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In Vivo Alteration in Protein Metabolism by Subacute Carbofuran Intoxication in the Freshwater Teleost, *Clarias batrachus*

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Carbamate pesticides, due to their lower persistence in nature, are extensively used to control a variety of agricultural and greenhouse pests. However, excessive use of these pesticides has increased disruption of ecological balances by adversely affecting the ecological status of the environment, particularly to aquatic species and several other non-target organisms (Sastry and Siddiqui 1982; Trotter et al. 1991; Singh et al. 2003)

Carbofuran (2, 3-dihydro – 2, 2-dimethyl –7 benzofuranyl methyl carbamate) is an organo-carbamate pesticide widely used as an insecticide to prevent damage due to crop pests. The accumulation of very low concentrations of carbofuran produced hypercholinergic activity of central and peripheral organs (Gupta and Kadel 1989) by inhibiting acetylcholinesterase at synapses in the brain and neuromuscular junctions (Yadav et al. 1998). Carbofuran has been shown to be very toxic to fish when entering water bodies after its application to crop plants (Caro et al. 1973; Parkin 1994; Saglio et al. 1996).

Earlier reports from this laboratory have shown that sub-acute concentrations of carbofuran ($1/10^{th}$ and $1/20^{th}$ of LC50; Singh et al. 2003) induced drastic alterations in fish behavior (Kumari et al. 1997), activities of acetyl cholinesterase (Yadav et al. 1998) and lactate dehydrogenase (Singh and Sharma 1998) in different tissues of Clarias batrachus. Some reports (Mukhopadhyay et al. 1982; Begum 2004) are available concerning the effect of sub-acute concentration of carbofuran on protein metabolism in different tissues of C. batrachus. The present communication deals with the effect of carbofuran on the activities of enzymes involved in protein metabolism in different tissues of C. batrachus exposed to sub-acute concentrations of carbofuran for two different incubation periods, i.e. 96 hr and 15 days.

MATERIALS AND METHODS

Healthy freshwater fish, Clarias batrachus (length 10 –12 cm, weight 25 – 30 g, and mixed same age group) were obtained from a fish-breeding farm at Faizabad, India. The fish were treated with potassium permanganate solution (0.5%, w/v) for five min to remove any dermal adherent. The fish were acclimatized in

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The free amino acid content in various tissues of carbofuran- treated fish was significantly increased as compared to that of control fish tissues. The level of amino acids increased with increasing exposure period and increasing carbofuran concentration in fish tissues.

The increase in free amino acid content was found to be greatest in fish brain followed by gill, muscle, and liver after 96 hr exposure at both concentrations of carbofuran (0.01 and 0.02 mg/L). The amino acid concentration was further increased in all tissues of carbofuran treated (0.01 mg/L) fish for 15 days in similar manner. The percent increase in free amino acid concentrations at 15 days was recorded as 27, 24, 13, 13, and 11 in fish brain, gill, liver kidney and muscle respectively. This level further increased when the higher concentration (0.02 mg/L) of carbofuran was added in the incubation medium (Table 1).

Table 1. Free amino acids (mg/g/wet wt) in the tissues of *C. batrachus* after different exposure periods to carbofuran.

Tissue	96 hr exposure period			15 days exposure period		
	Carbofuran mg/L			Carbofuran mg/L		
	0.00	0.01	0.02	0.00	0.01	0.02
Liver	9.84 ±0.02	10.05±0.03 (+2.13)	10.21±0.05 (+3.76)	9.72 ±0.05	10.94±0.07* (+12.55)	11.32±0.09* (+16.46)
Muscle	7.49 ±0.09	7.95±0.01 (+6.14)	8.29±0.07 (+10.68)	7.54 ±0.07	8.97±0.02 (+10.96)	9.65±0.01* (+27.98)
Gill	6.29 ±0.02	6.84±0.07 (8.74)	7.13±0.01 (13.35)	6.31 ±0.04	7.84±0.03* (+ 24.24)	8.18±0.09** (+ 29.63)
Brain	4.39 ±0.06	4.91±0.04 (+ 11.84)	5.11±0.02* (+16.40)	4.41 ±0.05	5.62±0.02** (+ 27.43)	6.27±0.03** (+42.17)
Kidney	5.32 ±0.04	5.67±0.03 (+5.7)	5.89±0.02 (+10.7)	5.41 ±0.03	6.19±0.01 (+12.6)	6.41±0.02* (+18.5)

Each value represents the mean ± S.E.M. of five different observations. Values in parenthesis are percent change over control. Significantly different at *$p<0.05$; **$p<0.01$; (Student's t-test)

The activity of transaminases (GOT and GPT) increased in tissues of carbofuran-treated fish for the 96 hr and 15 days incubation periods, but the enzyme activity was only significantly increased with 0.02 mg/L of carbofuran for the prolonged treatment period (15 days). Among the tissues, kidney appeared to be most highly affected followed by gill, muscle, brain and liver. The percent increase in the activity of GOT was 44, 38, 35, 33 and 26, respectively, at 0.02 mg/L carbofuran treatment for 15 days (Table 2).

GPT activity also increased in carbofuran treated fish and the data are presented in Table 3. At both carbofuran concentrations and treatment durations, the gills of the fish were maximally affected followed by kidney, muscle, brain and liver; the values of percent increase in these tissues at 0.02 mg/L of carbofuran concentration for 15 days exposure period were 55, 47, 46, 43 and 40 respectively (Table 3).

Table 2. Effect of carbofuran on the activity of glutamate oxaloacetate transaminase (GOT) in different tissues of *C. batrachus* exposed for 96 hr. and 15 days.

Tissue	96 hr exposure period			15 days exposure period		
	Carbofuran mg/L			Carbofuran mg/L		
	0.00	0.01	0.02	0.00	0.01	0.02
Liver	18.21 ±1.0	20.12±1.4 (+10.48)	21.19±0.5 (+16.36.)	19.13 ±0.1	22.31±0.8 (+16.62)	24.16±0.4** (+26.29)
Brain	9.74 ±1.0	10.54±0.9 (+8.21)	11.76±0.7 (+20.73)	9.41 ±0.6	11.92±0.6 (+26.67)	12.48±0.4* (+32.62)
Muscle	11.43 ±06	12.99±0.6 (+13.64)	13.44±0.9 (+17.58)	11.21 ±0.4	13.87±0.3* (+ 23.72)	15.11±0.2** (+ 34.79)
Gill	8.32 ±0.5	9.63±0.8 (+15.75)	10.39±0.4* (+24.82)	9.0 ± 0.3	11.14±0.1* (+ 23.77)	12.43±0.1** (+ 38.11)
Kidney	15.37 ±05	17.93±0.8 (+ 16.65)	19.89±0.5* (+29.41)	15.93 ± 0.1	20.54±0.1** (+ 28.93)	22.93±0.2*** (+43.94)

Unit expressed as µmoles pyruvate released/min/mg/protein. Each value represents the mean ± SEM of three different observations. Values in parentheses are percent change over control. Significantly different at *p<0.05; **p<0.01; ***p<0.001 (Student's t-test)

Table 3. Effect of carbofuran on the activity of glutamate pyruvate transaminase (GPT) in different tissues of *C. batrachus* exposed for 96 hr and 15 days.

Tissue	96 hr exposure period			15 days exposure period		
	Carbofuran mg/ L			Carbofuran mg/ L		
	0.00	0.01	0.02	0.00	0.01	0.02
Liver	14.11 ±0.6	16.35±0.8 (+15.87)	17.94±1.0 (+27.14)	15.01 ±0.1	18.31±0.2 (+ 21.98)	20.94±0.1** (+39.51)
Brain	6.13 ±0.2	7.01±1.2 (+14.35)	8.19±0.9 (+33.6)	6.74 ±0.4	8.72±0.8 (+29.37)	9.64±0.4** (+43.02)
Muscle	7.65 ±0.2	9.31±0.3 (+21.69)	10.27±0.7* (+34.2)	8.12 ±0.1	10.93±0.9* (+34.6)	11.89±0.7** * (+46.42)
Gill	6.45 ±0.3	8.15±1.1* (+26.35)	8.90±0.3** (+37.98)	6.93 ±0.3	9.47±0.3** (+36.65)	10.72±0.1** * (+54.68)
Kidney	13.13 ±0.7	16.41±1.2 (+24.98)	17.89±0.3* * (+36.25)	13.87 ±0.2	18.74±0.2* * (+35.11)	20.39±0.2** * (+47.01)

Unit expressed as µmoles pyruvate released /min/mg/protein. Each value represents the mean ± SEM of three different observations values in parentheses are percent change over control. Significantly different at *p<0.05; **p<0.01; ***p<0.001 (Student's t- test)

The activities of AAT and ALAT were monitored in control as well as carbofuran-exposed tissues and the results are presented in Tables 4 and 5, respectively. The activity of AAT in different tissues of control fish was found to

dechlorinated tap water for seven days under natural photoperiod and standard laboratory condition in 50 L glass aquaria (APHA 1991). The fish were fed with flour pellets and ground-dried shrimp; aquaria were cleaned and water was changed every day. Only healthy fish of both sexes were used in the experiment. The physico-chemical characteristics of the water used were temperature (24 ± 2.2 ^{0}C), pH (6.7 ± 0.3), DO (dissolved oxygen, 6.2± 0.4 ppm), alkalinity (96 ± 6.5 ppm), hardness as calcium carbonate (12 ± 7.1 ppm) and electrical conductivity (860 ± 42 um hos).

The LC50 value of carbofuran (98.8 % purity and acetone soluble) for C. batrachus has been determined in our laboratory (0.2 mg/L for 96 hr) (Singh et al. 2003). A stock solution of carbofuran was prepared in acetone and an appropriate volume of carbofuran was added in water (50 L glass aquarium) to give the desired concentration of carbofuran in the medium. Seven fish were transferred to each aquarium and maintained in six groups. Four groups were exposed to sublethal concentrations (0.01 and 0.02 mg/L) of carbofuran for 96-hr and 15 days exposure. The remaining two groups of fish were used as the control. An equal concentration of acetone was maintained in the control as was used for carbofuran treatment.

At the end of the experimental period, the fish were sacrificed and dissected. The tissues of liver, brain, gill, kidney and muscle were thoroughly washed in ice-cold normal saline (0.15 M). Each tissue was minced and a 10% homogenate (w/v) was prepared in cold sucrose solution (0.25 M) by means of a Potter-Elvehjam homogenizer, using a teflon coated pestle under ice–cold conditions. The homogenate was kept in cold for 30 min with intermittent stirring and then centrifuged at 10,000 x g for 15 min in a refrigerated high-speed centrifuge, and the supernatant was collected for assays of enzyme activity.

The activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were measured in cellfree homogenate of each tissue following the method of Reitman and Frankel as described by Bergmeyer (1965). The protein content in different tissues was estimated by the method of Lowery et al (1951), amino acid content by the method of Moore and Stein (1954) and aspartate aminotransferase (AAT) and alanine amino-transferase (ALAT) activity was estimated by the method of Bergmeyer (1965).

Data collected from three to five replicates were statistically analyzed and presented as mean ± S.E.M. Comparisons of the unpaired mean values between the experimental and respective controls were made using unpaired Student's t-test and differences with $P<0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

The free amino acid content in various normal and carbofuran-treated tissues was measured for 96 hr and 15 days of exposure and the data are presented in Table 1.

be maximal in the liver followed by muscle, brain and gill. When the fish were treated with carbofuran (0.01 and 0.02 mg/L) for different exposure periods (96 hr), the activity was found to increase in the following order liver > gill> brain >muscle (Table 4).

Table 4. AAT activity in the tissues of *C. batrachus* after exposure to carbofuran.

Tissue	96 hr exposure period			15 days exposure period		
	Carbofuran mg/ L			Carbofuran mg/ L		
	0.00	0.01	0.02	0.00	0.01	0.02
Liver	9.42± 0.04	10.29±0.02 (+9.23)	10.69±0.05 (+13.48)	9.46 ±0.3	11.02±0.02* (+16.49)	11.90±0.04*** (+25.79)
Muscle	8.23 ±0.02	8.44±0.04 (+2.55)	8.75±0.04 (+6.31)	8.20 ±0.06	9.01±0.02 (+9.87)	9.42±0.04* (+14.87)
Gill	6.42 ±0.03	6.99±0.03 (+8.87)	7.25±0.02 (+12.92)	6.49 ±0.05	7.65±0.06* (+17.87)	7.93±0.05** (+22.18)
Brain	7.45 ±0.02	7.64±0.03 (+2.55)	7.79±0.02 (+4.56)	7.43 ±0.02	7.92±0.02 (+6.59)	8.22±0.01* (+10.63)
Kidney	6.23 ±0.02	6.34±0.07 (+1.76)	6.39±0.05 (+2.56)	6.26 ±0.04	6.56±0.06 (+4.79)	6.79±0.03 (+8.46)

Each value represents the mean ± S.E.M. of five different observations. Value in parenthesis is percent change over control. Significantly different at *$p<0.05$; **$p<0.01$; ***$p<0.001$ (Student's t-test)

Table 5. ALAT activity in the tissues of *C. batrachus* after exposure to carbofuran.

Tissue	96 hr exposure period			15 days exposure period		
	Carbofuran mg / L			Carbofuran mg / L		
	0.00	0.01	0.02	0.00	0.01	0.02
Liver	8.94 ±0.02	9.23±0.04 (+3.24)	9.49±0.02 (6.15)	8.90±0.01	9.86±0.03* (+10.79)	10.14±0.04* (+13.93)
Muscle	6.94 ±0.03	7.12±0.06 (+2.59)	7.34±0.03 (+5.76)	6.97±0.02	7.64±0.02 (+9.61)	7.86±0.02* (+12.77)
Gill	7.83 ±0.01	7.99±0.02 (+2.04)	8.23±0.02 (+4.87)	7.85±0.02	8.61±0.03 (+8.82)	8.94±0.02* (+12.19)
Brain	6.12 ±0.03	6.27±0.03 (+2.45)	6.46±0.03 (+5.56)	6.09±0.04	6.94±0.04 (+13.96)	6.93±0.04* (+13.79)
Kidney	5.89 ±0.02	5.98±0.04 (+1.53)	6.08±0.05 (+3.22)	5.84±0.02	6.19±0.04 (+5.99)	6.28±0.06 (+7.53)

Each value represents the mean ± S.E.M. of five different observations. Values in parenthesis are percent change over control. Significantly different at *$p<0.05$ (Student's t-test)

The effect was more pronounced at the higher carbofuran concentration (0.02 mg/L) for the longer duration (15 days). AAT activity increased more during the 15 days exposure period but a different trend was obtained for the fish organs tested. The activity of ALAT also increased in different fish tissue at both carbofuran concentrations and exposure periods (Table 5).

After 96 hr exposure period, the rise in level of enzyme activity was recorded to be almost same in liver, muscle, gill and brain when compared to control. Similar results were obtained when the fish were treated with the higher concentration of carbofuran (0.02 mg/L) for longer period (15 days) of exposure (Table 5). However, the increase in the enzyme activity in fish tissues was about two times higher as compared to that observed with low concentration of the pesticide (Table 5). The activity of ALAT in the kidney of the fish remained only slightly affected. The results presented in Tables 4 and 5 indicated that the levels of enzyme activities for protein metabolism in the fish tissues increased with increasing pesticide concentration and duration of exposure.

The transaminases (GOT and GPT) are the key enzymes in fish that can be used as indicators of chemical pollution and also to diagnose the impact of sub-lethal toxicity of environmental contaminants in fish (Mukhopadhayay et al. 1982). In the present investigation, kidney of *C. batrachus* was the most affected organ as carbofuran induced a marked elevation in GOT activity in this tissue. This increase in GOT was attributed to pesticide toxicity caused by carbofuran (Mukhopadhayay et al. 1982). Gill et al (1990), however, have reported inhibition of GOT activity in liver and kidney and elevated activity in cardiac muscle of *P. conconius* exposed to aldicarb and organophosphate compounds.

The sharp increases in GPT activity in all the fish tissues tested from the carbofuran treated *C. batrachus* indicated the gill being maximally affected. These results were in agreement with the reports of Mukhopadhyay et al (1982) and Sharma (1999) on *C. batrachus* exposed to carbofuran and carbaryl. Some other workers have also reported increase in the activity of GPT in fish tissue due to intoxication of monocrotophos in *C. punctatus* (Samuel and Sastry, 1989) and malathion in C. straitus (Sadhu et al.1985). The increase in GOT and GPT activities in fish tissues due to carbofuran treatment suggested that there was an increase in the transamination process and also that protein was increasingly metabolized. This notion is further supported by the decrease in protein level under carbofuran stress (Singh and Sharma, 1998). Sharma (1999) has shown that carbaryl (carbamate) caused increased GOT activity in liver and serum of C. batrachus, suggesting probable alterations in the pathophysiological status of the liver as well as increased turnover of protein in C. batrachus due to pesticide toxicosis. The enhanced activities of transaminases (AAT and ALAT) provide the oxaloacetic acid and pyruvate, α-ketoglutarate and glutaric acid (the TCA cycle intermediates) which could be utilized to meet the increased energy demand during carbofuran induced stress condition (Begum, 2004). The above findings suggested that there is a significant change in protein metabolism during carbofuran toxicity in all the tissues of C. batrachus tested for the short (96 hr) and long (15-days) exposure periods. The total protein levels decreased (Singh and Sharma 1998), whereas free amino acid content increased after these treatment periods (present work). The decrease in protein content may be due to reduced protein synthesis and / or enhanced proteolysis during carbofuran toxicity. An increase in free amino acid content, which might have come as a result of tissue damage, is also suggestive of a decreased utilization of amino

acids (Sastry and Siddiqui 1984). The significant decreases in protein content with elevated level of free amino acids in the fish organs also indicate the activation of compensatory mechanisms in the fish to counter the sublethal toxic stress (Sreedevi et al. 1992). The results from present investigation demonstrated that carbofuran at low concentration caused significant alteration in the protein metabolism.

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