Iwata, Y.; Düsch, M.; Westlake, W. E.; Gunther, F. A. *Bull. Environ. Contam. Toxicol.* **1975**, *14*, 49.
Kilgore, W. W.; Li, M.; Mull, R.; Winterlin, W.; Borhani, N.; Kraus, J.; Kurtz, P. M.; Akesson, N.; Yates, W. Food Protection and Toxicology Center, University of California, Davis, CA, June 1977, preliminary report.
Knaak, J. B.; Peoples, S. A.; Jackson, T. V.; Fredrickson, A. S.; Enos, R.; Maddy, K. T.; Bailey, J. B.; Düsch, M. E.; Gunther, F. A.; Winterlin, W. L. *Arch. Environ. Contam. Toxicol.* **1978**, *7*, 465.
Maddy, K. T., California Department Food and Agriculture, personal communication, 1977.
Popendorf, W. J.; Leffingwell, J. T. *J. Agric. Food Chem.* **1978**, *26*, 437.
Spear, R. C.; Lee, Y.; Leffingwell, J. T.; Jenkins, D. *J. Agric. Food Chem.* **1978**, *26*, 434.
Spear, R. C.; Popendorf, W. J.; Leffingwell, J. T.; Milby, T. H.; Davies, J. E.; Spencer, W. J. *JOC, J. Occup. Med.* **1977a**, *19*, 406.
Spear, R. C.; Popendorf, W. J.; Spencer, W. F.; Milby, T. H. *JOC, J. Occup. Med.* **1977b**, *19*, 411.
Spencer, W. F.; Cliath, M. M.; Davis, K. R.; Spear, R. C.; Popendorf, W. J. *Bull. Environ. Contam. Toxicol.* **1975**, *14*, 265.
Spencer, W. F.; Iwata, Y.; Kilgore, W. W.; Knaak, J. B. *Bull. Environ. Contam. Toxicol.* **1977**, *15*, 656.
Winterlin, W.; Kilgore, W.; Mourer, C.; Mull, R.; Walker, G.; Knaak, J.; Maddy, K. *Bull. Environ. Contam. Toxicol.* **1978**, *20*, 255.

Received for review March 18, 1980. Accepted August 14, 1980. This research was supported in part by the National Institute of Environmental Health Sciences, Grant No. ES00534.

Metabolism of Deethylatrazine, Deisopropylatrazine, and Hydroxyatrazine by the Soluble Fraction (105000*g*) from Goose Liver Homogenates

Thomas S. Foster,* Shahamat U. Khan, and M. Humayoun Akhtar

Incubation of deethylatrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine] and deisopropylatrazine [2-chloro-4-(ethylamino)-6-amino-*s*-triazine] with the soluble fraction (105000*g*) from goose liver homogenates resulted in the formation of the corresponding hydroxy analogues. No dealkylation of hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] occurred when it was incubated with the enzyme preparation. The data suggest that, in the metabolism of atrazine by the soluble fraction from liver homogenates, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues rather than by partial N-dealkylation of hydroxyatrazine.

The metabolism of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] has been studied extensively in these laboratories. Residues of unchanged atrazine and some hydroxy and dealkylated metabolites were found to be present in excreta and various body tissues after chickens had been fed an atrazine-fortified ration (Foster and Khan, 1976; Khan and Foster, 1976). Partial N-dealkylation and hydrolysis were proposed as major pathways for atrazine metabolism in the chicken (Foster and Khan, 1976; Khan and Foster, 1976). Further investigations showed that the soluble fraction (105000*g*) from chicken liver homogenates contains a heat-labile, glutathione-dependent enzyme(s), which metabolizes atrazine in in vitro incubations (Foster et al., 1979). This was accomplished by conjugation with glutathione and subsequent hydrolysis with partial N-dealkylation to the hydroxy and dealkylated analogues. The major metabolic pathway was shown to proceed via enzymatic hydrolysis. Similar studies with enzyme preparations from goose, pig, and sheep liver homogenates indicated that in vitro metabolism of a mixture of atrazine and simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] proceeded via partial N-dealkylation accompanied by hydrolysis (Khan et al., 1979). However, hydrolysis to the corresponding hydroxy analogues appeared to be slower than partial N-dealkylation.

The present study is a continuation of these investigations concerning metabolism of *s*-triazines by an enzyme preparation from liver homogenates. It was of special interest to ascertain whether, in the in vitro metabolism of atrazine by the enzyme preparation, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues or by the partial N-dealkylation of hydroxyatrazine.

Three atrazine metabolites, namely, deethylatrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine], deisopropylatrazine [2-chloro-4-(ethylamino)-6-amino-*s*-triazine], and hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], were incubated with the soluble fraction (105000*g*) from goose liver homogenates. Reaction products were extracted and identified by gas chromatography (GC).

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada) and used as received. Reference standards of deethylatrazine, deisopropylatrazine, and hydroxyatrazine were gifts from Ciba Geigy Ltd., Switzerland.

Stock solutions of deethylatrazine (195.9 μg/mL) and deisopropylatrazine (202.0 μg/mL) in acetone and hydroxyatrazine (41.2 μg/mL) in methanol were stored in the dark at room temperature. Gas chromatographic analyses of these solutions under the conditions described below indicated a single peak in each case. All other chemicals were analytical grade or the purest grade available.

Enzyme Preparation. Livers were obtained from male Chinese geese after they had been killed by cervical dislocation. The soluble fraction (105000*g*) was prepared as described previously (Khan et al., 1979) and used as the enzyme preparation. It was prepared fresh on the day each series of incubations was carried out.

In Vitro Incubations. Incubations were carried out in 50-mL glass-stoppered Erlenmeyer flasks. A typical incubation mixture contained enzyme preparation (4.5

Animal Research Institute (T.S.F. and M.H.A.) and Chemistry and Biology Research Institute (S.U.K.), Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6.

0021-8561/80/1428-1083$01.00/0

Table I. Common Names, Chemical Names, Retention Times, and Thermionic Response of s-Triazine and Metabolites

common name	chemical name	retention time,[a] min	1/2 fsd,[b] ng
hydroxyatrazine	2-hydroxy-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine		
atratone[c]	2-methoxy-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine	4.2	10.0
atrazine	2-chloro-4-(ethylamino)-6-isopropylamino-1,3,5-triazine	5.7	6.8
deethylatratone[c]	2-methoxy-4-amino-6-(isopropylamino)-1,3,5-triazine	7.0	30.7
deisopropylatratone[c]	2-methoxy-4-(ethylamino)-6-amino-1,3,5-triazine	8.9	51.8
deethylatrazine	2-chloro-4-amino-6-(isopropylamino)-1,3,5-triazine	11.4	11.7
deisopropylatrazine	2-chloro-4-(ethylamino)-6-amino-1,3,5-triazine	15.0	15.5
ammeline	2-chloro-4,6-diamino-1,3,5-triazine	31.2	66.7

[a] 3% Carbowax 20M on an 80–100 mesh Chromosorb WHP column, 1.5 m × 0.4 cm i.d.; column and alkali flame ionization detector temperatures were 220 and 270 °C; nitrogen, hydrogen, and air flow rates were 60, 35, and 300 mL/min, respectively. [b] 50% full-scale deflection. [c] Prepared by methylation of the corresponding hydroxy analogues with diazomethane.

mL), reduced glutathione (GSH) (0.5 mL), deethylatrazine (195.9 μg) or deisopropylatrazine (202.0 μg) or hydroxyatrazine (82.4 μg), and 0.134 M phosphate buffer, pH 7.4 (0.5 mL). The atrazine metabolites were added prior to addition of enzyme preparation and after addition of buffer. The organic solvents from the stock solutions were removed under a gentle stream of dry nitrogen with warming to 37.5 °C. For control flasks where no enzyme was added, appropriate amounts of buffer were added to maintain a constant volume. GSH was prepared just prior to addition by mixing glutathione (0.0027 g) with 0.5 M sodium hydroxide (0.5 mL) and neutralizing the mixture with 0.134 M phosphate buffer, pH 7.4 (0.45 mL). The flasks were gassed with dry nitrogen, stoppered, and incubated for 5 h at 37.5 °C. The reaction was terminated by freezing in a dry ice–methanol mixture and immediately taken to dryness by freeze-drying.

Extraction of Reaction Products. The freeze-dried material was extracted in the incubation flasks with methanol (4 × 25 mL) by means of a magnetic stirrer (30 min/extraction). The extracts were filtered through Whatman No. 1 paper into round-bottomed flasks (300 mL). Incubation flasks were further rinsed with acetone (2 × 25 mL), and the rinse was added to the filter paper. The residue on the filter paper was washed with acetone (25 mL). The combined filtrate was taken to dryness on a rotary evaporator at room temperature.

The dried filtrate was redissolved in several portions of dried chloroform (5–10 mL) and placed on an acidic alumina column [20 g, acidic aluminum oxide, activity 1 (E. Merck, Darmstadt, Germany; Brinkmann Instruments, Rexdale, Ontario), 11 × 70 mm, prewashed with dried chloroform (200 mL)], which had been topped with 10 mm of anhydrous Na_2SO_4. The column was eluted with 600 mL of dried chloroform (eluate I). The incubation flasks were further rinsed with methanol (2 × 5 mL) which was placed on the column. The column was eluted with 10% (v/v) methanol in chloroform (290 mL) followed by 5% (v/v) H_2O in methanol (300 mL) and finally with 50 mL of acetone (eluate II).

Eluate I was taken to dryness on a rotary evaporator at room temperature. The residue was redissolved in methanol (2 mL), and aliquots were analyzed by gas chromatography (GC).

Eluate II was concentrated to about 1 mL on a rotary evaporator at room temperature, and an excess of freshly prepared ethereal solution of diazomethane (prepared from Diazald, Aldrich Co., Inc., Milwaukee, WI) was added until the yellow color persisted (Khan et al., 1975). The flask was stoppered, and the contents were allowed to stand overnight (16 h) at room temperature with occasional shaking. The mixture was taken to dryness in a gentle stream of dry nitrogen. The residue was dissolved in methanol (2 mL) and an aliquot analyzed by GC.

Table II. In Vitro Degradation of Hydroxyatrazine and Dealkylated Metabolites of Atrazine by the Goose Liver Soluble Fraction (105000g)[a]

	Deethylatrazine (195.9 μg/flask) deethylatrazine		deethylatratone[b]		recovery,
	μg	%	μg	%	%
no enzyme	186.1	95.0	9.2	4.7	99.7
enzyme	152.3	77.8	42.4	21.6	99.4

	Deisopropylatrazine (202.0 μg/flask) deisopropylatrazine		deisopropylatratone[c]		recovery,
	μg	%	μg	%	%
no enzyme	187.9	93.0	12.0	6.0	99.0
enzyme	169.4	83.9	28.9	14.3	98.2

	Hydroxyatrazine (82.4 μg/flask) atratone[d]		deisopropylatratone		recovery,
	μg	%	μg	%	%
no enzyme	76.2	92.5			92.5
enzyme	73.9	89.7			89.7

[a] Data indicate the reaction products measured by GC analyses after incubation of deethylatrazine, deisopropylatrazine, or hydroxyatrazine by the soluble fraction (105000g) from goose liver homogenates for 5 h under conditions described in the text. Data are means from duplicate incubations performed on different days and have been corrected for column recovery and methylation efficiency. [b] Deethylatratone data expressed as deethylatrazine. [c] Deisopropylatratone data expressed as deisopropylatrazine. [d] Atratone data expressed as hydroxyatrazine.

Methylation of the 2-hydroxy compounds with diazomethane resulted in low recoveries of the corresponding 2-methoxy analogues. Thus, only a 60% yield of atratone [2-methoxy-4-(ethylamino)-6-(isopropylamino)-s-triazine] from hydroxyatrazine and a 68% yield of the methoxy derivatives of hydroxydeethylatrazine and hydroxydeisopropylatrazine were obtained by the method employed. Furthermore, recoveries of hydroxyatrazine, deethylatrazine, and deisopropylatrazine from the acid alumina column by the technique outlined above were 60, 40, and 82%, respectively. Therefore, data were corrected accordingly for the poor methylation efficiency and low recovery from the column.

Gas Chromatography (GC). The gas chromatograph was a Pye, series 104, Model 64, fitted with an alkali flame ionization detector having an RbCl annulus. The column used was a 1.5 m × 0.4 cm i.d. glass tube packed with 3% Carbowax 20M coated on 80–100 mesh Chromosorb WHP. On-column injections were used; injector port temperature

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