tions) and possibly of acetate displacement at C-12 by the dichlorovinyl group followed by recombination and hydrolysis (during workup) at C-9 or C-10. Such triacetates or keto diacetates might be expected to have Rd's in the range found for these products.

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We thank E. O. Oswald, who aided in the collection of infrared and low-resolution mass spectra, and L. H. Keith and other members of his staff (Southwest Water Laboratory, EPA, Athens, Ga.), who obtained high-resolution mass spectra including those of the apparent thermal decomposition product(s) of **II**.

NOTE ADDED IN PROOF. Two-dimensional cochromatography of the six major products (three from each) of the reactions of metabolites **II** or **III** with Ac_2O and *p*-TsOH resulted in four spots, indicating that two products may be common to both metabolites. This is possible if epoxide ring opening (both cis and trans products) occurs in the case of **III** with epoxide ring opening and rearrangement occurring for **II**.

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Metabolism of Atrazine and 2-Hydroxyatrazine by the Rat

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Rats were given either ^{14}C-ring-labeled atrazine (**I**) or 2-hydroxyatrazine (**II**) as a single oral dose. Seventy-two hours after dosing the radioactivity was recovered, mainly in the urine (**I**, 65.5%; **II**, 78%) and feces (**I**, 20.3%; **II**, 5.5%). Less than 0.1% of the dose from either compound was detected in the expired air, and less than 0.1% of the ^{14}C from **II** was detected in the body tissues. The carcasses of the rats given **I** contained 15.8% of the dose, and the distribution of this radioactivity in selected tissues was determined. **II** and its two mono-*N*-dealkylated analogs (**VI** and **VII**) were identified in the urine from rats given **II**. These represented 88.4% of the urinary radioactivity. Nineteen urinary metabolites from **I** were separated by ion-exchange chromatography. Four of these (approximately 47% of the urinary radioactivity) were identified as **II**, **VI**, **VII**, and ammeline. Two additional metabolites of **I**, representing approximately 10% of the urinary radioactivity, were characterized by mass spectrometry.

Corn and sorghum metabolize atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, **I**, Figure 1) to 2-hydroxyatrazine (**II**), to both of the mono-*N*-dealkylated analogs of **II**, and to the glutathione and cysteine conjugates of atrazine (Shimabukuro, 1968; Shimabukuro *et al.*, 1970; Lamoureux *et al.*, 1970). Shimabukuro *et al.* (1970) have also shown that the plant residues of the glutathione conjugate and unchanged **I** decreased with time, while the residues of the 2-hydroxytriazines and the amounts of radioactivity unextractable with methanol increased with time after application of ^{14}C-labeled **I**. These studies suggested that mature plants would contain little, if any, unchanged atrazine, and that the diet of animals feeding on atrazine-treated crops would contain residues of 2-hydroxyatrazines.

Larson *et al.* (1971) compared the metabolism of 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine (**III**) with that of its

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2-hydroxy analog (IV) in the rat. III was extensively N-deethylated (60.7% of the dose), while none of IV was N-deethylated. Approximately 45% of III was totally N-dealkylated, with ammeline (2-hydroxy-4,6-diamino-*s*-triazine, V) being the major urinary metabolite. Ammeline was not present as a urinary metabolite of IV. The only similarity in the rat urinary metabolites of III and IV was the hydroxylation of the *sec*-butyl moiety; however, in the case of III, this hydroxylation was accompanied by N-deethylation. These studies demonstrated that the type of substitution on the 2 position of the triazine ring greatly influenced the metabolic fate of these triazines in the rat and suggested the need for a comparison of the metabolic fate of a 2-chlorotriazine herbicide with that of its 2-hydroxy analog.

This report concerns the metabolism of atrazine (I) and 2-hydroxyatrazine (II) by rats. The urinary and fecal metabolites of II were identified, and the major urinary metabolites of atrazine were either identified or characterized by mass spectrometry.

EXPERIMENTAL

General. The metabolism cages, collection of CO_2, preparation of samples, radioanalysis, combustion analysis of tissues and feces, and ion-exchange chromatographic methods have all been previously reported (Bakke *et al.*, 1967).

Animal Treatment. All rats were dosed by stomach tube with ethanolic solutions of either ^{14}C-ring-labeled atrazine or 2-hydroxyatrazine, and the urine and feces were collected daily. Fourteen rats (310 and 420 g) were each given 0.53 mg of ^{14}C-atrazine containing 3.54 μCi of ^{14}C. Two of these rats were each housed in all-glass metabolism cages and the CO_2 was collected as previously reported (Bakke *et al.*, 1967). At the end of 72 hr, these two rats were sacrificed and the total body radioactivity was determined. The remaining 12 rats were housed in stainless steel cages; the urine and feces were collected daily and tissues taken for analysis of radioactivity after sacrifice at 2, 4, and 8 days (four rats in each group). Eight rats (280 to 340 g) were each given 5.5 mg of ^{14}C-atrazine containing 0.5 μCi of ^{14}C. Two rats (300 and 360 g) were each given 0.5 mg of ^{14}C-hydroxyatrazine containing 0.50 μCi of ^{14}C and six rats were each given 5.0 mg of ^{14}C-hydroxyatrazine containing 0.38 μCi of ^{14}C.

Isolation of Metabolites. The metabolites were isolated from the 0- to 24-hr urine samples collected from the rats given the low specific activity atrazine and hydroxyatrazine. The same isolation procedure was used for both the atrazine and hydroxyatrazine metabolites.

The freeze-dried urine solids were dissolved in a minimum quantity of water (approximately 10 ml), and 3-ml aliquots were acidified to pH 3 with 6 *N* HCl just prior to application to the amino acid analyzer ion-exchange column (column A). The radioactive fractions collected from column I were separately freeze-dried and the residues taken up in 5 ml of water, acidified to pH 3 with 6 *N* HCl, and applied to a 1- × 20-cm column of AG-50X8 cation-exchange resin in the ammonium form (column B). The citrate present from the column A buffer was eluted with water and the radioactivity then eluted with 1 *N* NH_4OH. The recoveries from column B ranged from 71 to 95%. The citrate-free fractions were freeze-dried, and the residue from each fraction was dissolved in methanol and chromatographed on Whatman No. 1 paper. The chromatograms were developed in isoamyl alcohol–acetic acid–water (40:10:50, v/v/v). The radioactive fractions were extracted from the paper with methanol, the extracts were concentrated, and each was separately applied to a 0.9- × 100-cm column of methanol-equilibrated Sephadex LH-20. The recoveries from LH-20 were 95 to 98%. The radioactive fractions eluted from the LH-20 column with methanol were taken to dryness, and each was silanized by heating with 50 μl of *bis*-trimethylsilyl trifluoroacetamide containing 1% trimethylchlorosilane (Regisil). The silanized metabolites were gas chromatographed on a 6-ft, ⅛-in. i.d. glass column of 3% SE-30 on 60/80 mesh Chromosorb W in a Perkin-Elmer 801 gas chromatograph fitted with an effluent splitter. The carrier gas was helium at 30 ml per min. The temperature was programmed from 100 to 250°C at 10°C per min. The detector was located within the column oven, and its temperature varied with the oven temperature. The injector was maintained at 220°C. Ten percent of the effluent from the column went to the flame detector.

Table I. Recovery of Administered Radioactivity from Rats 72 Hr after a Single Oral Dose of Either Ring-^{14}C-Labeled Atrazine or 2-Hydroxyatrazine

	Atrazine-^{14}C,[a] %	2-HO-Atrazine-^{14}C,[b] %
Urine	65.5	78.0
Feces	20.3	5.5
CO_2	<0.1	<0.1
Body	15.8	<0.1
Total	101.7	83.5

[a] Dose, 0.53 mg. [b] Dose, 0.50 mg.

The elution of radioactivity from the gas chromatograph was monitored by trapping each peak in a glass tube and each tube was assayed for radioactivity.

Feces were extracted four times with methanol and the methanol extracts combined and taken to dryness. The residue was taken up in 5 ml of water, acidified to pH 3, and applied to column B. The radioactivity which eluted with 1 *N* NH_4OH was freeze-dried, derivatized with Regisil, and gas chromatographed.

Infrared spectra were obtained from samples in micro-KBr pellets (10 mg KBr; 2-mm diameter pellets) using a Perkin-Elmer 337 infrared spectrometer equipped with a 4× beam condenser. Mass spectra were obtained on samples trapped from the gas chromatograph using the solid sample inlet system of the Varian M-66 mass spectrometer equipped with a V-5500 control console.

RESULTS AND DISCUSSION

The rats given the 0.5-mg doses of either atrazine (I) or 2-hydroxyatrazine (II) excreted the radioactivity mainly in the urine (Table I), with 85 to 95% of that excreted in the urine appearing during the first 24 hr after dosing. The feces contained 20.3% of the dose from I and 5.5% of the dose from II. Less than 0.1% of the ^{14}C from either I or II appeared as $^{14}CO_2$ in the expired air.

At sacrifice, 3 days after dosing, the carcasses from the rats given the 0.5-mg doses of I contained 15.8% of the administered ^{14}C. Analysis for ^{14}C from I in various tissues (Table II) 2, 4, and 8 days after dosing with I showed that fat and muscle had lower residues than the other tissues examined, of which liver, kidney, and lung contained the higher residues. Liver, kidney, and the digestive tract with its contents showed the greatest decrease in residues with time.

Samples (160 to 200 mg) of the homogenized and freeze-dried carcass from rats dosed with II contained less than 15 cpm above background on 40-min counts, which is much lower

Table II. Radioactivity Detected in Rat Tissues after a Single Dose of Ring-^{14}C-Labeled Atrazine

Time of sacrifice, days	Tissue residues, ppm of atrazine-^{14}C equivalent (freeze-dried tissue basis)[a]							
	Liver	Brain	Heart	Lung	Kidney	Digestive tract	Omental fat	Leg muscle
2	3.5	1.6	2.5	3.0	4.0	5.8	0.5	0.6
4	2.6	1.4	1.6	2.3	2.3	0.9	0.1	0.5
8	1.7	1.1	1.4	2.0	1.7	0.9	0.1	0.5

[a] Averages of tissues from four rats.

Table III. Quantitation of the Rat Urinary Metabolites from Either Ring-^{14}C-Labeled Atrazine or 2-Hydroxyatrazine Separated by Ion-Exchange Chromatography and Chromatographic Data for the Isolated Metabolites

Fraction no. from Figure 2	% of radioactivity in the urine				R_f[a]	glc elution temp, °C	Structure, Figure 1
	Atrazine		2-OH-Atrazine				
	0.53 mg[b]	5.5 mg[b]	0.5 mg[b]	5.5 mg[b]			
1, 2, 3	3.0	5.7					
4, 5	2.0	<1	2.4	11.7			
6	1.0	2.0					
7	5.2	5.5			Origin	167 217 (212)[c] 230	V VIIIc[d] Not characterized
8	4.1	2.6					
9	2.4	3.7					
10	3.0	1.9					
11, 12	7.7	5.6			0.35	205	IXa and/or b[d]
13	33.0	54.0			Origin	166	V
14	3.6	1.7					
15	2.4	0.8					
16, 17, 18	12.7	8.5	88.4	72.3	0.34 0.5 0.75	178 177 186	VII VI II
19	4.1	0.9					
Total	84.1	92.9	90.8	84.0			

[a] Paper chromatography. [b] Doses given to each rat. [c] Glc elution temperature of the methylated and silanized metabolite. [d] Proposed structures.

than our defined detectability limits of twice background (31 cpm). The lack of tissue residue from **II** is of significance since Shimabukuro *et al.* (1970) have shown **II** to be a residue in atrazine-treated plants, and Harris (1967) has shown **II** to be a soil residue from atrazine-treated soils.

Figure 2 gives the elution pattern from column A of the radioactivity in the 0- to 24-hr urine samples from the rats given the 0.5-mg doses of **I**. At least 19 radioactive components were present. The quantitation of these metabolites and those in the urine after administration of the 5.5-mg doses of **I** and of both dose levels of **II** are listed in Table III. The data in Table III show that **II** was not converted to as many products as **I**, and that the major metabolite from **I** (fraction 13) was not present in the urine from animals dosed with **II**.

Table III also shows that the "high-dose" urines amples used for the isolation of atrazine and 2-hydroxyatrazine metabolites contained all the components present in urine from animals given lower doses.

The chromatographic data for all isolated metabolites are given in Table III. The mass spectra are given in Table IV.

The major fraction separated on column A from the 0–24 hr urine samples from rats given the 5.5-mg doses of **II** (eluting in the area of fractions 16 to 18 in Figure 2) separated into three components by paper chromatography. These were silanized, gas chromatographed, and identified by mass spectrometry to be unchanged compound **II**, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (**VI**), and 2-hydroxy-4-amino-6-ethylamino-*s*-triazine (**VII**) by comparison with known silanized compounds. Methanol extraction recovered 89.5% of the radioactivity applied to the paper (**II**, 42.3%; **VI**, 22.3%; **VII**, 24.8%; based on activity applied to the paper). The fraction eluted from column A in the area of fractions 4 and 5 (Figure 2) from this urine was not identified. It elutes from the column where acidic materials are expected and could be a conjugate or an acidic dealkylation intermediate.

Metabolites **II**, **VI**, and **VII** were found, by the same procedure, in fractions 16 to 18 (Figure 2) from the urine of rats given the 5.5-mg doses of **I**. Approximately 83% of the radioactivity applied to the paper chromatograms was recovered by methanol extraction (**II**, 8.3%; **VI**, 44.1%; **VII**, 30.0%; based on the activity applied to the paper). The percentage composition of the fractions eluted from the paper suggests that column A fractions 16, 17, and 18 contain, respectively, compounds **II**, **VI**, and **VII**.

The silyl derivative of the major metabolite in the urine from rats dosed with **I** (fraction 13, Figure 2) gave a mass spectrum identical with the silyl derivative of authentic ammeline (**V**, Bakke *et al.*, 1971). Ammeline was identified as the major rat and cow urinary metabolite of **III** (Larson *et al.*, 1971; Bakke *et al.*, 1971). Ammeline was not present as a metabolite of 2-hydroxyatrazine.

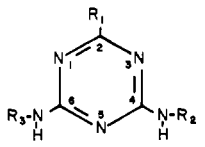
Structures **VIIIc** and either **IXa** or **IXb** were proposed for atrazine urinary fractions 7 and 12, respectively, based on the mass spectra obtained from their silyl derivatives.

The silyl derivative of atrazine fraction 12 (Figure 2) gave a mass spectrum (Table IV) with two sets of molecular ions ($M\cdot^+$ = 371 and 299) and fragment ions [$M\cdot^+ - 15$, and $M\cdot^+ - 117$; 117 = $COOSi(CH_3)_3$] separated by 72 amu (atomic mass units) corresponding to mono- and disilyl derivatives. The monosilyl derivative is presumed to result from hydrolysis

Table IV. Mass Spectra

m/e[a]	Relative abundance	$M^+ + 1$ intensity[b]	Fragment ion description
		Fraction 12, Figure 2 (structure **IXa** and/or **IXb**, Figure 1)	
		Disilyl	
371	4		$M\cdot^+$
356	12	29($C_{13}H_{26}N_5O_3Si_2$; 27.7)	$M\cdot^+ - CH_3$
254	100	25($C_{10}H_{16}N_5OSi$; 19.1)	$M\cdot^+ - COOSi(CH_3)_3$
		Monosilyl	
299	10		$M\cdot^+$
284	7		$M\cdot^+ - CH_3$
182	100		$M\cdot^+ - COOSi(CH_3)_3$
		Fraction 7, Figure 2 (structure **VIIIa**, Figure 1)	
		Trisilyl	
417	68.5	34($C_{14}H_{31}N_5O_4Si_3$; 33)	$M\cdot^+$
402	75		$M\cdot^+ - CH_3$
373*	6.5		$M\cdot^+ - CO_2$
358*	8		$M\cdot^+ - (CH_3 + CO_2)$
328	14.5		$M\cdot^+ - OSi(CH_3)_3$
312	13		$M\cdot^+ - [CH_3 + HOSi(CH_3)_3]$
300	100		$M\cdot^+ - COOSi(CH_3)_3$
255	32		
193.5	2		$M\cdot^+ - 2CH_3$
		Disilyl	
345	100	25($C_{11}H_{23}N_5O_4Si_2$; 24.5)	$M\cdot^+$
330	88		$M\cdot^+ - CH_3$
301*	20		$M\cdot^+ - CO_2$
286*	43		$M\cdot^+ - (CH_3 + CO_2)$
256	35		$M\cdot^+ - OSi(CH_3)_3$
240	55		$M\cdot^+ - [CH_3 + HOSi(CH_3)_3]$
228	70		$M\cdot^+ - COOSi(CH_3)_3$
157.5	12		$M\cdot^+ - 2CH_3$
		Monosilyl	
273	85	17($C_8H_{15}N_5O_4Si$; 16.0)	$M\cdot^+$
258	40		$M\cdot^+ - CH_3$
229*	25		$M\cdot^+ - CO_2$
214*	100		$M\cdot^+ - (CH_3 + CO_2)$
184	50		$M\cdot^+ - OSi(CH_3)_3$
168	55		$M\cdot^+ - [CH_3 + HOSi(CH_3)_3]$
156	38		$M\cdot^+ - COOSi(CH_3)_3$
		Fraction 7 $-CH_3$ (Methylated, disilyl fraction 7)	
359	62	26($C_{12}H_{25}N_5O_4Si_2$; 25.6)	$M\cdot^+$
344	53		$M\cdot^+ - CH_3$
328	21		$M\cdot^+ - CH_3O$
312	8		$M\cdot^+ - (CH_3 + CH_3OH)$
300	82		$M\cdot^+ - COOCH_3$
299	45		
286	100		$M\cdot^+ - Si(CH_3)_3$
270	9		$M\cdot^+ - OSi(CH_3)_3$
255	31		
164.5			$M\cdot^+ - 2CH_3$
		2-Chloro-4-trimethylsilyloxymethylamino-6-isopropyl-*s*-triazine	
289 (Cl)	20		$M\cdot^+$
288 (Cl)	2.5		$M\cdot^+ - H$
274 (Cl)	23		$M\cdot^+ - CH_3$
244 (Cl)	4		$274 - CH_2O$ and/or $M\cdot^+ - NHCH_2O$
216 (Cl)	3		$M\cdot^+ - Si(CH_3)_3$
200 (Cl)	21		$M\cdot^+ - OSi(CH_3)_3$
199 (Cl)	12		$M\cdot^+ - HOSi(CH_3)_3$
184 (Cl)	41		$274 - HOSi(CH_3)_3$
172 (Cl)	9		$M\cdot^+ - NHCH_2OSi(CH_3)_3 + H$
129.5 (Cl)	3		$M\cdot^+ - 2CH_3$
118	100		$[NHCH_2OSi(CH_3)_3]^+$
114.5 (Cl)	12.5		$244 - CH_3$
110	15		

[a] Fragments marked with an asterisk (*) are metastable transitions. [b] $M\cdot^+ + 1$ isotope peak intensities are listed relative to the associate peak being 100%. Values in parentheses are calculated for the empirical formulas given.



		R_1	R_2	R_3
I		Cl-	CH_3CH_2-	$(CH_3)_2CH$-
II		HO-	CH_3CH_2-	$(CH_3)_2CH$-
III		CH_3O-	CH_3CH_2-	$CH_3CH_2CH(CH_3)$-
IV		HO-	CH_3CH_2-	$CH_3CH_2CH(CH_3)$-
V		HO-	H-	H-
VI		HO-	H-	$(CH_3)_2CH$-
VII		HO-	CH_3CH_2-	H-
VIII	a.	HO-	HO-	$HOOCCH_2$-
	b.	HO-	$HOCH_2$-	HOOC-C(OH)-
	c.	HO-	H-	HOOC-CH-
IX	a.	HO-	CH_3CH_2-	$CH_3CH(COOH)$-
	b.	HO-	$HOOCCH_2$-	$(CH_3)_2CH$-

Figure 1. Structures of the compounds referred to in the text and tables

of the sample after elution from the gas chromatograph and not as a fragmentation mode of the disilyl derivative, since the relative intensities of the two sets of ions changed with residence time in the mass spectrometer, while the relative intensities within each set remained constant. The isotope peak intensity (m/e + 1) for the $M\cdot^+ - 15$ fragment from the $M\cdot^+$ at m/e 371 indicated that the fragment contained two silicon atoms and was in good agreement with the elemental formula $C_{13}H_{26}N_5O_3Si_2$. This would give the $M\cdot^+$ the elemental formula $C_{14}H_{29}N_5O_3Si_2$ and the unsilanized metabolite the elemental formula $C_8H_{13}N_5O_3$, which would have a molecular weight 30 greater than 2-hydroxyatrazine. The proposed elemental formula also contained two more oxygen atoms and two less hydrogen atoms than 2-hydroxyatrazine. This indicated oxidation of one of the alkylamino groups to an acid. This is supported by the presence of the base peak in both fragmentation patterns being at $M\cdot^+ - 117$ [117 = $COOSi(CH_3)_3$]. The mass spectrum suggested the metabolite to have either structure **IXa** or **IXb** (Figure 1). The infrared spectrum was of little value, indicating only the presence of the *s*-triazine ring. Strong triazine ring bands in the carbonyl region mask the carboxyl group frequencies.

Three radioactive silyl derivatives were separated by glc after derivatization of atrazine fraction 7. The fraction which eluted from the gas chromatograph at 167°C was identified as the trisilyl derivative of ammeline (V). Since ammeline eluted from column A at 540 to 570 ml (fraction 13, Figure 2) and fraction 7 eluted in the area where compounds more acidic than ammeline are expected, the ammeline in this fraction must have resulted from decomposition of some more acidic precursor or hydrolysis of an acidic conjugate after elution from column A. Ammeline represented approximately 16% of the radioactivity from fraction 7 trapped from the gas chromatograph. The third component eluting at 230°C (12%) was not identified.

The second component in atrazine fraction 7 eluted from the gas chromatograph at 217°C and represented approximately 61% of the radioactivity trapped from the gas chromatograph. The mass spectrum of this component contained three sets of molecular ions ($M\cdot^+$ = 417, 345, and 373), with the associated fragments given in spectrum 7 on Table IV. The probable origins of the fragment ions and the $M\cdot^+ + 1$

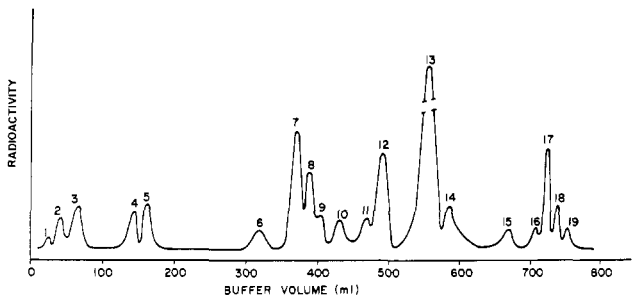


Figure 2. Ion-exchange chromatography of the metabolites from ring-^{14}C-labeled atrazine in rat urine

isotope peak intensities for the three molecular ions are also given in Table IV. The three sets of fragment ions which were separated by 72 amu indicated the presence of three different silyl derivatives of the metabolite and also showed that the sequential removal of two of the silyl groups did not qualitatively affect the fragmentation of the portion of the molecule which produced the fragment ions. As with atrazine fraction 12, these three sets of ions resulted from hydrolysis of silyl groups after trapping from the gas chromatograph. Each fragmentation pattern contained metastable transitions corresponding to the loss of 44 amu (44 = CO_2) from both the $M\cdot^+$ and the $M\cdot^+ - 15$ fragments. The expulsion of CO_2 has been reported as a fragmentation mode of trimethylsilyl benzoates (Teeter, 1962). This suggested the presence of a trimethylsilyl ester of a carboxylic acid, which is supported by the presence of large $M\cdot^+ - 117$ fragment ions in each spectrum [117 = $COOSi(CH_3)_3$].

The $M\cdot^+ + 1$ isotope peak intensities for the three molecular ions were in good agreement with those calculated for the mono-, di-, and trisilyl derivatives of a compound with an empirical formula $C_5H_7N_5O_4$. The calculated values for the expected $M\cdot^+ + 1$ isotope peak intensities are given in parentheses in Table IV.

A sample of this silanized metabolite, trapped from the gas chromatograph, was treated with methanol to remove the silyl groups, and then reacted with diazomethane and again silanized. The methylated and silanized derivative eluted from the gas chromatograph at 212°C and gave mass spectrum 7-CH_3 in Table IV. The molecular ion was at m/e 359 and the base peak was the loss of 59 amu [59 = $COOCH_3$] from the molecular ions. No metastable losses of 44 amu were observed and the $M\cdot^+ + 1$ isotope peak intensity for the molecular ion was in good agreement with that calculated for the disilyl derivative of a compound having the empirical formula $C_6H_9N_5O_4$. The molecular weight and the fragmentation pattern indicated this derivative to be the methyl ester corresponding to the above trisilyl metabolite.

Assuming metabolite 7 to be a carboxylic acid with the empirical formula $C_5H_7H_5O_4$ having the basic ring structure of a 2-hydroxy-4,6-diamino-*s*-triazine (elemental formula less one hydrogen = $C_4H_4N_5O_3$) with substitution at one or both of the amino groups would leave a fragment containing CH_3O unaccounted for in the structure. Three possible structures for this metabolite are given in Figure 1 (structures **VIIIa**, **b**, and **c**).

Mass spectrometry, without the known compounds, cannot distinguish which of the structures is present; however, model compounds tend to rule out two of the structures.

Neither silanized atrazine metabolite 12 (either structure **IXa** or **IXb**, Figure 1) nor the silylester of *N*-phenylglycine fragmented in the mass spectrometer to produce $M\cdot^+ - 89$

[89 = $OSi(CH_3)_3$] and $M^{\cdot+}$ − 105 [105 = CH_3 + $HOSi(CH_3)_3$] fragment ions, and neither spectrum contained metastable losses of 44 (CO_2) or 59 (CH_3 + CO_2) amu. This would tend to rule out the *N*-triazinyl glycine (**VIIIa**) structure for atrazine fraction 7.

The mass spectrum of the silyl ether of 2-chloro-4-hydroxymethylamino-6-isopropylamino-*s*-triazine (Allied Chemical Corp., No. ACD 15M-1) is given in Table IV with the probable sources of the fragment ions. The major fragment ions from this silyl ether can be attributed to fragmentations and/or rearrangements in the trimethylsilyloxymethylamino group. The major differences between this fragmentation pattern and that of the silanized rat atrazine metabolite 7 were the fragment ions corresponding to $M^{\cdot+}$ − 1 (*m/e* 288), $M^{\cdot+}$ − 90 (*m/e* 199), *m/e* 118, and the relatively large chlorine-containing doubly charged fragment ion at *m/e* 114.5. The $M^{\cdot+}$ − 45 ion most probably arose from the expulsion of the elements of formaldehyde from the $M^{\cdot+}$ − 15 precursor, as reported by Budzikiewicz *et al.* (1967) and the doubly charged ion (*m/e* 114.5) resulted from the loss of a methyl group from the $M^{\cdot+}$ − 45 fragment ion. The *m/e* 118 fragment, which was the base peak, had a mass corresponding to the trimethylsilyloxymethylamino group. None of the fragment ions corresponding to these fragmentation modes were observed in the mass spectrum of atrazine metabolite 7 (mass spectrum 7, Table IV). It was therefore concluded that the hydroxymethylamino group was not present, and that structure **VIIIc** was the most probable structure for atrazine fraction 7. Also, the presence of a hydroxymethylamino group would require the presence of an *N*-triazinyl carbamic acid which is too unstable to exist.

Methanol extracts of the feces from rats given hydroxyatrazine contained only hydroxyatrazine. The radioactivity in the feces from rats given atrazine could not be characterized due to the very poor yields upon extraction with chloroform, methanol, aqueous methanol, or acidified aqueous methanol.

Bohme and Barr (1967) reported the isolation and identification of five rat urinary metabolites of atrazine which all contained the 2-chloro group. Three of these corresponded to the 2-chloro analogs of ammeline (**V**) and the two mono-*N*-dealkylated 2-hydroxyatrazine metabolites (**VI** and **VII**) isolated by ion-exchange chromatography. None of the rat urinary metabolites of atrazine isolated in this study by ion-exchange chromatography contained the 2-chloro moiety. This was shown to result from hydrolysis of the 2-chloro group on column A during isolation. Both ^{14}C-atrazine and ^{14}C-2-chloro-4,6-diamino-*s*-triazine (**X**) were put through the isolation procedure. The ^{14}C from atrazine eluted quantitatively from the column at 710 to 730 ml and the ^{14}C from **X** eluted quantitatively with fraction 13 (ammeline). The mass spectra of these fractions after silanization and glc were identical with those of the silyl derivatives of 2-hydroxyatrazine and ammeline. This does not rule out the presence of 2-hydroxyatrazines in the urine as metabolites of atrazine, but it does demonstrate that the 2-chlorotriazines present in the urine had been converted to the 2-hydroxy analogs in a near quantitative yield, since the recoveries of radioactivity applied to column A ranged from 93 to 98%. The 98% recoveries were obtained when the total column effluent from one run was collected together and assayed.

The atrazine metabolites identified or characterized in this study represented N-dealkylation intermediates leading to ammeline and/or 2-chloro-4,6-diamino-*s*-triazine. Either or both of these compounds probably represent the terminal rat metabolites resulting from the ingestion of 2-chlorotriazine herbicides. The lack of $^{14}CO_2$ in the expired air from rats given ring-labeled atrazine indicated that the triazine ring was not metabolized by the rat.

The identification of 2-hydroxyatrazine (**II**) and its two mono-*N*-dealkylated analogs (**VI** and **VII**) as the major urinary metabolites from **II** demonstrated that the rat removes either the ethyl group or the isopropyl group from **II**, but not both, prior to its excretion in the urine. Removal of both groups would have given ammeline (**V**) as a urinary metabolite.

Previous work (Larson *et al.*, 1971) demonstrated that **V** was not a rat urinary metabolite of either **IV**, 2-hydroxy-4-amino-6-ethylamino-*s*-triazine (**VII**), or 2-hydroxy-4-amino-6-*sec*-butylamino-*s*-triazine (**XI**). However, in contrast with the rat metabolism of **II**, N-deethylation of **IV** was not observed, and both **VII** and **XI** were excreted unchanged in the urine (55 to 57% of the dose) and the feces (31 to 41% of the dose).

The evidence suggested that a 2-hydroxy-4,6-dialkylamino-*s*-triazine is sufficiently nonpolar to be absorbed. Removal of one of the alkyl groups gave either structures that are readily excreted by the kidney or structures that cannot be further dealkylated by the mammalian enzymes. The excretions of 30 to 45% of the administered 2-hydroxy-4-amino-6-alkylamino-*s*-triazines (**VII** and **XI**) in the feces (Larson *et al.*, 1971) within 24 hr of oral administration indicated that these compounds were poorly absorbed in the gastrointestinal tract.

The excretion or inability of the rat to metabolize these 2-hydroxy-4-amino-6-alkylamino-*s*-triazines could account for the large number of rat urinary metabolites (18 to 19) from the 2-chloro- and 2-methoxytriazine herbicides. With the 2-chloro or 2-methoxy group present, oxidations and/or dealkylations occur at one or both of the alkylamino groups until the chloro or methoxy group is hydrolyzed, then metabolic dealkylation can occur until the 2-hydroxy-4-amino structure is obtained, at which point the compounds are excreted in the urine, with the alkylamino group on the 6 position remaining at whatever oxidation state it was in when the 2-chloro or 2-methoxy group was removed. With the 2-chlorotriazines this theory is based on the assumption that all of the 2-hydroxytriazine metabolites are not artifacts of the isolation procedure.

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