control was turned off, column and detector temperatures were 220 and 270 °C, and the carrier gas (nitrogen), hydrogen, and air flow rates were 60, 35, and 300 mL/min, respectively.

RESULTS AND DISCUSSION

Under the GC conditions described, the compounds gave a 50% full-scale deflection ($^1/_2$ fsd) in the 6.8–66.7-ng range (Table I). The column separated the compounds with good resolution. It should be noted, however, that hydroxyatrazine and the hydroxy derivatives of the dealkylated metabolites of atrazine were converted to the corresponding methoxy derivatives prior to GC analyses.

GC analysis of the extracts from the incubation mixtures is shown in Table II. Recoveries in the range of 98.2–99.7% indicate that correction factors used for recoveries from the acid alumina column and methylation efficiencies were valid. The formation of ammeline (2-chloro-4,6-diamino-*s*-triazine) and its hydroxy analogue, 2-hydroxy-4,6-diamino-*s*-triazine, was not observed in any of the incubation mixtures. This is in accordance with previous findings (Foster and Khan, 1976; Khan and Foster, 1976; Foster et al., 1979; Khan et al., 1979) that the metabolism of atrazine involves only partial N-dealkylation.

Some hydrolysis of 2-chloro partially N-dealkylated metabolites to the corresponding hydroxy analogues was observed when they were incubated for 5 h in 0.134 M phosphate buffer, pH 7.4. This could possibly be attributed to chemical hydrolysis of the compounds at the neutral–alkaline pH of the medium (Armstrong et al., 1967). However, hydrolysis of the compounds was significantly more pronounced when the incubation involved the soluble fraction (105000*g*) from goose liver homogenates. Metabolic hydrolysis, which probably involves a dechlorinase, has been previously observed as being the predominant factor in the metabolism of atrazine by the soluble fraction from various liver homogenates (Foster et al., 1979; Khan et al., 1979).

In conclusion, it can be stated that the soluble fraction (105000*g*) from liver homogenates contains enzyme systems that metabolize atrazine by partial N-dealkylation and hydrolysis. In in vitro incubations with the enzyme preparation, hydrolysis predominates and results in the formation of hydroxyatrazine which does not undergo further degradation by dealkylation. However, partially N-dealkylated metabolites, deethylatrazine and deisopropylatrazine, are further hydrolyzed to the corresponding hydroxy analogues.

ACKNOWLEDGMENT

The skilled technical assistance of N. Zabolotny, W. R. McDowell, H. Lie, and F. X. Ryan is greatly appreciated.

LITERATURE CITED

Armstrong, D. E., Chesters, G., Harris, R. F., *Soil Sci. Soc. Am. Proc.* **31**, 61 (1967).
Foster, T. S., Khan, S. U., *J. Agric. Food Chem.* **24**, 566 (1976).
Foster, T. S., Khan, S. U., Akhtar, M. H., *J. Agric. Food Chem.* **27**, 300 (1979).
Khan, S. U., Foster, T. S., *J. Agric. Food Chem.* **24**, 786 (1976).
Khan, S. U., Foster, T. S., Akhtar, M. H., *Pestic. Sci.* **10**, 460 (1979).
Khan, S. U., Greenhalgh, R., Cochrane, W. P., *J. Agric. Food Chem.* **23**, 430 (1975).

Received for review March 27, 1980. Accepted June 25, 1980. Animal Research Institute Contribution No. 917 and Chemistry and Biology Research Institute Contribution No. 1162.

Metabolism of Carbamate Insecticide Thiofanox in Rats

Byong Han Chin,* Marilyn J. Tallant, Warren C. Duane, and Lloyd J. Sullivan

The metabolic fate of thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime was investigated in the rat using [^{35}S]-, [*S-methyl*-^{14}C]-, or [*N-methyl*-^{14}C]P. The overall recovery of the ^{35}S-, *S-methyl*-^{14}C-, and *N*-methyl-^{14}C- label was 91, 94, and 95% of the single dose, respectively. The majority of the dose was eliminated in the following day's urine after [^{35}S]P and [*S-methyl*-^{14}C]P administration. After [*N-methyl*-^{14}C]P administration, the majority of the dose was eliminated in the urine and CO_2 of expired air. Major metabolic pathways of P were oxidation (40%) and N-demethylation (35%) while S-demethylation (4%) was a minor pathway. The major oxidation product of P found in urine was its sulfoxide, 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime. Other minor urinary metabolites identified were oxime sulfoxide, oxime sulfone, and parent sulfone. Excretion of unidentified anionic products found in urine represents 20% of the dose.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-([methylamino)carbonyl]oxime, is a systemic and contact insecticide developed by Diamond Shamrock Corp. Metabolic studies in soils (Duane, 1974) and plants (Whitten and Bull, 1974) showed rapid oxidation of P to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime.

The purpose of this study was (a) to determine the extent of the distribution in selected tissues and the route of excretion of ^{35}S- and ^{14}C-labeled P following oral administration to rats and (b) to determine the relative amounts of possible metabolites in urine.

MATERIALS AND METHODS

Chemicals. Both radioactive and nonradioactive P were furnished by the T. R. Evans Research Center, Diamond Shamrock Corp., Painesville, Oh. The three positions of

Diamond Shamrock Corporation, T. R. Evans Research Center, Painesville, Ohio 44077 (B.H.C. and W.C.D.), and Carnegie-Mellon Institute of Research, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213 (M.J.T. and L.J.S.).

0021-8561/80/1428-1085$01.00/0

P labeled with radioisotopes were the sulfur, the *N*-methyl carbon, and the *S*-methyl carbon designated throughout this report as ^{35}S, *N*-methyl-^{14}C-, and *S*-methyl-^{14}C-, respectively. The corresponding specific activities were 17.0, 6.0, and 5.2 mCi/mmol with radiochemical purity of 98%. P_1, P_2, and three oximes, 3,3-dimethyl-1-(methylthio)-2-butanone oxime (O), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime (O_1), and 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime (O_2) were also furnished by Diamond Shamrock Corp. A commercial synthetic rat diet (Knaak et al., 1965) containing 10% alphacel, 4% vegetable oil, 27% vitamin-free casein, 40% sucrose, 4% salt mixture, and vitamins was obtained from Nutritional Biochemicals Corp., Cleveland, Oh.

Solubility and Stability of P. The water solubility of P was reported to be 5200 ppm (Chin et al., 1976). Therefore, 1000 ppm of P in water used for this study is well below the water solubility of P. These authors also reported that P in water was very stable even after 10 weeks of storage; therefore, the possibility of dosing of P_1 (oxidation product of P) to animals was negligible. The stability of P also applies to the stability of P in the synthetic diet (Knaak et al., 1965) used for this study.

Animal Procedures. Male and female Harlan–Wistar rats weighing 183–208 g from the Carnegie-Mellon University colony were used for this study. Prior to dosing, these rats were acclimated for 3 days in metal metabolism cages. The animals were allowed free access to water and a synthetic diet (Knaak et al., 1965). After being dosed, animals in ^{35}S studies were housed singly in metal metabolism cages which permitted the simultaneous and separate collection of urine and fecal material. The rats on ^{14}C studies were housed singly in glass metabolism cages which allowed the simultaneous and separate collection of CO_2, urine, and feces. All animals were transferred to clean metabolism cages daily.

Dosing Procedures. Throughout the study, the amount of radioactivity used per rat was 6–10 μCi. Two dosing procedures (A and B) were used.

Procedure A. Experiment 1. Three male and three female rats received a single dose of 0.5 mL of a water solution containing 0.5 mg of [^{35}S]P by gastric intubation. After the first day, the same rats were maintained on the synthetic diet containing 100 parts per million (ppm) of nonradioactive P for 14 days. Using the same rats, experiments 2 and 3 were conducted in order to study the effect of dietary inclusion of P on excretion pattern of [^{35}S]P in urine.

Experiment 2. On the 15th day, the animals were removed from the diet containing 100 ppm of P and fed a compound-free diet overnight. The second dose of 0.5 mg of [^{35}S]P was given orally on the 16th day, and animals were maintained on the diet containing 100 ppm of P for 10 days.

Experiment 3. On the 26th day, the animals were removed from the diet containing 100 ppm of P and fed a compound-free diet overnight. On the 27th day, the third dose of 0.5 mg of [^{35}S]P was given, and urine and feces were collected for 3 days.

Procedure B. A single dose of 0.5 mL of a water solution containing 0.5 mg of [*N-methyl*-^{14}C]P or [*S-methyl*-^{14}C]P was administered orally to each of four female rats on a synthetic diet (Knaak et al., 1965).

Sample Procedure for Urine and Feces. The urine and cage washings from the animals were collected daily and analyzed by liquid scintillation techniques according to the procedures of Knaak et al., (1965). All data were collected to 100% efficiency based upon quench urines prepared for the study. Decay corrections for sulfur-labeled compounds were based upon standard samples of the appropriate ^{35}S-labeled compound on the day of dosing.

Fecal samples were dried in an oven at 60 °C. The dried samples were weighed and ground and 0.5 g was weighed into cellulose bags prepared from dialysis tubing. One-half milliliter of a 10% (w/v) sucrose solution was added, the sample thoroughly wetted, and the bag closed and dried in an oven at 60 °C. The sucrose served as a binder for the powdered sample. A Paar double-valved oxygen bomb was used to combust the samples (Knaak et al., 1965). The gases produced by combustion of the ^{14}C-labeled feces sample and residual oxygen were passed through two absorption traps, each of which contained 15 mL of 2% ethanolamine in methoxyethanol. Two-milliliter samples of trapping reagent from each trap were analyzed separately by liquid scintillation counting.

For combustion of the ^{35}S-labeled feces sample, the bomb wall was first coated with a thin layer of Tergitol-TP-9 (Union Carbide Corp.). Then 2 mL of 1% sodium carbonate was placed in the bomb to wet the walls. After the combustion of the sample, the bomb was opened and washed 3 times with small amounts of distilled water. The washings were pooled, and 1-mL aliquots were counted by liquid scintillation counting.

Sample Procedures for Tissues. Animals on [*N-methyl*-^{14}C]P were anesthetized with ether and exsanguinated via heart puncture in the morning of the 14th day after dosing. All animals on [^{35}S]P and [*N-methyl*-^{14}C]P studies were killed on the third day after dosing. The rats were anesthesized with ether, and the blood was removed by heart puncture of the left ventricle. The wet weights of all selected tissues were obtained at necropsy. Except for blood, tissues were pooled by sex and were homogenized in acetone and centrifuged, and the supernatant was removed for counting. The residues were resuspended in acetone and centrifuged, the supernatant was removed, and the residues were dried for combustion. The combustion procedure used was the same as that for fecal samples. Duplicate samples of dried tissues were combusted where sufficient material was available.

Blood was centrifuged for 15 min at 600 *g*, and the plasma was separated from red cells. The plasma was analyzed directly by liquid scintillation counting while the red cells were dried and combusted. Residual tissues and carcasses were reduced to small pieces, frozen in dry ice, coground with dry ice in a Model ED-5 Thomas-Wiley mill, dried, and combusted (Knaak et al., 1965).

Ion-Exchange Chromatography. Three milliliters of the urine from first day samples, pooled by sex, was added to a 1.2 × 30 cm DEAE-cellulose (diethylaminoethylcellulose) column previously prepared by the method of Sullivan et al. (1972). The elution gradients consisted of 0.005–0.01, 0.01–0.05, and 0.05–0.1N ammonium formate. Three hundred milliliters of each concentration was used for each gradient. Approximately 450 4-mL fractions were collected, and every fifth fraction was analyzed for radioactivity by liquid scintillation counting techniques. Fractions comprising a peak were pooled and counted for quantitation. The neutral peak contained in 2–3 column void volumes was either continuously extracted for 24 h with diethyl ether and the ether phase examined by thin-layer chromatography or concentrated by azeotropic distillation at reduced pressure with 1-butanol and analyzed by silica gel column chromatography.

Silica Gel Column Chromatography. Three milliliters of urine from the first-day samples, pooled by sex, or concentrates of fractions from DEAE chromatography was

Table I. Excretion of Radioactivity following Oral Administration of Thiofanox to Rats[a]

	recovery, % of dose									
	[^{35}S]P[b]			[*S-methyl*-^{14}C]P[c]				[*N-methyl*-^{14}C]P[c]		
days after treatment	no. of expt	urine	feces	no. of expt	urine	feces	CO_2	no. of expt	urine	feces	CO_2
1	3	72.0	4.2	2	73.0	1.6	3.0	2	48.7	3.0	17.5
2	3	9.6	0.9	2	10.8	0.3	0.7	2	3.1	0.8	6.7
3	3	1.5	0.3	2	2.1	0.1	0.2	2	0.7	0.4	5.8
4	2	0.5	0.1	1	1.0	0.1	0.1	2	0.9	0.2	0.8
5-7	2	1.1		1	0.8			2	1.4	0.4	1.5
8-12								2	1.2		1.4
total		85.0	5.5		87.7	2.1	4.0		56.0	4.8	33.7

[a] Each rat was dosed orally with 0.5 mg of thiofanox. [b] A group of three male and three female rats was used in each experiment. [c] A group of four female rats was used in each experiment.

added to 3 g of Davison 923 silica gel which had been deactivated by water and oven dried at 120 °C until ~4% water remained. The sample and silica gel were dried by azeotroping the water with 1-butanol at reduced pressure. The temperature of the sample was never allowed to exceed 37 °C. Dried acetonitrile was added to the sample and then distilled off at reduced pressure to produce a free flowing powder. The dried gel was then added to the top of a 2.5 × 28 cm column of deactivated silica gel (80 g) previously packed as a slurry in isooctane (2,2,4-trimethylpentane). Elution was accomplished by using a series of linear gradients in the order isooctane–isopropyl ether, isopropyl ether–acetonitrile, and acetonitrile–methanol. Each gradient consisted of 500 mL of the first-named solvent in the mixing chamber and an equal volume of the second-named solvent in the reservoir of the gradient device (Knaak et al., 1965). Approximately 250 fractions were analyzed for radioactivity by liquid scintillation counting techniques. The fractions comprising a resolved peak were pooled, and the solvent was removed by distillation under reduced pressure. Major peaks were further analyzed by thin-layer chromatography.

Thin-Layer Chromatography. Brinkman precoated plates of silica gel G which have nominal thicknesses of 250 μm were used without activation. The solvent system for single-dimensional analysis was chloroform–ethyl acetate–1-butyl ether–dioxane at a 4:2:2:2 ratio.

Urine samples were continuously extracted with diethyl ether for 24 h. The ether phase was dried over anhydrous sodium sulfate and concentrated by distillation under reduced pressure. The concentrate was spotted on thin-layer plates by using a microsyringe.

Standard determinations were performed by using authentic samples of a number of compounds which were potential metabolites. The following were the mean R_f values in the 4:2:2:2 single-dimensional analysis for these compounds.

chemical name	symbol	R_f
3,3-dimethyl-1-(methylthio)-2-butanone oxime	O	0.89
3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)-carbonyl]oxime	P	0.80
3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime	O_2	0.75
3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime	P_2	0.61
3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime	O_1	0.50
3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)-carbonyl]oxime	P_1	0.33

With each single-dimensional analysis, standards were spotted on either side of the unknown. Half-centimeter strips were removed from the middle line of the plate and analyzed for radioactivity by liquid scintillation counting techniques. The remainder of the plate was developed with iodine vapor to visualize the standards for confirmation of compound identity.

RESULTS

Elimination in Urine, Feces, and Carbon Dioxide. The excretion data of [^{35}S]P, [*S-methyl*-^{14}C]P and [*N-methyl*-^{14}C]P are given in Table I for urine, feces, and CO_2. Average recoveries of radioactivity for these three labels were 91–95%. Results in column 1 represent average excretion results of [^{35}S]P from three different experiments. Excretion results of three experiments were combined because recoveries of [^{35}S]P of naive animals (experiment 1) and of animals maintained on a diet of P (experiments 2 and 3) were similar. Total urinary excretions average 85 and 88% for [^{35}S]P and [*S-methyl*-^{14}C]P, respectively. The compound for both labels was excreted rapidly for the first 2 days with a tailing effect thereafter. Daily excretions fell to less than 1% after the third day, but detectable radioactivity (0.04% of dose) was found for up to 16 days. Fecal excretion represented 2–6% of the dosages. Carbon dioxide excretion with the [*S-methyl*-^{14}C]P label amounted to only 4% of the dose.

Total urinary excretion for [*N-methyl*-^{14}C]P studies averaged 56% of the dose while carbon dioxide excretion was 34% of the dose, indicating extensive N-demethylation of this compound.

Distribution of Residues in Tissues and Blood. On the third day following the dose of [^{35}S]P (experiment 3), male and female rats were killed, and 18 selected tissues plus plasma and red blood cells in these rats were analyzed for radioactivity. The results of these determinations are given in Table II. The amounts remaining in the animals by tissue distribution studies were 1–2% of the dose. Tissues from the females that contained greater than 0.1 ppm were the large intestine, the stomach, and the thyroid. All other tissues in the females had less than 0.07 ppm. For the males, the small and large intestines, liver, and kidney had larger values than the females. The majority of the tissues from males contained less than 0.1 ppm. The highest values as ppm in the whole organ tissue were found in the thyroid of both sexes.

The quantitative results obtained from silica gel column chromatography of urine from rats dosed with [^{35}S]P, [*S-methyl*-^{14}C]P, or [*N-methyl*-^{14}C]P are given in Table III as percent of the urine and as percent of the dose. The typical silica gel chromatogram of urine from rats treated with [^{35}S]P is shown in Figure 1.

Thin-layer chromatographic analyses showed that peak

Table II. Distribution of Radioactive Equivalents of Thiofanox in Tissues and Blood

	[^{35}S]P[a]			
	female		male	
tissue	% dose	ppm[b]	% dose	ppm
bone	0.004	0.04	0.003	0.03
brain	0.006	0.02	0.010	0.03
eye	0.001	0.03	0.0001	0.01
fat, back	0.002	0.01	0.003	0.01
fat, renal	0.002	0.02	0.002	0.01
heart	0.005	0.03	0.008	0.03
intestine, small	0.137	0.07	0.326	0.11
intestine, large	0.170	0.17	0.356	0.27
kidney	0.023	0.06	0.128	0.23
liver	0.069	0.02	0.215	0.06
lung	0.011	0.03	0.010	0.03
muscle	0.003	0.02	0.004	0.02
ovary	0.002	0.06		
spleen	0.005	0.03	0.005	0.02
stomach	0.083	0.16	0.075	0.11
testes			0.014	0.02
thyroid	0.02	0.66	0.001	0.28
carcass	0.58	0.04	0.840	0.04
plasma[c]	0.013	0.03	0.017	0.04
red blood cells[c]	0.022	0.03	0.038	0.04
total	1.14		2.06	

[a] Third day after dose of [^{35}S]P, three rats for each sex. [b] Parts per million. [c] ppm as mass/unit volume.

1 cochromatographed as O, the oxime of P. Peak 2 cochromatographed as a mixture of P and O_2, the oxime of P_2. The identity of peak 3 is unknown. Peak 3 was found with [^{35}S]- and [*S-methyl*-^{14}C]P but not with [*N-methyl*-^{14}C]P. Peak 4 cochromatographed as P_2 and excreted as a minor metabolite from rats dosed with all three labels. Peak 5 is postulated to be a hydroxylated P_2 because of the increased polarity (dielectric property) over P_2 as reflected in the silica gel system. The identity of peak 6 is unknown. Peak 7 is chromatographed as a O_1. With all three labels, 36–41% of the dose was excreted as P_1 (peak 8), a major metabolite. Total amounts of radioactivity found in peaks 9, 10, and 11 in [^{35}S]- and [*S-methyl*-^{14}C]P urine were 15% of the dose while these peaks in [*N-methyl*-^{14}C]P urine were 1% of the dose.

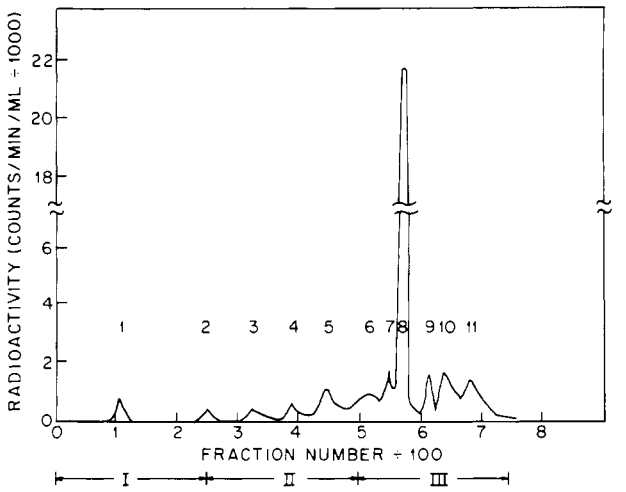

Figure 1. Silica gel chromatography of urinary metabolites of [^{35}S]thiofanox. Elution program: (I) isooctane–isopropyl ether; (II) isopropyl ether–acetonitrile; (III) acetonitrile–methanol. Metabolites are identified in Table III.

Knaak et al. (1965) utilized DEAE-cellulose chromatography to separate anionic from nonionic metabolites. This methodology was applied to the analysis of the urine obtained in this study. The data are given in Table IV for percent in the urine or percent of dose for the three labels. The quantity of peak A (nonionics) and peaks B and C (anionics) are in good agreement with percentages for, [^{35}S]P and [*S-methyl*-^{14}C]P. Peak D (anionics) is found only with [^{35}S]P.

DISCUSSION

The majority of a single dose of P with a ^{35}S- or *S*-methyl-^{14}C label is excreted in first-day urine with a small percentage (~4%) in the feces. Less than 1% of the dose with either label was excreted on the fourth day after dose. Studies with [^{35}S]P showed that feeding rats on a diet containing 100 ppm of P over a 24-day period had no effect on the urinary excretion. Studies utilizing [*N-methyl*-

Table III. Silica Gel Chromatographic Separation of First-Day Urine of Rats Dosed with [^{35}S]P, [*S-methyl*-^{14}C]P, or [*N-methyl*-^{14}C]P

			[^{35}S]P[a]		[*S-methyl*-^{14}C]P[b]		[*N-methyl*-^{14}C]P[b]	
fraction	peak no.	tentative identifn	% urine	% dose	% urine	% dose	% urine	% dose
90-130	1	O	2.3	1.6	2.3	1.7	0	0
231-290	2	P; O_2	0.7	0.5	0.6	0.4	0	0
291-340	3	unknown	1.1	0.8	0.9	0.6	0	0
376-415	4	P_2	2.4	1.7	1.4	1.0	0.6	0.2
416-480	5	unknown	6.4	4.6	3.9	2.9	1.0	0.5
481-540	6	unknown	8.9	6.4	10.0	7.3	5.2	2.4
541-555	7	O_1	4.4	3.1	2.2	1.6		
556-600	8	P_1	50.7	36.5	51.6	37.7	87.9	41.1
601-625	9	unknown	4.2	3.0	3.1	2.2	0	0
626-665	10	unknown	8.1	5.8	7.0	5.1	2.4	1.1
666-730	11	unknown	8.7	6.4	13.0	9.5	2.9	1.4

[a] Average of three male rats. [b] Average of four female rats.

Table IV. DEAE-cellulose Chromatography of First-Day Urine from Rats Dosed with P[a]

		[^{35}S]P		[*S-methyl*-^{14}C]P		[*N-methyl*-^{14}C]P	
fraction no.	peak	% urine	% dose	% urine	% dose	% urine	% dose
5-30	A	73.2	52.7	79.5	58.0	98.1	47.8
31-105	B	20.0	14.4	20.5	15.0	1.9	0.9
106-130	C						
330-410	D	6.8	4.9	0	0	0	0

[a] Average of three female rats.

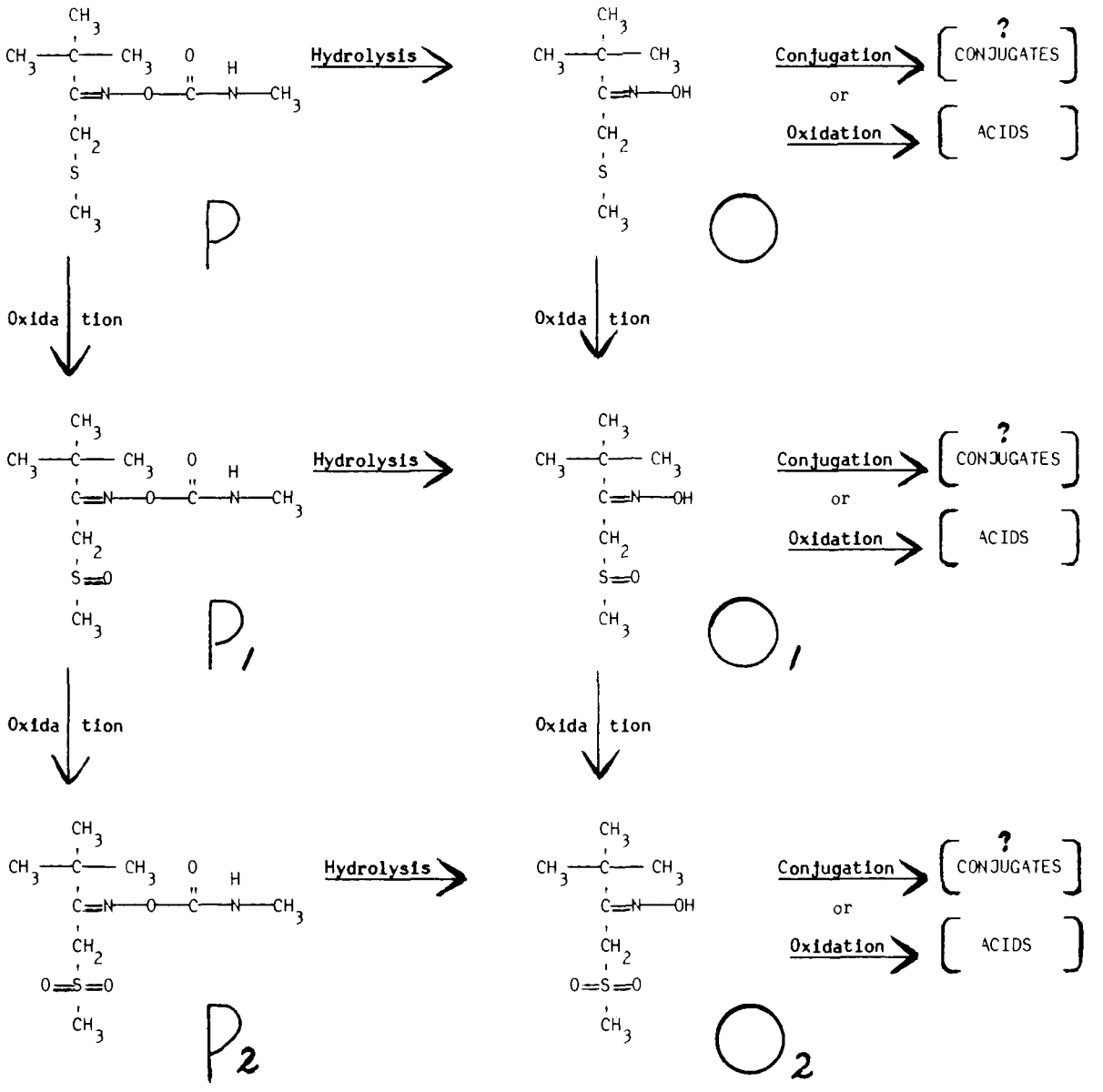

Figure 2. Metabolic pathway of thiofanox in the rat.

^{14}C]P demonstrated that significant N-demethylation (or hydrolysis) occurred with CO_2 evolution averaging 34% of the dose. The S-demethylation was a minor pathway for this compound because only 4% of [*S*-methyl-^{14}C]P was evolved as CO_2.

Tissue residue studies with [^{35}S]P showed that the radioactivity was distributed throughout the tissues studied. Expressed as ppm in each tissue, the highest values were found in the thyroid glands for both sexes. General tissue distribution of radioactivity, including fat, indicates partition of P to the metabolically active nonfatty organs and low body accumulation. Both male and female rats had similar amounts of P in the plasma, the red blood cells, the stomach, and the intestinal tract. As contrasted to females, the males showed a higher percentage of the dose in kidneys and liver and a slightly lower percentage in most other tissues. This may be an indication of a sex difference in response between male and female rats.

The metabolic pathway of P in the rat as determined by chromatographic methods is depicted in Figure 2. The probable pathways are given as open structures with possible pathways in brackets. Silica gel column chromatography resulted in the best overall analytical scheme for quantitation. The results for the first-day urine indicated that the major degradation pathway is oxidation of P to P_1 and excretion in the urine. Results from these metabolic studies on thiofanox are in general agreement with those found by Knaak et al. (1966) for aldicarb, 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, in the rat. Also, some further oxidation of P_1 to P_2 and its hydrolysis product O_2 takes place. The unknown anionic products probably are conjugates of O, O_1, or O_2 based on the chromatographic elution characteristics of DEAE-cellulose chromatography. They are not conjugates of intact carbamates because significant amounts of peaks B and C were found with [^{35}S]P and [*S-methyl*-^{14}C]P but only a small percentage was found as peaks B and C with [*N-methyl*-^{14}C]P. The anionic components of the urine are possibly conjugates of O_2 as reported for methomyl, *S*-methyl *N*-[(methylcarbamoyl)-oxy]thioacetimidate (Knaak, 1971).

LITERATURE CITED

Chin, W. T.; Duane, W. C.; Meeks, R. L.; Stallard, D. E. *J. Agric. Food Chem.* **1976**, *24*, 1001.

Duane, W. C. "The Metabolism of DS-15647 in Soils", Technical Bulletin, Diamond Shamrock Corp., Cleveland, OH, 1974.

Knaak, J. B. *Bull. W.H.O.* **1971**, *44*, 121–131.
Knaak, J. B.; Tallant, M. J.; Bartley, W. J.; Sullivan, L. J. *J. Agric. Food Chem.* **1965**, *13*, 537–543.
Knaak, J. B.; Tallant, M. J.; Sullivan, L. J. *J. Agric. Food Chem.* **1966**, *14*, 573–578.
Sullivan, L. J.; Eldridge, J. M.; Knaak, J. B.; Tallant, M. J. *J. Agric. Food Chem.* **1972**, *20*, 980–985.
Whitten, C. J.; Bull, D. L. *J. Agric. Food Chem.* **1974**, *22*, 234.

Received for review March 17, 1980. Accepted August 26, 1980.

Metabolism of the Herbicide Buthidazole in Corn Seedlings and Alfalfa Plants

Ching C. Yu, Yousef H. Atallah,* and David M. Whitacre

[^{14}C]Buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-^{14}C-yl]-4-hydroxy-1-methyl-2-imidazolidinone] was absorbed from nutrient solution and translocated by corn seedlings. Most of the radiocarbon in seedlings could be extracted by ethanol. Unextracted radiocarbon gradually increased to ~19% at day 25; however, acid hydrolysis released almost all of the conjugated metabolites in solids. Buthidazole was slowly but steadily metabolized. Unchanged buthidazole constituted 96% of the total radiocarbon at day 1, decreasing to 62% at day 25. The major degradation pathways were conjugation, hydroxylation, and N-demethylation. Alfalfa plants translocated and metabolized buthidazole more quickly and extensively than did corn seedlings. Unchanged buthidazole constituted 15% of total radiocarbon in alfalfa plants after 2 days but only 4% after 16 days. The major degradation pathway in alfalfa was hydroxylation of the imidazolidinone ring, followed by ring opening and subsequent N-demethylation and hydrolysis.

The herbicide buthidazole, code name VEL-5026, has shown promise for industrial vegetation control and for control of broadleaf and grassy weeds in tolerant crops. The mode of action, basis of selectivity, and metabolic fate of buthidazole have been reported for several crop and weed species (Hatzios, 1979; York, 1979; Hilton and Nomura, 1979). Although only gradually metabolized by tolerant crops, buthidazole was rapidly metabolized and eliminated by lactating cows and laying hens (Atallah et al., 1980) and small mammals (Yu and Atallah, 1976). The study reported here was undertaken to determine the uptake, translocation, and metabolism of buthidazole in corn seedlings (*Zea mays* L.) and alfalfa plants (*Medicago sativa* L.)—both of which have tolerance to this herbicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-^{14}C-yl]-4-hydroxy-1-methyl-2-imidazolidinone] was synthesized by Velsicol Chemical Corp., Chicago, IL, and had a specific activity of 10.25 mCi/mmol and a radiochemical purity of greater than 98% by thin-layer chromatography. Buthidazole and seven model metabolites (Table I) were also synthesized by Velsicol; each was greater than 90% pure.

Treatment of Plants. Pioneer corn seeds (Minnesota 4201) were planted in vermiculite, nurtured with 50% Hoagland nutrient solution 1 (Hoagland and Arnon, 1938), and maintained under Gro-Lux lamps at 25 °C. Hoagland nutrient solution 1 consists of the following: potassium acid phosphate, 1 mM; potassium nitrate, 5 mM; calcium nitrate, 5 mM; magnesium sulfate, 2 mM; boron, 0.5 ppm; manganese, 0.5 ppm; zinc, 0.05 ppm; copper, 0.02 ppm; molybdenum, 0.05 ppm; iron, 1 ppm. After 8 days, corn seedlings (12–15 cm tall) were carefully separated from vermiculite under water and were individually placed in 24-mL glass scintillation vials each containing 1.1 ppm of [^{14}C]buthidazole in 10 mL of 50% Hoagland solution 1 (total of 9.3×10^5 dpm). Twenty-one corn seedlings were exposed for 24 h in this manner and then were transferred individually to fresh vials containing 20 mL of 50% Hoagland solution. The day of transfer to buthidazole-free solution was referred to as day 0; three plants were harvested for analysis at days 0, 1, 2, 4, 8, 14, and 25.

Additional corn seedlings were treated to generate sufficient quantitites of metabolites for mass spectrometric analysis. Routinely, 10 seedlings were placed individually in vials containing 20 mL of 50% Hoagland solution with 50 ppm of [^{14}C]buthidazole (7.6×10^5 dpm). In one case, five seedlings were placed individually in vials containing 20 mL of Hoagland solution 1 fortified with 200 ppm of buthidazole; seedlings thus treated were exposed for 3 days and then transferred to buthidazole-free 50% Hoagland solution for 20 days. Seedlings from each test were composited prior to analysis.

In another test designed to characterize unextractable radiocarbon in plant solids, six corn seedlings were placed individually in vials containing 20 mL of 50% Hoagland solution with 1.7 ppm of [^{14}C]buthidazole (3×10^6 dpm). The seedlings were held in the radiocarbon solution continuously for 21 days and then combined as a composite for analysis.

Established alfalfa sets (Funk's F-261, 34% California, 33% Washington, and 33% Idaho) in their second year of growth were obtained from Velsicol Agriculture Research Center, Woodstock, IL, in November. Alfalfa shoots were cut at the soil surface and were individually placed in vials containing 20 mL of full-strength Hoagland solution fortified with 0.8 ppm of [^{14}C]buthidazole (1.4×10^6 dpm). The plants were maintained continuously in the radiocarbon solution under Gro-Lux lamps at 25 °C until harvested in duplicate at days 2, 5, 6, 9, 12, and 16.

Thin-Layer Chromatography (TLC). Precoated silica gel G TLC chromatoplates (Macherey-Nagle and Co., distributed by Brinkmann Instruments, Inc.) were used for the separation of metabolites. Both one-dimensional

Research and Development Department, Velsicol Chemical Corporation, Chicago, Illinois 60611.

0021-8561/80/1428-1090$01.00/0