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Environmental Contamination and Toxicology

Ammonia Metabolism in Freshwater Teleost, *Clarias batrachus* (Linn.) on Exposure to Trichlorfon

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The indiscriminate and extensive use of insecticides to protect crops poses a serious threat to humans and the surrounding environment. The pesticides which are liberated into aquatic environment have a deterious effect on fish and subsequently to man (Metelev et al. 1983). Kilgore and Mingyuli (1975) emphasized that the concentration of pesticide residues was found to be more in aquatic ecosystem rather than the terrestrial ecosystem. The freshwater fishes constitute an important link in food chain and their pollution by insecticides imbalances the aquatic ecosystem. Trichlorfon, is an organo phosphate compound being widely used in India to control agricultural pests. The sublethal exposure of OP compounds have produced several changes in energy metabolism of fish (Rani et al. 1989). The freshwater teleost, Clarias batrachus, is an edible fish and is considered to be economically important in pisiculture industry. The present study was undertaken to identify the different shifts involved in ammonia metabolism in liver, brain, and gill tissues of the insecticide exposed fish.

MATERIALS AND METHODS

Healthy freshwater fish, C.batrachus, weighing around 20±2g and measuring 18±4 cm in length were collected and acclimatized for 10 days to laboratory conditions in a cement tank under running tap water. The fish were fed ad libitum with groundnut cake and white of the egg, but starved 1 d prior to experiment. Technical grade (90% purity) sample of trichlorfon (O,O-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate) was used in sublethal concentration. The LC50 value of trichlorfon at 48 hr calculated by the method of Finney (1964) was found to be 6 mg/L. The fish were exposed to a sublethal concentration of 2 mg/L for 48 hr and 96 hr. The control fish were maintained with 0.1 mL acetone since it was used for dissolving trichlorfon. After removing the fish at stipulated time interval, liver, brain and gill tissues were quickly isolated and kept in ice-jacketed petri-dishes for biochemical estimations. The total protein content was determined by Folin-phenol method (Lowry et al. 1951),

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Table 1. Different test conditions and concentrations for enzyme assayed at temperature 29°C.

Enzyme	pH	Substrate concentration	Enzyme concentration	Buffer concentration
Acidic Protease	3.0	10 mg (Denatured Hb)	35 mg	100 µmoles (Citrate buffer)
Neutral Protease	7.0	10 mg (Denatured Hb)	35 mg	100 µmoles (Phosphate buffer)
Alkaline Protease	9.0	10 mg (Denatured Hb)	35 mg	100 µmoles (Biocarbonate buffer)
GDH	7.4	50 µmoles (Sodium Glutamate)	30 mg	100 µmoles (Phosphate buffer)
AMP Deaminase	6.8	10 µmoles (AMP)	30 mg	50 µmoles (Succinate buffer)
Adenosine Deaminase	6.5	30 µmoles (Adenosine)	30 mg	50 µmoles (Phosphate buffer)
Glutaminase	4.9	40 µmoles (Glutamine)	25 mg	50 µmoles (Sodium citrate buffer)

free amino acids by ninhydrin method (Moore and Stein 1954), ammonia by nesslerization (Bergmeyer 1965), glutamine by acid hydrolysis method described by Colowick and Kaplan (1967) and urea by diacetyl monoxime method (Natelson 1971). The different test conditions of the enzyme assays are given in Table 1.

For assaying acidic, neutral and alkaline proteases, 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. A clear cell free supernatent was used for the assay of proteases by the method of Davis and Smith (1955). Acid protease activity was assayed at pH 3.0 using citrate buffer, neutral protease at pH 7.0 using phosphate buffer and alkaline protease activity at pH 9.0 with carbonate-bicarbonate buffer and 10 mg of denatured haemoglobin protein was used as substrate.

To assay glutamate dehydrogenase (GDH), 10% tissue homogenates were prepared in ice cold 0.25 M sucrose solution and centrifuged at 5000 rpm for 15 minutes. A clear cell free supernatent was used for the assay of GDH by the method of Lee and Lardy

.Table 2. Levels of total proteins, free amino acids, ammonia glutamine and urea in tissues of control and trichlorfon expose Clarias batrachus.

Parameters	Tissues	Control	Trichlorfon treated fish	
			48 h	96 h
Total proteins (mg/100 mg wet wt of tissue)	Liver	18.90±0.33	11.60±0.31 PC=-38.62	11.19±0.19 PC=-36.98
	Brain	12.98±0.16	8.16±0.04 PC=-37.13	8.64±0.0(PC=-33.43
	Gill	7.99±0.13	6.01±0.28 PC=-24.78	6.22±0.14 PC=-22.15
Free Amino acids (µmol/ gm wet wt of tissue)	Liver	815±40.0	1113±40.0 PC=+36.56	1086±90.0 PC=+33.25
	Brain	589±50.0	789±80.0 PC=+33.96	728±40.0 PC=+23.60
	Gill	488±50.0	680±40.0 PC=+39.34	646±20.0 PC=+32.38
Ammonia (µmol/gm wet wt of tissue)	Liver	19.92±2.08	28.96±2.10 PC=+45.38	25.63±3.23 PC=+28.66
	Brain	12.66±0.37	8.11±0.04 PC=-35.94	8.89±0.04 PC=-29.78
	Gill	8.09±0.99	5.64±0.77 PC=-30.66	5.81±0.04 PC=-28.18
Glutamine (µmol/gm wet wt of tissue)	Liver	136.85±15.32	198±16.07 PC=+46.50	186±16.98 PC=+37.83
	Brain	101.84±5.42	131.37±9.16 PC=+29.00	126.80±9.3 PC=+24.51
	Gill	33.59±4.47	48.05±4.77 PC=+43.05	42.53±4.36 PC=+26.62
Urea (µmol/gm wet wt of tissue)	Liver	13.56±0.94	16.96±0.96 PC=+25.07	16.05±1.99 PC=+18.36
	Brain	10.17±0.93	13.21±0.88 PC=+29.89	12.06±1.88 PC=+18.58
	Gill	5.75±0.78	3.27±0.11 PC=-43.13	3.42±0.22 PC=-40.52

Each value is mean ± SD of 6 observations. All values are statistically significant from control at 1% level ($P<0.01$). PC denote percent change over control. * Not significant.

(1965). In addition to substrate, buffer and enzyme, 0.1 µ mole of NAD^+ and 2 µ moles of INT were added to the reaction mixture

For the assay of adenosine monophosphate (AMP) deaminase 10% tissue homogenates were prepared in ice cold distilled wate and centrifuged at 3000 rpm for 15 minutes. The clear supernaten was used for the assay of enzyme (Weil-Malherbe and Gree

Table 3. Activities of proteases (acid, neutral and alkaline) in tissues of control and trichlorfon exposed Clarias batrachus.

Parameters	Tissues	Control	Trichlorfon treated fish	
			48 h	96 h
Acid protease (µmol of tyrosine equivalents/ mg protein/h)	Liver	1.36±0.20	1.81±0.22 PC=+33.09	1.67±0.19 PC=+22.79
	Brain	1.19±0.18	1.58±0.22 PC=+32.77	1.45±0.20 PC=+21.85
	Gill	0.63±0.06	0.83±0.11 PC=+31.75	0.77±0.10 PC=+22.22
Neutral protease (µmol of tyrosine equivalent/ mg protein/h)	Liver	1.84±0.20	2.46±0.28 PC=+33.70	2.31±0.25 PC=+25.54
	Brain	1.50±0.20	1.98±0.21 PC=+32.00	1.83±0.20 PC=+22.00
	Gill	0.85±0.08	1.04±0.10 PC=+22.35	1.02±0.09 PC=+20.00
Alkaline protease (µmol of tyrosine equivalents/ mg protein/h)	Liver	1.52±0.20	2.04±0.25 PC=+34.21	1.89±0.20 PC=+24.34
	Brain	1.26±0.18	1.69±0.20 PC=+34.13	1.59±0.18 PC=+26.19
	Gill	0.75±0.08	0.96±0.09 PC=+28.00	0.90±0.08 PC=+20.00

Each value is mean ±S.D. of 6 observations. All values are statistically significant from controls at 1% level ($P < 0.01$). PC denotes percent change over control.

1955 modified by Wagelin et al. 1978). The reaction mixture contained buffer, substate, enzyme, 5 µmoles ATP, 2 µmoles $MgCl_2$ and 1 µmole EDTA.

For assaying adenosine deaminase activity by the method of Agarwal and Parks (1978), 10% tissue homogenats were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes to obtain a clear supernatent which was used as enzyme source.

To assay the activity of glutaminase by the method of Alton-Meister (1955), 10% tissue homogenates were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 minutes. The supernatent was used as enzyme source.

RESULTS AND DISCUSSION

Table 2 shows that the toxic stress of trichlorfon to fish has led to the decrease in tissue protein fractions. Probably proteins may be utilized to meet the energy demands during OP toxicity. Liver seems to be highly affected in the exposed fish since it is the most important metabolic organ. The free

Table 4. Activities of glutamate dehydrogenase, AMP deaminase, adenosine deaminase and glutaminase in tissue of control and trichlorfon exposed Clarias batrachus.

Parameters	Tissues	Control	Trichlorfon exposed fish 48 h	96 h
Glutamate dehydrogenase (μmole of formazan formed/mg protein/h)	Liver	0.65±0.080	0.90±0.01 PC=+38.46	0.84±0.09 PC=+29.23
	Brain	0.21±0.050	0.29±0.01 PC=+38.09	0.25±0.01 PC=+23.81
	Gill	0.07±0.009	0.12±0.01 PC=+71.42	0.10±0.01 PC=+42.86
AMP deaminase (μmole of ammonia formed/mg protein/h)	Liver	0.47±0.018	0.74±0.04 PC=+58.67	0.65±0.04 PC=+40.69
	Brain	0.32±0.016	0.51±0.02 PC=+57.23	0.49±0.03 PC=+51.69
	Gill	0.17±0.014	0.27±0.02 PC=+56.32	0.26±0.02 PC=+50.00
Adenosine deaminase (μmole of ammonia formed/mg protein/h)	Liver	0.16±0.020	0.26±0.02 PC=+59.04	0.24±0.02 PC=+56.63
	Brain	0.09±0.02	0.16±0.01 PC=+71.58	0.15±0.01 PC=+66.32
	Gill	0.07±0.010	0.12±0.01 PC=+62.03	0.10±0.01 PC=+56.96
Glutaminase (μmole of ammonia released/mg protein/h)	Liver	1.49±0.120	2.23±0.16 PC=+49.66	2.18±0.18 PC=+46.31
	Brain	1.26±0.100	2.01±0.15 PC=+59.52	1.95±0.12 PC=+54.76
	Gill	0.92±0.100	1.46±0.10 PC=+58.70	1.40±0.08 PC=+52.17

Each value is mean ±S.D. of 6 observations. All values are statistically significant from controls at 1% level ($P < 0.01$). PC denotes percent change over control.

amino acid pool was increased in the tissues of fish during exposure to trichlorfon. The enhanced free amino acid levels may be channelled for energy synthesis and other metabolic reactions (Kovacs and Seglen 1981). The ammonia levels in tissues other than liver were decreased. The increased level of ammonia in liver tissue could be due to the fact that it is the site for ammonia production, utilization and detoxification by converting ammonia to glutamine and urea (Goldstein et al. 1982). The decrease in ammonia levels in brain and gill tissues suggest the removal and excretion of ammonia from these two tissues by the process of diffusion. The decrease in brain ammonia might be its conversion to non-toxic glutamine as it is evident from increased levels of glutamine. The three tissues recorded an increase in glutamine levels. The formation of glutamine in

brain is considered to be an important method for the removal of toxic ammonia (Dabrowsk and Wlasow 1986). An increase in urea levels were observed which were significant at 48 hr but slightly affected at 96 hr of exposure to trichlorfon. The high levels of urea in liver suggests the detoxification of excess ammonia to urea in this tissue.

Table 3 and 4 show the enzyme activities of acid, neutral and alkaline proteases, glutamate dehydrogenase, AMP and adenosine deaminases and lastly glutaminase which were found to be increased in liver, brain and gill tissues of the fish exposed to trichlorfon for 48 hr and 96 hr. The increased activity of acid, neutral and alkaline proteases indicates increased protein degradation to yield excess energy to overcome the toxic impact. The enhancement in the activity of GDH due to trichlorfon toxicity was observed in liver, brain, and gill tissues. This indicates higher oxidation of amino acids to combat the toxic effect of OP compound. The higher activity of GDH may result in efficient operation of oxidative deamination under toxic impact of trichlorfon. A significant increase was noted in both AMP and adenosine deaminase activities in tissues of exposed fish. This may be due to stimulation of proteases which modulate the AMP deaminase activity (Raffin 1981). The enhanced glutaminase activity in toxic fish suggests increased production of ammonia by the synthesis of nucleotides through the activity of glutaminase (Lowenstein 1972). The elevation in glutaminase activity can be regarded as an adaptive measure in ammoniogenesis of trichlorfon exposed fish. The present study concludes that trichlorfon, in sublethal concentration alters tissue ammonia metabolism in C.batrachus. As a consequence of trichlorfon toxicity, the fish shifts to alternate methods of metabolism to overcome the toxic stress and maintain its survival in the polluted environment.

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