Preparation and Detection of Derivatives of Temik and Its Metabolites as Residues

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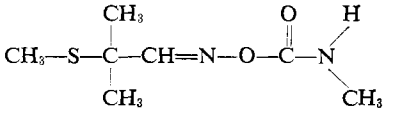
Metabolism of Temik leads predominantly to the sulfoxide and sulfone, both toxicologically important. Of less importance are the oxime analogs of the three carbamoyl oximes, known as metabolites of Temik. The primary concerns of a residue study of Temik are isolation and detection of the carbamoyl oximes. The oximes present in the sample are removed by acid hydrolysis, which is also employed for cleanup. Further cleanup with a column packed with silica gel also allows preliminary separation of the compounds. For quantitative determination, gas-liquid chromatography utilizes a column packed with Carbowax 20M and SE-30 on Gas Chrom Q and equipped with a Dohrmann Model T-300-P microcoulometric titration cell. Because of the wide spectrum of retention times, the analysis is carried out at column temperatures ranging from 80° to 190° C. Excellent peak shape and resolution are obtained. Peak area is proportional to amount of injection, and response is linear to at least 1.0 μg. Spiked and treated samples of sugar beets were also analyzed. Recoveries of 90 to 104% were observed, and the minimum detectable amount was found to be 0.01 p.p.m.

Temik [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl) oxime] is an experimental systemic pesticide exhibiting excellent performance against many species of insects, mites, and nematodes. It is also known as UC21149, and its structural formula is:



The insecticidal and acaricidal properties of Temik were first reported by Weiden *et al.* (1965). Its toxicities for rats and different species of insects were also mentioned. Payne *et al.* (1966) reported the synthesis of a series of trisubstituted acetaldehyde *O*-(methylcarbamoyl) oximes, and their physical properties and insecticidal activities. Temik was pronounced to be the most generally effective contact and systemic insecticide. Temik sulfoxide and Temik sulfone (Table I) were included in this paper, which also reported the hydrolysis and thermal decomposition of Temik. Synthesis of C^{14}-labeled Temik was reported by Bartley *et al.* (1966). The C^{14}-labeled Temik sulfoxide was prepared by peracid oxidation of labeled Temik.

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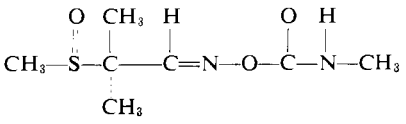
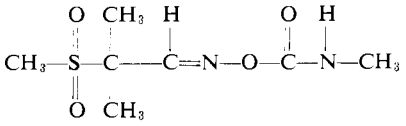
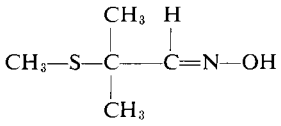
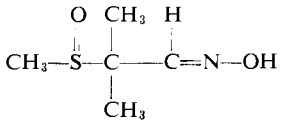
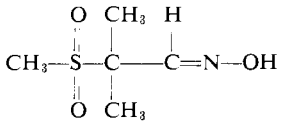
Studies of metabolism of Temik in cotton plant and housefly by Metcalf *et al.* (1966), in insect pests of cotton by Bull *et al.* (1967), in cotton plants and soil by Coppedge *et al.* (1967), and in rats by Knaak *et al.* (1966) and Andrawes *et al.* (1967) have shown similar results. Regardless of the biological system to which it is applied, Temik is readily and completely oxidized to its sulfoxide; further oxidation to its sulfone is slow. Temik sulfoxide and Temik sulfone are further hydrolyzed to the oximes, which are the degradation products and are nontoxic (Table I).

A colorimetric method for the determination of Temik residues in fruits and vegetables, reported by Johnson and Stansbury (1966), involves both acid and base hydrolysis to liberate hydroxylamine, which is then oxidized to nitrous acid by iodine. Finally the nitrous acid is determined by diazotization. However, this method is nonspecific and cannot be used to determine the individual metabolites. A GLC method for the determination of Temik and its metabolites has been reported by Maitlen *et al.* (1968). A gas chromatograph equipped with a commercial flame photometric detector specific for sulfur was employed.

Since the oximes are nontoxic, it is not necessary to include them in an analytical procedure. However, any acceptable procedure for the determination of Temik must also include Temik sulfoxide and Temik sulfone and should be specific for each of these two metabolites as well as the parent compound. The method described is a gas-liquid chromatographic method for Temik and its significant metabolites.

Table I. Chemical Names and Formulas of Important Temik Metabolites

Abbreviated Name	Chemical Name	Formula
Temik sulfoxide	2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl) oxime	
Temik sulfone	2-Methyl-2-(methylsulfonyl)-propionaldehyde *O*-(methylcarbamoyl) oxime	
Temik oxime	2-Methyl-2-(methylthio)-propionaldehyde oxime	
Temik sulfoxide oxime	2-Methyl-2-(methylsulfinyl)-propionaldehyde oxime	
Temik sulfone oxime	2-Methyl-2-(methylsulfonyl)propionaldehyde oxime	

EXPERIMENTAL

Chemicals. Standards of Temik and its metabolites were used without further purification. One microgram per microliter stock solutions were prepared by weighing an exact amount of the pure compound and dissolving it in chloroform. Sample solutions were prepared from stock solutions in concentrations of 200, 50, and 10 ng. per μl., respectively.

Extraction. Tops and roots of the sugar beet samples submitted by the Entomology Department were analyzed separately. Two portions of 50 grams of the chopped foliage or 100 grams of the chopped roots were blended with 125 or 175 ml. of chloroform, respectively. One portion was used for the determination of Temik and the second portion for the sulfoxide and the sulfone. Two separate analyses were necessary because of the vast differences in the solubilities and other related properties of Temik and the two metabolites. Then 150 grams of sodium sulfate were added to the foliage samples and 175 grams were added to the root samples. The mixtures were blended at high speed for 60 seconds. The procedure was repeated with two additional portions of chloroform.

The extract was transferred to a 500-ml. Erlenmeyer flask attached to a vacuum rotatory evaporator and immersed in a 50° C. water bath. The mixture was concentrated to 2 ml. and then evaporated to complete dryness with a stream of nitrogen.

Cleanup and Derivatization. To each flask containing the dry residue from the chloroform extracts, 40 ml. of 0.1*N* hydrochloric acid was added. The mixture was heated in a boiling water bath for 10 minutes with occasional swirling to help in loosening the lumps and dissolving the desired constituents. Then the flask was cooled to room temperature. At this point each oxime had been converted to its aldehyde, and the carbamoyl oximes had been extracted into the aqueous solution.

Each sample used for the determination of Temik was transferred to a clean flask after filtering through a coarse sintered glass funnel to remove the solid materials. The apparatus was rinsed twice with distilled water. Twenty milliliters of 1.0*N* sodium hydroxide were added to the acid solution. The resultant basic solution was reheated in the boiling bath for 2 minutes, cooled to room temperature, and transferred to a separatory funnel. It was extracted with three 50-ml. portions of chloroform, which was dried by the use of anhydrous sodium sulfate. The final solution was evaporated to a small volume by using the vacuum rotatory evaporator and was transferred to a graduated sedimentation tube. Then, it was evaporated to exactly 0.10 ml. with a stream of nitrogen. This concentrated solution was ready for further cleanup or for GLC analysis.

Each acid solution used for the determination of Temik sulfoxide and Temik sulfone was transferred to a separatory funnel after filtering through a coarse sintered glass funnel. The apparatus was rinsed twice with distilled water. The solution was extracted with two 50-ml. portions of cyclohexane to remove chlorophyll and fatty materials, with three 50-ml. portions of chloroform. The chloroform solution was dried with anhydrous sodium sulfate, and collected in a round-bottomed flask. This final solution was concentrated to a small volume by using the vacuum rotatory evaporator and transferred to a sedimentation tube, where it was evaporated to exactly 0.20 ml. with a stream of nitrogen. Thus it was ready for further cleanup or GLC analysis.

The above procedures separated the desired compounds

from most samples. However, when further cleanup became necessary, the procedure reported by Kadoum (1967) was employed with some modification. The elution column used in his procedure consisted of approximately 3 inches of plain silica gel packed in a disposable pipet. Because of the slowness in the elution rate, the columns used in the present study (Figure 1) were operated under reduced pressure to assure an elution rate of 0.2 ml. per minute.

The column was prewetted with three 0.5-ml. portions of benzene. Before the last portion of the solvent passed into the sand, 0.1 ml. of the concentrated chloroform extract was added to the top of the column, and was eluted first with three 0.5-ml. portions of benzene (fraction I). Before the last portion of the eluent passed into the sand, the collecting test tube was replaced and the eluent was changed to collect three 0.5-ml. portions each of a 3-to-1 mixture of benzene and methanol (fraction II). The same procedure was repeated with a 1-to-1 benzene-methanol mixture (fraction III), a 1-to-3 mixture of benzene and methanol (fraction IV), and pure methanol (fraction V). The last fraction was driven out by three additional portions of the same solvent. Each fraction was collected in a separate graduated test tube, and evaporated to 1.0 ml. with a stream of nitrogen.

Fractions I and V from the above operation were discarded because they did not contain any of the compounds included in this study. Fractions II, III, and IV from the base-hydrolyzed sample could be used for the determination of the oximes of Temik sulfoxide and Temik sulfone in addition to Temik oxime. This offers an excellent method for confirming the results obtained from analysis of the carbamates themselves. For eluents obtained from samples treated with acid only, only fractions III and IV were analyzed for Temik sulfoxide and Temik sulfone. Table II summarizes the information on the elution pattern of the separated components.

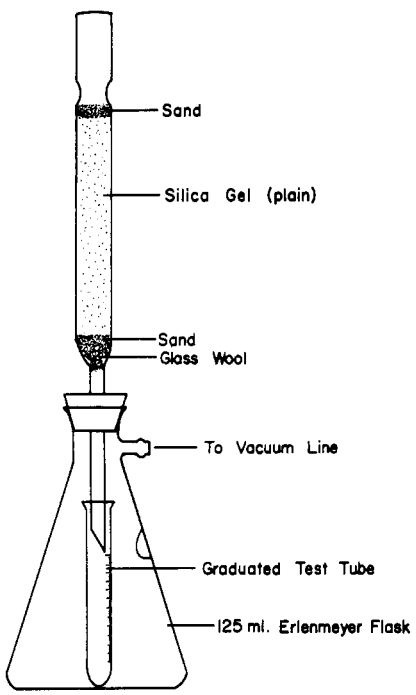


Figure 1. Elution chromatographic column

Gas Chromatograph. A Dohrmann microcoulometric gas chromatograph consisted of a Model C-200 coulometer, a Model T-300-P titration cell for determining sulfur, a Model P-100 furnace control unit, and a Model G-100 gas chromatograph. The gas chromatograph was connected to a recorder with a range of 1.0 mv. It was operated at a speed of 0.5 inch per minute.

Gas Chromatograph Analysis. The gas chromatograph was set up according to the following conditions:

Column.	Glass, 5 feet × 6-mm. O.D.
Packing.	A 1-to-1 mixture of 5% Carbowax 20M and 10% SE 30 on 100/120-mesh Gas Chrom Q
Carrier gas.	Nitrogen at 120 ml./min.
Combustion gas.	Oxygen at 120 ml./min.
Column temperatures.	80° C. for Temik, 165° C. for Temik sulfone, 190° C. for Temik sulfone oxime, and 135° C. for all others.
Block temperatures.	135° C. for Temik, 200° C. for Temik sulfone, 240° C. for Temik sulfone oxime, and 190° C. for all others.
Bias reading.	160 mv.

The column was conditioned overnight at 135° C. Standards were run frequently. Five microliters of the final sample solutions were injected into the gas chromatograph for analysis and the resultant "peak" areas were measured. When necessary, these solutions were further concentrated or diluted. A Filotechnical Salmoiraghi Model 236A optical planimeter was used to measure the peak areas.

RESULTS AND DISCUSSION

Gas chromatographic retention data on Temik and its metabolites are listed in Table II, which also shows the column chromatographic fractions in which these compounds can be found. Since the retention times of Temik oxime, the sulfoxide, and the sulfoxide oxime are close together, and these compounds cannot be separated by column chromatography, it is best to remove the oximes prior to the actual analysis. This study utilized acid hydrolysis to convert the oximes quantitatively to their respective aldehydes (Johnson and Stansbury, 1966), which do not interfere with the analysis. It has been reported

Table II. Gas Chromatographic Retention Times and Elution Chromatographic Fractions[a]

Compound	Column Temp., °C.	Retention Time, Min.	Column Fraction Where Found
Temik	80	2.8	II
Temik oxime	135	3.6	II, III
Temik sulfoxide oxime	135	4.7	II, III
Temik sulfoxide	135	5.7	III
	150	3.3	
Temik sulfone	165	4.0	III, IV
Temik sulfone oxime	150	5.5	
	190	2.8	III

[a] Gas chromatographic conditions and column chromatographic conditions described in text.

that carbamates are stable under acid conditions (Cassil and Cullen, 1968; Coppedge *et al.*, 1967) but are rapidly hydrolyzed to form their oximes under basic conditions (Johnson and Stansbury, 1966). Heating in aqueous acid solution also extracts the desired compounds from the crop materials, because Temik and its metabolites are soluble in water while the crop materials are not. After heating with the dried chloroform extract of sugar beets leaf samples and filtering through coarse sintered glass funnel, the acid solutions appear to be only very slightly green.

Solubility studies of Temik and its metabolites indicated that Temik is soluble in cyclohexane, mixed hexane, ethyl ether, chloroform, alcohols, water, etc. Temik sulfoxide and Temik sulfone are practically insoluble in hydrocarbons and ethers, but are very soluble in chloroform, alcohols, and water. Extraction studies (Table III) showed that Temik sulfoxide and Temik sulfone remained in the aqueous layer when the 0.1*N* acid solutions were extracted with cyclohexane, mixed hexane, or ethyl ether, while Temik itself was partitioned into the organic layer in every case. Extraction of the aqueous solutions with chloroform partitioned all of the carbamates into the organic layer. These properties made it possible to extract chlorophyll and other crop materials from the acid solutions with cyclohexane, and to extract the insecticides from both samples and aqueous solutions with chloroform.

Despite the fact that Temik was extracted by cyclohexane along with the contaminants, attempts to analyze this insecticide after the solvent was evaporated and followed by base hydrolysis did not yield satisfactory results. Apparently, Temik was lost during evaporation. Similar results were obtained when mixed hexane or ethyl ether was tested in place of cyclohexane. Therefore, Temik was analyzed apart from its metabolites, and was determined as its oxime for two reasons. First, because of its short retention time, Temik must be determined at a temperature below 80° C., allowing enough time for the solvent to be vented from the gas chromatograph before the insecticide itself elutes from the column. However, at temperatures below 80° C., other contaminants from the samples may not be eluted out prior to Temik itself and their responses would cover the response from Temik. Second, Temik is detected with very poor response compared with its metabolites, including Temik oxime, and this can affect the over-all sensitivity of the method. Since Temik is metabolized rapidly to its oxidation products and is rarely found as a residue (Metcalf *et al.*, 1966), the alternate procedures are rarely used for residue analysis. The primary concern of a residue analysis for Temik is to isolate and detect its sulfoxide and sulfone.

Acid hydrolysis provided an efficient method of cleanup for both the tops and roots of sugar beets, and when additional cleanup was needed, column chromatography was employed. In that case, the base-hydrolyzed sample was

Table III. Separation and Recovery of Temik and Metabolites by Selective Extractions[a]

Solvent Systems	Compounds Studied: Temik[b]	Temik sulfoxide	Temik sulfone
Cyclohexane	1000	5.0	<0.05
0.1*N* HCl	<0.05	1000	1000
Mixed hexanes (b.p. 42° C.)	1000	6.0	<0.05
0.1*N* HCl	<0.05	1000	1000
Ethyl ether	1000	8.0	6.25
0.1*N* HCl	<0.05	1000	1000

[a] Quantities in micrograms.
[b] Temik determined as its oxime.

Table IV. Separation and Recovery of Temik, Temik Sulfoxide, and Temik Sulfone from Associated Oxime of Each Compound

P.P.M. Added		P.P.M. Found[b]		% Recovery[a]
Temik	Temik oxime	Temik[b]	Temik oxime	Temik
0.50	0.50	0.48	[c]	96
0.20	0.20	0.20	[c]	100
0.10	0.10	0.095	[c]	95
0.05	0.05	0.052	[c]	104
0.02	0.02	0.02	[c]	100
0.01	0.01	0.0096	[c]	96
Temik sulfoxide	**Temik sulfoxide oxime**	**Temik sulfoxide**	**Temik sulfoxide oxime**	**Temik sulfoxide**
0.50	0.50	0.48	[c]	96
0.20	0.20	0.20	[c]	100
0.10	0.10	0.10	[c]	100
0.05	0.05	0.048	[c]	96
0.02	0.02	0.019	[c]	95
0.01	0.01	0.0099	[c]	99
Temik sulfone	**Temik sulfone oxime**	**Temik sulfone**	**Temik sulfone oxime**	**Temik sulfone**
0.50	0.50	0.46	[c]	92
0.20	0.20	0.20	[c]	100
0.10	0.10	0.095	[c]	95
0.05	0.05	0.046	[c]	92
0.02	0.02	0.018	[c]	96
0.01	0.01	0.009	[c]	90

[a] Recovery for the oximes was practically zero.
[b] Determined as oxime after removal of latter.
[c] No Temik oxime, sulfoxide oxime, or sulfone oxime detected, or less than 0.01 p.p.m.

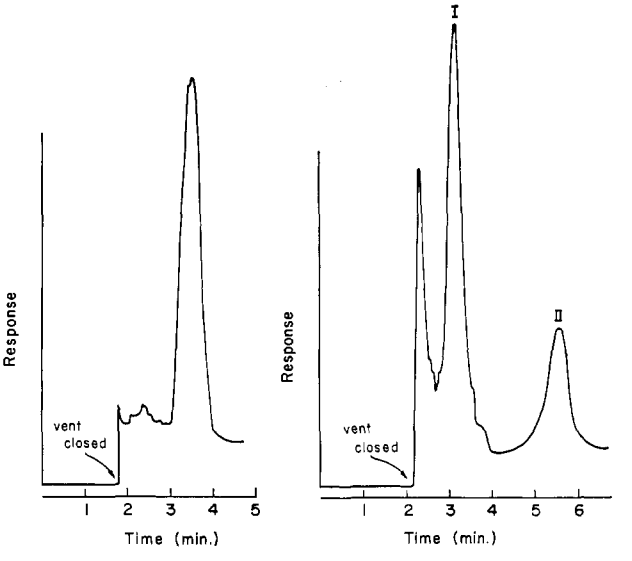


Figure 2. Gas chromatograms of spiked sugar beet foliage

Left. 0.05 p.p.m. of Temik after conversion to oxime
Right. 0.05 p.p.m. of Temik sulfoxide (I) and Temik sulfone (II)

Table V. Gas Chromatographic Determination of Temik and Metabolites in Chloroform Extracts of Sugar Beets

Amount of Sample, G.	Time since Application of Insecticide, Days	Found, P.P.M.		
		Temik[a]	Temik sulfoxide	Temik sulfone
	Sugar Beet Foliage			
50	7	0.8	1.2	ND[b]
		0.7	1.1	ND
	14	ND[b]	0.98	ND
		ND	0.86	ND
	21	ND	1.13	ND
		ND	0.80	ND
	29	ND	1.20	0.07
		ND	1.10	0.07
	35	ND	0.20	0.05
		ND	0.62	0.06
	42	ND	0.82	0.10
		ND	0.62	0.10
	161 (harvest time)	ND	ND	ND
	161	ND	ND	ND
	Sugar Beet Root			
100	7	ND	0.08	ND
		ND	0.06	ND
	14	ND	0.05	ND
		ND	0.05	ND
	21	ND	0.05	ND
		ND	0.05	ND
	29	ND	ND	ND
		ND	ND	ND
	35	ND	0.05	ND
		ND	ND	ND
	42	ND	ND	ND
		ND	ND	ND
	161 (harvest time)	ND	ND	ND
	161	ND	ND	ND

[a] Temik determined as oxime.
[b] None detected, or less than 0.01 p.p.m.

again cleaned up in a separate column apart from the sample treated with only dilute acid. Excellent results and recoveries were obtained from the cleanup procedures employed. Analysis of the spiked samples showed over 90% recoveries of the residues (Table IV).

As temperature programming was not available, it was necessary to determine Temik (as its oxime) and Temik sulfoxide at 135° C., and Temik sulfone at 165° C. with the gas chromatographic system used and the microcoulometric detector. However, when it was not necessary to determine Temik, it was possible to determine Temik sulfoxide and Temik sulfone at 150° C. (Figure 2). Standard curves were constructed by plotting peak area against the amount of compound. A straight line was observed for every compound from 50 ng. to at least 3 μg.

Results of the analysis of treated samples of sugar beets are listed in Table V. The plants were sampled six times at an interval of 7 days, and the last samples were collected at the time of harvest. No residue was found in the untreated samples. In the treated samples Temik sulfoxide was found to be predominant, with much less Temik sulfone detected. Trace amounts of Temik were found in the samples collected 7 days after application of the insecticide, and none was found in the samples collected after that time. This observation agrees with that made by Maitlen *et al.* (1968). The minimum detectable amount was found to be 0.01 p.p.m. for each of the compounds studied (Table IV).

ACKNOWLEDGMENT

The authors thank the Union Carbide Corp., New York, N.Y., for gifts of a grant-in-aid and standards of chemicals used in this investigation.

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Received for review July 10, 1968. Accepted October 14, 1968. Division of Agricultural and Food Chemistry, 155th Meeting, ACS, San Francisco, Calif., April 1968.