Bull. Environ. Contam. Toxicol. (1989) 43:199–206

Environmental Contamination and Toxicology

Atrazine Biodegradation in Rats—A Model for Mammalian Metabolism

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s -triazine) as a selective herbicide is used in agriculture without strict interdiction, that leads to its presence in raw and industrial food products, as well as in agricultural watersheds (Muir et al. 1978). Because of its low chemical reactivity and insolubility in water, atrazine is very stable, its residue and metabolites were found in ground after several months of application (Frank and Sirons 1985).

The metabolism of chloro-s-triazine herbicides has not been extensively investigated in mammals. Firstly, the metabolism of s-triazines in mammals was studied in the rabbit and rat, including the chloro-s-triazines: atrazine, simazine and propazine (Bohme and Bar 1967). It was suggested that N-dealkylation occured readily, that the triazine ring was not cleaved, and that the carbon chlorine bond was rather stable. Subsequent studies showed that atrazine and hydroxyatrazine were rapidly excreted by the rat (Bakke et al. 1972), and that the type of substitution on position 2 of the triazine ring greatly influenced the metabolic fate of these triazines in the rat.

The metabolism of atrazine and six possible metabolites by rat liver subcellular fractions was studied **in vitro** (Dauterman and Muecke 1974). It was found that dealkylation reaction predominated the conjugation reaction with glutathione, and that the isopropyl group was more easily dealkylated than the ethyl group. This investigation showed that atrazine was involved in the reaction of dealkylation in the microsomal fraction and the conjugation with glutathione in the soluble fraction of the rat liver. The authors did not observe any evidence for the dechlorination of the chloro-s-triazines to hydroxy-s-triazines.

The present study was undertaken to investigate the biodegradation of atrazine at the subcellular level after the oral treatment of the rat. The aim of the study was to detect atrazine residue and its metabolites in rat tissues, specifically the most responsible tissues for atrazine metabolism - the liver and kidney, and

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especially to investigate if atrazine or its metabolites could pass the brain barrier.

MATERIALS AND METHODS

Analytically pure samples of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and its metabolites: atratone (2-methoxy-4-ethylamino-6-isopropylamino-s -triazine), deethylatratone (2-methoxy-4-amino-6- isopropylamino-s-triazine), deethylatrazine (2-chloro-4 -amino-6-isopropylamino-s-triazine), 2-methoxy-4,6-diamino -s-triazine and deisopropylatrazine (2-chloro-4-ethylamino -6-amino-s-triazine) were obtained from Ciba Geigy, Basel. All solvents were of pesticide grade, kept in dark bottles over anhydrous sodium sulphate, and were used without further purification. Diazomethane, used for the methylation of atrazine hydroxy-metabolites, was prepared according to the method published elsewhere (Becker 1973).

Male rats of the Fisher strain, aged 90 days (approximately 250 g of body weight), were maintained in a 12 hr light and 12 hr dark environment, in temperature and humidity controlled rooms, and on ad libitum food and water. The animals were treated orally by a stomach tube with atrazine dissolved in paraffine oil (15 or 30 mg in 0.5 ml, daily for 7 days). The control group received the same volume of paraffine oil only. The rats were sacrified 24 hr after receiving the last dose of the pesticide. Tissue samples (liver, kidney and brain) were collected and immersed in cold distilled water. The tissues were slightly dried on filter paper, excess fat surroundings were removed and after weighing, the samples were freeze-dried and kept at -20 ^{o}C until analysis.

The freeze dried tissues were pooled from each group of rats, cut into small pieces, homogenized, and then extracted with methanol (1:5, w/v) for 3 hr at room temperature and with constant shaking. The extract was filtered, the sample residues were washed with methanol (3 x 50 ml), and the combined filtrate dried on a rotary evaporator at room temperature. The dried residue was dissolved in chloroform, and in portions of 5-10 ml, the chloroform solution was transfered on an acidic alumina column (aluminium oxide, per Brockmann, activity degree II-III, 24 mm x 140 mm). The column was topped with 10 mm of anhydrous sodium sulphate and pre-washed with chloroform. First, the column was eluated with 250 ml of chloroform (eluate 1), and then with 250 ml of methanol (eluate 2). Eluate 1 was concentrated to a volume of about 10 ml on a rotary evaporator at room temperature, and it was finally dried with a stream of dry nitrogen. The dry residue was dissolved in hexane and an aliquot of this solution was injected into the gas chromatograph. Eluate 2 was concentrated to a volume of about 10 ml on a rotary evaporator at room temperature, and an excess of freshly prepared diazomethane solution was added until reaching the stable yellow color. The flask was stoppered and the contents allowed to stand over night with occasional shaking. The mixture was dried in a stream of dry nitrogen, the residue was dissolved

in hexane, and an aliquot of hexane solution was injected into the gas chromatograph.

The gas chromatography was carried out with a Varian Model 3760 equiped with a flame ionization detector (FID). The operation conditions were: injector temperature 180°C, detector temperature 230°C, carrier gas flow 30 ml N_2/min, siliconized glass column (200 x 0,4 cm) and stationary phase 3% Carbowax 20 M on Chromosorb WHP (80-100 mesh). The sensitivity of detection was up to 200 ng of atrazine or metabolite.

Gas chromatography - mass spectrometry (GC-MS) analysis was performed by a Hewlet-Packard Model 5985B with 50 m column of fused silica with methyl silicone. The column was programmed from 30 to 260°C: isothermal 0,5 min, then 20°C/min up to 4.5 min and from 4.5 min 10°C/min up to 260°C. The mass spectra were recorded at 70 eV, 300 Amp and 2.4 kV. The samples were analyzed under identical conditions and the mass spectra of the metabolites were compared with those of the reference compounds.

RESULTS AND DISSCUSION

The gas chromatographic response of atrazine and its metabolites in the mixture is shown in Table 1.

Table 1. Chemical names and retention time of atrazine and its metabolites

Common name	Chemical name	Retention time R_t	Rel R_t
Atratone	2-methoxy-4-ethylamino-6-isopropylamino-s-triazine *	5.33	0.72
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-s-triazine	7.44	1.00
Deethylatratone	2-methoxy-4-amino-6-isopropylamino-s-triazine *	8.40	1.13
Deethylatrazine	2-chloro-4-amino-6-isopropylamino-s-triazine	14.47	1.94
	2-methoxy-4,6-diamino-s-triazine *	17.30	2.33
Deisopropylatrazine	2-chloro-4-ethylamino-6-amino--s-triazine	18.80	2.53

* Compounds prepared by methylation of the corresponding hydroxy analogues with diazomethane

The column appears to separate the most of compounds with the good resolution. However, it should be noted that hydroxy atrazine and its dealkylated analogues were methylated to the corresponding methoxyderivatives prior to the determination on the gas chromatograph.

The typical gas chromatogram of the extracts of the brain is shown in Figure 1 which represents the gas chromatograms of the chloroform eluate of the rat male brain, from control and treated animals. In the chloroform extract of the brain from atrazine treated male rats, a peak of deethylatrazine is evident, as well as a peak of unchanged atrazine residue, but to a smaller degree.

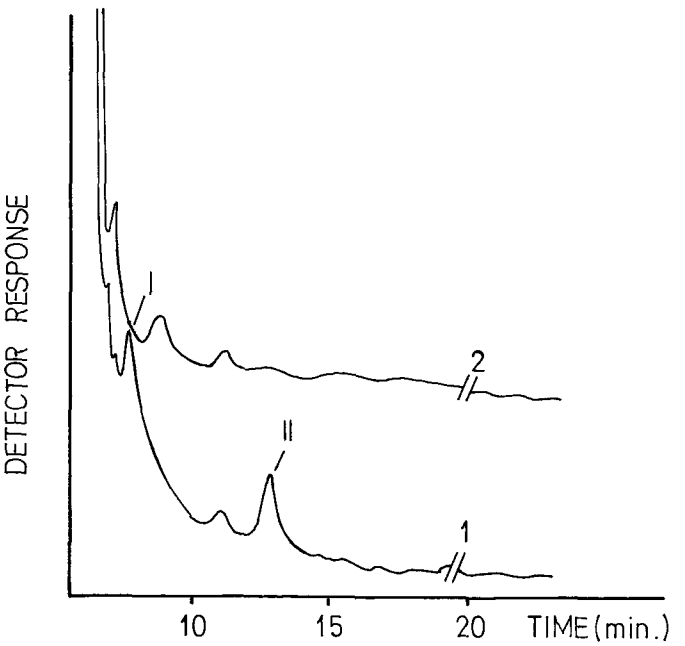

Figure 1. Gas chromatograms of the brain extract from male rat;(1) treated rats with atrazine (chloroform extract) and (2) control (chloroform extract); peak number (I) atrazine and (II) deethylatrazine.

Table 2 represents the detected residues of atrazine and five atrazine metabolites in the tissues of the liver, kidney and brain from male rats treated with two doses of atrazine (6 or 12 mg/100 gr body weight daily during 7 days). However, the recoveries of hydroxyatrazine were low due to the poor efficiency of the methylation (approximately 30 to 50%). Since a considerable variation occured in the recoveries of the hydroxy analogues, the data reported as residues of atrazine hydroxy metabolites should only be regarded as qualitative. The data from Table 2 show that unchanged atrazine was detected in all tested tissues, and the highest content was found in the kidney, a lower concentration in the liver and the lowest in the brain. Deethylatrazine was

detected at the kidney and brain level. Another dealkylated atrazine metabolite, deizopropylatrazine, was detected in the liver and kidney of the rats treated with atrazine (Table 2).

Table 2. Residues of atrazine and its metabolites in male rat after **in vivo** treatment

Atrazine[a]	Organ or tissue	Wet wt[b] (g)	Residues, ppm[c]					
			Atrazine	Deethylatrazine	Deisopropylatrazine	Hydroxyatrazine	Hydroxydeethylatrazine	Hydroxydiaminoatrazine
6	liver	70.00	2.30	ND	4.70	0.22	ND	0.24
6	kidney	25.00	4.00	6.20	2.40	ND	ND	ND
6	brain	18.20	0.87	2.08	ND	ND	ND	ND
12	liver	70.30	3.10	ND	4.80	0.75	ND	0.40
12	kidney	24.50	7.50	9.80	5.10	ND	0.18	ND
12	brain	19.70	1.67	2.73	ND	ND	ND	ND

a mg/100 g bw daily during 7 days
b Wet weight of organs and tissues from ten adult male rats pooled for residue determination
c Calculated to wet weight basis
ND not detectable

The results presented in Table 2 for the detected hydroxy analogues of atrazine, suggest that even with respect to the loses during methylation, these metabolites are less prevalent in the tested tissues. Hydroxyatrazine and hydroxy-diaminoatrazine were found in the liver, and hydroxy-deethylatrazine only in the methanol eluate of the kidney. None of atrazine hydroxy analogues was detected in the brain.

The identities of unchanged atrazine and metabolites in the extracts were confirmed finally by GC-MS analysis. Figure 2 represents a mass spectra of the main atrazine metabolite in the brain, deethylatrazine. A GC-MS analysis of the brain extract gives the major peak of 2-chloro-4-amino-6-izopropyl-amino-s-triazine (deethylatrazine) with a retention time of 14.47 min. The mass spectra of deethylatrazine gives a molecular ion at m/e 187, a base peak at m/e 172 ($M^+ - CH_3$) and an ion at m/e 145 ($M^+ - CH_2CH{=}CH_2$). The mass spectrum of this compound (B on Figure 2) found in the brain chloroform extract was identical to the mass spectrum of deethylatrazine standard (A on Figure 2), respectively.

Our data suggest that dealkylated biodegradation products of atrazine or unchanged atrazine are mainly present in the liver, kidney and brain of the atrazine treated rats . The established and possible pathways of atrazine in the rat, as a model for atrazine biodegradation in mammals, is presented in Figure 3. The

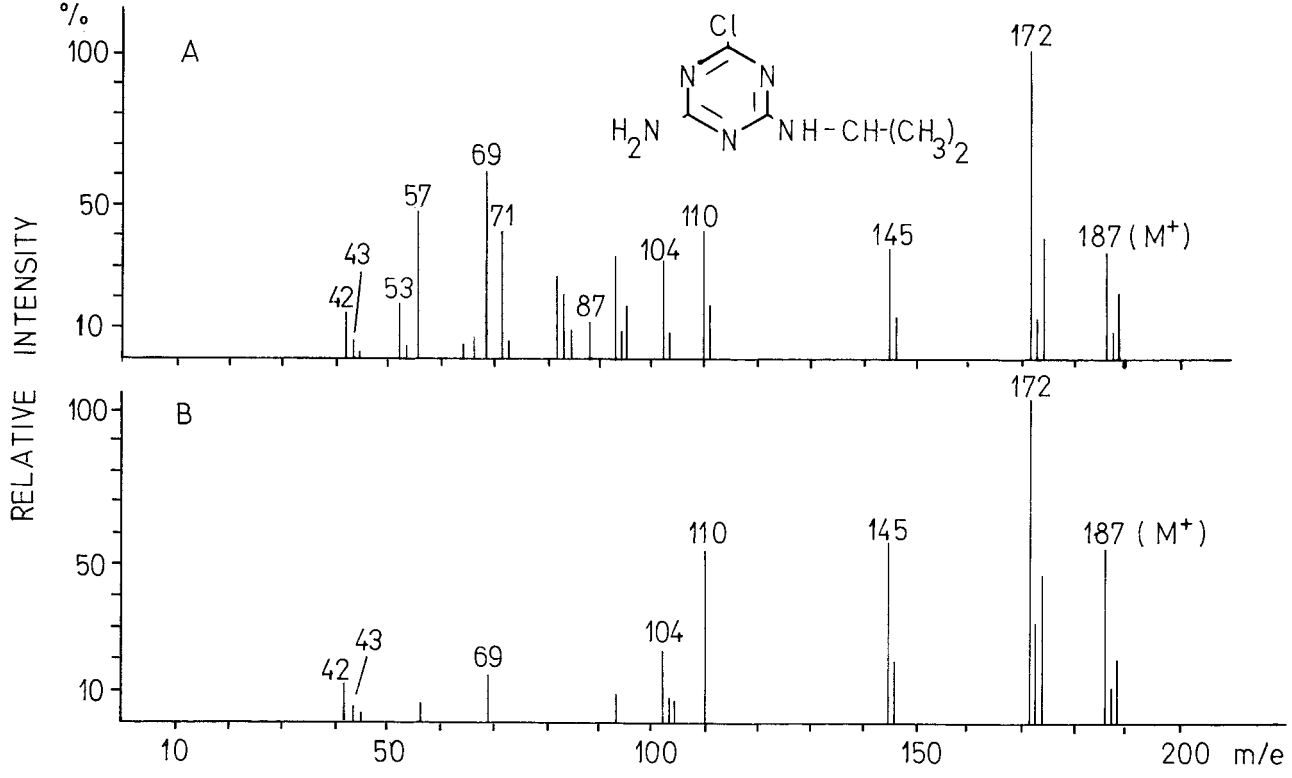

Figure 2. Mass spectra of deethylatrazine (2-chloro-4-amino- 6-izopropyl-amino-s-triazine); (A) standard and (B) chloroform extract of the brain from rat treated with atrazine.

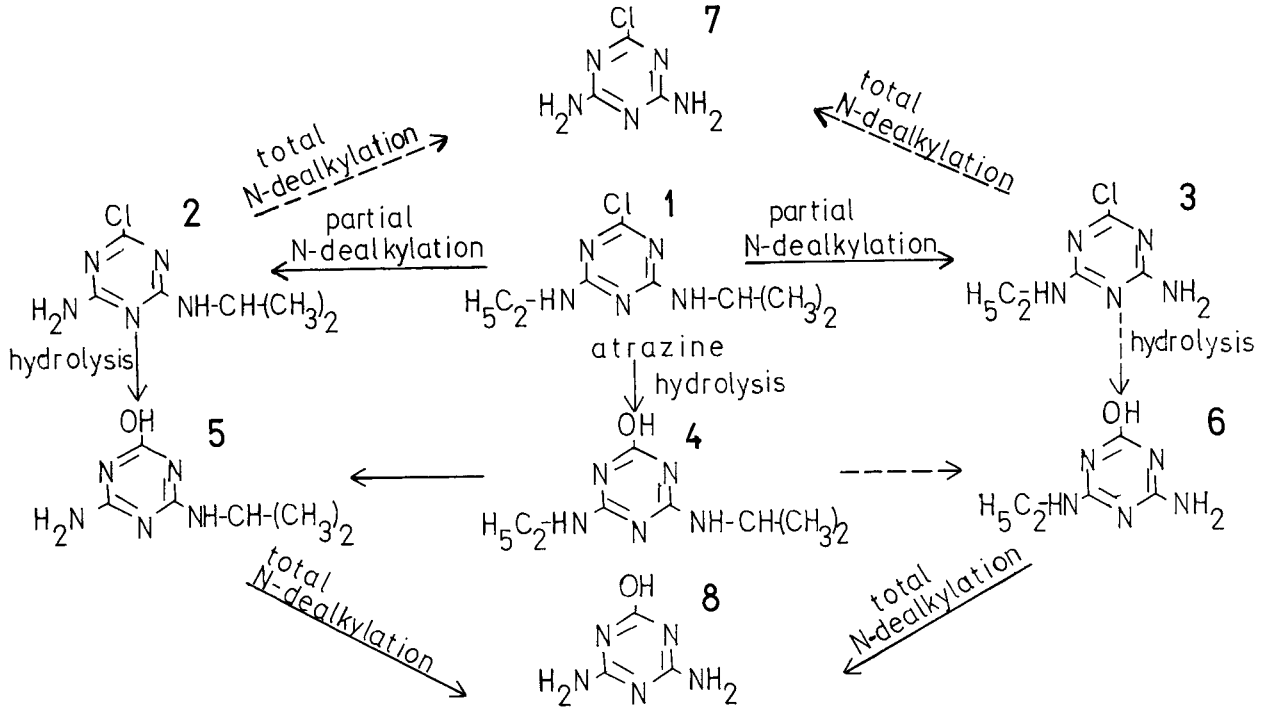

Figure 3. Atrazine biodegradation in rat male tissues after treatment with atrazine (1); deethylatrazine (2), deisopropylatrazine (3), hydroxyatrazine (4), hydroxy-deethylatrazine (5), hydroxy-deisopropylatrazine (6), 2-chloro-diaminoatrazine (7), hydroxy-diamino-atrazine (8).
_____ established pathway, -------- possible pathway.

partial and total N-dealkylation of lateral alkylamino groups in the position 4 and 6 and hydroxylation in position 2 of s-triazine ring is the established way of atrazine biodegradation in the rat. The main atrazine pathways in chicken tissues, the brain was not studied, were N-dealkylation and predominantly hydroxylation, because residues of hydroxy metabolites were detected in higher quantities versus 2-chloro-metabolites (Khan and Foster 1975). The present data suggest that the dominant ways of atrazine biodegradation in the rat are partial N-dealkylation followed by hydroxylation (Figure 3) with the possible total N-dealkylation of atrazine.

In addition to our previous study concerning the atrazine influence on the crucial biochemical processes in the brain (Kniewald et al. 1979), our studies on the influence of the identified atrazine metabolite - deethylatrazine in the brain, in comparison to atrazine, suggested the importance of determining the toxic influence of biodegradable pesticide products on the hormonal balance at the neuroendocrine level in mammals (Kniewald et al. 1987).

Acknowledgments. Supported in part by the Self-Managed Community of Interest for Scientific Research of SR Croatia and Yugoslav-USA Joint Fund for S&T Cooperation Grant No PN 649.

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Received August 9, 1988; accepted December 5, 1988.