The Metabolism of 2-Methyl-2-(methylthio)propionaldehyde O-(Methylcarbamoyl)oxime in the Rat

J. B. KNAAK, MARILYN J. TALLANT, and L. J. SULLIVAN

Mellon Institute, Pittsburgh, Pa.

The metabolic fate of *S*-methyl-C^{14}, *tert*-butyl-C^{14}, and *N*-methyl-C^{14} Temik [2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime] was investigated in the rat. The over-all recovery of the *S*-methyl, *tert*-butyl, and *N*-methyl label was, respectively, 95, 96 and 72% of the doses. Tissue residues (8 to 10% of the dose) were found only in the case of *N*-methyl labeled Temik. Seventy per cent of an oral dose of Temik was excreted as 2-methyl-2-(methylsulfinyl)propionaldehyde oxime and 2-methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime. The remaining metabolites appeared to be acids formed by further oxidation of the oxime. In conjunction with the metabolic studies, in vivo cholinesterase depression and recovery curves were obtained with plasma, red blood cells, and brain. At an oral dose of 0.33 mg. per kg., the cholinesterase activity of Temik-treated rats recovered from inhibition two hours prior to the rats treated with 2-methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime.

UNION CARBIDE (*14*) recently introduced a new oxime substituted carbamate insecticide Temik [2 - methyl - 2 - (methylthio)propionaldehyde *O*-(methylcarbamoyl) oxime]. Temik is a proposed trademark for a 10% formulation of UC 21149. Previous metabolism studies involving carbamate insecticides have dealt primarily with the fate of substituted aryl *N*-alkyl carbamates (*5, 10, 11, 15*). The nature of the metabolic products derived from these carbamates largely depends on the nature of the phenol and the in vivo stability of the carbamate to hydrolysis.

Since the chemistry of Temik indicated a susceptible point of attack on the sulfur atom, the investigation of the metabolism of this compound in the rat required study of the oxidation products of Temik as an alternative to conjugation of this carbamate or its hydrolytic products (*1, 13*).

Methods

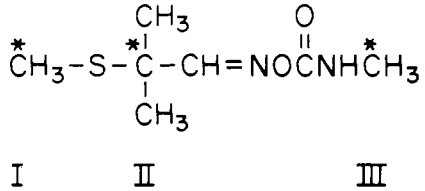
Chemicals. Temik was labeled separately with C^{14} (*4*) in three different positions (Figure 1). The synthesized C^{14} compounds were 2-methyl-2-(methyl-C^{14} - thio)propionaldehyde *O* - (methylcarbamoyl)oxime—compound I, 4.94 mc. per mmole—2-methyl-2-(methylthio)propionaldehyde-2-C^{14} *O*-(methylcarbamoyl)oxime—compound II, 3.9 mc. per mmole—and 2-methyl-2-(methylthio)propionaldehyde *O*-(methyl-C^{14}-carbamoyl)oxime—compound III, 7.2 mc. per mmole.

Compounds I, II, and III will be referred to hereafter as *S*-methyl, *tert*-butyl, and *N*-methyl labeled Temik, respectively. 2 - Methyl - 2 - (methyl-C^{14} - thio)propionaldehyde oxime (*S*-methyl labeled oxime) and 2-methyl-2-(methylthio)propionaldehyde - 2 - C^{14} oxime (*tert*-butyl labeled oxime) were synthesized by Bartley *et al.* (*4*). The specific activities of *S*-methyl-C^{14} and *tert*-butyl-C^{14} oximes were 7.0 mc. per mmole and 5.6 mc. per mmole, respectively.

Labeled 2 - methyl - 2 - (methylsulfinyl)propionaldehyde *O* - (methyl-C^{14} - carbamoyl)oxime (*N* - methyl-C^{14} Temik sulfoxide) was synthesized by Bartley *et al.* (*4*).

Nonlabeled Temik, oxime [2-methyl-2-(methylthio)propionaldehyde oxime] and methylacrylonitrile were supplied by Union Carbide Olefins Division, South Charleston, W. Va.

2 - Methyl - 2 - (methylthio)propionitrile (nitrile) was prepared (*3*) by adding oxime (0.75 mole) dissolved in toluene dropwise with stirring to a solution of dimethylaniline (0.75 mole) and phosgene (1.11 moles) in 200 ml. of toluene. Intermittent cooling was employed to maintain the reaction temperature at 25° to 30° C. After addition of the oxime, an additional quantity of dimethylaniline (0.75 mole) dissolved in toluene was added and the mixture stirred for 1.5 hours at 25° C. Dimethylaniline was removed from the toluene layer with water and dilute HCl. Trace quantities of HCl were removed from the toluene layer with 5% (w./v.) $NaHCO_3$. The organic phase was dried using anhydrous Na_2SO_4



I II III

Figure 1. Structural formula of Temik and position of C^{14}

and concentrated at 50° to 60° C. at 110 mm. to a yellow oil. Distillation on a 36-inch spinning band column gave a 64% yield of the nitrile, b.p. 69° to 70° C. at 40 mm.

2 - Methyl - 2 - (methylsulfonyl)-propionitrile, 2 - methyl - 2 - (methylsulfinyl)propionaldehyde oxime (oxime sulfoxide), and 2-methyl-2-(methylsulfonyl)propionaldehyde oxime (oxime sulfone) were prepared (*3*) from their corresponding precursors, the nitrile and oxime, by peracetic acid oxidation. A typical oxidation was carried out by treating approximately 0.1 mole of the oxime or nitrile dissolved in 50 to 75 ml. of ethyl acetate dropwise with 18.9% peracetic acid—0.1 mole for the sulfoxide and 0.2 mole for the sulfone—in ethyl acetate. The addition was carried out over a one-hour period with cooling (25° to 30° C.) and stirring. The mixture was allowed to stir overnight at room temperature. 2-Methyl-2-(methylsulfonyl)propionitrile, oxime sulfoxide, and oxime sulfone were isolated by cooling, flooding the reaction mixture with hexane, and filtering off the white solids. 2 - Methyl - 2 - (methylsulfonyl)-propionitrile was recrystallized from hot carbon tetrachloride as white needles having a melting point of 62° to 63° C., while oxime sulfoxide and oxime sulfone were recrystallized from a hot toluene-hexane solution to give white needles melting at 107° to 108° C. and 131° to 131.5° C., respectively.

2 - Methyl - 2 - (methylsulfinyl)-propionaldehyde *O*-(methylcarbamoyl)-oxime—Temik sulfoxide—and 2-methyl-2 - (methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime—Temik sulfone—were synthesized from recrystallized Temik in a manner previously described for the sulfoxide and sulfone of the oxime and nitrile. The sulfoxide of Temik (m.p. 108° to 110° C.) was recrystallized from benzene–ethyl acetate

(95 to 5) and the sulfone of Temik (m.p. 132° to 133° C.) from ethyl acetate.

Elemental and spectral analyses (infrared and NMR) were obtained for the nonlabeled nitrile, oxime sulfoxide, oxime sulfone, Temik sulfoxide, and Temik sulfone.

Cholinesterase Studies. Male rats (Carworth Farms-Elias stock) weighing 150 grams were treated orally and intraperitoneally with Temik dissolved in 50 μl. of 95% ethanol and Temik sulfoxide dissolved in 50 μl. of water at the levels indicated in Figure 2. Control animals for the Temik studies received 50 μl. of ethanol. Three to six rats were treated and killed at the times indicated in Figure 2 and at eight hours. Twenty control animals were used for each Temik study—oral, intraperitoneal—while six control animals were used for each Temik sulfoxide study—oral, intraperitoneal.

For acetylcholinesterase assay, 0.75 ml. of plasma, 0.5 ml. of hemolyzed cells (*8*), or 0.75 ml. of a brain preparation (*7*) containing microsomal and soluble cholinesterase, were added to 8.0 ml. of 0.9% sodium chloride in a reaction vessel kept at 38° C. The pH was adjusted to 7.4 with 0.02*N* NaOH, and 0.2 ml. of 3% acetylcholine bromide was added. The hydrolysis rate of this compound was followed by means of an automatic recording titrator (*9*).

The cholinesterase values obtained in these studies were analyzed by statistical methods. Bartlett's method (*2*) was used to test variances for homogeneity. When the variances were homogeneous, an analysis of variance was made. Duncan's multiple range test (*6*) was used to delineate differences between treatment means. When variances were heterogeneous, the same procedure was followed because the replicates in each time interval generally were too few to allow individual *t*-tests to be used accurately.

Excretion of Temik-C^{14} and Oxime-C^{14}. *S*-Methyl-C^{14}, *tert*-butyl-C^{14}, and *N*-methyl-C^{14} Temik, dissolved in polyethylene glycol 400, were individually administered (oral) to rats, eight animals per labeled compound, in 50-μg. doses (0.33 mg. per kg.) using the weighed syringe technique. This dose was selected on the basis of the red cell and brain cholinesterase depression and recovery data given in Figure 2. Ten milligrams (66 mg. per kg.) of *S*-methyl-C^{14} and *tert*-butyl-C^{14} oxime were individually administered to rats (four animals per label) as previously described for Temik.

Male rats (Carworth Farms-Elias stock) weighing approximately 150 grams were used and maintained on a commercial synthetic diet to facilitate the collection of fecal material. The animals were housed individually in glass metabolism cages, which permitted the separate and simultaneous collection of urine, feces, and respiratory CO_2. The daily urine, fecal, and CO_2 samples from animals administered *S*-methyl and *tert*-butyl labeled Temik and oxime were analyzed according to the method of Knaak *et al.* (*10*) for C^{14} over a four-day period. The daily urine and fecal samples from animals receiving *N*-methyl labeled Temik were collected and analyzed for C^{14} over an 11-day period, while respiratory CO_2 from each animal was trapped and analyzed for C^{14} over a four-day period.

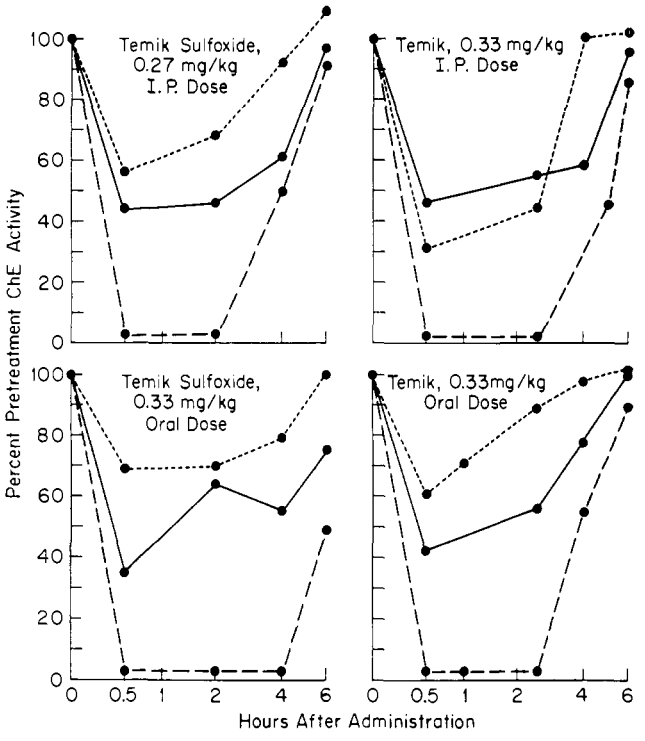


Figure 2. Effect of Temik and Temik sulfoxide on rat cholinesterase in vivo

---- Brain homogenate
—— Red blood cells
— — Plasma

At the termination of the C^{14} studies, the animals were killed and the carcasses (two animals per labeled compound) analyzed for residual radioactivity according to Knaak *et al.* (*10*) method.

Analysis of the Urinary Metabolites of Temik-C^{14} and *tert*-Butyl-C^{14} Oxime. The first-day urines, obtained in the excretion studies, were used as a source of metabolites for the following chromatographic studies.

Silica Gel Column Chromatography. A 1.5 × 24 cm. column was packed with 36 grams of deactivated silica gel (Davison 923, high purity) as a slurry in 1-to-1 dioxane–iso-octane. Deactivation consisted of saturating the gel with water and air drying it on a glass plate to a free flowing powder. Two-milliliter volumes of urine containing the metabolites of *S*-methyl-C^{14}, *tert*-butyl-C^{14}, or *N*-methyl-C^{14} Temik were individually absorbed on 3 to 5 grams of silica gel previously saturated with water and air dried. The water was evaporated slowly at 40° C. until the gel appeared to be dry. The metabolites on silica gel were added to the top of the column as a slurry in 1-to-1 dioxane–iso-octane and were eluted from the silica gel column as indicated in Figure 4. Four-milliliter fractions were collected, and every fifth fraction was analyzed by liquid scintillation counting techniques.

The urinary metabolites of *tert*-butyl-C^{14} oxime were chromatographed on silica gel as previously described for the metabolites of Temik.

For the identification of the metabolites, Temik-C^{14} (*tert*-butyl and *N*-methyl labels), *tert*-butyl labeled oxime, *N*-methyl labeled Temik sulfoxide, and the peracetic acid oxidation products of *tert*-butyl labeled Temik, and oxime containing *tert*-butyl-C^{14} Temik sulfoxide, *tert*-butyl-C^{14} Temik sulfone, *tert*-butyl-C^{14} oxime sulfoxide, and *tert*-butyl-C^{14} oxime sulfone were chromatographed under similar conditions.

Ion Exchange Chromatography. Two-milliliter volumes of urine containing the metabolites of *S*-methyl and *tert*-butyl labeled Temik were chromatographed individually on a 1.5 × 24 cm. column of diethylaminoethyl (DEAE)-cellulose using the procedure of Knaak *et al.* (*10*) and on a 1.5 × 24 cm. column of Dowex 1 times 8, 200 to 400 mesh, in the acetate form using three linear gradients (piperazine–acetate buffer, pH 6.0) as indicated in Figure 5. Volumes of 300 ml. were used in both the mixing chamber and the reservoir of the gradient device (*9*). Column flow rates (4.0 ml. per minute) were controlled by an all-Teflon microbellows pump (Research Appliance Co., Pittsburgh, Pa.). Four-milliliter fractions were collected, and 1.0 ml. was analyzed for radioactivity by liquid scintillation counting.

The urinary metabolites of *tert*-butyl-C^{14} oxime were chromatographed on DEAE-cellulose and Dowex 1 times 8 (acetate form) as previously described for the metabolites of Temik.

THIN LAYER CHROMATOGRAPHY. Thin layer chromatoplates were prepared according to the method of Lees and DeMuria (*12*) using a slurry of silica gel G (Brinkmann Instruments, Westbury, N. Y.) in water. The plates were activated for 2 hours at 120° C. Two hundred μg. of labeled or nonlabeled Temik, oxime, oxime sulfoxide, or oxime sulfone, and Temik sulfoxide or Temik sulfone were separately applied to the chromatoplate as a 1% solution in dioxane. Development was accomplished using the solvent systems indicated in Table I. The dioxane-methanol system was used to elute the polar sulfoxides. The compounds developed up the plate in an order similar to their elution from the deactivated silica gel column. All operations were performed in a standard dry box previously swept for 2 hours with 2.0 liters of CO_2 per minute. The chromatoplates were exposed to CO_2 for 2 hours prior to the application of Temik, derivatives or metabolites. The chromatographic solvents were exposed to CO_2 at the time the plates were placed in the elution jars. After chromatography, nonlabeled materials were detected with iodine vapor and radioactive material by scintillation counting of successive 1.0 cm. squares of gel.

Urinary C^{14} metabolites, C and D, off the silica gel column, contained in 20 to 50 μl. of solvent in the peak tubes, were rechromatographed on thin layer plates along with oxime sulfoxide and Temik sulfoxide as controls. The C^{14} metabolites appearing in urine were chromatographed under similar conditions. Radioactive metabolites were detected by scintillation counting and the nonlabeled standards by iodine vapor.

GAS CHROMATOGRAPHY. Temik, oxime, and their neutral sulfur oxidation products, the sulfoxide and sulfone, were studied by gas chromatography. Table II gives the retention times for the principal decomposition products of Temik and related materials. The identity of the decomposition products was determined by cochromatography of the precursor with known chemicals. Of the compounds studied, the oxime was the only compound which chromatographed without decomposition. A Barber-Colman 5000 gas chromatograph equipped with a radioactive monitor and flame detector using a 10 to 1 split in column effluent was fitted with a glass column, 2.43 meters × 6 mm. (I.D.), packed with 10% Carbowax 20-M on Applied Science Gas Chrom Q, 80 to 100 mesh. Operating conditions were: injection port 350° C., oven temperature programmed from 75° to 225° C., a flow rate of 80 ml. per minute. Under these conditions, the thermal decomposition products would be qualitatively, but not quantitatively, related to their respective precursor, i.e., Temik or related products.

Urines from animals receiving *tert*-butyl labeled Temik were gas chromatographed under similar conditions, and the radioactive decomposition products were related to their C^{14} precursors—metabolites.

Results

Cholinesterase Studies. Figure 2 shows the effect with time of Temik and Temik sulfoxide on plasma, red blood cells, and brain cholinesterase values. Cholinesterase values at the various time intervals after dose were compared statistically, and a summary of the results are given in Table III. The treatment variances were for the most part homogeneous (X^2), while the analysis of variance indicated significant differences of the cholinesterase values

Table I. Average R_f's in Two Solvent Systems from Temik and Related Compounds on Silica Gel G Thin Layer Plates

Compound	Solvent System 1:1 Hexane-dioxane	Solvent System 9:1 Dioxane-methanol
2-Methyl-2-(methylthio)propionaldehyde oxime	0.77	0.74
Temik	0.62	0.71
2-Methyl-2-(methylsulfonyl)propionaldehyde oxime	0.57	0.70
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	0.31	0.66
2-Methyl-2-(methylsulfinyl)propionaldehyde oxime	0.23	0.56
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	0.10	0.46

Table II. Retention of Principal Products of Temik and Related Compounds on a Carbowax 20M Gas Chromatographic Column

Compounds	Principal Decomposition Products	Retention in Time, minutes	Retention in Temp., °C.
Temik	2-Methyl-2-(methylthio)propionitrile	14.6	119.0
2-Methyl-2-(methylthio)propionaldehyde oxime	2-Methyl-2-(methylthio)propionaldehyde oxime	35.6	181.8
2-Methyl-2-(methylsulfinyl)propionaldehyde oxime	Methacrylonitrile	3.76	86.3
2-Methyl-2-(methylsulfonyl)propionaldehyde oxime	2-Methyl-2-(methylsulfonyl)propionitrile	42.8	203.5
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Methacrylonitrile	3.76	86.3
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	2-Methyl-2-(methylsulfonyl)propionitrile	42.8	203.5

Table III. Comparison of the Cholinesterase Values for Temik and Temik Sulfoxide

Compound, Dose, and Route	Tissue	Homogeneity of Variance, X^2	Analysis of Variance, F	Minimum Time Interval, Hours: Significantly different[b] from controls	Minimum Time Interval, Hours: Not significantly different[b] from controls
Temik	Plasma	2.71	7.8	5.0	6.0
0.33 mg. per kg.	Red blood cells	7.19	17.7	5.0	6.0
I.P.	Brain	18.0[a]	76.7	2.5	4.0
Temik sulfoxide	Plasma	0.23	5.8	4.0	6.0
0.27 mg. per kg.	Red blood cells	1.59	12.9	4.0	6.0
I.P.	Brain	5.94	29.3	2.0	4.0
Temik	Plasma	10.96[a]	4.9	5.0	6.0
0.33 mg. per kg.	Red blood cells	6.40	31.4	5.0	6.0
Oral	Brain	11.41	4.2	1.0	2.5
Temik sulfoxide	Plasma	4.56	40.7	6.0	8.0
0.33 mg. per kg.	Red blood cells	3.18	7.4	6.0	8.0
Oral	Brain	5.63	14.3	4.0	6.0

[a] Variances were heterogeneous.
[b] Fiducial limit of $P = 0.05$ used.

obtained at one time interval with values at every other time interval. Table III gives the minimum time interval for which the cholinesterase values are significantly different and those not significantly different from the controls.

The sulfoxide of Temik could not be administered intraperitoneally to male rats at 0.33 mg. per kg. without causing severe cholinesterase poisoning symptoms and, in some cases, death. Red cell and brain cholinesterase values were depressed to 40 and 35% of pretreatment values 0.25 hour after dose.

Excretion of Temik-C^{14} and Oxime-C^{14}. The excretion of *S*-methyl-C^{14}, *tert*-butyl-C^{14}, and *N*-methyl-C^{14} labeled Temik is given in Figure 3 for urine, feces, and CO_2. Each study represents the average daily excretion of eight animals over a period of four days. The excretion of *S*-methyl-C^{14} and *tert*-butyl-C^{14} Temik essentially was complete in four days while excretion of *N*-methyl-C^{14} Temik was not complete. An additional 2.0% of the *N*-methyl label was excreted in urine and feces between the fourth and 11th day. The average per cent of the dose excreted as $C^{14}O_2$ for the *S*-methyl and *tert*-butyl labeled Temik was 1.1 and 0.5%, respectively. The over-all recovery of the dose for the *S*-methyl, *tert*-butyl (after four days), and *N*-methyl (after 11 days) labeled Temik was 95.2, 96.0, and 71.8%, respectively.

The carcasses of the animals administered the *S*-methyl and *tert*-butyl labeled Temik were found to contain less than 0.1% of the dose after 4 days, while the carcasses of the animals administered *N*-methyl labeled Temik were found to contain 8 to 10% of the dose after 11 days. The C^{14} residues found in the animals after 11 days brought the over-all recovery of the *N*-methyl label to approximately 80%.

The excretion of *S*-methyl-C^{14} and *tert*-butyl-C^{14} labeled oxime is given in Figure 3 for urine, feces, and CO_2. Each study represents the average daily excretion of four animals over a 4-day period. The C^{14} from the oxime—*S*-methyl and *tert*-butyl labels—was distributed between urine, feces, and CO_2 similarly to the *S*-methyl and *tert*-butyl labels of Temik, and the rate of excretion of the two labels parallel the rates obtained from Temik.

Nature of the Urinary Metabolites of Temik-C^{14} and *tert*-Butyl-C^{14} Oxime. Figure 4 gives the results obtained with the urinary metabolites of C^{14} labeled Temik — *S* - methyl, *tert* - butyl, and *N*-methyl—on a silica gel column. Four distinct groups of materials were found by chromatography on deactivated silica gel—group I, metabolites A, B and C; group II, metabolite D; group III, metabolites E and F; group IV, metabolite(s) G.

The metabolites appearing in group I were least polar materials and were identified as A, oxime; B, oxime sulfone; C, oxime sulfoxide. The position and order of elution of these compounds were established by chromatographing known compounds under identical column conditions. Under these conditions, Temik, cochromatographs with the oxime (A), and Temik sulfone chromatographs between the oxime sulfone (B), and sulfoxide (C). No metabolites corresponding to Temik and Temik sulfone were observed with *N*-methyl labeled Temik.

Metabolite D, group II, was identified as Temik sulfoxide. The position and order of elution of Temik sulfoxide were established by separately chromatographing *N*-methyl labeled Temik sulfoxide and the peracetic acid oxidation products of *tert*-butyl labeled Temik.

The identity of the metabolites in group III, E and F, and in group IV, G, presently is unknown.

Table IV lists the metabolites (Figure 4) in order of their elution and gives their percentages relative to the C^{14} recovered from the column. The percentages found in various urine samples for metabolite C, oxime sulfoxide, and D, Temik sulfoxide, varied as indicated while their combined total (metabolite C + D) remained close to 70%.

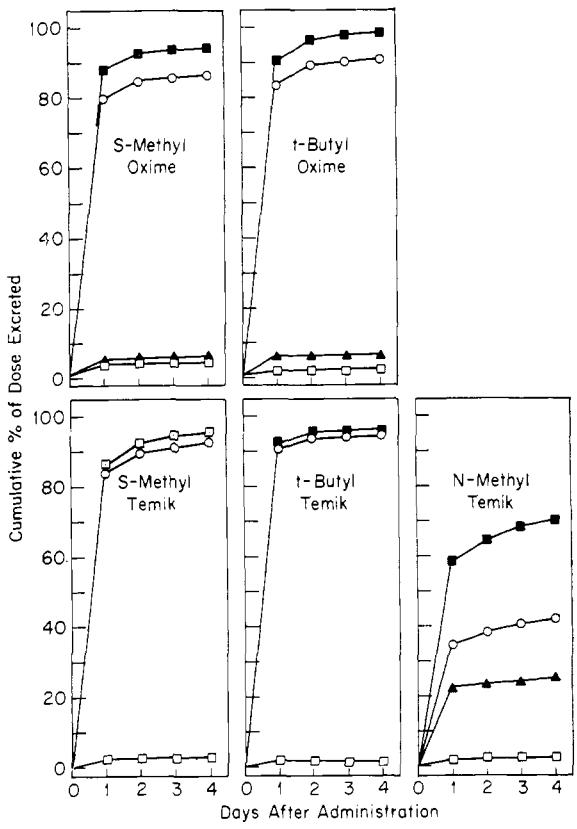


Figure 3. Excretion of Temik-C^{14} and 2-methyl-2-(methylthio)propionaldehyde oxime-C^{14} by the rat

C^{14} dose equivalents in ■, urine, feces and CO_2; ○, urine; ▲, CO_2; □, feces. Male rats (150 grams) were orally administered 50 μg. of Temik (8 animals per label) and 10 mg. of oxime (4 animals per label)

Table IV. Urinary Metabolites of Temik-C^{14} Excreted by the Rat and Separated by Silica Gel Column Chromatography

	Metabolites[a]	Metabolites of Total[b] C^{14} Recovered from Column, % I	II	III
A	2-Methyl-2-(methylthio)propionaldehyde oxime	1.0	0.2	0.0
B	2-Methyl-2-(methylsulfonyl)propionaldehyde oxime	2.0	3.0	4.3[d]
C	2-Methyl-2-(methylsulfinyl)propionaldehyde oxime	31.0	33.0	
D	2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	35.5	38.0	82.2
E	Unidentified metabolite	8.0	11.0[c]	3.3
F	Unidentified metabolite	7.5		5.6
G	Unidentified acids or other polar metabolites	15.0	14.8	4.6

[a] Listed in order of elution.
[b] I. 2-Methyl-2-(methyl-C^{14}-thio)propionaldehyde *O*-(methylcarbamoyl)oxime.
II. 2-Methyl-2-(methylthio)propionaldehyde-2-C^{14} *O*-(methylcarbamoyl)oxime.
III. 2-Methyl-2-(methylthio)propionaldehyde *O*-(methyl-C^{14}-carbamoyl)oxime.
All urines were pooled collections made from 4 animals, 24 hours after an oral dose of 50 μg. per animal.
[c] Average value for E and F.
[d] Average value for B and C.

tert-Butyl-C^{14} oxime was metabolized and excreted in the urine as oxime sulfoxide (~30%) and unknown acids (~47%).

On DEAE-cellulose, 75 to 80% of the urinary metabolites of *S*-methyl and *tert*-butyl labeled Temik and 47% of the metabolites of *tert*-butyl labeled oxime chromatographed as neutral compounds. The remaining C^{14} metabolites of Temik and oxime chromatographed as unresolved components directly after the neutrals and prior to butyric-C^{14} acid. On DEAE-cellulose butyric acid chromatographs prior to acetic acid.

Figure 5 gives the results obtained with the urinary metabolites of C^{14} labeled Temik—*S*-methyl and *tert*-butyl—and *tert*-butyl labeled oxime on Dowex 1. Approximately 70% of the Temik-C^{14} metabolites chromatographed as neutral compounds, but only 44% of the oxime metabolites chromatographed as neutral compounds. The remaining C^{14} metabolites chromatographed as weak acids. Table V gives the percentages obtained for these acids, and Figure 5 indicates their chromatographic location. The chromatogram shows the location of nine different acids. Metabolites C, E, H, and J may be identical, respectively, to metabolites D, F, G, and I, since changes in column operating conditions often cause shifts in the retention time of acidic components. If these shifts occurred, the acidic metabolites of *S*-methyl-C^{14} Temik are C-D, E-F, G-H, and I-J. Metabolite B was not found in the urine of animals administered *S*-methyl labeled Temik.

By use of this grouping procedure, the urinary metabolites of *tert*-butyl-C^{14} oxime are C-D, E-F, G-H, and I-J.

Metabolite C from the silica gel column chromatographed on thin layer plates similarly to a known sample of oxime sulfoxide, and metabolite D chromatographed as Temik sulfoxide. The C^{14} metabolites in unfractionated urine chromatographed as two major spots corresponding to oxime sulfoxide and Temik sulfoxide. Small amounts of other radioactive materials were present but were not identified.

First-day urine containing *tert*-butyl labeled Temik metabolites gave de-

Table V. Urinary Metabolites of Temik-C^{14} and 2-Methyl-2-(methylthio)propionaldehyde-2-C^{14} Oxime Excreted by the Rat and Separated by Ion Exchange Chromatography on Dowex 1 (Acetate Form)

	Metabolites of Total[b] C^{14} Recovered from Column, %		
Metabolites[a]	*I*	*II*	*III*
A Neutral compounds	71.1	69.3	43.8
B Acidic unknowns	0.0	10.0	0.0
C Acidic unknowns	7.4	0.0	0.0
D Acidic unknowns	0.0	3.9	8.9
E Acidic unknowns	3.2	0.0	0.0
F Acidic unknowns	0.0	2.5	12.0
G Acidic unknowns	0.0	0.0	10.3
H Acidic unknowns	8.9	5.4	0.0
I Acidic unknowns	0.0	0.0	25.0
J Acidic unknowns	9.4	8.9	0.0

[a] Listed in order of elution.

[b] I. 2 - Methyl - 2 - (methyl - C^{14}-thio)propionaldehyde *O*-(methylcarbamoyl)oxime.
II. 2 - Methyl - 2 - (methylthio)-propionaldehyde - 2 - C^{14} *O*-(methylcarbamoyl)oxime.
III. 2 - Methyl - 2 - (methylthio)-propionaldehyde - 2 - C^{14} oxime.

All urines were pooled collections made from 4 animals, 24 hours after an oral dose of 50 μg. per animal for Temik and 10 mg. per animal for the oxime.

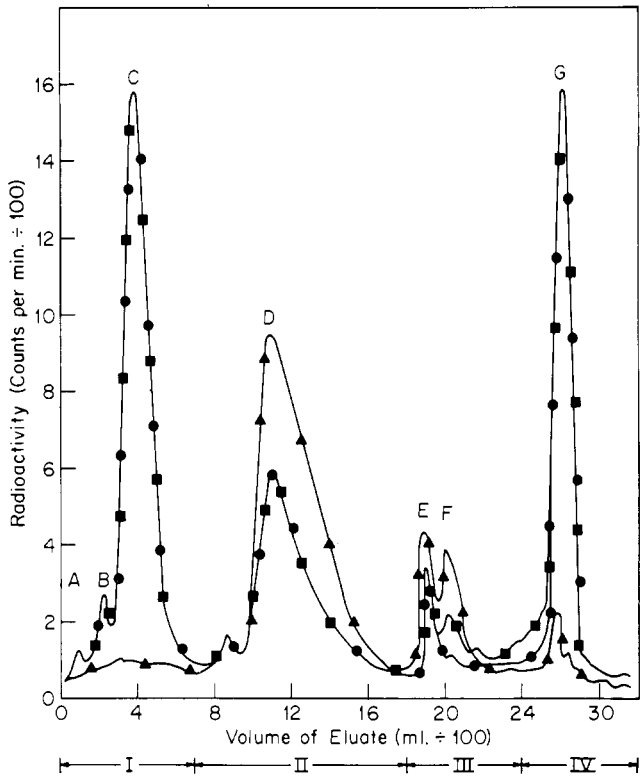


Figure 4. Silica gel chromatography of urinary metabolites of Temik-C^{14}

■. *S*-Methyl-C^{14} Temik
●. *tert*-Butyl-C^{14} Temik
▲. *N*-Methyl-C^{14} Temik
Metabolites are identified in Table IV

Elution program:
I. 1 : 1 dioxane–iso-octane
II. 3 : 1 dioxane–iso-octane
III. 95 : 5 dioxane-water
IV. 90 : 5 : 5 dioxane–water–glacial acetic acid

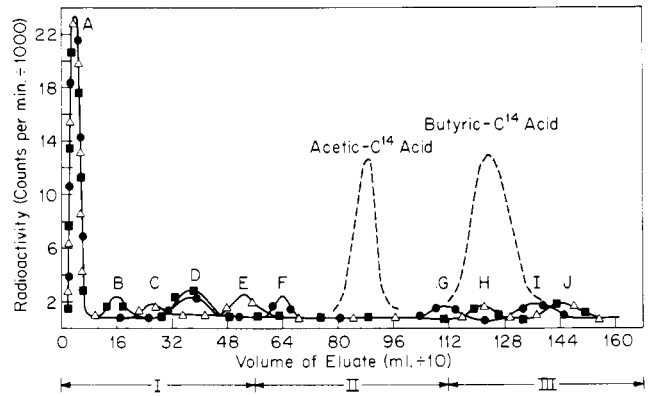


Figure 5. Chromatography of the urinary metabolites of Temik-C^{14} and oxime-C^{14} on Dowex 1 (acetate form)

△. *S*-Methyl-C^{14} Temik
■. *tert*-Butyl-C^{14} Temik
●. 2-Methyl-2-(methylthio)propionaldehyde-2-C^{14} oxime
Gradient elution program:
I. 0.01M piperazine-acetate, pH 6.0, to 0.05M piperazine-acetate, pH 6.0
II. 0.05M piperazine-acetate, pH 6.0, to 0.1M piperazine-acetate, pH 6.0
III. 0.1M piperazine-acetate, pH 6.0, to 0. 5M piperazine-acetate, pH 6.0

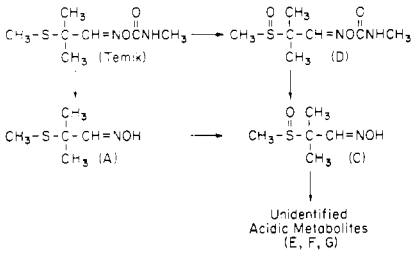


Figure 6. Metabolic fate of Temik in the rat

A. 2-Methyl-2-(methylthio)propionaldehyde oxime
C. 2-Methyl-2-(methylsulfinyl)propionaldehyde oxime
D. Temik sulfoxide, 2-methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime
Letters refer to metabolites in Figure 4

composition products by gas chromatography equivalent to oxime sulfoxide and Temik sulfoxide. Only trace quantities of the nitrile related to Temik [2 - methyl - 2 - (methylthio)propionitrile] were found, and no C^{14} peak was detected corresponding to the oxime, oxime sulfone, or Temik sulfone.

Discussion

The oral LD_{50} value for Temik is approximately 1.0 mg. per kg. in the rat. Because of the oral toxicity of Temik, the study of its metabolism at some selected dose below its LD_{50} value was essential. Cholinesterase activity levels in the brain, red cells, and plasma were used to select the dose (0.33 mg. per kg.) and set the C^{14} specific activity requirements of *S*-methyl, *tert*-butyl, and *N*-methyl labeled Temik. Cholinesterase recovery studies with Temik showed that, at the selected dose (0.33 mg. per kg.), rat brain and red cell cholinesterase values are back to pretreatment values by six hours.

Temik was rapidly absorbed from the gastrointestinal tract and excreted in urine and respiratory CO_2. Excretion of the *S*-methyl and *tert*-butyl labels was essentially complete (90%) at the end of 24 hours but the *N*-methyl label was 60% complete. Small quantities of the *N*-methyl label (0.2% of dose per day) were excreted up to 11 days in urine and feces. *S*-Methyl-C^{14} or *tert*-butyl-C^{14} residues could not be detected in the animal carcass after four days. *N*-methyl-C^{14} residues (8 to 10% of the dose) were found after 11 days in the carcass. *S*-Methyl and *tert*-butyl labeled oximes were readily absorbed, metabolized, and excreted in the urine of the rat.

Temik is metabolized in the rat (Figure 6) and excreted in first-day urine as oxime sulfoxide (~30%), Temik sulfoxide (~40%), and a number of other polar compounds (5 to 9) believed to be acids (~30%). Evidence for the presence of trace quantities (~1%) of Temik in urine was obtained only by gas chromatography, and evidence for the presence of trace quantities (1 to 2%) of oxime and oxime sulfone was obtained only by silica gel chromatography. To substantiate their presence fully, additional evidence is needed. The importance of small quantities of these materials in the over-all metabolic scheme, although unknown, is probably small. No evidence was obtained for the presence of Temik sulfone by either gas, silica gel, or thin layer chromatography.

During these metabolic studies, difficulties were encountered in obtaining reproducible results on thin layer plates with Temik and its derivatives in air. When Temik was chromatographed in air, a series of compounds were found between the origin and Temik. On thin layer chromatographic plates and in air the oxime sulfoxide was converted to oxime sulfone and unidentified products. Under similar conditions, the sulfoxide of Temik was hydrolyzed and yielded oxime sulfoxide. Temik and its derivatives were stable when run in a dry box containing CO_2.

Temik and sulfoxide was a more potent in vivo inhibitor of plasma, red cell, and brain cholinesterase than Temik. At 0.33 mg. per kg., cholinesterase activity in Temik treated animals (oral) returned to pretreatment cholinesterase values two hours prior to Temik sulfoxide treated animals.

Acknowledgment

The authors thank Sarah J. Kozbelt and Jane M. Eldridge for their skilled technical assistance and Carrol S. Weil for the statistical analysis.

Literature Cited

(1) Barnsley, E. A., Thompson, A. E. R., Young, L., *Biochem. J.* **90,** 588 (1964).
(2) Bartlett, M. S., *Proc. Roy. Soc. (London)* **A 160,** 268 (1937).
(3) Bartley, W. J., Union Carbide Olefins Division, South Charleston, W. Va., personal communication, 1965.
(4) Bartley, W. J., Heywood, D. L., Steele, T. E. N., Skraba, W. J., J. AGR. FOOD CHEM. **14,** 604 (1966).
(5) Dawson, J. A., Heath, D. F., Rose, J. A., Thain, E. M., Ward, J. B., *Bull. World Health Organization* **30,** 127 (1964).
(6) Duncan, D. B., *Biometrics* **13,** 164 (1957).
(7) Goldberg, M. E., Johnson, H. E., Knaak, J. B., Smyth, H. F., Jr., *J. Pharmacol. Exptl. Therap.* **141,** 244 (1963).
(8) Knaak, J. B., O'Brien, R. D., J. AGR. FOOD CHEM. **8,** 198 (1960).
(9) Knaak, J. B., Stahmann, M. A., Casida, J. E., *Ibid.*, **10,** 154 (1962).
(10) Knaak, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., *Ibid.*, **13,** 537 (1965).
(11) Krishna, J. G., Casida, J. E., *Ibid.*, **14,** 98 (1966).
(12) Lees, T. J., Demuria, P. J., *J. Chromatog.* **8,** 108 (1962).
(13) March, R. B., Metcalf, R. L., Fukuto, T. R., Maxon, M. G., *J. Econ. Entomol.* **48,** 355 (1955).
(14) Weiden, M. H. J., Moorefield, H. H., Payne, L. K., *Ibid.*, **58** (1) 154 (1965).
(15) Williams, E. A., Meikle, R. W., Redemann, C. T., J. AGR. FOOD CHEM. **12,** 457 (1964).

Received for review April 6, 1966. Accepted July 25, 1966.