Temik-S^{35} Metabolism in a Lactating Cow

H. Wyman Dorough and G. Wayne Ivie

A lactating Jersey cow fed Temik-S^{35} [2-methyl-2-(methylthio-S^{35}) propionaldehyde *O*-(methylcarbamoyl)oxime] as a single dose of 0.1 mg. per kg. of body weight eliminated over 96% of the administered radioactivity from the body within 540 hours after treatment. The percentages of the total dose detected in the urine, milk, and feces were 90.2, 3.0, and 2.9, respectively. Milk sampled 3 hours after treatment contained the maximum concentration of residues, 62 p.p.b. Temik equivalents, but had declined to 10, 1, and 0.1 p.p.b. after 84, 276, and 540 hours, respectively. The radiolabeled residues consisted primarily of organic solvent–extractable products. Those materials extractable with organic solvents were comprised largely of 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime, 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime, 2-methyl-2-(methylsulfinyl)propionaldoxime, and 2-methyl-2-(methylsulfonyl)propionitrile. When rats were fed milk containing Temik-S^{35} metabolites daily for 9 days, approximately 90% of each dose was excreted in the urine within a 24-hour period. The nature of the metabolites in the urine was similar to those in the milk diet.

The *O*-(methylcarbamoyl)oximes were first introduced as a new type of carbamate pesticide in 1965 (Weiden *et al.*). One of the most promising materials in this group is Temik [2-methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl)oxime]. It is active as a contact poison against many insect species and is highly effective as a plant systemic insecticide (Payne *et al.*, 1966).

Because it possesses rather unique biological properties, the metabolism of Temik has been extensively investigated in mammals (Andrawes *et al.*, 1967; Knaak *et al.*, 1966), plants (Coppedge *et al.*, 1967; Metcalf *et al.*, 1966), and insects (Bull *et al.*, 1967; Metcalf *et al.*, 1966). The Temik molecule undergoes both hydrolytic and oxidative pathways in the organisms studied thus far. Sulfur oxidation of Temik to form the sulfinyl (Temik sulfoxide) and sulfonyl (Temik sulfone) derivatives of the carbamate results in compounds which are still toxic and possibly more stable in vivo than the parent compound. Hydrolysis of Temik or its toxic metabolites, Temik sulfoxide and Temik sulfone, produces compounds significantly less toxic than the intact carbamates.

A pesticide with the broad spectrum of activity (insecticidal, acaricidal, and nematocidal) which has been observed with Temik has an excellent potential for use on a variety of crops. Some of these crops could be used as feed for dairy animals. To ensure its safe use and to develop proper analytical procedures, it is necessary to know the level and chemical nature of residues in the milk of animals exposed to Temik.

METHODS AND MATERIALS

Temik-S^{35} (specific activity 45.7 mc. per mmole) was crystallized from a hexane-ether mixture and its purity determined by thin-layer chromatography (TLC) and by radioautography. Radioactive purity of the Temik-S^{35} administered to the cow was 99%.

For treatment, the purified product was dissolved in 200 ml. of acetone, and the radioactive solution was mixed with 2 pounds of crushed grain. Immediately after the solvent had evaporated (approximately 20 to 30 minutes with mixing), the cow was fed the treated grain. The feeding container was washed with acetone, and the unconsumed Temik-S^{35} was determined by radioassay. An earlier small scaled mixing of radioactive material and feed showed that there was no loss of Temik following the complete mixing process.

The total amount of Temik consumed by the cow was 40 mg. or a dose of 0.1 mg. per kg., based on the body weight of the animal. The total intake of radioactivity was 1.6×10^{10} c.p.m. No ill effect to the cow was noted following the administration of the labeled Temik.

Milk, urine, and feces were collected at 3, 6, and 12 hours after treatment and then at 12-hour intervals thereafter until no radiolabeled residues could be detected. Aliquots of milk and urine were radioassayed at the time the samples were taken. The feces, as well as the remainder of the milk and urine, were frozen until time of analysis.

All radioactive measurements were accomplished using a Packard Tri-Carb Model 3365 liquid scintillation counter. The counting efficiency was adjusted to 70%, and the necessary quench corrections were made utilizing automatic external standardization. Total radioactivity in the milk and urine samples was determined by adding 0.2 ml. of these materials to scintillation vials for direct counting. Radioactive residues in the feces were quantitated by oxygen combustion of approximately 1.0 gram (wet weight) in a Parr bomb, rinsing the bomb with water, and counting 0.2-ml. aliquots of the aqueous wash. The quantity of radiolabeled metabolites in milk and feces solids following extraction also was determined by oxygen combustion.

Silica gel G thin-layer plates (0.3 mm. thick) were used to separate Temik and those metabolites in milk, urine, and feces that were extractable into organic solvents. The chromatograms were developed two dimensionally with the first solvent system consisting of a 2 to 1 ether-hexane mixture containing 20% acetone and the second system consisting of a 2 to 1 methylene chloride–acetonitrile mixture. Radioactivity on the chromatograms was located by radioautography and then quantitated by

Department of Entomology, Texas A&M University, College Station, Tex. 77843

scintillation counting of the gel containing the labeled products.

For metabolite identification, a mixture of known compounds considered as possible Temik metabolites (Table I) was added to organic extracts of the milk, urine, or feces and their cochromatography with the labeled unknowns on TLC determined. The standards were detected by spraying the plates with a 1% aqueous solution of potassium permanganate. If cochromatography of one of the standards with a radioactive metabolite was indicated, the metabolite was isolated in sufficient quantity, when possible, to allow additional studies of its identity using techniques described by Andrawes *et al.* (1967).

Methods of extraction and clean-up of the milk, urine, and feces were the same as those reported earlier (Dorough, 1967). Because certain Temik metabolites did not partition from water into chloroform readily, several chloroform extractions of the aqueous phases were required. Those radioactive residues extracted into chloroform were termed organo-extractables, those residues in the water termed water-solubles, and that radioactivity remaining in the milk and feces solids was termed unextractables.

A portion of the 3-hour milk sample, containing 62 p.p.b. Temik equivalents, was concentrated to approximately one-half volume by freeze drying, and aliquots were fed to two rats daily for 9 days. Three and one-half milliliters of the milk concentrate containing residues equivalent to 1 μg. of Temik were given daily by stomach tube to each rat. The combined urine from the two animals was collected 24 hours after each treatment and at 24-hour intervals for 5 days following the last treatment. Analysis of the urine was accomplished by direct counting of 0.2-ml. aliquots and by examining the organo-extractables on TLC.

RESULTS AND DISCUSSION

The major route of elimination of the Temik-S^{35} dose administered to the lactating cow was by way of the urine (Figure 1). Approximately 83% of the dose was present in urine collected within 24 hours after treatment and had increased to 90% of the dose after 540 hours.

Radioactive residues from the Temik-S^{35} treatment were present in milk collected after 3 hours and continued to be detectable in all samples taken through a 540-hour period after treatment. Total radioactivity in these milk samples accounted for 3.02% of the administered dose. However, the pattern of excretion was such that residues were extremely small in individual milk samples.

Only 2.85% of the Temik-S^{35} dose was eliminated from the cow in the feces. Excretion by this route was complete after 192 hours.

Total recovery of the Temik-S^{35} treatment in the urine, milk, and feces was 96.06% after 540 hours. The fact that radiolabeled residues were detected in the milk and urine for a long period following treatment indicated that Temik was persistent in the animal. Actually, the indicated persistence might have resulted in part from the sensitive method used to detect residues. With the milk and urine, 0.2-ml. aliquots were assayed directly for radioactivity. Based on a minimum of 20 c.p.m. above background as representing the presence of true residues, it was possible, because of the high specific activity of the Temik-S^{35}, to detect as little as 1×10^{-7}% of the administered dose in a single aliquot of the milk or urine. This extreme sensitivity allowed quantitation of residues which otherwise could not have been considered because of the dilution factor that is inherent in such studies.

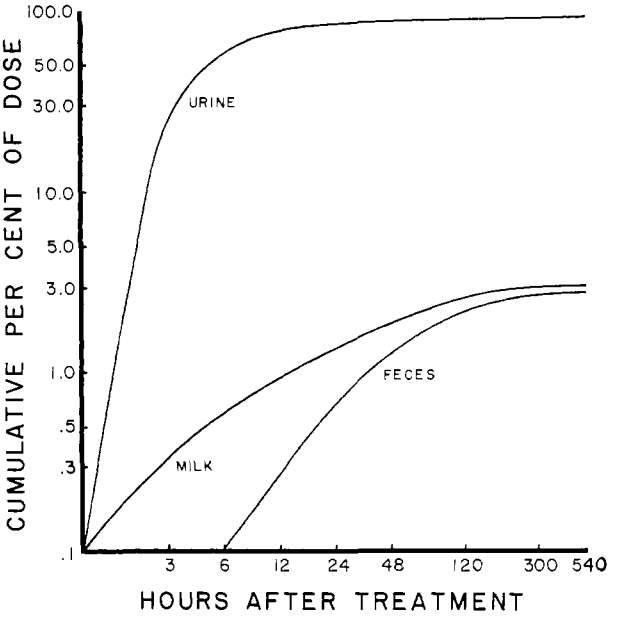

Figure 1. Elimination of radioactivity in the urine, milk, and feces of a cow treated with Temik-S^{35}

Table I. Standards Used in Study of Temik Metabolism by Lactating Cow

Chemical Name	Structure	Abbreviation[a]
2-Methyl-2-(methylthio-S^{35})propionaldehyde *O*-(methylcarbamoyl)oxime	$CH_3S^{35}C(CH_3)_2CH{=}NOC(O)NHCH_3$	Temik-S^{35} (T)
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	$CH_3S(O)C(CH_3)_2CH{=}NOC(O)NHCH_3$	Temik sulfoxide (T-SO)
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	$CH_3S(O)_2C(CH_3)_2CH{=}NOC(O)NHCH_3$	Temik sulfone (T-SO_2)
2-Methyl-2-(methylthio)propionaldoxime	$CH_3SC(CH_3)_2CH{=}NOH$	Temik oxime (O)
2-Methyl-2-(methylsulfinyl)propionaldoxime	$CH_3S(O)C(CH_3)_2CH{=}NOH$	Oxime sulfoxide (O-SO)
2-Methyl-2-(methylsulfonyl)propionaldoxime	$CH_3S(O)_2C(CH_3)_2CH{=}NOH$	Oxime sulfone (O-SO_2)
2-Methyl-2-(methylsulfinyl)propionitrile	$CH_3S(O)C(CH_3)_2C{\equiv}N$	Nitrile sulfoxide (N-SO)
2-Methyl-2-(methylsulfonyl)propionitrile	$CH_3S(O)_2C(CH_3)_2C{\equiv}N$	Nitrile sulfone (N-SO_2)

[a] Abbreviations in parentheses are designations used in tables.

Whole milk containing S^{35} residues was extracted with acetonitrile and chloroform and the radioactivity characterized as organo-extractables, water-solubles, and unextractables. The aqueous layer of the milk was extracted with chloroform until no additional radiolabeled materials could be removed (Dorough, 1967).

Radioassay of the three milk fractions showed that most of the radioactivity in the milk was extractable into organic solvent (Table II). In the 3-hour milk sample, there were 62 p.p.b. Temik equivalents present. Of these, 41.4 p.p.b. were organo-extractables, 17.1 p.p.b. water-solubles, and 3.5 p.p.b. could not be extracted from the milk. Total residues in the milk declined rapidly with the organo-extractables comprising an increasing percentage as the time after treatment increased. No water-soluble metabolites were detectable after 60 hours, and the unextractables could not be detected in samples collected after 120 hours.

The organo-extractables were spotted on TLC and the chromatograms developed two dimensionally. Radioautography of the plates containing the 3-hour milk extract resulted in 12 darkened areas on the film (Figure 2). Using cochromatographic techniques, seven of the 12 Temik metabolites were tentatively identified (Table III). Identification of each product was further confirmed by chemical degradation and other means (Andrawes *et al.*, 1967). Table III shows that most of the metabolites were excreted in the milk for a relatively short time after treatment. Only two products, unknown 5 and nitrile sulfone, were present after 96 hours.

Each sample of urine collected during the first 540 hours after treatment was extracted with chloroform, and the amount of radioactivity in the aqueous and organic solvent phases was determined. In these samples, approximately 20 to 30% of the total radioactive content of the urine remained in the water layer after extraction. The organo-extractables from the urine were applied to TLC, and attempts were made to determine the identity of metabolites using the same techniques as described for analysis of milk. The same metabolites detected in the milk were present in the urine (Table IV). The most persistent metabolite was nitrile sulfone, which was detectable in all samples through 540 hours.

Very little of the administered dose of Temik-S^{35} was eliminated from the cow in the feces (Figure 1). For this reason, only fecal samples collected at 24, 36, and 48 hours after treatment were extracted and the nature of the radiolabeled residues examined. The feces were extracted with acetonitrile and chloroform in a manner similar to the extraction of milk. Thin-layer chromatography and radioautography were used to analyze the organo-extractables. This fraction contained five metabolites of Temik (Table V) Temik sulfoxide was the most abundant metabolic product in the organic solvent extract of the 24-hour feces, but after 48 hours nitrile sulfone was the most persistent material. Unlike the milk and urine, Temik was detected in the feces. Water-soluble Temik-S^{35} metabolites made up about 32% of the radioactivity in the 24-hour feces but had declined to 8% by 48 hours. The unextractable materials were of similar magnitude in the three fecal samples.

In rats fed daily doses of the Temik-S^{35} metabolites in milk, the daily excretion of the radiolabeled materials in the urine was about 90% of the total dose administered. After treatment ceased, the urine continued to contain small quantities of radioactivity for 5 days. At this time, 96% of the total Temik-S^{35} milk metabolites were excreted from the rats without undergoing a great deal of further metabolism or degradation (Table VI). There was a general decrease in the amount of organo-extractable metabolites with a corresponding increase in the water-

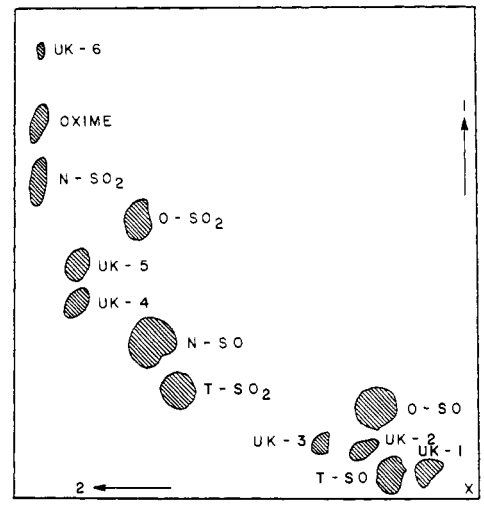

Figure 2. Drawing of a radioautogram showing separation of Temik-S^{35} metabolites by two-dimensional thin-layer chromatography

Developed in first dimension with 2 to 1 ether-hexane + 20% acetone and in second dimension with 2-to-1 methylene chloride–acetonitrile

Table II. Quantity and Partitioning Characteristics of Residues in Milk of Cow Fed Temik-S^{35}

Hours after Treatment	P.P.B. Temik-S^{35} Equivalents Present as Organo-extractables	Water-solubles	Un-extractables	Total
3	41.4	17.1	3.5	62.0
6	29.5	12.3	3.1	44.9
12	16.8	4.3	5.7	26.8
24	15.1	1.8	3.7	20.6
36	14.0	0.5	1.9	16.4
48	12.6	0.3	1.2	14.1
60	11.0	0.1	0.7	11.8
72	10.2	0	0.7	10.9
84	9.1	0	0.7	9.8
96	8.8	0	0.3	9.1
108	7.8	0	0.2	8.0
120	7.2	0	0.1	7.3
132	6.0	0	0	6.0
144	5.4	0	0	5.4
168	4.3	0	0	4.3
192	3.0	0	0	3.0
240	2.1	0	0	2.1
276	1.0	0	0	1.0
360	0.5	0	0	0.5
408	0.4	0	0	0.4
456	0.3	0	0	0.3
504	0.2	0	0	0.2
540	0.1	0	0	0.1

Table III. Tentative Identification and Relative Magnitudes of Organo-Extractable Metabolites in Milk of Cow Fed Temik-S^{35}

Hours after Treatment	Per Cent of Total S^{35}-Organo-Extractables in Milk Present as Following Metabolites[a]											
	UK-1	T-SO	UK-2	O-SO	UK-3	T-SO_2	N-SO	UK-4	UK-5	O-SO_2	N-SO_2	O
3	2.21	16.41	0.67	34.27	1.91	6.07	28.33	0.44	0.85	1.83	6.21	0.80
6	5.06	5.98	1.65	28.88	0.47	1.91	32.60	1.32	0.99	1.42	18.57	1.15
12	3.64	0.85	0.71	8.32	0.35	0.61	30.56	0	3.82	0.49	47.90	2.75
24	0.79	0	0	0.61	0.30	0	9.43	0	4.14	0	82.83	1.90
36	0	0	0	0	0	0	2.78	0	4.78	0	90.58	1.86
48	0	0	0	0	0	0	0.75	0	4.49	0	94.76	0
72	0	0	0	0	0	0	0.21	0	4.84	0	94.95	0
96	0	0	0	0	0	0	0.15	0	4.62	0	95.23	0
144	0	0	0	0	0	0	0	0	3.66	0	96.34	0
192	0	0	0	0	0	0	0	0	2.33	0	97.67	0
276	0	0	0	0	0	0	0	0	0.96	0	99.04	0
300	0	0	0	0	0	0	0	0	0.30	0	99.70	0
312 to 540	0	0	0	0	0	0	0	0	0	0	100.00	0

[a] UK: Metabolites of unknown identity; see Table I for metabolite designations.

Table IV. Organo-Extractable Urinary Metabolites from Cow Fed Temik-S^{35}

Hours after Treatment	Per Cent of Total S^{35}-Organo-Extractables in Urine Present as Following Metabolites[a]											
	UK-1	T-SO	UK-2	O-SO	UK-3	T-SO_2	N-SO	UK-4	UK-5	O-SO_2	N-SO_2	O
3	1.79	57.80	1.37	26.11	0.00	5.48	4.80	0.00	0.00	2.13	0.38	0.14
6	2.37	38.46	0.52	18.88	0.32	1.91	9.67	2.46	3.33	7.67	11.12	3.29
12	4.11	25.76	0	3.73	0.71	1.09	20.21	3.44	3.89	2.05	33.03	1.98
24	2.89	4.16	0	2.19	0.89	0.91	31.32	5.04	3.51	2.67	44.89	1.53
36	2.13	1.57	0	1.51	1.41	0	17.76	4.47	4.19	1.31	64.08	1.39
48	1.76	1.44	0	1.80	1.47	0	10.26	3.72	4.00	0.73	73.80	1.02
72	0.22	0.40	0	0.20	3.52	0	1.72	0	3.64	0	90.30	0
96	0	0	0	0	3.02	0	0.98	0	2.70	0	93.30	0
120	0	0	0	0	1.03	0	0.21	0	1.54	0	97.22	0
132	0	0	0	0	0.64	0	0	0	1.27	0	98.09	0
168	0	0	0	0	0	0	0	0	0.32	0	99.68	0
180 to 540	0	0	0	0	0	0	0	0	0	0	100.00	0

[a] UK: Metabolites of unknown identity; see Table I for metabolite designations.

Table V. Nature of Radiolabeled Residues in Selected Fecal Samples from Cow Fed Temik-S^{35}

Radioactivity Detected as	Per Cent of Total Radioactivity in Fecal Sample Present as Indicated Metabolite		
	24 hours	36 hours	48 hours
Unextractables	18.45	25.85	16.55
Water-solubles	32.45	13.95	8.50
Temik	30.78	12.11	2.23
Temik sulfoxide	8.09	3.76	3.72
Temik sulfone	3.95	4.32	2.71
Oxime	2.19	3.38	7.87
Oxime sulfoxide	4.09	1.59	0.61
Nitrile sulfone	0	35.04	57.81

soluble materials. One product, unknown 6 (Figure 2, Table VI), was present in the rat urine although its presence had not been detected in the cow's milk or urine.

Apparently the metabolic pathway of Temik in dairy animals is the same as that reported in other organisms. Carbamate metabolites, Temik sulfoxide and Temik sulfone, are produced as a result of oxidation of the sulfur atom. These active metabolites (Metcalf *et al.*, 1966) are formed rapidly by the cow but are quickly eliminated from the body, primarily by way of the urine. The Temik molecule and its carbamate metabolites undergo hydrolysis to form several products (Table IV) which are subsequently excreted from the body. Metabolism and elimination of the administered Temik-S^{35} were, for the most part, very rapid. There was, however, a very slow release of those radioactive materials remaining in the animal after 48 hours (Figure 1). This pattern of excretion was observed in rats treated orally with Temik (Andrawes *et al.*, 1967).

The level of radiolabeled residues in the milk following the Temik-S^{35} treatment was extremely low. The highest concentration of Temik equivalents in a single milk sample was only 62 p.p.b. (Table II). This level was detected in milk sampled 3 hours after treatment and may not reflect the quantity of residues in the milk had a normal milking schedule been maintained. In fact, the Temik equivalents in the combined milk collected during the first 12 hours equaled only 45 p.p.b. The shorter milking intervals were necessary to obtain maximum amounts of metabolites in the minimum amount of milk.

Although residues of Temik-S^{35} in milk were low when considered on a part-per-billion basis, approximately 3% of the total dose was eliminated in the milk. Obviously, the residues were secreted over a long period of time. Natural dilution of residues resulted from this pattern of slow release in the milk.

Metabolites of Temik in the milk (Table III) were the same as those detected in the urine (Table IV). Coppedge *et al.* (1967) reported the formation of the identified products in soil and plants. Unknown metabolic products

Table VI. Metabolites Detected in Urine of Rats Fed Milk from Cow Treated with Temik-S^{35}

Metabolites	3-Hour Milk[a]	Per Cent of Total Radioactivity in Sample Present as Indicated Metabolite: Days 1	2	3	4	5	6	7	8	9[b]	10	11–14
Water-solubles	20.60[c]	56.75	53.46	54.81	51.29	54.44	53.77	52.20	50.55	53.26	52.62	51.98
Unknown 1	1.49	3.17	2.36	2.45	4.57	4.66	5.09	4.02	4.55	3.12	2.90	0
Temik sulfoxide	13.02	2.79	2.05	2.03	3.35	3.08	3.11	3.06	2.00	2.12	2.85	0
Unknown 2	0.53	1.57	1.85	1.37	0.95	1.13	1.25	1.08	1.26	1.74	0.93	0
Oxime sulfoxide	27.20	10.02	12.85	8.39	13.71	10.27	12.44	13.10	13.36	12.12	7.69	2.10
Unknown 3	1.52	0.69	0.71	0.84	0.61	0.78	1.22	0.72	0.53	0.77	0.61	0
Temik sulfone	4.82	0	0	0	0	0	0	0	0	0	0	0
Nitrile sulfoxide	22.48	9.55	9.30	9.44	8.69	8.45	7.51	8.42	8.64	10.80	8.42	6.70
Unknown 4	0.35	0	0	0	0	0	0	0	0	0	0	0
Unknown 5	0.67	0	0	0	0	0	0	0	0	0	0	0
Oxime sulfone	1.45	1.32	2.57	1.29	1.36	1.77	1.38	1.62	2.58	2.63	1.88	0
Nitrile sulfone	5.24	3.28	7.11	7.74	6.13	6.81	7.54	7.33	8.76	8.08	13.20	37.12
Oxime	0.63	6.10	5.33	7.65	6.81	5.79	4.31	5.01	4.78	3.35	5.11	2.10
Unknown 6	0	4.76	2.41	3.99	2.53	2.82	2.38	3.44	2.99	2.01	3.79	0

[a] Three-hour milk sample from cow treated with Temik-S^{35} that was used to feed rats for 9 days.
[b] Last day rats received treatment.
[c] Includes unextractable metabolites.

in this study were not compared with those unknowns reported by Coppedge and coworkers. These two studies show something of the number and nature of metabolites that must be considered in developing a workable method of Temik residue analysis.

Data collected from a single dose of Temik-S^{35} to a cow serve primarily as a guide line for more complete studies that must be conducted once there is a practical method of residue analysis available. In addition, such a study could indicate that further development of a pesticide would be unwise because of residual hazards. Nothing in the present study suggests that the latter is true of Temik. The carbamate and its metabolites are present in milk at very low levels and, if they were consumed by other animals, the residues probably would be excreted from the body rather rapidly. Final evaluation of the safe use of Temik must come from additional research findings. This study indicates that the compound merits the required efforts.

LITERATURE CITED

Andrawes, N. R., Dorough, H. W., Lindquist, D. A., *J. Econ. Entomol.* **60,** 979 (1967).
Bull, D. L., Lindquist, D. A., Coppedge, J. R., J. AGR. FOOD CHEM. **15,** 610 (1967).
Coppedge, J. R., Lindquist, D. A., Bull, D. L., Dorough, H. W., J. AGR. FOOD CHEM. **15,** 902 (1967).
Dorough, H. W., J. AGR. FOOD CHEM. **15,** 262 (1967).
Knaak, J. B., Tallant, M. J., Sullivan, L. J., J. AGR. FOOD CHEM. **14,** 573 (1966).
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).
Payne, L. K., Jr., Stansbury, H. A., Weiden, M. H., J. AGR. FOOD CHEM. **14,** 356 (1966).
Weiden, M. H., Moorefield, H. H., Payne, L. K., *J. Econ. Entomol.* **58,** 154 (1965).

Received for review January 8, 1968. Accepted February 29, 1968. Approved for publication by the Director, Texas Agricultural Experiment Station. Study supported in part by U.S. Public Health Service Grant No. ES-00085 and Regional Project S-22. Radioactive Temik and its metabolite standards supplied by Union Carbide Corp., Olefins Division, South Charleston, W. Va.