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Subacute Atrazine Treatment Effects on Rat Renal Functions

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Weed control by means of herbicides is one of the major practices in modern agriculture. In particular S-triazines herbicides are widely used and among them, atrazine (2-chloro-4-ethylamine-6-isopropylamine-s-triazine) is the most commoly used as a selective herbicide for the control of annual grasses and broad-leaf weeds in corn and sorghum. Consequently, the residues of these products are found in these cereals (Norris and Fong 1983) leading to the constant ingestion of residual quantities of the chemicals, posing a hazardous problem for the health of man and animals.

The atrazine effects in vegetables are well known (Jones and Winchell 1984). Photosynthetic apparatus is the main target of atrazine action (Esser 1975), but like other inhibiting photosynthetic herbicides, atrazine also inhib its RNA, protein and lipid synthesis (Ashon et al 1977). However, the effects in mammals are still largely unknown. Analysis of atrazine residues, effected by Bakke et al (1972) in rats, shown that the kidney, the principal elim ination route of this pesticide, was one of the organs with the highest levels of atrazine.

Hence, the present study was undertaken to evaluate the toxic effects of atrazine on rat renal functions.

MATERIALS AND METHODS

Male albino rats (Wistar strain) weighing 240–280 g were used. Animals were maintained on a standard rat pellet diet and had free access to water. There were four experimental groups with ten animals in each one. Each group

of treated animals daily received 10, 20,40 and 60 mg of atrazine per 100 g of body weigh respectively for fourteen days. Doses were administered with arabic gum by oral tube. Animals in the control group received arabic gum only.

The animals were daily housed in a metabolism cage to collect the urine. The sodium, potassium and chloride levels of the urine were determined daily. Creatinine and albumin levels were also analysed in the urine. At the end of the experiment, the animals were fasted overnight and sacrificed under light anesthesia. Previously, blood was drawn for determining creatinine, lactate dehydrogenase and α-hydroxybutyrate dehydrogenase levels in serum.

Creatinine was assayed by the colorimetric method described by Jaffe (1896). Albumin was determined as described by Rennie (1975). The activities of lactate dehydrogenase and α-hydroxybutirate dehydrogenase were assayed as described by Wroblewnsky (1958) and Rosalky (1963) respectively. The sodium and potassium levels were determined by emission flame photometry and chloride level by the potenciometric method.

All chemicals used were of analytical grade. The creatinine, lactate dehydrogenase and α-hydroxybutirate dehydrogenase kits were from Boehringer-Mannheim.

Data shown in this study were statistically evaluated by Student's t-test.

RESULTS AND DISCUSSION

Renal excretion of pesticides is one of the common elimination routes of pesticides, but little information is available about the interactions of these toxics with the renal function. Atrazine is accumulated by the rat kidney as described by Bakke et al (1972) who also demonstrated that the atrazine is primarily excreted via the kidney in the rat, and a minor atrazine excretion occurs via the bile. In the present study, the effects of acute atrazine exposure on rat renal functions were investigated.

The results presented in Table 1 show that the subacute atrazine exposure in rats significatively increased the elimination of sodium, potassium and chloride. A similar

Table 1 Effects of subacute exposure on renal uptake of electrolytes in rats

	sodium	potassium	chloride
	meq/Kg body wt./24 h		
Control	0.27±0.02	0.53±0.04	0.46±0.01
Group 1 (10 mg)	1.00±0.05	0.71±0.06	1.02±0.04
Group 2 (20 mg)	1.41±0.06	1.35±0.04	1.89±0.07
Group 3 (40 mg)	2.26±0.05	1.75±0.07	2.44±0.32
Group 4 (60 mg)	2.78±0.07	2.45±0.07	2.83±0.13

Values are mean ± SD

increase in the eliminations of these electrolytes was also observed in persons occupationally exposed to pesticides (Morgan and Roan 1969), and in rats after chronic exposure to chloro-organic derivatives (Reuber 1980).

The anomalies observed in the increase of electrolyte levels in urine could be attributed to an alteration in the reabsorption mechanism that occurs in the renal tubules. It has been documented that modifications in the process of absorption cause a considerable increase of electrolytes in urine, and they are normaly described in experimental nephritis (López-Campos et al 1978).

The disappearance of creatinine is used as an approximate index of glomerular filtration rate.Marked changes in renal blood flow are also reflected by changes in glomerular filtration (Kurtzman 1977). Therefore, the effect of atrazine on the disappearance of creatinine from plasma was determined in order to estimate changes in renal hemodynamics subsequent to herbicide intoxication.These results are presented in Table 2. There was a decrease in the levels of creatinine clearance in atrazine exposed rats compared to control, but the decrease is not significant in the group treated with 10 mg of atrazine.

Proteinuria has been considered an important sign of renal disfunction since 1827. In normal conditions only a minor

Table 2 Effect of subacute atrazine exposure on creatinine clearance in rats

Group	Creatinine Clearance (ml/min)	p value
Control	0.46 ± 0.03	
Group 1 (10 mg)	0.41 ± 0.04	NS
Group 2 (20 mg)	0.32 ± 0.02	0.005
Group 3 (40 mg)	0.24 ± 0.03	0.001
Group 4 (60 mg)	0.19 ± 0.02	0.001

Values are mean ± SD
NS = not significant

protein fraction of low molecular weight may easily pass the glomerular membrane, and later, it is reabsorbed by the renal tubules (Pitts 1976). In the present study a significant increase in urine protein level was observed in atrazine exposed animals (Table 3). These results suggest that lesions are produced or on the glomerular membrane with an increase of the permeability for plasma protein or as subsequent decrease in the reabsorption in renal tubules

Table 3 Effect of subacute atrazine exposure on protein levels in urine in rats

Group	Proteins (mg/dl)	p value
Control	1.57 ± 0.15	
Group 1 (10 mg)	2.49 ± 0.27	0.01
Group 2 (20 mg)	5.23 ± 0.25	0.001
Group 3 (40 mg)	7.59 ± 0.21	0.001
Group 4 (60 mg)	9.61 ± 0.28	0.001

Values are mean ± SD

due to a tubular ephitelium lesions. A similar proteinuria was also observed in persons intoxicated with paraquat (Vaziri et al 1979).

Another measure also considered, are the levels of the different activities of the lactate dehydrogenase isoenzymes, since this isoenzymes are relatyvely abundant in the renal tissue, and especially in the proximal convoluted tubules. This study of the different activities is interesting because this product affects the liver also. So, we must distinguish between changes due to the activities of renal origin and those due to activities of the liver. This is possible if we take as a base the following facts: the activities of renal origins show proportions among the different isoenzymes ($LDH_{total}/HBDH = 2$) very similar to those of the normal serum ($LDH_t/HBDH = 2.02$), while the activities originating from the liver are very different demonstrating a high proportion of the LD_5 isoenzyme and very little α-hydroxybutyrate dehydrogenase activity (LD_1). The relation between both activities in liver tissue is close to 10.

In table 4, we can see the activities of the lactate dehydrogenase isoenzyme in the different groups of treated rats. There is a large increase in the total activity and the comparative study with HBDH actibity shows that the relation between the different isoenzymes is practically main

Table 4 Effect of subacute atrazine exposure on the activities of lactate dehydrogenase and α-hydroxibutirate dehydrogenase in rats.

Group	Activity LDH_{total}	Activity HBDH	LDH/HBDH
Control	1.420 ± 17	703 ± 73	2.02
Group 1 (10 mg)	1.761 ± 66	874 ± 34	2.01
Group 2 (20 mg)	2.277 ± 43	1.146 ± 18	1.98
Group 3 (40 mg)	2.835 ± 52	1.364 ± 52	2.07
Group 4 (60 mg)	3.066 ± 31	1.452 ± 57	2.11

Enzymatic activities U.I./l
Temperature 37ºC.

tained in serum. So, we can deduce that almost the whole of the lactate dehydrogenase activity increase was due to the enzyme of renal origin, because in the other case the LDH_t/HBDH ratio would have augmented clearly.

The results presented in this communications indicate that the exposure to this pesticide may produce alterations in renal functions. Since the normal route of atrazine elimination is through the kidney, the nephrotoxic properties of the herbicide may affect not only its own excretion but also increase its own toxicity in the kidney and other organs.

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