Residues of Atrazine (2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and Its Metabolites in Chicken Tissues

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Standard laying ration fortified with atrazine at the 100-ppm level was fed to chickens for 7 days followed by nonfortified ration for a further 7-day period. The chickens were killed and the concentrations of residues in various body tissues and organs were determined. A relatively high concentration of unchanged atrazine (38.8 ppm) was found in abdominal fat. Metabolites identified in tissues and organs were: hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine), deethylhydroxyatrazine (2-hydroxy-4-amino-6-isopropylamino-*s*-triazine), and deethylatrazine (2-chloro-4-amino-6-isopropylamino-*s*-triazine). The highest concentration of hydroxyatrazine (16.2 ppm) and deethylhydroxyatrazine (15.5 ppm) was present in the liver. Partial N-dealkylation and hydrolysis were major pathways of atrazine metabolism in the chicken. Dealkylation occurred mainly at the ethylamino group resulting in the 2-chloro-(or 2-hydroxy)-4-amino-*s*-triazine structure with the isopropylamino group remaining at the 6 position.

In recent years numerous studies have been concerned with pesticide residues in both poultry and eggs. Residues of chlorinated insecticides in tissues and eggs of chickens fed rations containing pesticides have been reported (Liska et al., 1964; Stadelman et al., 1965; Cummings et al., 1966; Ritchey et al., 1967). These studies were useful in evaluating the hazard of insecticides to poultry consuming contaminated feed. While various workers have reported on mammalian metabolism of herbicidal *s*-triazines (St. John et al., 1965; Bohme and Bar, 1967; Bakke et al., 1967, 1971, 1972; Robbins et al., 1968; Larson and Bakke, 1971, 1975), no attention was given to the metabolism of these compounds in chickens. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), an *s*-triazine herbicide, is used extensively in the culture of corn. Since ground corn constitutes about 50% of many poultry rations, it is possible that chickens may consume large amounts of the herbicide via contaminated ration. Thus, there seemed to be a need to investigate the metabolism of atrazine in chickens.

In an earlier study carried out in this laboratory, residues of atrazine were not detected in eggs, abdominal fat, and minced tissues of chickens which had been fed the herbicide at a level of 0.5 ppm in the diet (Foster et al., 1972). It was thought that either atrazine disappeared rapidly from chicken tissues, or the experimental techniques were not sensitive enough to detect the low levels of the herbicide which may have been present. Consequently, a further study of the metabolism of atrazine was made in chickens which were fed a standard laying ration which contained the herbicide at the 100-ppm level. It was realized that this was a high level of contamination and in practice would only arise as the result of an accident. It was hoped, however, that this level would result in residues much above the minimal levels which can be detected by gas chromatography and sufficient for purposes of identification by mass spectrometry. Residues of unchanged atrazine and some metabolites, namely, hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine), deethylatrazine (2-chloro-4-amino-6-isopropylamino-*s*-triazine) and possibly deethylhydroxyatrazine (2-hydroxy-4-amino-6-isopropylamino-*s*-triazine) were present in excreta collected 24 h after chickens had been fed the fortified ration (Foster and Khan, 1976). Furthermore, some residues of atrazine and metabolites continued to appear in the excreta up to 4 days after the chickens were returned to a nonfortified ration. It was suspected, therefore, that the high levels of atrazine fed to the chickens resulted in some buildup of residues in various body tissues. The investigation reported here is an extension of this study. Residues of atrazine and metabolites extracted from various tissues and organs were identified by gas chromatography–mass spectrometry.

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EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade and used as received. Reference standards of atrazine and metabolites (Table I) were gifts from Ciba-Geigy Limited, Switzerland.

Animal Treatment and Collection of Body Tissues. Six Single Comb White Leghorn hens, 1.5 years old, were kept in individual laying cages under conditions described previously (Foster et al., 1972). The chickens were fed a standard laying ration containing 100 ppm of atrazine (Atrex 80W, Ciba-Geigy Corporation) for 7 days. Although no accurate record of total food consumption was kept, it was estimated that each chicken consumed about 60–65 mg of atrazine in diet during this period. The chickens were then fed a nonfortified ration for a further 7 days and killed and the heart, liver, kidneys, lungs, gizzard, oviduct, crop, and intestines plus samples of leg muscle, breast muscle, and abdominal fat were removed. Abdominal fat was stored at −20 °C until analyzed. Skin and fat were carefully removed from tissues and organs by dissection. The crop, gizzard, intestines, and oviduct were opened, freed of undigested food, excreta, eggs, and other foreign objects, and washed in distilled water. Excess moisture was removed by blotting before weights were obtained. The samples were then freeze-dried and kept at −20 °C until analyzed.

Determination of Residues. (i) *Organs and Tissues.* The freeze-dried organ or tissue sample, pooled from 3 chickens, was extracted with methanol (1:5, w/v) on a mechanical shaker for 2 h at room temperature. The extract was filtered, the sample residue washed with methanol (3 × 50 ml), and the combined filtrate evaporated to dryness on a rotary evaporator at room temperature. The dried residue was dissolved in several portions of chloroform (5–10 ml) and placed on an acidic alumina column (aluminum oxide, acidic Woelm, activity 1, 20 g, 24 mm × 70 mm) topped with 10 mm of anhydrous

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

Common name	Chemical name	Peak no. (Fig. 1)	Retention time,[a] min	$^1/_2$fsd,[b] ng
Atratone	2-Methoxy-4-ethylamino-6-isopropylamino-*s*-triazine	1	4.7	8.0
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine	2	6.7	4.7
Hydroxyatrazine	2-Hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine			
Deethylatratone[c]	2-Methoxy-4-amino-6-isopropylamino-*s*-triazine	3	7.9	11.1
Deisopropylatratone[c]	2-Methoxy-4-ethylamino-6-amino-*s*-triazine	4	9.9	22.5
Deethylatrazine	2-Chloro-4-amino-6-isopropylamino-*s*-triazine	5	13.6	5.2
c	2-Methoxy-4,6-diamino-*s*-triazine	6	16.9	25.0
Deisopropylatrazine	2-Chloro-4-ethylamino-6-amino-*s*-triazine	7	17.8	26.3
Ammeline	2-Chloro-4,6-diamino-*s*-triazine	8	37.3	40.0

[a] 3% Carbowax 20M column. [b] 50% full scale deflection. [c] Prepared by methylation of the corresponding hydroxy analogues with diazomethane.

Na_2SO_4 and pre-washed with chloroform. 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 on a rotary evaporator at room temperature and finally taken to dryness with a stream of dry air. The residue was dissolved in hexane and an aliquot of this solution was injected into the gas chromatograph.

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

(ii) *Abdominal Fat.* The fat sample (10 g) was blended at high speed with a mixture (500 ml) of acetonitrile–water (9:1, v/v) until all the sample dissolved. The extract was then concentrated to about 100 ml on a rotary evaporator at room temperature and washed with hexane (3 × 50 ml) to reduce the fat content. Gas chromatography of the hexane washings did not show the presence of atrazine or metabolites thereby indicating no loss of the residues. The hexane washings were discarded. The extract was taken to dryness by occasional addition of small volumes of absolute alcohol and evaporation on a rotary evaporator. The dried residue was dissolved in several portions (5–10 ml) of chloroform and chromatographed on an acidic alumina column according to the procedure described above, and an aliquot was analyzed by gas chromatography.

Determination of Residues in Body Tissues Fortified with Atrazine and Hydroxyatrazine. The tissue and organ samples were fortified with a mixture of atrazine and hydroxyatrazine at 0.1- and 0.5-ppm levels. The solvent was allowed to evaporate and the sample mixed thoroughly. Further processing of the samples was done as described above.

All samples were analyzed in duplicate and average values are reported. Residues levels in the tissue and organ samples are reported on a wet weight basis.

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 two columns used were: (a) 1.5 m × 0.4 cm i.d. glass tube packed with 3% Carbowax 20M coated on 80–100 mesh Chromosorb WHP, and (b) 1.5 m × 0.4 cm i.d. glass tube packed with 0.3% ethylene glycol adipate (EGA) on 80–100 mesh Chromosorb WHP. The injector port temperature was turned off and the detector temperature was 270 °C.

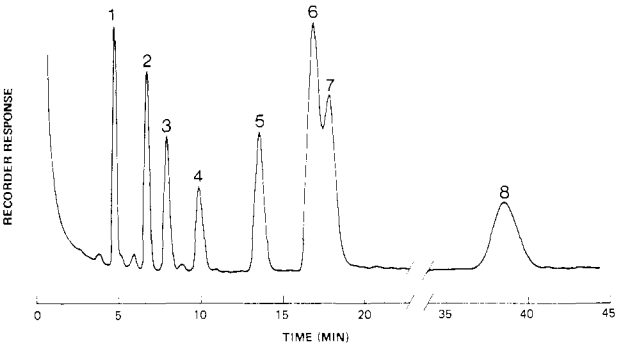

Figure 1. Gas chromatograms of: (1) atratone; (2) atrazine; (3) deethylatratone; (4) deisopropylatratone; (5) deethylatrazine; (6) 2-methoxy-4,6-diamino-*s*-triazine; (7) deisopropylatrazine; and (8) ammeline. GC conditions: glass column, 1.5 m × 0.4 cm i.d. packed with 3% Carbowax 20M on Chromosorb WHP; on-column injection, 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.

The nitrogen carrier gas, hydrogen, and air flow rates were 60, 35, and 300 ml/min, respectively.

The concentrations of atrazine and metabolites in various extracts were determined on column a at 220 °C. Both columns a and b were used for the identification of metabolites in tissues and organs. Column b was programmed from 90 to 190 °C at a rate of 6 °C/min.

Gas Chromatography–Mass Spectrometry (GC–MS). A Finnigan Model 3100 mass spectrometer connected to a Finnigan Model 9500 gas chromatograph by means of a jet separator was used. The mass spectrometer was interfaced with a Model 6100 computer-controlled data acquisition system. A 1.5 m × 0.4 cm i.d. glass column packed with 0.3% EGA on 80–100 mesh Chromosorb WHP was used for gas chromatographic separation. The column was programmed from 90 to 190 °C at a rate of 6 °C/min and the helium flow rate was 30 ml/min. The mass spectra were recorded at 70 eV. A synthetic mixture of the suspected metabolites was prepared from the reference compounds and the mass spectra obtained. The samples were analyzed under identical conditions and the mass spectra of the metabolites were compared with those of the reference compounds.

RESULTS AND DISCUSSION

The gas chromatographic response of the synthetic mixture on column a is shown in Figure 1. Under the GC conditions described, the compounds gave a 50% full scale deflection ($^1/_2$fsd) in the 4.7 to 40.0 ng range (Table I). The column appears to separate most of the compounds with good resolution. It should be noted, however, that

Table II. Weights of Organs and Tissues and Residues of Atrazine and Metabolites in Hens Fed a Diet Containing 100 ppm of Atrazine

Organ or tissue			Residues, ppm[b]			
Name	Wet wt,[a] g	Dry wt,[a] g	Atrazine	Deethyl-atrazine	Hydroxy-atrazine	Deethylhydroxy-atrazine
Gizzard	20.2	5.3	[c]	2.33	0.52	0.30
Crop	5.1	1.1				
Intestine	17.3	4.0		0.47	0.59	
Liver	47.8	13.5			16.18	15.50
Kidney	11.1	2.6			4.28	2.29
Heart	9.1	2.6			0.70	
Lung	14.0	3.0			0.35	
Oviduct	52.1	11.7			2.49	
Leg muscle	23.0	5.9	0.04	0.04	0.94	1.81
Breast muscle	26.0	6.5		0.32		0.78
Abdominal fat			38.82	1.55	0.69	

[a] Average weight of each organ or tissue from three hens which was pooled for residue determination. [b] Converted to wet weight basis. [c] None detected.

hydroxyatrazine and its dealkylated analogues were converted to the corresponding methoxy derivatives prior to GC.

Recoveries of atrazine from the organ and tissue samples at 0.1- and 0.5-ppm fortification levels ranged from 90 to 102%. However, the recoveries of hydroxyatrazine were low and ranged from 35 to 54% due to the poor efficiency of the methylation. Since a considerable variation occurred in the recoveries of the hydroxy analogue, the data reported for residues of hydroxy metabolites should only be regarded as qualitative. It should be pointed out that preliminary experiments showed very little loss of methoxy analogues, such as atratone (2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine), during the extraction procedure.

Gas chromatographic analysis of the extracts of various tissues and organs on columns a and b showed peaks having retention times identical with those of the reference standards. Typical gas chromatograms of extracts of gizzard, kidney, and liver obtained on column a are shown in Figure 2. The identities of unchanged atrazine and metabolites in the extracts were confirmed by co-chromatography with authentic standards and finally by GC–MS analysis. A GC–MS of the major peak with a retention time of 6.7 min, observed in the gas chromatogram of the extract of abdominal fat, showed a molecular ion at m/e 215, a chlorine isotopic peak ($M^{\cdot+}$ + 2), a base peak at m/e 200 ($M^{\cdot+} - CH_3$), and an ion at m/e 173 ($M^{\cdot+} - CH_3CH{=}CH_2$). The spectrum was consistent with the mass spectrum of authentic atrazine. The mass spectra of metabolites represented by GC peaks at retention times of 4.7 and 7.9 min (peaks I and II, Figure 2) exhibited molecular ions at m/e 211 and 183, respectively. Furthermore, the molecular ions decomposed with loss of both CH_3 ($M^{\cdot+} - 15$) and $CH_3CH{=}CH_2$ ($M^{\cdot+} - 42$) to give the m/e 196 and 169, and m/e 168 and 141 ions, respectively, with further fragmentation analogous to that observed for authentic 2-methoxy derivatives of hydroxyatrazine and deethylhydroxyatrazine. A GC–MS of peak III (Figure 2) showed a molecular ion at m/e 187, a chlorine isotopic peak ($M^{\cdot+}$ + 2), a base peak at m/e 172 ($M^{\cdot+} - CH_3$), and an ion at m/e 145 ($M^{\cdot+} - CH_3CH{=}CH_2$). The mass spectrum of this compound was identical with that of authentic deethylatrazine. In view of the foregoing, metabolites represented by peaks I, II, and III (Figure 2) were identified as atratone, deethylatratone, and deethylatrazine, respectively.

A significant concentration of unchanged atrazine was present in the abdominal fat (Table II) even though the chickens were returned to a noncontaminated diet for 7 days. It appears that the fatty tissues stored part of atrazine and prolonged its persistence in the chicken. Bohme and Bar (1967) and Larsen and Bakke (1975) observed that rat and rabbit urinary metabolites from the 2-chloro-*s*-triazines were all 2-chloro analogues of their respective parent molecules and none of the metabolites were found to contain the 2-hydroxy moiety. Total N-dealkylation, partial N-dealkylation, and N-dealkylation with *N*-alkyl oxidation were suggested as the major routes of the metabolism of 2-chloro-*s*-triazines metabolism in rat and rabbits. It was shown, however, that unchanged atrazine, deethylatrazine, and hydroxyatrazine residues were present in the excreta from chickens fed atrazine at the 100-ppm level in a standard laying ration (Foster and Khan, 1976). This indicated that the metabolism of atrazine in the chicken proceeded mainly via partial

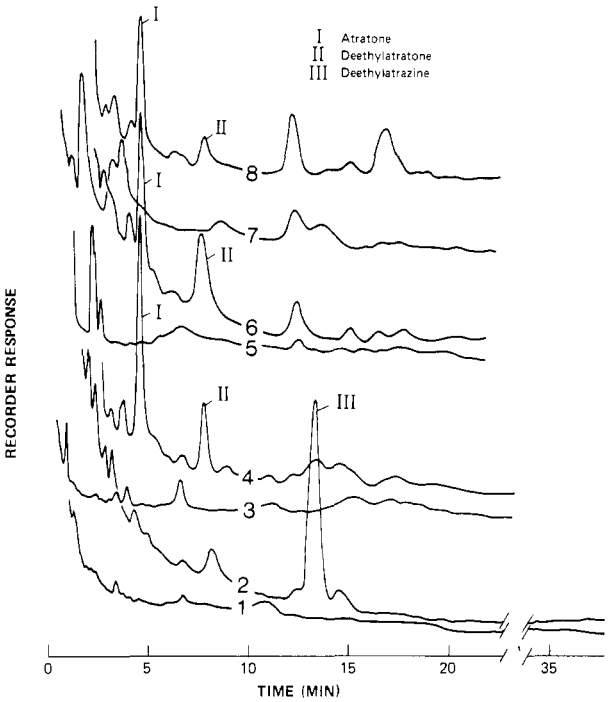

Figure 2. Gas chromatograms of extracts of organs and tissues from hens: (1) gizzard (control hens, chloroform eluate); (2) gizzard (treated hens, chloroform eluate); (3) gizzard (control hens, methanol eluate); (4) gizzard (treated hens, methanol eluate); (5) kidney (control hens, methanol eluate); (6) kidney (treated hens, methanol eluate); (7) liver (control hens, methanol eluate); and (8) liver (treated hens, methanol eluate); I, atratone; II, deethylatratone; III, deethylatrazine.

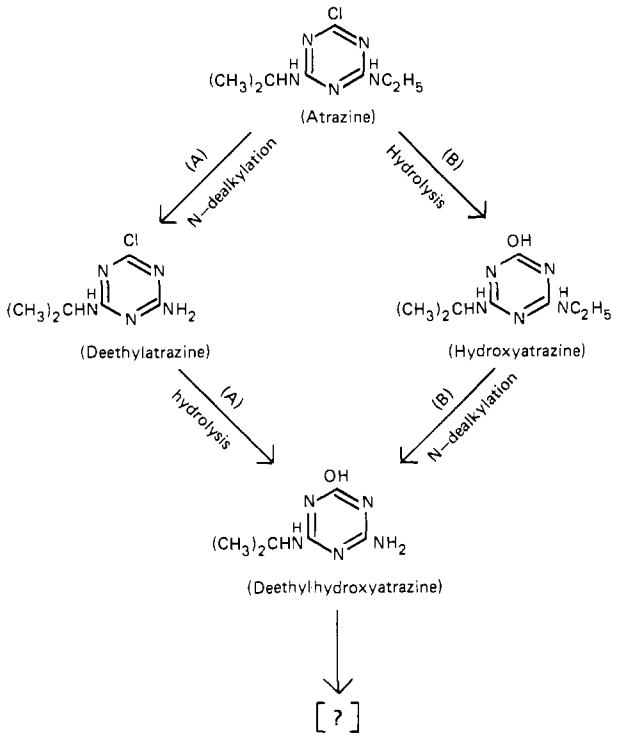

Figure 3. The metabolic pathways of atrazine in the chicken.

N-dealkylation accompanied by hydrolysis. The data presented in this paper are consistent with these findings. It is of interest to note that atrazine given to rats was converted to the 2-hydroxy analogues in a near quantitative yield, thereby indicating hydrolysis to be the major route of metabolism (Bakke et al., 1972). However, in a later study the 2-hydroxy compound was found to be an artifact resulting from hydrolysis of the corresponding 2-chloro analogues during ion-exchange chromatography (Larsen and Bakke, 1975). In our study hydroxyatrazine and its deethylated analogue appear to be the major metabolites of atrazine in the chicken. These metabolites were present in considerably higher concentrations in liver (Table II). It appears that chickens are capable of metabolizing atrazine by at least two different pathways (Figure 3). Pathways A and B were both partially active as evidenced by the presence of residues of metabolites containing both 2-chloro and 2-hydroxy moieties in gizzard, intestine, leg muscle, breast muscle, and abdominal fat. However, pathway B contributed significantly to the accumulation of hydroxy metabolites in liver, kidney, heart, and lung. It is also evident that metabolic N-dealkylation occurred mainly at the ethylamino group which resulted in the 2-chloro-(or 2-hydroxy)-4-amino-*s*-triazine structure with the isopropylamino group remaining at the 6 position. The absence of 2-chloro-(or 2-hydroxy)-4,6-diamino-*s*-triazine indicated that complete N-dealkylation of atrazine does not occur in chickens. The subsequent metabolism of the partially N-dealkylated metabolites containing 2-chloro or 2-hydroxy moieties is still unknown. Further studies utilizing [^{14}C]atrazine (ring labeled) are currently in progress.

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LITERATURE CITED

Bakke, J. E., Larson, J. D., Price, C. E., *J. Agric. Food Chem.* **20**, 602 (1972).
Bakke, J. E., Robbins, J. D., Feil, V. J., *J. Agric. Food Chem.* **15**, 628 (1967).
Bakke, J. E., Robbins, J. D., Feil, V. J., *J. Agric. Food Chem.* **19**, 462 (1971).
Bohme, C., Bar, F., *Food Cosmet. Toxicol.* **5**, 23 (1967).
Cummings, J. G., Zee, K. T., Turner, V., Quinn, F., *J. Assoc. Off. Agric. Chem.* **49**, 354 (1966).
Foster, T. S., Khan, S. U., *J. Agric. Food Chem.* **24**, 566 (1976).
Foster, T. S., Morley, H. V., Purkayastha, R., Greenhalgh, R., Hunt, J. R., *J. Econ. Entomol.* **65**, 982 (1972).
Larsen, G. L., Bakke, J. E., *J. Agric. Food Chem.* **23**, 388 (1975).
Larson, J. D., Bakke, J. E., *Proc. N. Dakota Acad. Sci.* **24**, 178 (1971).
Liska, B. J., Langlois, B. E., Mostert, G. C., Stadelman, W. J., *Poultry Sci.* **43**, 982 (1964).
Ritchey, S. J., Young, R. W., Essary, E. O., *J. Food Sci.* **32**, 238 (1967).
Robbins, J. D., Bakke, J. E., Feil, V. J., *J. Agric. Food Chem.* **16**, 698 (1968).
St. John, L. E., Jr., Ammering, J. W., Wagner, D. G., Warner, R. G., Lisk, D. J., *J. Dairy Sci.* **48**, 502 (1965).
Stadelman, W. J., Liska, B. J., Langlois, B. E., Mostert, G. C., Stemp, A. R., *Poultry Sci.* **44**, 435 (1965).

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