated cotton leaves

Solid line N-$C^{14}H_3$ label, dotted line $C^{14}H_3S$ label, dashed line tert-C^{14} label. Solvent in tubes: 1–15, ether-hexane; 16–25, chloroform; 26–40, ethyl acetate; 41–60, methanol; 61–65, water

(A-83%, B-74%, C-72%) with a small amount having been further oxidized to Temik sulfone (IV) (A-6%, B-6%, C-5%). After 13 days, the Temik sulfoxide was still the major C^{14} metabolite (A-77%, B-81%, C-78%) and the Temik sulfone had increased (A-18%, B-13%, C-12%). Metabolite II does not contain the N-$C^{14}H_3$ group and cannot be Temik which was completely oxidized in previous experiments within 4 to 9 days. This metabolite appears to be the primary hydrolysis product, Temik oxime. Metabolite III also does not contain the N-$C^{14}H_3$ group and is probably the hydrolysis product of Temik sulfone or Temik oxime sulfone. This compound is less polar than the hydrolysis product of Temik sulfoxide or Temik oxime sulfoxide (Table I). The latter compound (metabolite VII) elutes from the column in methanol along with Temik sulfoxide and can be distinguished by thin layer chromatography with SBE solvent, by its failure to give the ninhydrin test, and its absence from the N-C^{14} label. The identity of the water eluting VIII is not known, but the ester linkage appears to be intact and this represents only a small percentage of C^{14} (at 4 days A = 6%, B = 7%, C = 9%; at 13 days A = 5%, B = 6%, C = 10%). This fraction may contain conjugates of carbamate metabolites.

Confirmatory experiments were conducted by repeating the treatment of isolated leaves with the three labeled Temiks exactly as above and studying thin layer chromatograms of extracts. After 4 days, extraction with 70% ethanol showed 90 to 99% of the C^{14} as Temik sulfoxide (R_f 0.15–0.16 SBE and 0.42–0.44 AB). The remainder was present as unchanged Temik (R_f 0.63–0.65 SBE) and as a trace of Temik sulfone (R_f 0.73–0.76 AB). After 13 days, the C^{14} of homogenates was partitioned between $HCCl_3/H_2O$: N-$C^{14}H_3$ 3.5; $C^{14}H_3S$ 4.2; and *tert*-C^{14} 2.7. Thin layer chromatography of the chloroform with solvent AB gave two ninhydrin positive spots with all three labels: R_f 0.35-N-$C^{14}H_3$ 70%, $C^{14}H_3S$ 66%, *tert*-C^{14} 83% (Temik sulfoxide) and R_f 0.64 (Temik sulfone). The presence of only two radioactive spots in all three labels was confirmed by radioautography. The radioactivity in the water phase was located in a single spot at R_f 0.1 in each of the three labels.

To relate the rate of metabolism of Temik to the total lifetime of the cotton plant, large plants with developing squares and bolls were injected with 5 mg. of the three C^{14}-labeled Temiks and elution chromatograms were made at 13 and 36 days. The amounts of radioactivity in the leaves were much lower than in the previous experiments, and the elution patterns were correspondingly simpler. After 13 days about 99% of the C^{14} radioactivity was present in the methanol eluate. Thin layer chromatography showed that this was 89% Temik sulfoxide (R_f 0.2 SBE, ninhydrin positive) and 11% Temik sulfoxide oxime (R_f 0.4 solvent SBE with *tert*-C^{14}). There was a trace of C^{14} in the ethyl acetate eluate as Temik sulfone and a trace in the water eluate (Table IV).

After 36 days in the plant, most of the C^{14} radioactivity from N-C^{14} and *tert*-C^{14} labels was in the methanol eluate (N-$C^{14}H_3$-82%, *tert*-C^{14}-80%) and a much smaller amount (N-$C^{14}H_3$ and *tert*-C^{14} 13%) was present in the ethyl acetate eluate as Temik sulfone. Another small amount (N-$C^{14}H_3$ 5%, *tert*-C^{14} 8%) was present in the water eluate. Thin layer chromatography of the methanol eluate revealed Temik sulfoxide (R_f 0.15–0.2) and Temik sulfoxide oxime (R_f 0.4 in *tert*-C^{14} label) in a ratio of about 85 to 15%.

These experiments were continued through 56 days at which time the $HCCl_3/H_2O$ partition coefficients of leaves and seeds as shown in Table IV still indicated the presence of appreciable amounts of chloroform partitioning C^{14}, and thin layer chromatography showed the presence of Temik sulfoxide.

Plant Metabolism of Temik Sulfoxide. Two chromatographically pure preparations were available for study with N-C^{14} and *tert*-C^{14} labels. The latter preparation (1.34 μc. per mg.) was much more radioactive than the former (0.075 μc. per mg.). These were administered to isolated cotton leaves through uptake of aqueous solution. The elution chromatograms after 4 and 13 days at 65° F. are shown in Figure 1. At 4 days, only a trace amount of the C^{14} activity was in the ethyl acetate eluate. This was clearly the Temik sulfone (R_f 0.36 SBE). The C^{14} fraction eluting in methanol from the *tert*-C^{14} label was about 63% Temik sulfoxide (R_f 0.15 SBE) and the remainder Temik sulfoxide oxime (R_f 0.4). At 13 days, with the *tert*-C^{14} label, the ethyl acetate eluting Temik sulfone formed 10% of the total C^{14} activity. The methanol eluting C^{14} (82%) was shown by thin layer chromatography to consist of about 64% Temik sulfoxide (R_f 0.15 SBE, ninhydrin positive) and 36% Temik sulfoxide oxime (R_f 0.4).

With the N-C^{14} label at 4 days (Figure 1), a trace of Temik sulfone was observed with most of the activity in the methanol eluate (R_f 0.16, SBE) as Temik sulfoxide. At 13 days a trace of Temik sulfone was observed in the ethyl acetate. Most of the activity eluted in methanol (R_f 0.23 SBE) as Temik sulfoxide.

Plant Metabolism of Temik Sulfone. Two preparations of Temik sulfone with N-$C^{14}H_3$ and *tert*-C^{14} labels were used with isolated cotton leaves to further elucidate the metabolism of Temik. Results of a typical set of several experiments are shown in the 4- and 13-day elution chromatographs of Figure 1. Two peaks of elution were found in ethyl acetate and in methanol with both labels. The identity of the ethyl acetate eluate as Temik sulfone was confirmed by its R_f values of 0.63–0.65 in thin layer chromatography in solvent AB, R_f values of 0.34–0.35 in solvent SBE, its ninhydrin positive reaction, and a single spot by radioautography, in both C^{14} labels.

Thin layer chromatography showed the methanol eluate to be a mixture of two major components R_f 0.35–0.40 and R_f 0.1 in solvent SBE. After 13 days with the *tert*-C^{14} label, the spot R_f 0.39 contained 71% of the total activity and with the N-$C^{14}H_3$ label 98% of the total activity. These two materials seem to form in the leaf rather than by alteration on the column, as thin layer chromatography of the leaf extracts at 4 days after uptake of Temik sulfone showed N-$C^{14}H_3$ label 90–93% Temik sulfone and *tert*-C^{14} label 80–98% Temik sulfone (R_f 0.34–0.36 SBE solvent). At 13 days, the leaf extracts contained N-$C^{14}H_3$ label 77% Temik sulfone (R_f 0.36) and *tert*-C^{14} label 71–86% Temik sulfone (R_f 0.35). The remainder of the radioactivity was mostly at R_f 0.1. The authors suggest that this material is Temik sulfoxide, which has the proper elution and thin layer chromatographic properties, contains both radiolabels, and is ninhydrin positive. This could only be formed by reduction of the

Table IV. Translocation and Metabolism of Temik in Mature Cotton Plants

Days after Treatment	Label	P.P.M.	$HCCl_3/H_2O$ Partition of C^{14}	Metabolites Found as % Total C^{14}		
				SO	SO_2	SO oxime
13-leaf	N-C^{14}		0.64	84	0.7	
	$C^{14}H_3S$		0.92	61	7.0	
	tert-C^{14}		0.37	49.5	16.3	5.4
36-leaf	N-C^{14}		0.40	80.0	15.0	. . .
	$C^{14}H_3S$		0.28			
	tert-C^{14}		0.37	65.5	14.3	13.0
56-leaf	N-C^{14}	25.7	0.012			
	tert-C^{14}	35.4	0.22	50	50[a]	
56-seed	N-C^{14}	2.12	0.23			
	tert-C^{14}	0.57	0.038			

[a] See footnote Table III.

Temik sulfone in the plant, and such reactions have not yet been described. There is the possibility of formation of the $N \rightarrow O$ or N-CH_2OH derivatives of Temik sulfone as described by Dorough and Casida for carbaryl (*3*). These would be more polar and would also contain both radiolabels. These points require further investigation.

At both 4 and 13 days, a small shoulder appeared in the *tert*-C^{14}–labeled elution chromatograms (Figure 1) at the chloroform–ethyl acetate interface. This, probably, represents the hydrolysis product Temik oxime sulfone (metabolite III of Figure 1). A summary of the plant metabolism of Temik is shown in Figure 2.

Metabolism of Temik in Insects

Production of $C^{14}O_2$. Experiments were carried out with topical applications of Temik N-$C^{14}H_3$, $C^{14}H_3S$, and *tert*-C^{14} labels to the female housefly *Musca domestica* S_{NAIDM} and carbamate resistant R_{MIP} strains. Following application, the flies were immediately placed in a metabolism chamber, and the $C^{14}O_2$ released was trapped over 24 hours and determined by liquid scintillation counting (*6*). The data obtained are shown in Table V. The values obtained for release of $C^{14}O_2$ as per cent of total Temik absorbed vary somewhat with the dosage (as the compound is very toxic to flies) and with the fly strain. However, the information shows quite conclusively that about two times as much $C^{14}O_2$ is released from the Temik N-C^{14} as from the *tert*-C^{14} or $C^{14}H_3S$ labels. This suggests that a small proportion of the Temik is oxidized at the N-CH_3 group and then converted to formaldehyde and ultimately to CO_2 as Dorough and Casida (*3*) have suggested for carbaryl.

The production of as much as 5% $C^{14}O_2$ from the Temik $C^{14}H_3S$ indicates the S-demethylation of Temik followed by oxidation to $C^{14}O_2$. This type of metabolic demethylation has been shown by Mazel, Henderson, and Axelrod (*4*) to occur with S-methyl cysteine and methylmercaptan which, when incubated with rat liver microsomes, produced formaldehyde. Both N-dealkylation and S-dealkylation are well known functions of the action of the "mixed function" oxidases (*2*) and may be expected to occur readily in the housefly because of its high oxidase activity.

Internal Metabolism. Fifty female S_{NAIDM} flies were topically treated each with 1.6 to 1.8 μg. of Temik with N-C^{14}, *tert*-C^{14}, and $C^{14}H_3S$ labels. After 4 hours, the surface was washed thoroughly in 20 ml. of acetone and the washed flies homogenized in 50 ml. of 100% ethanol. The ethyl alcohol extract was introduced then into the chromatographic column and eluted as described under Plant Metabolism. The per cent penetration of the Temik as measured from the surface wash was N-$C^{14}H_3$-72%, $C^{14}H_3S$-75%, and *tert*-C^{14}-68%. The elution of the C^{14} metabolites is shown in Figure 2. From 98 to 99% of the internal C^{14} in the flies (amount applied – surface wash and fecal excretion) was recovered from the column with each of the three labeled Temiks. The identity of II (Temik), IV (Temik sulfone), and VI (Temik sulfoxide) was confirmed with thin layer chromatography. Peak I is unlabeled in N-CH_3 and possibly is Temik oxime. Peak III is unknown.

The Temik sulfoxide represents the greater part of the total C^{14}, (N-$C^{14}H_3$-75%, $C^{14}H_3S$-60%, *tert*-C^{14}-65%) and the Temik sulfone much less (N-C^{14}-H_3-6%, $C^{14}H_3S$-21%, *tert*-C^{14}-10%). The water eluting C^{14} (N-$C^{14}H_3$-19%, $C^{14}H_3S$-19%, and *tert*-C^{14}-25%) is relatively higher than in the cotton plants (compare Figures 1 and 3). Thus, although the definition of the minor eluates is less precise owing to the lesser amounts of radioactivity in the housefly experiments, apparently, the oxidative metabolism of Temik in the housefly is very similar to that in the cotton plant.

Treatment of 50 female S_{NAIDM} flies with 1.0 μg. of *tert*-C^{14} Temik sulfoxide for 4 hours, followed by thorough washing of the surface in acetone as above, indicated that only 8.0% of the Temik sulfoxide had penetrated. This, compared to the average of 72% penetration of Temik, shows the effect of the greater sulfoxide polarity upon the solubility of the compound in the insect cuticle and accounts for the decreased topical toxicity of the sulfoxide as shown in Table II. After 4 hours, 4.6% of the total dosage had appeared in the feces.

An identical experiment with *tert*-C^{14} Temik sulfone applied to 50 female S_{NAIDM} flies for 4 hours showed 36% penetration of the Temik sulfone, as compared with 8.0% for Temik sulfoxide, and 72% for Temik. These rates of penetration are in accord with the $HCCl_3/H_2O$ partition values for the compounds as shown in Table I. With the Temik sulfone, 6.7% of the total dosage had appeared in the feces.

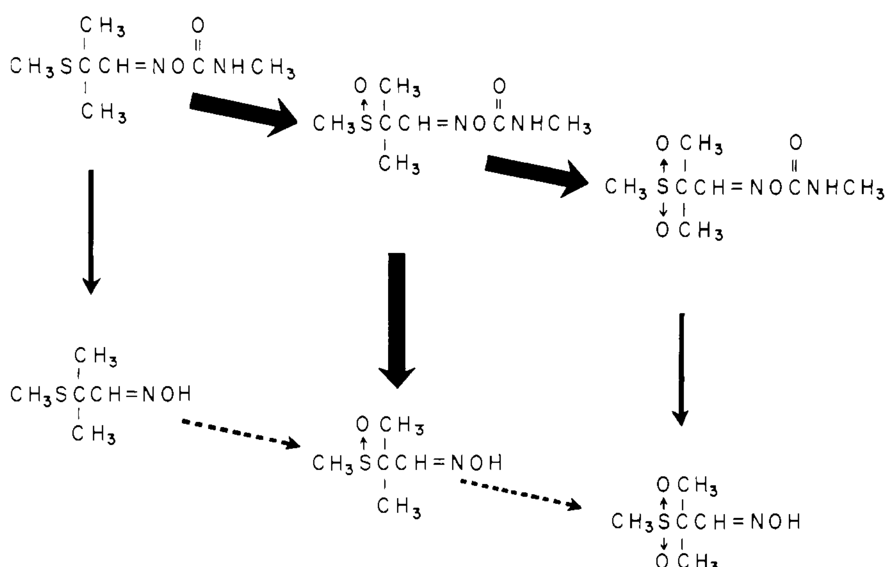


Figure 2. Pathways of Temik metabolism in cotton

Broad arrows represent major pathways, dotted arrows hypothetical pathways of minor importance

Table V. Metabolism of C^{14} Temik to $C^{14}O_2$ in the Housefly

Compound	Strain	Dosage, μG./Female	Absorbed 24 Hours, %	C^{14} as Per Cent of Amount Absorbed		
				CO_2	Fecal	Internal
N-$C^{14}H_3$	S_{NAIDM}	0.28	67.4	5.6	15.2	79.1
	R_{MIP}	0.28	88.2	9.0	41.7	49.2
	S_{NAIDM}	1.8	71.3	3.0	7.6	89.3
	R_{MIP}	1.8	79.5	6.7	21.8	66.4
$C^{14}H_3S$	S_{NAIDM}	0.25	60.5	5.0	24.8	70.2
	R_{MIP}	0.17	86.5	2.6	49.1	48.3
	S_{NAIDM}	1.7	65.6	1.2	12.6	86.2
	R_{MIP}	1.7	72.6	0.9	24.7	74.4
tert-C^{14}	S_{NAIDM}	0.20	59.7	5.1	31.0	63.9
	R_{MIP}	0.16	83.2	2.6	49.4	48.0
	S_{NAIDM}	1.6	65.5	1.1	6.7	92.3
	R_{MIP}	1.6	72.9	1.3	13.5	85.2

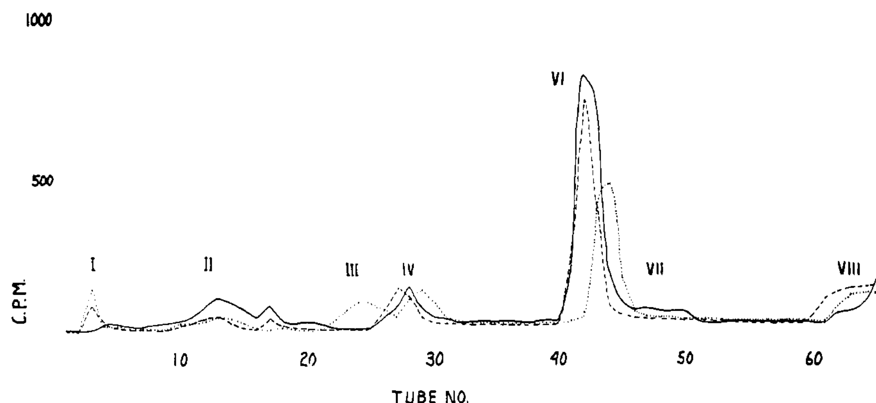


Figure 3. Elution chromatography of C^{14} Temik metabolites in the housefly

Legend as in Figure 1

Table VI. Metabolism of C^{14} Temik in R_{MIP} Housefly

Compound		% Absorbed C^{14} in Feces at 24 hr.
N-$C^{14}H_3$ as	Temik	1
	Temik-SO	28
	Temik-SO_2	72
$C^{14}H_3S$ as	Temik	3
	Temik-SO	23
	Temik-SO_2	74
tert-C^{14} as	Temik	1
	Temik-SO	23
	Temik-SO_2	76

Thin layer chromatography of the fly homogenate in 50% ethanol with solvent SBE showed a trace of activity at R_f 0.4 (Temik sulfone), another at R_f 0.06 ninhydrin positive, and a third at R_f 0.7 which might be the hydrolysis product Temik oxime sulfone.

Because of the poorer penetration of Temik sulfoxide and sulfone into the housefly, obtaining enough internal radioactivity from topical applications was difficult. Therefore, the two compounds were made up as 1–2% sugar baits and 50 to 100 mg. were fed to 20 female flies. After 24 hours, the flies were homogenized in 50% ethanol or acetonitrile, and the extract was concentrated and subjected to thin layer chromatography using SBE solvent. The *tert*-C^{14} Temik sulfoxide treatment produced a spot containing 90% of the C^{14} at R_f 0.15, ninhydrin positive, which was the original Temik sulfoxide and a spot with 10% of the C^{14} at R_f 0.4 which was probably Temik sulfone. The excreta contained only Temik sulfoxide.

With the sugar baits of Temik sulfone, after 24 hours, the internal C^{14} was located in three spots, R_f 0.18, R_f 0.28, and R_f 0.42 with SBE solvent. The last of these contained 47% of the activity with N-$C^{14}H_3$, 83% with $C^{14}H_3S$, and 44% with *tert*-C^{14} Temik, was apparently unchanged Temik sulfone. The others, present with all three labels, are of unknown constitution. Their R_f values are not readily identifiable with any of the primary oxidation or hydrolysis products of Table I unless reduction to Temik sulfoxide (R_f 0.15) has occurred.

Excretion of C^{14} Metabolites. Following topical treatment of houseflies with C^{14} Temik N-C^{14}, *tert*-C^{14}, and $C^{14}H_3S$, a large proportion of the C^{14} is liberated in the feces over 24 hours as shown in Table VI. The percentage of the absorbed dosage thus excreted varied with the total dosage and with the fly strain, being much higher at lower dosages and with resistant flies. However, where the flies survived over the 24-hour period, from 40 to 60% of the total dosage was excreted in the feces.

The nature of the fecal metabolites of Temik was explored by treating approximately 150 to 200 female R_{MIP} houseflies with 0.016-0.018 μg. of Temik N-C^{14}, *tert*-C^{14}, or $C^{14}H_3S$ and collecting the feces for 24 hours. Both the acetone extracts of the feces and the homogenates of the houseflies in ethanol were concentrated in air streams and analyzed by thin layer chromatography, first by using SBE to separate Temik (R_f 0.6–0.7) from Temik sulfoxide and sulfone (R_f 0.1–0.4) and then by rechromatographing the first half of the thin layer plate using AB to separate Temik sulfoxide (R_f 0.4) from Temik sulfone (R_f 0.68). This method gave excellent separations of the three metabolites which are shown in Table V as percentages of the total C^{14} in the feces. The patterns for the three labeled Temiks were almost identical and showed only a very small amount of Temik remaining after 24 hours and the major excretory product to be Temik sulfone. No evidence of hydrolysis of Temik, Temik sulfoxide, or Temik sulfone was obtained.

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