Metabolic Fate of Abate Insecticide in the Rat

Roger C. Blinn

The fate of tritium-labeled Abate insecticide, *O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate, was determined when administered orally in sesame oil to rats. Fecal and urinary routes were the principal means of elimination over a 48- to 72-hour period. Gross radioactivity levels in the blood peaked between 5 and 8 hours after ingestion and dissipated with a half life of approximately 10 hours. Appreciable residual levels of radioactivity were found only in the gastrointestinal tract and fatty tissue. In feces and in fatty tissue the radioactivity was due principally to unchanged Abate insecticide, with minor amounts of its sulfoxide derivative also present. While traces of Abate were found in urine, the principal urinary metabolic products were sulfate ester conjugates of the phenolic hydrolysis products 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol, and 4,4'-sulfonyldiphenol.

Abate insecticide, the active ingredient of which is the *O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene ester of phosphorothioic acid (also referred to as Compound CL 52,160), is registered for the control of mosquito and midge larvae. The low toxicity of Abate to animals (Gaines *et al.*, 1967) and to humans (Laws *et al.*, 1967) and the low concentrations required in water to control mosquito and midge larvae (Bowman and Orloski, 1966) suggest the possibility of its use in potable water.

In a study of the metabolic fate of Abate on bean leaves (Blinn, 1968), it was found that these residues were rather stable chemically and consisted principally of unchanged Abate. The major degradation product found was the sulfoxide derivative, with minor amounts of the sulfone derivative, its unsymmetrical mono-oxono analog, and the symmetrical dioxono analog of Abate. Glucosidic conjugates of the phenolic hydrolysis products, 4,4'-thiodiphenol, 4,4'-sulfinyldiphenol, and 4,4'-sulfonyldiphenol, were increasingly evident with time.

To aid evaluation of the toxicological significance of these residues, the metabolic fate of tritium-labeled Abate in a representative mammal, the rat, was studied. Because the absence of a gall bladder in the rat made the collection of bile inconvenient, a limited study was conducted with guinea pigs to evaluate the role of biliary excretion in the elimination of Abate and its metabolites.

EXPERIMENTAL RESULTS AND DISCUSSION

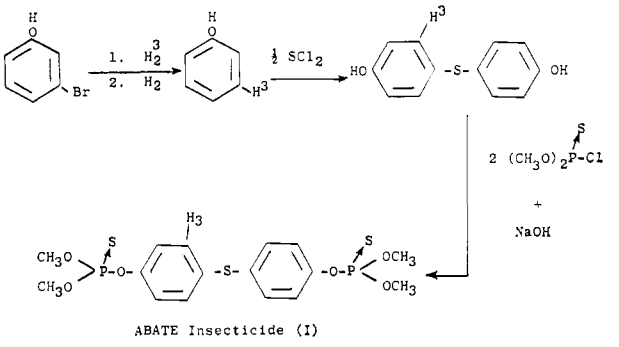
Radiotracer. *O,O,O',O'*-Tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate (Compound I) was prepared with the tritium label as shown in Figure 1 (Wagner, 1966) and used throughout this study. This preparation had a specific activity of 288 mc. per mmole and its radiopurity was established (Blinn, 1968).

Standard Separation, Counting, and Identification Procedures. Standard procedures for sample combustion, liquid scintillation counting, thin-layer chromatography, multiple internal reflectance spectroscopy, autoradiography, and scanning of developed chromatograms have been described (Blinn, 1968). Aliquots of urine were used directly for liquid scintillation counting; frozen tissue or feces were chopped finely and extracted several times with methanol, and aliquots of the methanolic extract were subjected to liquid scintillation counting. The radioactive content of the dried marc from the extraction procedure was determined by Schöniger flask combustion.

Metabolic Study Using Rats. TREATMENT OF ANIMALS. Elimination and Tissue Residue Studies. Male Sprague-Dawley rats weighing 150 to 200 grams were given, by gavage, dosages of tritium-labeled Compound I dissolved in sesame oil (Table I). The animals were immediately placed in stainless steel metabolism cages (Acme Metal Products, Inc.). Liberal amounts of rat chow and drinking water were provided. Urine and feces were separated and collected at intervals. A dry ice–chilled tube was used as the collection reservoir for the urine to minimize chemical degradation of the radiolabeled metabolic products. At the end of the test period, the rats were sacrificed by carbon dioxide asphyxiation. Liver, kidney, stomach, intestines, muscle, and fat were removed. All urine, feces, and tissue were stored at −20° F. until processed for radioactivity assay or for extraction.

Blood Studies. Separate studies were designed to elucidate the role of blood in the metabolic scheme. Two of these studies involved sacrificing groups of dosed rats at intervals and collecting the blood by heart puncture. The other study, designed to eliminate individual variation, was conducted on a single rat, collecting 0.4-ml. samples of blood from the tail vein at appropriate intervals.

Aliquots of fresh blood were smeared on combustion paper and, after drying, assayed for radioactivity by com-



ABATE Insecticide (I)

Figure 1. Synthetic route for tritium-labeled Abate

Metabolism Laboratory, Agricultural Division, American Cyanamid Co., Princeton, N. J. 08540

bustion. The remaining sample was then used immediately for further evaluation.

Extraction Procedures. Frozen tissues and feces were chopped finely and extracted repeatedly with a large excess of methanol in a blender. Fresh blood was extracted successively with several volumes each of acetonitrile, acetone, ethyl ether, and chloroform. Urine was acidified to about pH 1 and extracted with several equal volumes of chloroform and then with ethyl ether. After gross radioactivity was determined in each extract, the solvent was removed by evaporation under vacuum at 40° C. in a rotary evaporator and the residues were partitioned between equal volumes of chloroform and water.

Hydrolysis Procedures. Acid Hydrolysis. Aliquots of the water-soluble radioactive extracts were hydrolyzed in a reducing environment by heating with an equal volume of 3*N* hydrochloric acid solution for an hour on a steam bath. The reducing atmosphere was provided by adding about 0.5 gram of granular tin to the acid solution, and was used to minimize air oxidation of phenolic hydrolysis products. After being heated, hydrolyzate solutions were cooled, filtered, and extracted four times each with equal volumes of chloroform and ethyl ether. The combined extracts were evaluated for radioactivity and then subjected to various chromatographic procedures.

Enzymatic Hydrolysis. To indicate the nature of the water-soluble radioactive compounds, aliquots were incubated with enzyme preparations at 37° C. for 72 hours in pH 5.0 acetate buffer solution. The incubates were then extracted with chloroform and ethyl ether as described for acid hydrolysis. The enzyme preparations used were bovine liver glucuronidase (Calbiochem β-grade), mollusk β-glucuronidase (Calbiochem β-grade), and limpet phenol sulfatase (Sigma Type II). The last two enzyme preparations possessed both glucuronidase and sulfatase activity. To achieve selective glucuronidase activity, enzymatic incubations were performed in the presence of 0.1*M* phosphate solution; to achieve selective sulfatase activity, the enzyme solutions were temporarily lowered to pH 2.2 prior to incubation at pH 5.0 (Sigma Chemical Co., 1967).

Metabolic Study Using Guinea Pigs. Male English shorthaired guinea pigs weighing 350 to 400 grams were given tritium-labeled Compound I by gavage (Table I). Each group of three animals was immediately placed in a large stainless steel metabolism cage and supplied with liberal amounts of guinea pig chow and drinking water. At intervals the three guinea pigs of a group were sacrified; blood was removed by heart puncture and bile removed from the excised gall bladders. Total radioactivity content of the water-diluted biliary fluid was determined by direct liquid scintillation counting, while that of blood, urine, and feces was determined as described for rats. Aliquots of 0.1 ml. of blood and bile were shaken thoroughly with 10 ml. of hexane to determine extractable radioactivity.

RESULTS AND DISCUSSION

Rate of Elimination of Radioactivity. Table I demonstrates the ability of the rat to eliminate in the urine and feces all but traces of radioactivity resulting from dosage with tritium-labeled Compound I. Increasing the dosage rate did not significantly change the elimination pattern. However, the amount of sesame oil used to administer the

Table I. Elimination Pattern of Tritium-Labeled Abate from Rats and Guinea Pigs

No. of Animals	Dosage μg./animal	Dosage Mg./kg.	Sesame Oil, Ml./Animal	Hours	4 Urine	4 Feces	7 Urine	7 Feces	24 Urine	24 Feces	31 Urine	31 Feces	48 Urine	48 Feces	54 Urine	54 Feces	72 Urine	72 Feces	96 Urine	Total Urine	Total Feces	Total Urine + feces
												Per Cent of Dosage										
												RAT										
1	970	5.0	0.5				6.3	15.6	21.1	32.3	2.9	5.0	2.8	12.0	...	...	...	...	...	33.1	64.9	98.0
1	1,357	6.2	0.9				6.1	...[a]	16.0	15.5	12.1	13.0	6.5	20.5	0.6	1.5	1.6	1.8	...	42.9	52.3	95.2
3	3,164	15.8	0.8				3.2	...	24.6	...[b]	8.3	...[b]	6.9	...[b]	1.4	...	2.1	...	...	46.5	35.6[b]	...
4	9,168	45.8	0.4				...	...	28.5	...	...	...	5.2	...	...	...	2.3	...	1.0[c]	37.0	...	...
												GUINEA PIG[d]										
9	11,888	31.9	0.4		0.1	Trace	1.5	6.0	5.2	62.9	...	...	...	...	...	...	...	...	...	6.7	68.9	75.6

[a] Not collected until 24 hours.
[b] 24-, 31-, and 48-hour feces combined and counted. Other samples not evaluated for radioactivity.
[c] Only urine collected and counted.
[d] Each value a composite from three animals. Feces and urine collected for only specified period. No attempt made to complete elimination study or to determine radioactivity in gastrointestinal tract or other tissues.

dosage appeared to affect the urine-feces ratio, probably by increasing the absorption of Compound I from the digestive tract. The total amount of radioactivity eliminated was over 95%, regardless of the amount of sesame oil used.

Table I also shows the initial rate of radioactivity elimination by guinea pigs, which have a much higher rate of fecal elimination of radioactivity than rats. This suggests that Compound I is less effectively absorbed in the guinea pig digestive system.

Distribution of Radioactivity in Tissues, Blood, and Bile. The residual radioactivity found in the various rat tissues resulting from the treatment with tritium-labeled Compound I is presented in Table II. Only fatty tissues and the alimentary system showed appreciable residue levels. Since fecal elimination of radioactivity after 72 hours was demonstrable (Table I), it is not surprising that residual amounts of the unassimilated radiolabeled dosage were found in the stomach and the gastrointestinal tract.

The radioactivity concentration levels in the rats' blood increased rapidly for 5 to 8 hours after treatment, then declined with an average half life of 10 hours (Figure 2). The curve resulting from the withdrawal of blood from the tail vein of a single animal is in good agreement with those resulting from the sacrifice of several animals at each interval.

A comparison of the blood values for the guinea pig and the rat (Figure 2) clearly demonstrates the much lower concentrations found for the guinea pig. The high radioactivity level in the bile suggests that the biliary metabolic route may be a significant factor in the total guinea pig metabolic scheme (Table III).

Nature and Identity of Radioactive Compounds. BLOOD AND BILE. Thin-layer chromatography of the chloroform-soluble portion of extractable radioactivity from rat blood showed the presence of only one compound, identified by cochromatography as Compound I. This extractable radioactivity amounted to about 24% of the total blood radioactivity 2 hours after treatment and dropped to about 7% after 48 hours. In contrast, Compound I in guinea pig blood declined from about 12% at 4 hours posttreatment to 1.4% at 24 hours.

Both acid and enzymatic hydrolysis of the water-soluble radioactive materials in rat blood resulted in the appearance of 4,4′-thiodiphenol (III) and 4,4′-sulfinyldiphenol (IV). The proportion of III in blood rose from about 7% at 2 hours posttreatment to about 20% at 8 hours, and remained at this percentage for the remaining 40 hours of the study. The proportion of IV rose from about 20% at 2 hours posttreatment to about 40% at 5 hours and then declined to about 10% at 48 hours. The remaining very polar water-soluble radioactive materials in blood could not be identified because of the complexity of the mixture.

Less than 1% of the radioactivity in the biliary fluid was extractable into hexane and was not investigated further.

FATTY TISSUE. All of the radioactivity extracted from fatty tissue partitioned into chloroform. Two-dimensional silica gel thin-layer chromatography of the extract, after diminishing co-extracted fats by partitioning from hexane into methanol-water (87.5 to 12.5) and treating with Nuchar C-190-N decolorizing charcoal, yielded only two radioactive compounds. These were identified by thin-layer cochromatography as Compound I and its sulfoxide derivative (II).

FECES. Approximately 93% of the radioactivity in the feces was extractable into methanol, and about 87% of this radioactivity partitioned into chloroform. Silica gel column chromatography of this extract, using 150-ml. portions of 0, 2.5, 5, 10, 15, 20, and 25% methanol in methylene chloride, yielded five radioactive peaks. Three

Table II. Radioactivity Residues in Tissues (P.P.M.[a]) Resulting from Treatment of Rat with Tritium-Labeled Abate

Tissue	Dosage and Hours after Treatment: 970 μg./rat, 48 hr.	1357 μg./rat, 72 hr.
Fat	1.25	1.75
Intestines	...	0.51
Kidneys	0.07	0.09
Liver	0.17	0.19
Muscle	0.03	0.02
Stomach	0.04	0.24

[a] Calculated as Abate insecticide.

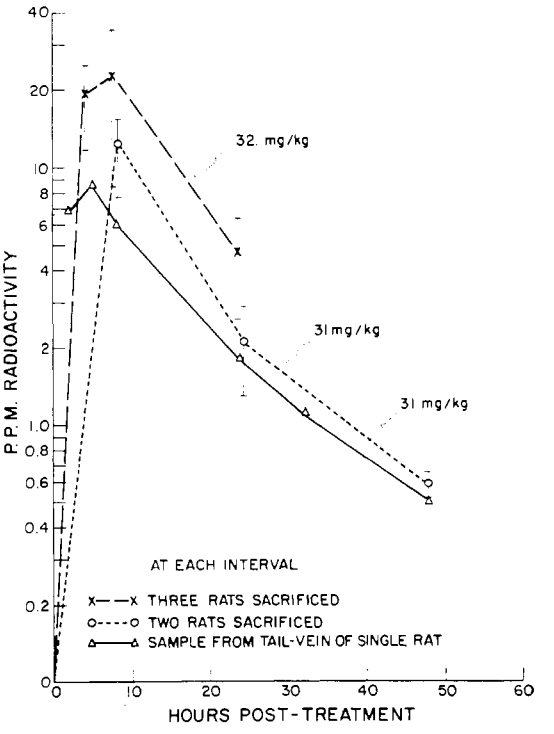


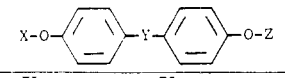
Figure 2. Concentrations of radioactivity found in blood of rats treated with tritium-labeled Abate

Table III. Radioactivity Levels in Blood and Bile from Guinea Pigs Treated with Tritium-Labeled Abate

Hours Posttreatment	Radioactivity, P.P.M.[a]: Blood	Bile	Ratio[b]
4	0.21	21.6	103
8	0.66	59.5	90
24	0.23	10.5	46

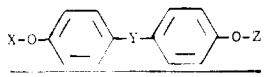
[a] Calculated as Abate insecticide.
[b] Bile/blood ratio.

Table IV. Relative Amounts of Radioactive Compounds in Rat Feces



X	Y	Z	Compound No.	Hours after Dosage 24	31	48	54	72	Total
				Per Cent of Dosage					
$(CH_3O)_2$-P(S)	S	$(CH_3O)_2$-P(S)	I	12.8	8.9	8.2	0.4	0.2	30.5
$(CH_3O)_2$-P(S)	SO	$(CH_3O)_2$-P	II	1.6	1.8	6.1	0.5	0.7	10.7
H	S	H	III	0.5	0.9	3.1	0.3	0.5	5.3
H	SO_2	H	V						
H	SO	H	IV	0.6	1.4	3.2	0.3	0.4	5.9
			Total	15.5	13.0	20.6	1.5	1.8	52.4

Table V. Relative Amounts of Radioactive Materials in Composite Rat Urine Samples



X	Y	Z	Compound No.	Per Cent of Radioactivity in Composite Sample: Sample A[a]	Sample B[b]
H	S	H	III	82	67
H	SO	H	IV	6	10
H	SO_2	H	V	2	2
Unknown compounds				10	21
			Total	100	100

[a] Composition of Sample A: 7 hr., 34%; 24 hr., 26%; 48 hr., 40%. This composite represented 8.8% of dosage given rat.
[b] Composition of Sample B: 31 hr., 49%; 48 hr., 51%. This composite represented 6.6% of dosage given rat.

Table VI. Percentage of Extractable Radioactivity after Enzymatic Incubation of Rat Urine Conjugates

Enzyme	Inhibition of Glucuronidase Activity	Indication of Reactivity: Gluc.[a]	Sulf.[b]	% Extracted[c]
None	No	Neg.	Neg.	15.6
Sulfatase				
Limpet	No	Pos.	Pos.	89.1
	Yes	Neg.	Pos.	83.1
Glucuronidase				
Bovine liver	No	Pos.	Slight	29.3
Mollusk	No	Pos.	Pos.	94.5

[a] Glucuronidase activity inhibited by temporarily adjusting pH to 2.2. Reactivity indicator 0.1*M* phenolphthalein β-glucuronide.
[b] Reactivity indicator 0.1*M* phenolphthalein sulfate.
[c] Combined chloroform and ethyl ether extract from incubate.

were identified by comparative infrared characterization as Compound I, its sulfoxide derivative (II), and 4,4′-sulfinyldiphenol (IV). The remaining peaks were a mixture of compounds, only two of which were identified as 4,4′-thiodiphenol (III) and 4,4′-sulfonyldiphenol (V). At least five unidentified radioactive compounds were separated, but not elucidated further because of their occurrence in such minor concentrations that sufficient material could not be isolated for further characterization.

The relative amounts of the various radioactive compounds found in feces varied with the exposure period after treatment (Table IV). The relative amounts of the unknown materials were not determined because of the complexity of the mixture and the small quantities of each present.

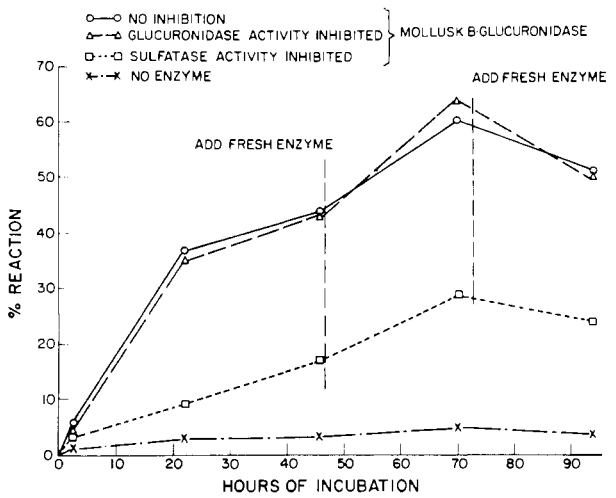


Figure 3. Rate of enzymatic hydrolysis of urinary conjugates in fresh urine from rats treated with tritium-labeled Abate

URINE. Approximately 17% of the urinary radioactivity was extractable from acidified unhydrolyzed urine into chloroform and ethyl ether. Four compounds representing 67% of this radioactivity were identified as Compound I, 4,4′-thiodiphenol (III), 4,4′-sulfinyldiphenol (IV), and 4,4′-sulfonyldiphenol (V).

Columnar silica gel chromatography of the 2.5% methanol-acetone extract of lyophilized urine, using 250-ml. portions of 2.5, 5, 10, and 25% methanol in acetone, resulted in three radioactive peaks. Two of these peaks, representing 74% of the urinary radioactivity, were each separated by thin-layer chromatography into at least five ill-defined radioactive zones. The third peak was not investigated further.

Acid hydrolysis of urine, however, resulted in the extractability of about 80% of the urinary radioactivity into chloroform and ether. Silica gel column chromatography of this extract, using 90-ml. portions of 2.5, 5, 10, 15, and 20% methanol in methylene chloride, yielded four radioactive peaks. The peak comprising 75% of the extracted urinary radioactivity, after two silica gel column chromatographic isolations, was identified by comparative infrared characterization as 4,4′-thiodiphenol (III). Isolation by column chromatography was necessitated by the instability of III on either silica gel or alumina thin-layer plates,

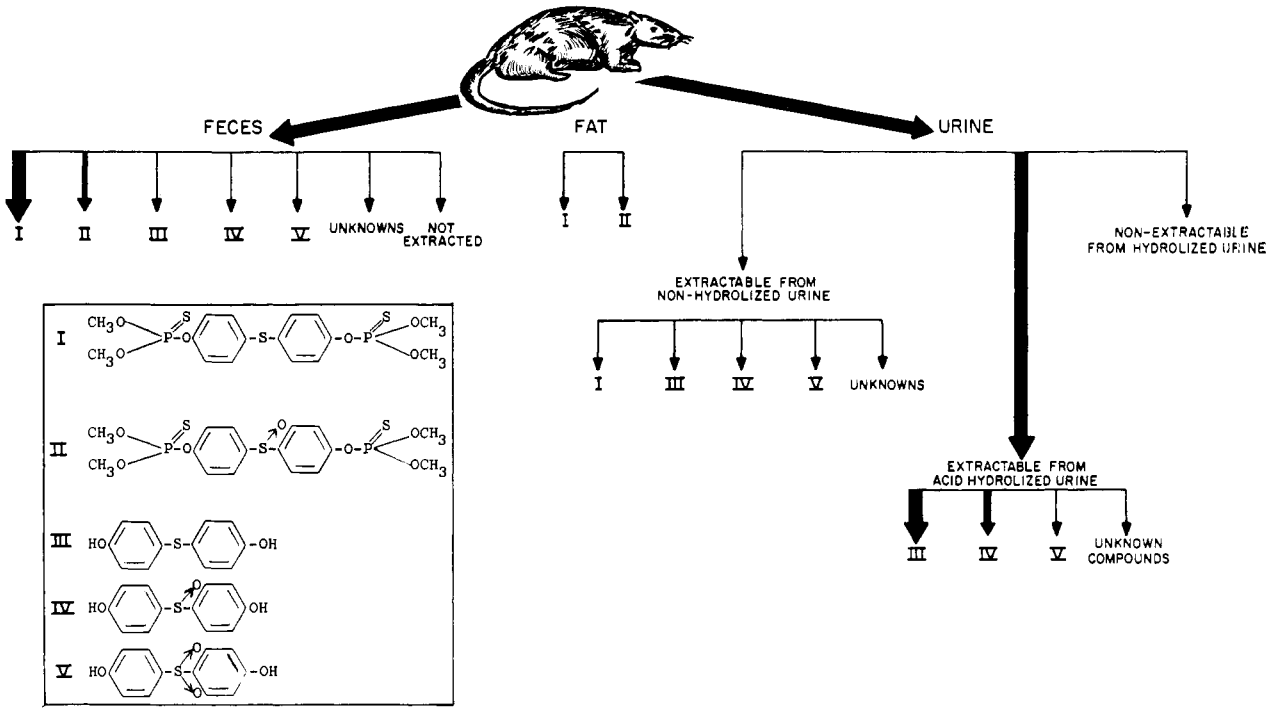


Figure 4. Distribution and metabolic pattern for Abate in the rat

as evidenced by the appearance of yellow coloration on standing, by serious losses of III, and by the appearance of other materials upon elution and rechromatography.

A second chromatographic peak from hydrolyzed urine, comprising about 8% of the extracted urinary radioactivity, was identified by comparative infrared characterization as 4,4′-sulfinyldiphenol (IV). A third peak comprising about 2% of the extracted urinary radioactivity was identified as 4,4′-sulfonyldiphenol (V). The remaining 11% of the extractable radioactivity from hydrolyzed urine was composed of at least three compounds which were not identified.

No extensive study was made to determine the relative amounts of the various phenolic compounds resulting from acid hydrolysis of urine samples collected at various time intervals posttreatment; however, the composite values listed in Table V are included as illustrative of the variability encountered.

The nature of the conjugates found in urine was indicated by incubation with the various glucuronidase and sulfatase preparations described in the experimental section. The results (Table VI) of these enzyme reactions with the previously described chromatographed conjugates clearly show that hydrolytic reactivity is associated with sulfatase activity, since inhibition of the glucuronidase activity had little effect on hydrolysis. The rate of enzymatic hydrolysis of urinary conjugates in fresh urine is presented in Figure 3. The enzyme used was mollusk β-glucuronidase which had either its glucuronidase or its sulfatase activity selectively inhibited. These data demonstrate that inhibition of sulfatase activity severely decreases the rate of conjugate hydrolysis, strongly suggesting that the conjugates are sulfate esters of the hydrolysis products of Compound I and its oxidation products. The data in Figure 3 also indicate the presence in rat urine of natural inhibitors of the enzymatic reaction and the instability of the released phenols to the long-term incubation conditions. These factors must be considered when interpreting data from enzymatic incubation of urine.

CONCLUSIONS

The distribution and metabolic pattern for tritium-labeled Abate insecticide in a rat biosystem are represented in Figure 4. Unchanged Abate (I) constitutes the principal material found in fecal matter, which undoubtedly has not been assimilated during passage through the gastrointestinal tract. Other elimination products found in feces include the sulfoxide derivative (II) and phenolic hydrolysis products. While traces of Abate (I) are found in urine, sulfate ester conjugates of phenolic hydrolysis products comprise the bulk of the urinary elimination products. The presence or absence of the postulated desmethyl Abate in urine could not be established, but it might be among the very polar unidentified compounds found on thin-layer chromatograms. Over 80% of the radioactivity administered to the rat has been identified, and the 13% of extractable unidentified radioactivity is distributed among 10 or more components.

LITERATURE CITED

Blinn, R. C., J. Agr. Food Chem. **16,** 441 (1968).
Bowman, J. S., Orloski, E. J., *Mosquito News* **25,** 557 (1966).
Gaines, T. B., Kimbrough, R., Laws, E. R., Jr., *Arch. Environ. Health* **14,** 283 (1967).
Laws, E. R., Jr., Morales, F. R., Hayes, W. J., Jr., Joseph, C. R., *Arch. Environ. Health* **14,** 289 (1967).
Sigma Chemical Co., St. Louis, Mo., "Sigma Price List," March 1967.
Wagner, F. A., Jr., American Cyanamid Co., Princeton, N. J., unpublished data, 1966.

Received for review July 2, 1968. Accepted October 3, 1968.