Metabolism of Atrazine by the Chicken

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Atrazine fed at 100 ppm in a standard laying ration for 7 days to hens did not produce any visible adverse physiological changes or evoke any symptons of toxicity. There was no effect on egg production or egg weight. No residues of atrazine or its metabolites were detected in eggs. However, unchanged atrazine (I) and metabolites deethylated atrazine (II), hydroxyatrazine (V), and possibly deethylated hydroxyatrazine (VI) were present in excreta collected 24 h after hens had been fed the fortified ration. Some residues of atrazine and its metabolites continued to appear in the excreta up to the 4th day after the hens had been returned to the noncontaminated diet. It is possible therefore that there had been some buildup of residues in the various tissues. It appears that the metabolism of atrazine in the hen proceeds mainly via partial N-dealkylation accompanied by hydrolysis. Metabolic dealkylation occurs at the ethylamino group resulting in metabolites II and VI.

The metabolism of atrazine in plants, especially corn and sorghum, has been thoroughly investigated (Plaisted and Thornton, 1964; Negi et al., 1964; Davis et al., 1965; Shimabukuro, 1967, 1968; Shimabukuro et al., 1970, 1971; Lamoureux et al., 1970, 1972; Roeth and Lavy, 1971). Three basic reactions have been identified in the metabolism: hydrolysis of the 2-chloro group; N-dealkylation of the side chains; and displacement of the 2-chloro group with glutathione or γ-glutamylcysteine (conjugation).

A number of investigations have been concerned with the metabolism of *s*-triazines in animals. Palmer and Radeleff (1964, 1969) presented data on the toxicity of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] and other triazine herbicides to cattle, sheep, and chickens and concluded that if the compounds were used as recommended, they constituted very little hazard to animals. These findings were confirmed for cattle and sheep under regular grazing conditions by Johnson et al. (1972). St. John et al. (1964) reported that a small amount of atrazine was excreted unchanged in the urine of the dairy cow. Bakke et al. (1967) observed that, in the rat, ^{14}C ring-labeled propazine [2-chloro-4,6-bis(isopropylamino)-*s*-triazine] and prometone [2-methoxy-4,6-bis(isopropylamino)-*s*-triazine] were rapidly absorbed, metabolized, and excreted in the urine and feces. When the label was in the isopropyl side chain, approximately 50% of the radioactivity evolved as $^{14}CO_2$ which indicated extensive dealkylation. They also reported at least 11 unidentified metabolites from prometone and 18 from propazine. Bohme and Bar (1967) isolated and identified several metabolites of atrazine, propazine, prometone, prometryne [2,4-bis(isopropylamino)-6-methylthio)-*s*-triazine], and simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine], from rat and rabbit urine and reported that the most common metabolic pathway was dealkylation. Robbins et al. (1968) isolated 16 metabolites of propazine from goat urine and reported that $^{14}CO_2$ studies in sheep indicated that dealkylation was also one of several detoxification mechanisms in the ruminant. Larson et al. (1971) identified the rat urinary metabolites of the triazine, GS-14254 [2-methoxy-4-(ethylamino)-6-(*sec*-butylamino)-*s*-triazine], 4 of which accounted for 91% of those excreted in the urine. Bakke et al. (1971) characterized 7 of 19 urinary and 2 fecal metabolites of GS-14254 in the cow and goat. The cow and goat urinary metabolites were isolated in a pattern qualitatively similar to that of rat urine metabolites. The parent herbicide was not detected in either the urine or the feces. Bakke et al. (1972) identified 4 of 19 urinary metabolites of atrazine in the rat and characterized 2 additional metabolites by mass spectrometry. More recently, Larsen and Bakke (1975) reported metabolism of cyprazine [2-chloro-4-(cyclopropylamino)-6-(isopropylamino)-*s*-triazine] by the rat. Four urinary and two fecal metabolites were identified.

In addition, Hutson et al. (1970) and Crayford and Hutson (1972) were able to show that the herbicide, 2-chloro-4-(ethylamino)-6-(1-cyano-1-methylethylamino)-*s*-triazine, was metabolized in the rat mainly via N-deethylation and conjugation with glutathione to yield mercapturic acid in the urine. These findings were confirmed by the in vitro studies of Dauterman and Muecke (1974) on the metabolism of atrazine and six possible metabolites by rat liver subcellular fractions.

Atrazine is used extensively in the culture of corn. Ground corn constitutes 50% of many poultry rations. Previous investigations in our laboratories showed no atrazine residues in eggs, abdominal fat, or minced tissues of chickens which had been fed atrazine at a level of 0.5 ppm in the diet (Foster et al., 1972). This investigation was undertaken to study the metabolism of atrazine by the laying hen. Metabolites in excreta or eggs were extracted and identified by GC–MS (gas chromatography–mass spectrometry).

EXPERIMENTAL SECTION

Animal Treatment. Six Single Comb White Leghorn hens 1.5 years old, at approximately 70% production, were kept in individual laying cages under conditions which have been previously reported (Foster et al., 1972) and were fed a standard laying ration containing 100 ppm of atrazine (Atrex 80W; Ciba-Geigy Corporation) for 7 days followed by the noncontaminated ration for a further 7 days. Excreta and eggs were collected on a 24-h basis. Eggs were stored at 4 °C and excreta at −20 °C in closed glass jars during the collection period. Both eggs and excreta were freeze-dried and stored in closed glass jars at −20 °C until analyses were performed.

Chemicals. All solvents were of pesticide grade and used as received. Analytically pure samples of atrazine (I) and atratone [2-methoxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] were obtained from Ciba Geigy. Hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] (V) was prepared by acid hydrolysis of atrazine according to Gysin and Knusli (1960). Other metabolites, deethylated atrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine] (II), deisopropylated atrazine [2-chloro-4-amino-6-(ethylamino)-*s*-triazine] (III), and

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Table I. Weight Changes, Egg Production, and Egg Weights

Hen no.	Body wt, g		Egg data[a]		
	Initial	Final	Pretreatment	Treatment	Posttreatment
			7[b]	7	7
1	1890	1800	52.2 (48.2-54.2)[c]	51.2 (49.5-53.8)	51.0 (49.7-53.0)
			6	6	6
2	1960	1710	52.9 (50.2-58.4)	51.7 (49.5-53.8)	52.1 (49.7-58.5)
			7	7	7
3	1720	1710	51.4 (42.2-58.4)	50.2 (49.5-53.8)	50.0 (49.7-58.5)
			6	7	7
4	1800	1730	52.9 (50.2-58.4)	50.6 (47.9-53.8)	50.6 (46.0-51.3)
			7	6	7
5	1950	1840	52.2 (48.2-58.4)	50.6 (48.3-53.8)	50.7 (49.7-51.3)
			5	7	7
6	1750	1590	51.7 (49.1-58.4)	51.3 (49.5-53.8)	51.1 (49.7-53.8)

[a] During the pretreatment period, all hens were fed a standard laying ration for 7 days, during the treatment period, a standard laying ration containing 100 ppm of atrazine for 7 days, and during the posttreatment period, a standard laying ration for 7 days. [b] Number of eggs. [c] Egg weight (grams), mean followed by range in parentheses.

deethylated atratone [2-methoxy-4-amino-6-(isopropylamino)-s-triazine] were obtained through the courtesy of D. C. G. Muir, McGill University, Montreal, Quebec, Canada.

Determination of Residues in Eggs. The freeze-dried sample (30 g) was blended with methanol (100 ml) for 15 min at room temperature and the resultant mixture filtered. The sample residue was washed with methanol (100 ml) and the combined filtrate evaporated to dryness on a rotary evaporator. The dried residue was redissolved in several portions of chloroform (5–10 ml) and placed on an acidic alumina column (aluminum oxide, acidic Woelm, activity 1, and previously washed with chloroform, 20 g, 24 mm × 70 mm) which had been topped with 10 mm of anhydrous Na_2SO_4. The column was first eluted with 200 ml of dried (anhydrous Na_2SO_4) chloroform (eluate I) and then with 200 ml of methanol (eluate II).

Eluate I was concentrated to about 10 ml under reduced pressure at room temperature on a rotary evaporator. It was finally taken to dryness with a stream of dry air, redissolved in several portions (2 ml) of hexane, and transferred to a column of silica gel (activated silica gel, previously washed with hexane, 7 g, 24 mm × 70 mm) which had been topped with 10 mm of anhydrous Na_2SO_4. Interfering co-extractants were eluted with 10% diethyl ether in hexane (50 ml). The column was then eluted with 30% diethyl ether in hexane (100 ml) and the eluate concentrated to 5 ml on a rotary evaporator at room temperature. An aliquot of this solution was injected into the gas chromatograph.

Eluate II was concentrated to about 2 ml on a rotary evaporator at room temperature and an excess of freshly prepared diazomethane solution (prepared from Diazald, Aldrich Company Inc., Milwaukee, Wis.) was added until the yellow color persisted (Khan et al., 1975). The flask was stoppered and the contents allowed to stand at room temperature for about 3 h with occasional shaking. The mixture was then taken to dryness in a stream of dry air. The residue was dissolved in hexane (5 ml) and analyzed by gas chromatography.

Freeze-dried eggs collected on one day of each of the control, treatment, and recovery periods were analyzed individually in order that individual hen variation could be assessed. The remainder were pooled (5–6 eggs) on a daily basis and ground in a glass mortar with a glass pestle until thoroughly mixed and the aliquots were analyzed.

Determination of Residues in Excreta. The freeze-dried sample (10 g) was extracted with methanol (100 ml) in a mechanical shaker for 2 h at room temperature. The mixture was filtered, the sample residue was washed with methanol (100 ml), and the combined filtrate evaporated to dryness on a rotary evaporator at room temperature. Further processing of the dried extract was done as described above for egg samples except that no silica gel column cleanup was required for the resultant eluate I.

In order to assess individual hen variation, the freeze-dried excreta from the control (7 days prior to treatment) period was pooled for each hen, ground in a glass mortar with a glass pestle until thoroughly mixed, and analyzed. The freeze-dried excreta from the remainder of the experiment (7 days treatment and 7 days recovery) was pooled (6 hens) on a daily basis, thoroughly mixed as described above, and analyzed.

Determination of Residues in Eggs and Excreta Fortified with Atrazine and Metabolites. A freeze-dried egg sample from the control period was fortified with a mixture of atrazine and hydroxyatrazine at 0.1-, 0.5-, or 1.0-ppm levels. Similarly, a freeze-dried excreta sample from the control period was fortified with a mixture of atrazine, hydroxyatrazine, and deethylated atrazine at 0.1-, 0.5-, or 1.0-ppm levels. In all cases the solvent was allowed to evaporate and the sample mixed thoroughly. Further processing of the sample was done as described above.

All samples were analyzed in duplicate and average values are reported. Residues are reported on a freeze-dried basis.

Gas Chromatography. The gas chromatograph was a Pye Series 104, Model 64, fitted with an alkali flame ionization detector (AFID) having a RbCl Annulus. The column was a 1.5 m × 0.4 cm i.d. glass tube packed with 3% Carbowax 20M coated on 80–90 mesh Chromosorb WHP. The operating conditions were: on-column injections; injector port temperature control turned off; column and detector temperatures, 220 and 270 °C, respectively; nitrogen carrier gas flow rate, 60 ml/min; hydrogen gas flow rate, 35 ml/min; and air flow rate, 300 ml/min; chart speed, 10 mm/min.

Mass Spectrometry. To obtain a mass spectrum, an aliquot of the solution containing approximately 1–3 μg of atrazine or metabolites was injected into a Finnigan Model 9500 gas chromatograph connected by a jet separator to a Finnigan Model 3100 mass spectrometer which was interfaced with a Model 6100 computer-controlled data acquisition system.

RESULTS AND DISCUSSION

No deaths or unusual symptoms were recorded during the experiment which indicates that the laying hen can tolerate 100 ppm of atrazine for at least 7 days. The losses

Table II. Recovery of Atrazine, Hydroxyatrazine, and Deethylated Atrazine from Fortified Samples of Eggs and Excreta

Sample	Fortification, ppm			Recovery, %[a]		
	Atrazine	Hydroxy-atrazine	Deethylated atrazine	Atrazine	Hydroxy-atrazine	Deethylated atrazine
Eggs	0.1	1.0		92	35	
	0.5			103		
Excreta	0.1	0.5	0.1	97	44	91
	0.5	1.0	0.5	96	41	88

[a] Average of duplicate analyses.

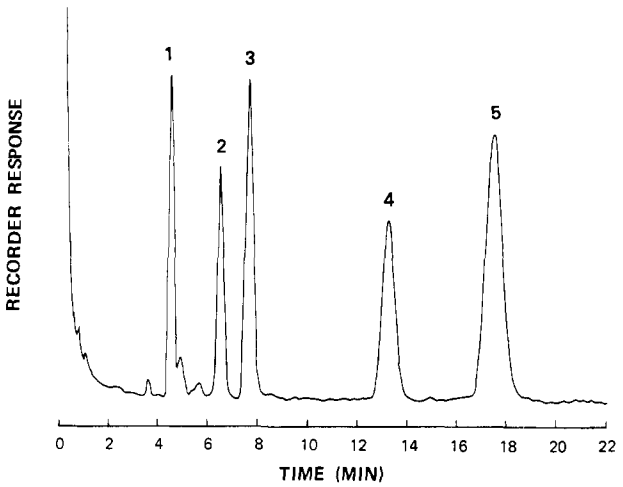

Figure 1. Gas chromatogram of (1) atratone (8.0 ng); (2) atrazine (3.5 ng); (3) deethylated atratone (11.3 ng); (4) deethylated atrazine (5.2 ng); and (5) deisopropylated atrazine (22.5 ng). GC conditions: glass column, 1.5 × 0.4 cm i.d., packed with 3% Carbowax 20M on Chromosorb WHP; on column injections; injector port temperature turned off; column and detector temperatures, 220 and 270 °C, respectively; carrier gas (nitrogen), hydrogen, and air flow rates, 60, 35, and 300 ml/min, respectively; chart speed, 10 mm/min.

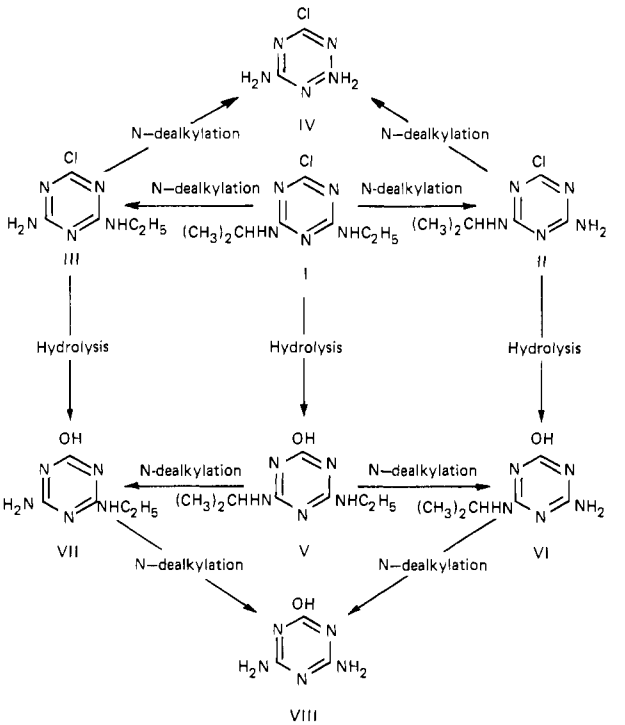

Figure 2. Possible metabolism of atrazine in laying hens.

in body weight shown in Table I are consistent with losses which occur during periods of high production. However, under the conditions of the experiment, it cannot be stated unequivocally that the weight loss was not partially the effect of feeding atrazine in the ration. Atrazine, at 100 ppm, did not affect the numbers of eggs laid nor were there any significant changes in egg weight (Table I).

The gas chromatographic response of atrazine and its metabolites on a 3% Carbowax 20M column using the AFID is shown in Figure 1. The column separated all the compounds with good resolution. It should be noted that hydroxyatrazine and its dealkylated analogue were converted to the corresponding methoxy derivatives prior to gas chromatography. Under the GC conditions described, the compounds give a 50% full scale deflection (0.5 fsd) in the 4.7 to 11.1 ng range with the exception of deisopropylated atrazine (26.3 ng).

The applicability of the method described above was tested for the determination of atrazine, hydroxyatrazine, and deethylated atrazine at 0.1-, 0.5-, or 1.0-ppm levels in freeze-dried samples of eggs and excreta. Recoveries of atrazine and deethylated atrazine from the fortified samples ranged from 88 to 103% (Table II). The recovery of hydroxyatrazine was very low and ranged from 35 to 44% due to the poor efficiency of the methylation of the hydroxy analogues. It should be noted, however, that in a preliminary experiment it was determined that very little loss of methoxy analogues would be experienced during the extraction technique. The recoveries of atratone and deethylated atratone from excreta, fortified at the 0.5-ppm level, ranged from 92 to 97%.

The possible reactions involved in the metabolism of atrazine in laying hens are summarized in Figure 2. The metabolites of atrazine (I) are the hydroxylated analogue of I, namely hydroxyatrazine (V), and N-dealkylation intermediates of I and V, which lead to 2-chloro-4,6-(diamino)-*s*-triazine (IV) and ammeline (VIII), respectively. Either or both of the latter compounds (IV and VIII) may be the terminal metabolites of the metabolism of I in the laying hen. In this study, no residues of atrazine or metabolites were detected in any of the egg samples. Figure 3 shows the gas chromatographic response of extracts of eggs from hens fed the fortified ration. Similarly, no atrazine or metabolites were present in excreta from hens fed the standard laying ration for 7 days during the pretreatment period (Table III). However, unchanged compound I and metabolites II, V, and possibly VI were present in the excreta collected 24 h after the hens had been fed the standard laying ration containing 100 ppm of atrazine. Typical gas chromatograms of extracts of excreta are shown in Figures 4 and 5. A few unknown peaks appeared in the chromatograms due to coextractives but they did not interfere with the peaks for atrazine or the metabolites.

The designated peaks were identified by comparing their retention times and mass spectra with those of the reference standards and by co-chromatography. A GC–MS of peaks 1 and 2 (Figure 4b) showed parent ions at m/e 215 and 187, with intense fragment ions at $M^{\cdot+} - 15$ (loss of methyl group with isopropyl group), confirming the identity of compounds I and II, respectively. Similarly,

Table III. Residues of Atrazine and Metabolites in Excreta from Hens Fed a Standard Laying Ration and a Standard Laying Ration Containing 100 ppm of Atrazine[a]

Treatment	Hen no.	Days after feeding	Atrazine	Deethylated atrazine	Hydroxy-atrazine	Deethylated hydroxy-atrazine
Standard laying ration	1	7[b]				
	2	7				
	3	7				
	4	7				
	5	7				
	6	7				
Standard laying ration + 100 ppm of atrazine	1-6[c]	1	0.31	2.03	0.50	0.08
	1-6	2	0.32	2.19	0.68	0.11
	1-6	3	0.37	2.84	0.79	0.16
	1-6	4	0.42	3.52	0.86	0.16
	1-6	5	0.45	1.71	1.13	0.19
	1-6	6	0.48	3.25	1.02	0.20
	1-6	7	0.52	3.05	1.12	0.22
	1-6	1	0.12	0.38	0.90	t[d]
Standard laying ration	1-6	2	0.06	0.03	0.54	t
	1-6	3			0.24	t
	1-6	4			t	
	1-6	5				
	1-6	6				
	1-6	7				

[a] Residues reported in parts per million based on freeze-dried weights. [b] Excreta for 7 days pooled. [c] Excreta for 6 hens pooled. [d] t = residue present in trace amounts.

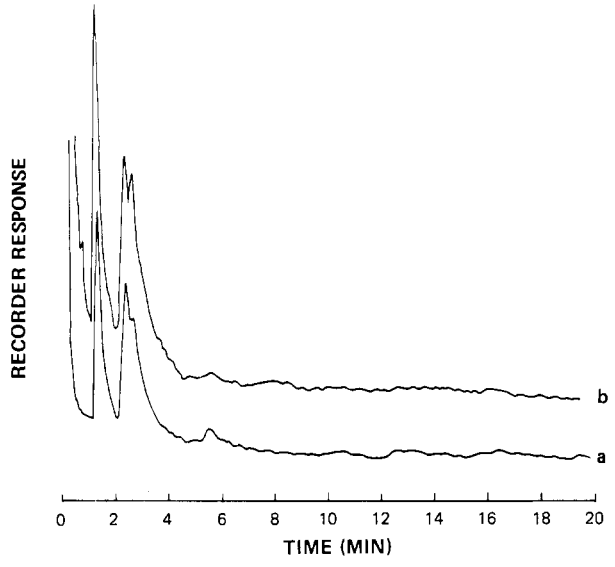

Figure 3. Gas chromatograms of extracts of eggs from hens 5 days after they were fed a standard laying ration containing 100 ppm of atrazine: (a) chloroform eluate; (b) methanol eluate. GC conditions same as in Figure 1.

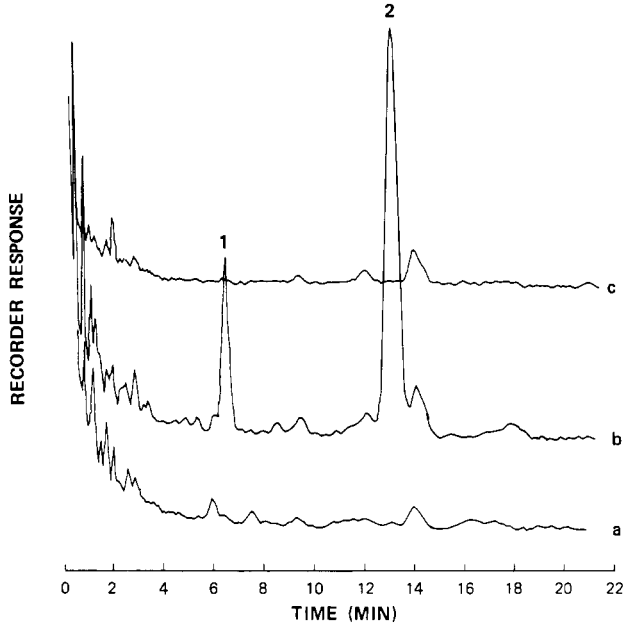

Figure 4. Gas chromatograms of extracts (chloroform eluates) of excreta from hens (a) on the 4th day of the pretreatment period, i.e. fed a standard laying ration; (b) on the 4th day of the treatment period, i.e. fed a standard laying ration containing 100 ppm of atrazine; and (c) on the 3rd day of the recovery period, i.e. fed a standard laying ration; peak number (1) atrazine; (2) deethylated atrazine. GC conditions are the same as in Figure 1.

a GC–MS of peak 1 (Figure 5b) gave mass spectra identical with the 2-methoxy derivative of authentic hydroxyatrazine ($M\cdot^+ = 211$, $M\cdot^+ - 15 = 196$). On the basis of gas chromatographic retention times on various columns (Carbowax 20M, SE-30, OV-17, EGA), peak 2 (Figure 5b) was assigned to compound VI. However, the concentration of the metabolite in the sample was too low for unambiguous confirmation by GC–MS.

The major metabolite, deethylated atrazine (II), tended to reach a plateau in the excreta by the 4th day on the fortified diet and could not be detected on the 3rd day after return to the noncontaminated ration. Hydroxyatrazine (V) was the next most prominent metabolite. Its excretion levels reached a plateau by the 5th day on the diet containing 100 ppm of atrazine, but could be detected for a longer time than the other metabolites after return to the standard ration. Some atrazine (I) was not metabolized and was excreted unchanged. Deethylated hydroxyatrazine (VI) was measured in small but significant amounts and plateaued on the 5th day of the feeding trial.

Compounds II, III, and IV were isolated and identified in the urine from rats given I (Bohme and Bar, 1967). However, none of the rat urinary metabolites was found to contain the 2-hydroxy moiety. On the other hand, Bakke et al. (1972) separated 19 metabolites from rat urine given I as a single oral dose. Four of these were identified as V, VI, VII, and VIII. According to these investigators, compound I, given to the rats, had been converted to the 2-hydroxy analogues in a near quantitative yield. Bakke et al. (1972) also identified 2-hydroxyatrazine (V) and its two mono-N-dealkylated analogues (VI and VII) as the

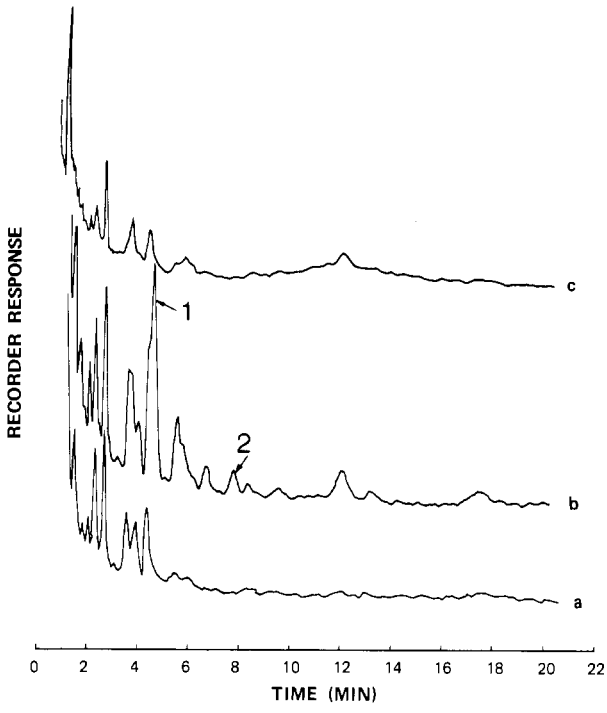

Figure 5. Gas chromatograms of extracts (methanol eluates) of excreta from hens (a) on the 4th day of the pretreatment period, i.e. fed a standard laying ration; (b) on the 4th day of the treatment period, i.e. fed a standard laying ration containing 100 ppm of atrazine; and (c) on the 3rd day of the recovery period, i.e. fed a standard laying ration; peak number (1) atratone; (2) deethylated atratone. GC conditions are the same as in Figure 1.

major metabolites in the urine of rats given V as a single oral dose. More recently, however, Larsen and Bakke (1975) concluded that the hydrolysis of the 2-chloro group does not appear to be the major route of metabolism in the rat. Furthermore, they suggested that the 2-hydroxy compounds isolated as metabolites of atrazine by Bakke et al. (1972) were artifacts resulting from hydrolysis of the corresponding 2-chloro analogues during ion-exchange chromatography. The metabolites of atrazine identified in excreta in our study contained both 2-chloro and 2-hydroxy moieties (Table III). This suggests that some atrazine was hydrolyzed by the hens to the 2-hydroxy analogue. Furthermore, it is evident that in the hen, mainly the ethyl group is removed from I and V. Removal of the isopropyl group or both alkyl groups from I and V would have resulted in the production of II and VII, or IV and VIII, respectively.

In this experiment, no attempt was made to ascertain whether any of the metabolites had been conjugated with glutathione to form mercapturic acids as has been shown in *s*-triazine metabolism in plants and rats.

In view of these findings, it appears that the major route of atrazine metabolism in the hen is via partial N-dealkylation and hydrolysis. In addition with the 2-chloro or 2-hydroxy group present, metabolic dealkylation occurs at the ethylamino group which results in the 2-chloro- (or 2-hydroxy-) 4-amino-*s*-triazine structure with the isopropylamino group remaining at the 6 position. Some residues of both atrazine and metabolites continued to appear in the excreta up to the 4th day after the hens had been returned to the noncontaminated diet (Table III). It is, therefore, possible that there had been some buildup of residues in various tissues. This aspect of the study is currently underway. It is realized that, in this experiment, the hens were fed a ration containing an exceptionally high level of atrazine. However, the presence of phytotoxic metabolites, especially compound II (Sirons et al., 1973), in the excreta could be of concern where soils have received large applications of broiler and laying hen house litter in order to improve the soil and crop production and yield (Perkins et al., 1964).

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