Determination of Residues of 2-Methyl-2-(methylthio)propionaldehyde *O*-(Methylcarbamoyl)oxime (UC-21149, Temik), Its Sulfoxide, and Its Sulfone by Gas Chromatography

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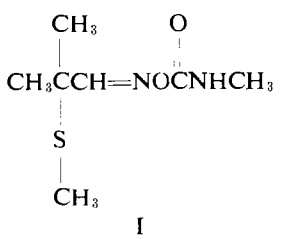
Residues of 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime (UC-21149, Temik), its sulfoxide, and its sulfone were determined in oranges, apples, sugar beets, and potatoes by gas chromatography with a commercial flame photometric detector highly specific for sulfur. Liquid chromatography of the plant extracts on a Florisil–Nuchar C 190N column served both as a satisfactory cleanup and as a method of separating the three compounds. Gas chromatographic analysis of the sulfoxide and sulfone fractions was accomplished on a packing with a mixed liquid phase (DC 200 and Carbowax 20M). Temik, because of its short retention time, was oxidized to its sulfone with a solution of hydrogen peroxide in acetic acid before gas chromatography. Temik oxidizes readily to its sulfoxide in plants, and even in fortified extracts; therefore, extracts should be analyzed without delay. As little as 2 ng. of the pure standards or 0.01 p.p.m. in crops was detected.

The systemic insecticide, 2-methyl-2-(methylthio)-propionaldehyde *O*-(methylcarbamoyl)oxime (Union Carbide UC-21149) is an experimental insecticide manufactured as a 10% granular formulation under the trade name Temik. Most investigators refer to pure UC-21149 as Temik and have based the names of its derivatives on Temik. In this paper, this practice is continued.

Metcalf *et al.* (1966) have shown that Temik (I) was metabolized in houseflies, *Musca domestica* L., and in cotton to Temik sulfoxide (II), Temik sulfone (III), Temik oxime, Temik sulfoxide oxime, and Temik sulfone oxime. The carbamoyloximes—Temik, its sulfoxide, and its sulfone—



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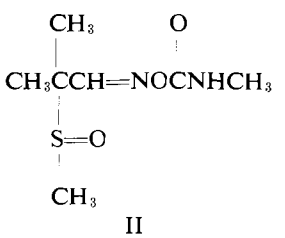

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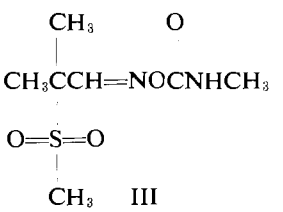

III

have high anticholinesterase activity, but the corresponding oximes have less than one thousandth as much. These investigators further showed that Temik sulfoxide, the most potent cholinesterase inhibitor of the group, is responsible for the high systemic activity and the long term persistence of insecticidal activity after application of Temik.

Bull *et al.* (1967) have recently presented data on the fate of Temik in insect pests of cotton [boll weevil, *Anthonomus grandis* Boheman, and tobacco budworm, *Heliothis virescens* (F.)] and have cited some of the more recent references on the chemistry and activity of the compound in other biological systems.

A sensitive analytical method applicable to a variety of crops was needed to separate and determine residues of the highly active cholinesterase inhibitors without interference from the relatively inactive oximes. The colorimetric method of Johnson and Stansbury (1966), which was developed before the nature and toxicity of the metabolites was established, has marginal sensitivity for Temik sulfoxide (which is 76 times as strong an anticholinesterase as Temik). The colorimetric method could be developed

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to include the toxic metabolites, but since it is not specific, interference from the nontoxic metabolites would be expected. Furthermore, in some of the crops of interest to the authors, experience with the colorimetric method at the Yakima laboratory showed that it was necessary to develop new cleanup procedures for each crop, and these procedures were very laborious.

The gas chromatographic flame photometric detector of Brody and Chaney (1966) appeared to be well suited for the analysis, since it can detect sulfur compounds like Temik, its sulfoxide, and its sulfone with good sensitivity and outstanding selectivity. The method finally adopted consisted of a simultaneous cleanup and separation of Temik, its sulfoxide, and its sulfone by liquid chromatography, and then a gas chromatographic (GLC) analysis with the flame photometric detector. Since the retention time of Temik was too short to allow it to separate sufficiently from the solvent peak, Temik was determined as the sulfone after quantitative conversion to this compound by hydrogen peroxide–acetic acid oxidation. The same cleanup procedure was used without modification for each crop. The method will detect 2 ng. of the pure standards and as little as 0.01 p.p.m. in crops.

EXPERIMENTAL

Solvents and Reagents. Distill the following solvents in glass: chloroform, acetone, petroleum ether (b.p. 60°–90° C.), and acetonitrile.

Solvent mixture I. A 1 to 9 (v./v.) mixture of acetone–petroleum ether.

Solvent mixture II. A 3 to 7 (v./v.) mixture of acetone–petroleum ether.

Temik and other standard solutions. Dissolve 0.1000 gram of pure Temik (Temik and its derivatives were supplied by the Union Carbide Corp., New York, N. Y.) in 1.0 liter of acetone, and dilute 4 ml. of this solution to 200 ml. with acetone (1 ml. contains 2.0 μg. of Temik). Prepare standard solutions of Temik sulfone, Temik sulfoxide, Temik oxime, and Temik sulfoxide oxime in the same manner. The Temik sulfone oxime was prepared by oxidizing some of the standard solution of Temik sulfoxide oxime with the oxidizing reagent as described later.

Florisil (60- to 100-mesh; 2.7% weight lost after heating for 20 hours at 110° C.) The Floridin Co., Tallahassee, Fla.

Nuchar C 190N. Fisher Scientific Co., Pittsburgh, Pa.

Adsorbent mixture I. Mix together equal parts of Nuchar C 190N, aluminum oxide (J. T. Baker Chemical Co., Phillipsburg, N. J.; not chromatographic grade), Florisil, 1 to 1 magnesium oxide (J. T. Baker Chemical Co.) and Hyflo Supercel (Johns-Manville Products Corp., New York, N. Y.).

Adsorbent mixture II. Mix together anhydrous sodium sulfate and Celite 545 (Johns-Manville) in a 2 to 1 ratio.

Oxidizing reagent. Prepare a 2 to 1 (v./v.) mixture of glacial acetic acid–30% hydrogen peroxide fresh daily.

Apparatus. Flame photometric detector (Melpar Corp., Falls Church, Va., now available from MicroTek Instrument Co., Baton Rouge, La.) with the 394-mμ filter (to determine sulfur compounds), attached to an Aerograph Hy-Fi gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) and equipped with a 10-mv. recorder.

Gas chromatographic column. Pack a 122-cm. × 0.175-cm. I.D. stainless steel or aluminum column with a 1 to 1 mixture of 5% Carbowax 20M on 60- to 80-mesh Gas-Chrom Q (Applied Science Lab., State College, Pa.) and 10% DC 200 on the same support.

Sample Preparation and Extraction. Finely chop the sample, and blend 150 grams in a 500-ml. Waring Blendor for 4 minutes with 450 ml. of chloroform, 4 grams of adsorbent mixture I, and 60 ± 10 grams of anhydrous sodium sulfate. Filter the solution through a plug of glass wool into a flask containing 60 ± 10 grams of sodium sulfate, shake, and filter through sodium sulfate until 300 ml. are collected. Add 2 ± 0.5 grams of adsorbent mixture II, evaporate the solution to dryness in a warm water bath (40° C.) with the aid of a stream of air (all subsequent evaporations are made in the same manner), and dissolve the residue in 15 ml. of acetonitrile. With a glass stirring rod, loosen the residue from the bottom of the flask, and filter the mixture into a test tube through a funnel tightly plugged with cotton. Rinse the flask and funnel with four 10-ml. portions of acetonitrile; evaporate the resultant solution to dryness, and dissolve the residue in 25 ml. of solvent mixture I.

Liquid Chromatography and Oxidation of Temik. Transfer the solution and rinsings of the tube with 25 ml. of solvent mixture I to a 28.5-cm. × 1.6-cm. I.D. glass chromatographic column prepared (bottom to top) with 5 grams of Florisil, 1 gram of Nuchar, and 10 grams of Florisil. (Use suction when packing the column, and plug both ends with cotton to hold the adsorbents in place.) As the last of the solution sinks into the column, add 60 ml. of solvent mixture I and allow it to flow through the column. The eluate to this point constitutes fraction I. It contains only the Temik oxime and may be discarded if desired. Change the receiving flask, and elute the Temik and Temik sulfone oxime with an additional 125 ml. of solvent mixture I; the eluate is fraction II. In a like manner, elute the Temik sulfone and the Temik sulfoxide oxime from the column with 180 ml. of solvent mixture II to give fraction III. Finally, elute the Temik sulfoxide from the column with 125 ml. of acetone (fraction IV). Evaporate chromatographic fractions II, III, and IV to dryness, dissolve each residue in 2.0 ml. of acetone, decant each into a small vial, and refrigerate until analysis.

Since lots of adsorbents occasionally vary, the chromatographic column used to separate Temik and its metabolites should be checked with known mixtures of the pure standard solutions, and adjustments should be made in elution volumes, if necessary.

Determine Temik sulfone oxime (fraction II), Temik sulfone (fraction III), and Temik sulfoxide (fraction IV) by injecting the indicated fraction into the gas chromatograph. After the Temik sulfone oxime has been determined (a negligible volume is used in the GLC), transfer the remainder of fraction II to a test tube, rinse the vial with chloroform, and evaporate the combined liquids to dryness. Add the oxidizing reagent (5.0 ml.), heat the solution in an oil bath at 75° C. for 20 minutes, and cool in a water bath at room temperature; then transfer the solution to a separatory funnel. Rinse the test tube with 50 ml. of distilled water and add it to the separatory funnel. Extract the solution in the funnel three times with 25-ml.

portions of chloroform. Dry the combined chloroform extracts by filtering through sodium sulfate. Evaporate the solution to dryness, dissolve the residue in 2.0 ml. of acetone, decant the solution into a small vial, and refrigerate it until analysis. After analysis, subtract the amount of Temik sulfone oxime previously determined from the total amount found in this analysis to determine the amount of Temik (as its sulfone).

Gas Chromatography. Inject portions of the cleaned-up extracts (ranging from 0.5 to 6.0 μl.) into the gas chromatograph. Adjust the injection port and the column oven temperatures to 160° and 115° C., respectively, and the nitrogen (carrier gas) flow rate to 156 ml. per minute. Operate the hydrogen-rich flame of the photometric detector with a hydrogen flow rate of 200 ml. per minute, and an oxygen flow rate of 44 ml. per minute.

RESULTS AND DISCUSSION

Before extraction, samples of apples, oranges (rind and pulp), potatoes, and sugar beets (foliage and roots) were fortified with 0.01, 0.05, 0.10, and 0.50 p.p.m. of Temik, its sulfoxide, and its sulfone. The samples were also fortified with the three oximes to determine whether the oximes would interfere in the analysis. The results are summarized in Tables I and II. Recoveries of the three insecticidal components are satisfactory. Those of the oximes are low; this is due in part to their incomplete extraction from the plant materials and incomplete recovery in the liquid chromatography. [A substantial fraction of the oximes (ca. 40%) was not recovered when the pure standards were subjected to the liquid chromatography.] As previously stated, the determination of the noninsecticidal oximes is not included in this analysis.

A comparison of GLC retention times of the compounds is given in Table III. Retention times of Temik sulfone and Temik sulfone oxime were the same, that of Temik sulfoxide oxime was slightly greater than that of Temik sulfoxide, and the retention time of Temik oxime was significantly greater than that of Temik, which was too short for quantitative analysis.

Knaak *et al.* (1966) reported on the GLC pyrolysis products of Temik and its metabolites. They found that Temik sulfone and Temik sulfone oxime formed 2-methyl-2-(methylsulfonyl)propionitrile, while Temik sulfoxide and Temik sulfoxide oxime formed methacrylonitrile. Temik formed 2-methyl-2-(methylthio)propionitrile. In

Table I. Recovery of Temik, Its Sulfoxide, and Its Sulfone from 100-Gram Crop Samples Fortified at Several Levels

	Added, P.P.M.			Recovery, %[a]		
Crop	Temik	Temik sulfoxide	Temik sulfone	Temik	Temik sulfoxide	Temik sulfone
Apple	0	0	0	ND	ND	ND
	0.50	0.50	0.50	96	98	113
	0.10	0.10	0.10	96	113	105
	0.05	0.05	0.05	93	114	128
	0.01	0.01	0.01	87	109	100
Orange Rind	0	0	0	ND	ND	ND
	0.50	0.50	0.50	87	110	84
	0.10	0.10	0.10	90	108	99
	0.05	0.05	0.05	80	114	88
Pulp	0	0	0	ND	ND	ND
	0.50	0.50	0.50	98	105	100
	0.10	0.10	0.10	107	92	92
	0.05	0.05	0.05	80	114	110
	0.01	0.01	0.01	74	89	96
Potato	0	0	0	ND	ND	ND
	0.50	0.50	0.50	96	98	88
	0.10	0.10	0.10	87	140	102
	0.05	0.05	0.05	70	130	118
Sugar beet Root	0	0	0	ND	ND	ND
	0.50	0.50	0.50	107	107	97
	0.10	0.10	0.10	92	135	135
	0.05	0.05	0.05	83	107	106
	0.01	0.01	0.01	80	101	94
Foliage	0	0	0	ND	ND	ND
	0.50	0.50	0.50	92	110	98
	0.10	0.10	0.10	100	110	112
	0.05	0.05	0.05	72	104	140

[a] ND indicates none detected or less than 0.01 p.p.m.

Table II. Recovery of the Oxime Metabolites of Temik from 100-Gram Crop Samples

	Added, P.P.M.			Recovery, %[a]		
Crop	Temik oxime	Temik sulfoxide oxime	Temik sulfone oxime	Temik oxime	Temik sulfoxide oxime	Temik sulfone oxime
Orange pulp	0	0	0	ND	ND	ND
	1.0	1.0	1.0	46	35	16
Sugar beet root	0	0	0	ND	ND	ND
	2.0	2.0	2.0	16	52	8
Potato	0	0	0	ND	ND	ND
	2.0	2.0	2.0	23	38	36

[a] ND indicates none detected or less than 0.03 p.p.m.

Table III. Recoveries and Gas Chromatographic Retention Times of Temik and Its Metabolites and the Liquid Chromatography Fractions in Which the Compounds Were Detected

		Per Cent Recovered from Liquid Chromatography Fraction			
Compound	Retention Time, Min.	I	II	III	IV
Temik[a]	<0.8[b]	ND	94	ND	ND
Temik sulfoxide[a]	2.0	ND	ND	ND	97
Temik sulfone[a]	4.3	ND	ND	91	ND
Temik oxime[c]	1.5	20	ND	ND	ND
Temik sulfoxide oxime[a]	2.1	ND	ND	43	ND
Temik sulfone oxime[d]	4.3	ND	22	ND	ND

[a] ND indicates none detected—i.e., less than 2.5 ng. or 2.5% recovery of the amount analyzed.
[b] Temik was determined as its sulfone.
[c] ND indicates none detected—i.e., less than 0.4 ng. or 3.6% recovery of the amount analyzed.
[d] ND indicates none detected—i.e., less than 5.0 ng. or 3.3% recovery of the amount analyzed.

the authors' laboratory, Temik sulfone and Temik sulfone oxime had the same retention time as authentic 2-methyl-2-(methylsulfonyl)propionitrile. Since Knaak *et al.* (1966) did not report the sulfur-containing product of Temik sulfoxide and Temik sulfoxide oxime, the authors' results cannot be compared with theirs. In the case of Temik, the authors appear to have obtained more extensive pyrolysis since 2-methyl-2-(methylthio)propionitrile would be expected to have a longer retention time than was found.

Table III shows that there is no danger of the oximes interfering with the corresponding carbamoyloximes despite the similarity of the retention times. Liquid chromatography efficiently separated the oximes from the carbamoyloximes with which they could interfere. The only interference that could occur is circumvented by subtracting the original amount of Temik sulfone oxime in fraction II (before oxidation) from the total amount found after oxidation. The difference is the amount of Temik sulfone derived from Temik.

Table IV illustrates the ease with which Temik spontaneously oxidizes to its sulfoxide in plant extracts. Even samples that were analyzed within 1 to 1.5 days after preparation showed significant conversion of Temik to the sulfoxide. The total recoveries from these samples were essentially 100%, but samples that had stood for seven days gave low total recoveries. Longer storage of extracts probably permits hydrolysis of Temik and its sulfoxide to the oximes. However, in these older samples the oximes could not be detected, a result that is not surprising considering the low recovery rates of the oximes from extracts (Table II) and the low levels of residues that were analyzed (0.50 and 0.10 p.p.m.). Obviously, the samples should be analyzed as quickly as possible after extraction, but even with this precaution, some of the Temik will be found as the sulfoxide. Indeed, the results of Metcalf *et al.* (1966) suggest that Temik is so easily oxidized, it may not generally be found in a crop in the unoxidized form. They found that after four days cotton plants contained only 5 to 18% of the total Temik moieties as the parent Temik. The authors' results with oranges showed the complete absence of Temik 100 days after its application, even in samples that contained appreciable sulfoxide (12.5 p.p.m.).

Table V shows the relatively slow oxidation of the sulfoxide to the sulfone in plant extracts. These samples were analyzed three days after preparation. Again, the results of these stability studies are in agreement with those of Metcalf *et al.* (1966).

The recoveries listed in Table I were determined by the simultaneous addition of Temik, its sulfoxide, and its sulfone to the samples before extraction. Completeness of extraction of the compounds from field-grown or other crops should be checked. Some of the Temik was undoubtedly recovered as the sulfoxide. This facile conversion explains the generally high recoveries of sulfoxide and the low recoveries of Temik.

The essentially quantitative oxidation of Temik or its sulfoxide to the sulfone is illustrated in Figure 1. Theoretically 0.050 p.p.m. of Temik upon oxidation will give 0.058 p.p.m. of Temik sulfone. Although Temik sulfoxide can be analyzed directly, its oxidation to the sulfone offers further verification of its identity.

Figure 2 gives the standard curves for Temik, its sulfoxide, and its sulfone over a concentration range of 2 to 10 ng. The range could be extended, but it is inconvenient to do so. Standards should be run each day, and it is less laborious to use a small concentration range and to adjust aliquot or dilution for the standardized range. Figure 3 shows the freedom from interference in the cleaned-up apple extracts. The chromatograms of the extracts from the other crops were of similar quality.

Oxidizing agents other than the hydrogen peroxide–acetic acid combination were tried. Acidic aqueous permanganate or hydrogen peroxide produced only partial oxidation of Temik. *m*-Chloroperbenzoic acid was a satisfactory oxidizing agent, but the trace quantities of the acid remaining after the oxidation reduced the sensitivity of the gas chromatographic column after several analyses. The hydrogen peroxide–acetic acid oxidizing agent used in our method left trace quantities of acetic acid in the samples, but these did not interfere with the gas chromatographic analysis.

Several liquid phases were tested in an attempt to increase the retention time of Temik relative to Temik sulfoxide and sulfone. High polarity phases, such as 10%

Table IV. Determination of Temik, Its Sulfoxide, and Its Sulfone in Crop Extracts Fortified with Temik Only

Crop	P.P.M. Added to 100-Gram Sample[a,b]	Recovery, %		
		Temik	Temik sulfoxide	Temik sulfone
Apple	0.50	80	5.7	ND[c]
	0.10	98	7.3	ND
Orange rind	0.50	58	5.0	ND
	0.10	72	21	ND
Orange pulp	0.10	100	9.3	ND
Potato	0.50	78	13	ND
	0.10	52	50	ND
Sugar beet root	0.50	65	5.7	ND
	0.10	95	11	ND
Sugar beet foliage	0.50	73	16	ND
	0.10	92	10	ND

[a] The 0.50 p.p.m. extracts were allowed to stand seven days before cleanup and analysis. [b] The 0.10 p.p.m. extracts were analyzed after 1 to 1.5 days. [c] ND indicates none detected (<4.0%).

Table V. Determination of Temik, Its Sulfoxide, and Its Sulfone in Crop Extracts Fortified with Temik Sulfoxide Only and Analyzed Three Days Later

Crop	P.P.M. Added to 100-Gram Sample	Recovery, %[a]		
		Temik	Temik sulfoxide	Temik sulfone
Apple	0.10	ND	98	ND
Orange rind	0.10	ND	98	6.0
Potato	0.10	ND	88	6.7
Sugar beet foliage	0.10	ND	103	ND

[a] ND indicates none detected or less than 2.0 ng. (<4.0%).

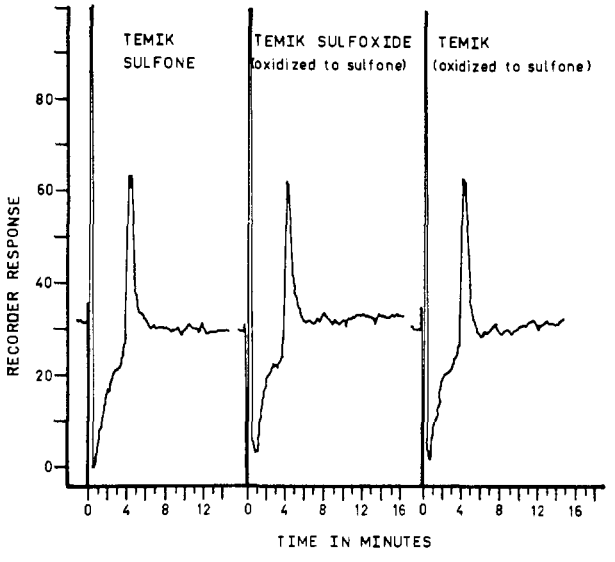

Figure 1. Comparison of equimolar solutions of Temik sulfone with oxidized solutions of Temik and Temik sulfoxide

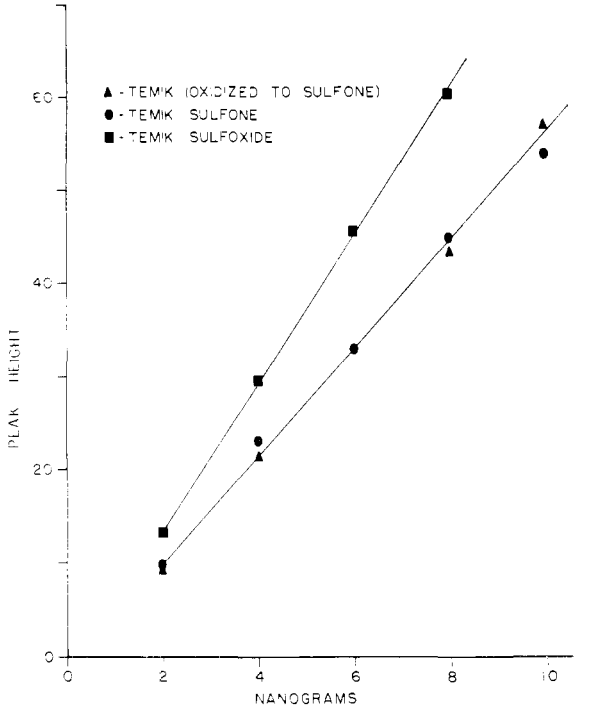

Figure 2. Standard curves for Temik (oxidized to sulfone), Temik sulfoxide, and Temik sulfone

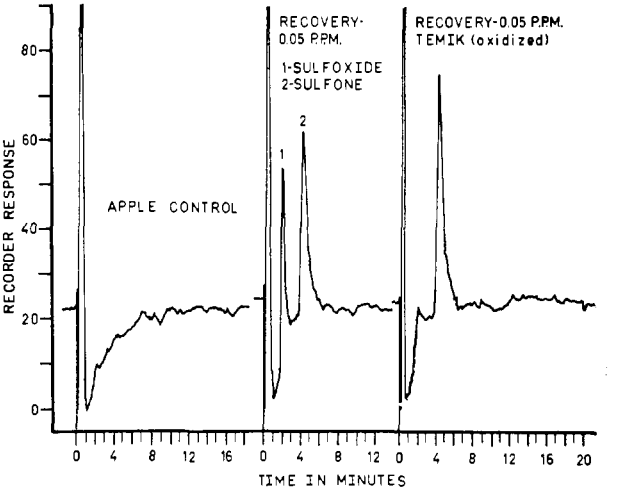

Figure 3. Gas chromatograms of apple extracts

QF-1-0065, 10% HI-EFF-3B, and 5 to 10% HI-EEF-2BP on Gas-Chrom Q or Anakrom ABS were tried without success. In these trials, the column was 183 cm. × 0.175 cm. I.D. and oven temperatures ranged from 75° to 180° C. Although the lower oven temperatures increased the retention time of Temik slightly, the solvent (acetone) in which the Temik was dissolved continued to partially mask the Temik peak.

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