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Toxic Effects of Acrylamide in a Freshwater Fish, *Heteropneustes fossilis*

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Ecological studies are now greatly concerned with toxic effects of xenobiotics on aquatic fauna. Fish, an important source of protein for humans form the main fauna of the aquatic ecosystem. They have been observed to serve as indicators of water pollution (Doudoroff et al. 1951; APHA 1975). Acrylamide (ACR) a neurotoxic vinyl monomer (Spencer and Schaumburg 1974) has been found to be present in our aquatic and terrestrial environment (Igisu et al. 1975) due to its widespread applications (NIOSH 1976; Tilson 1981). Polyacrylamides used as soil stabilizers and water flocculents contain unreacted acrylamide which contaminates water bodies and affects aquatic organisms (Edwards 1975; Brown et al. 1982; Prasad 1982).

It has been shown that acrylamide is a cumulative neurotoxic agent and can conjugate with glutathione enzymically and nonenzymically. The enzymic conjugation is catalysed by a cytosolic enzyme, glutathione S-transferase (Dixit et al. 1980). Laboratory animals exposed to acrylamide exhibited a decrease in glutathione level and in the activity of glutathione-S-transferase of brain and liver (Dixit et al. 1981a,b; Mukhtar et al. 1981). Various electrophiles are reported to be detoxified as they form conjugates with glutathione (Reed and Beatty 1981). The inhibition of glutathione-S-transferase by acrylamide, which catalyzes its conjugation with glutathione, may lead to the accumulation of the monomer and its enhanced neurotoxicity. At present little is known about the toxic effects of acrylamide in fishes. The present study deals with the determination of median lethal concentration (LC50) of acrylamide and its effect on the hepatic glutathione and glutathione-S-transferase levels of the fresh water fish, Heteropneustes fossilis.

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MATERIALS AND METHODS

Live healthy fish Heteropneustes fossilis obtained from the river Gomti at Lucknow, were transported to the laboratory in natural water and treated with potassium permanganate solution (2 mg/L) to remove ectoparasites, fungi etc. The fish were allowed to rest for 96-h in large glass aquaria containing dechlorinated tap water to acclimatise them to laboratory environment. Fish of weight (80-110 g) and length (16-20 cm), were selected for the experiment. The fishes were starved for 48-h before the experiment. Fishes from the same group kept under normal conditions were used as controls. Static bioassay tests were used for the experiment (Doudoroff et al. 1951; APHA 1975). The mortality data were analysed according to the method of Finney (1952) for calculating LC50 values and their 95% confidence limits.

The fish were divided into two groups:

Group I	(A)	:	Controls, kept in water only.
	(B)	:	1/5 of 24-h LC50 for 24-h only.
	(C)	:	1/3 of 24-h LC50 for 24-h only.
Group II	(A)	:	Controls, injected i.p. with 0.15 M NaCl.
	(B)	:	Acrylamide (10 mg/kg bw) in 0.15 M NaCl.
	(C)	:	Acrylamide (50 mg/kg bw) in 0.15 M NaCl.
	(D)	:	Acrylamide (100 mg/kg bw) in 0.15 M NaCl.

Estimation of glutathione:Fish were taken out from the test solution or water after 24-h. They were washed with distilled water and their liver was dissected out and kept in 0.7% ice cold saline. Liver was homogenized in 4 volumes of ice cold 0.1 M sodium phosphate buffer pH 7.4 containing 0.15 M potassium chloride and the protein precipitated by the addition of equal volume of 10% TCA. Glutathione was estimated by the method of Ellman (1959).

Assay of glutathione-S-transferase activity:The supernatants of liver homogenates obtained after centrifugation at 9,000xg for 15 min were further centrifuged at 104,000xg for 1 h in a MSE Superspeed 75 Ultracentrifuge to recover organelle free cytosol, which was used as the enzyme source. Glutathione-S-transferase activity towards 1-chloro-2, 4-dinitrobenzene as substrate was determined by the method of Habig et al. (1974). Protein was determined by the method of

Lowry et al. (1951) using bovine serum albumin as reference standard.

RESULTS AND DISCUSSION

The physicochemical characteristics of water observed throughout the experiment, are summarized in Table 1.

Table 1. Physico-chemical characteristics of test water

Test	Temperature (°C)	Dissolved Oxygen (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH
Acrylamide	20-25	6.25±0.35	129±1.4	114±8.4	7.7±0.28

Data represents mean ± S.D. of five samples.

The 24- and 48-h LC50 values of acrylamide for *H. fossilis* were found to be 104.13 and 86.81 mg/L (Table 2). After 10-h of exposure to 24-h LC50 the fish showed, signs of excitation, irritability, gulping of air at surface and erratic swimming. Prior to death, the tail and fins appeared to be paralysed. The dorsal fin had collapsed and there appeared some loss of sensitivity to sound and body orientation. The no effect level of acrylamide was 15 mg/L for a period of 48-h.

Table 2. LC50 values of acrylamide to *H. fossilis*

Time (h)	Number of test animals	LC50±S.E. (mg/L)	95% confidence limits (mg/L)
24	180	104.13±1.7	100.51 - 111.61
48	180	86.81±1.0	80.06 - 94.15

Exposure of *H. fossilis* to 1/3 of 24-h LC50 (34.7 mg/L) for a period of 24-h only resulted in significant fall of hepatic glutathione and glutathione-S-transferase levels (Table 3). However, exposure to 1/5 of 24-h LC50 (20.8 mg/L) for a period of 24-h only did not lead to any significant change, as compared to controls (Table 3) A dose dependent decrease of glutathione and glutathione-S-transferase activity was noted in the fishes after i.p. administration of acrylamide (Table 4).

Table 3. Effect of acrylamide exposure on hepatic glutathione content and glutathione-S-transferase activity of H. fossilis

Group	Glutathione (umol/g fresh weight)	Glutathione-S-transferase (nmol conjugate/ min/mg protein)
Control	3.00 ± 0.15	388 ± 9
Treated (1/5 LC50)	2.88 ± 0.11	371 ± 10
Treated (1/3 LC50)	2.32 ± 0.10*	293 ± 10**

Data represent mean ± S.E. of 3 values.
*P < 0,02; **P < 0.01
Five fish were taken for each set and exposed to 1/5 and 1/3 LC50 (24-h) for a period of 24-h only.

Table 4. Effect of a single i.p. dose of acrylamide on hepatic glutathione content and glutathione-S-transferase activity of H.fossilis

Treatment (mg ACR/kg bw)	Glutathione (umol/g fresh wt)	Glutathione-S-transferase (nmol conjugate/ min/mg protein)
Control	3.06 ± 0.16	378 ± 10
10	2.76 ± 0.10	287 ± 12*
50	2.01 ± 0.05*	188 ± 7*
100	1.05 ± 0.03*	114 ± 6*

Data represents mean ± S.E. of five animals sacrificed 24-h after injection.
*P < 0.001; when compared to control (Student's 't' test).

Although significant quantities of acrylamide have been detected in various water ways, its LC50 concentrations are not available. Several workers have reported different LC50 values for different fishes. Morris and Penzenstadler (1978) have observed that 124 mg ACR/L was the 96-h LC50 of acrylamide, while

the no effect level was 56 mg ACR/L for fathead minnow. Edwards (1975) reported that 50 mg ACR/L had no effect on goldfish upto 30 days. Direct comparison of these values is not possible as different species of fishes and routes of exposure have been used by the workers. The present study reveals 24- and 48-h LC50 values of acrylamide for H. fossilis to be 104 and 86 mg/L while no effect was observed at 15 mg/L.

The neurotoxic effects of acrylamide have been described in humans and some animals (Tilson 1981). Exposure to 50 mg/kg/day of acrylamide for a period of 8 days caused hind limb paralysis in rats (Dixit et al.1981a). In the present observations signs of neurotoxicity in fishes were noted in the form of collapse of fins and loss of tail movement, after exposure of H. fossilis for 24-h to LC50 of acrylamide. However, at 1/5 of 24-h LC50 these signs and symptoms were not apparent but interestingly the fishes exposed to this concentration showed decreased levels of glutathione and glutathione -S-transferase. It thus means that a longer period of interval is required for the manifestation of neuromuscular symptoms. Acrylamide is a cumulative neurotoxin (McCollister et al. 1964; Kaplan et al. 1973) and activity of glutathione-S-transferase which plays a critical role in detoxification is reported to be depressed in mammals before the development of the signs of paralysis (Dixit et al. 1981a). It is quite likely that gradual accumulation of acrylamide may also take place in the aquatic organisms by similar mechanisms, resulting in neurotoxicity and ultimate fatality.

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