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Endosulfan-mediated Biochemical Changes in the Freshwater Fish *Clarias batrachus*

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Objective Endosulfan is an extremely toxic organochlorine pesticide to aquatic organisms which might be hampering fish health through impairment of metabolism sometimes leading to death. So an experimental protocol was designed to look at endosulfan effects on a number of selected biochemical endpoints as well as to develop a mechanistic understanding of biochemical effects of endosulfan in freshwater fish. **Methods** The adult freshwater catfish *Clarias batrachus* were collected and acclimatized to laboratory condition for two weeks prior to experimentation. The toxicity bioassay test of commercial grade endosulfan (35% EC) was conducted for 21 days to determine its initial lethal concentration. The fish were exposed to sublethal concentration of endosulfan (0.06 mg/L) for 21 days. Pesticide – withdrawal experiments were also performed to study recovery. Protein synthesis inhibitors were injected to know the possible mechanism of recovery. The specimens of *C. batrachus* were sacrificed and brain, liver and caudal white skeletal muscle were removed. Tissues were homogenized and fractions were obtained by differential centrifugation. The activities of citrate synthase (CS), glucose 6-phosphate dehydrogenase (G6-PDH) and lactate dehydrogease (LDH) were assayed spectrophotometrically. Similarly, DNA, RNA and protein content were measured as per standard procedure. **Results** The exposure of sublethal concentration of endosulfan decreased the activity of citrate synthase (CS) and glucose 6-phosphate dehydrogenase (G6-PDH) in the brain, liver and skeletal muscle of the freshwater catfish, *C. batrachus*. The brain lactate dehydrogenase (LDH) activity was also reduced in response to endosulfan toxicity. The maximum reduction in activities of these enzyme was 34%-43%. Withdrawal of endosulfan restored the enzyme activity to control level in all the three tissues. The recovery in enzyme activity appears to be due to dissociation of endosulfan or its metabolite(s) from the enzyme molecules and/or fresh synthesis of enzymes. The treatment of actinomycin D or cycloheximide partially inhibited the withdrawal-dependent increase in enzyme activity. This substantiates *de novo* synthesis of enzyme during recovery period. Since the reduction in enzyme activity was more pronounced in response to actinomycin D, endosulfan might be inhibiting the transcription process. But endosulfan did not produce any significant effect on DNA content and RNA/DNA. However, the RNA and protein contents of brain, liver and skeletal muscle decreased significantly in tissues. The maximum decrease in RNA and protein was approximately 30%-37%. Withdrawal of endosulfan from the medium for 21 days restored the RNA, and protein contents nearly to their control levels. The treatment of actinomycin D or cycloheximide partially inhibited the withdrawal- dependent increase in these macromolecular contents. This effect was more pronounced in case of actinomycin D which again supports the possibility of endosulfan-induced inhibition at transcription level. **Conclusion** The present study suggests endosulfan-induced impairment of metabolism in fish, which appeared to be due to inhibition of transcription at some unknown points.

Key words: Catfish; Endosulfan; CS; G6-PDH; LDH; DNA; RNA; RNA/DNA ratio and protein

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INTRODUCTION

A large number of pesticides are drained in water bodies where fish encounter with them and develop various metabolic abnormalities. They accumulate in fish and affect human health too via ecological cycling and biological magnification. Endosulfan toxicity produces changes in concentration of protein, glycogen and lipid in *Channa punctatus*[1], *Barilius bendelisis*[2] and *Cyprinus carpio*[3]. Enzyme-mediated toxicological responses may be pesticide, enzyme, tissue or species-specific[4]. It has been reported that the activities of cytoplasmic malate dehydrogenase, mitochondrial malate dehydrogenase and lactate dehydrogenase decrease in liver and muscle of fish exposed to endosulfan[5]. The reduction observed in activities of enzymes may be due to nonproductive binding of endosulfan or its metabolite(s) with the enzyme molecules[6]. Recently, endosulfan-mediated changes in biochemical physiology of fish has also been reviewed[7].

Toxicity of endosulfan to non-target animals have been thoroughly reviewed by Naqvi and Vaishanavi[8]. Aquatic animals have been known to accumulate considerably greater quantities of endosulfan[8-10]. Endosulfan has been found to be generally more toxic to fish than invertebrates[11]. Several workers have estimated median lethal concentration (LC_{50}) of endosulfan for fish. It has been suggested that LC_{50} of endosulfan for most of the fishes may vary from 0.0006 to 10 ppm[12,13]. Devi *et al*.[14] demonstrated LC_{50} (96 h) value of 4.8 ppb for *Channa punctatus*. Whereas Arora and Kulshrestha[15] described LC_{50} value of 0.00075-0.001 ppm for this species. Bashamodhideen *et al*.[16] reported LC_{50} range of 0.346-0.424 ppm for the fish *Catla catla*. While Kumar[17] showed LC_{50} value of 0.3 ppm for *Lepidocephalichthys thermalis.* Rao and Murthy[18] found LC_{50} value of 1.2-3.0 ppm of endosuflan for *Anabus testudinus*.

Endosulfan is a central nervous system (CNS) poison and moderately hazardous[19]. This organochlorine pesticide has gained significance as a potential environmental pollutant due to its wide spread use in the control of agricultural insect pests[11]. Fishes are extremely sensitive to endosulfan and mortality has been reported a number of times as a result of endosulfan leakage into rivers[8]. Endosulfan is also included in the EPA's list of priority pollutant. Therefore, it was considered as a matter of warranted interest to elucidate biochemical effects of endosulfan in fish. The hypothesis was that endosulfan might be hampering fish health through impairment of metabolism which sometimes leads to death. So an experimental protocol was designed to look at endosulfan effects on a number of selected biochemical endpoints as well as to develop a mechanistic understanding of biochemical effects of endosulfan in freshwater fish. This work will add to the knowledge of mode of action of endosulfan at enzymatic and metabolic level. On this basis, one can further investigate and establish endosulfan-mediated effects at gene physiology level in fish.

MATERIALS AND METHODS

Fish

The adult freshwater catfish *C. batrachus* (75-80 g weight, 18-19 cm length) were collected from different fish markets from commercial vender. They were acclimatized to laboratory condition in plastic container using tap water (pH 7.6-7.9) for two weeks prior to experimentation. Fish were fed on minced goat liver on alternate day during acclimatization. However, feeding was stopped during experimentation in order to avoid the effect of

reduced feeding on metabolism.

Chemicals

Acetyl CoA, $MgCl_2$, nitrobenzoic acid (DTNB), DNA, Folin-Ciocalteau, reagent, glucose 6-phosphate, imidazole, acetone, endosulfan (35% EC), nicotinamide adenine dinucleotide (β-NADH), nicotinamide adenine dinucleotide phosphate (β-$NADP^+$), orcinol, oxaloacetate, RNA, sodium carbonate, sodium chloride, sodium citrate, sodium potassium tartrate, sodium pyruvate, tris (hydroxymethyl amine), diphenylamine, KCl and copper sulphate. All chemicals were of analytical grade and procured from Sisco Research Laboratory, Mumbai, India.

Toxicity Test

The toxicity bioassay test of commercial grade endosulfan (35% EC) was conducted for 21 days to determine its initial lethal concentration ($LC_{(I)}50$). During bioassay the morality in control groups was adjusted according to the method of Ludke *et al*.[20]. The LC_{50} was calculated by probit analysis method. It was estimated as 0.6 mg/L for 21 days. Accordingly, the sublethal concentration for endosulfan was selected as 0.06 mg/L. A fresh medium of endosulfan was prepared by changing water on alternate day. The entire work was carried out in two separate phases.

Exposure Phase

The fish were exposed to sublethal concentration of endosulfan (0.06 mg/L) for 21 days. Endosulfan was dissolved in acetone and the control group fish were exposed to water containing 0.01% acetone. Pilot experiment did not show any significant effect of 0.01% acetone exposure on biochemical changes in fish. This concentration of solvent was taken for experimental work. The fish used in the exposure phase were divided into two groups, each containing six fishes. One group served as control and the other group was exposed to 0.06 mg/L endosulfan for 21 days. Sampling was done on day 4, 7, 14 and 21. The groups of exposure phase were as given below.

Group A, Control exposed to acetone vehicle

Group B, Exposed to endosulfan for 4, 7, 14 and 21 days

Recover Phase

A total 36 fish were exposed to 0.06 mg/L of endosulfan for 21 days. A parallel control group (A) containing six fish was also maintained. Then six fish were sacrificed for endosulfan exposed group (B) and the remaining were divided into 5 groups each containing six individuals. Fish of these groups (C to E) were kept in control water for 7, 14 and 21 days to recover from the toxic effect. Two groups (F and G) recovered for 14 days were injected protein synthesis inhibitors viz., group F received actinomycin D (20 μg/100 g) and group G received cycloheximide (200 μg/100 g) on alternate day for 7 days. The groups of recover phase were as follows:

Group A, Control exposed to acetone

Group B, Exposed to endosulfan (END) for 21 days

Group C, END-withdrawal (END-W) for 7 days (7 D)

Group D, END-W for 14 days (14 D)

Group E, END-W for 21 days (21D)

Group F, END-W (14 D) + actinomycin D

Group G, END-W (14 D) + cycloheximide

Protein synthesis inhibitors (actinomycin D and cycloheximide) were injected to know the possible mechanism of recovery.

Enzyme Extraction

The specimens of *C. batrachus* were sacrificed by decapitation. The brain, liver and caudal white skeletal muscle were removed and washed in 0.6% saline. They were cleaned and weighed rapidly. A 10% homogenate (w/v) was prepared in tris-HCl buffer (0.1 mol/L tris-chloride, pH 7.5) using Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 700 g for 15 min in a high speed refrigerate centrifuge. The supernatant was decanted and centrifuged at 12 000 g for 20 min to get the mitochondrial pellet. The resulting supernatant was taken as the cytoplasmic fraction for the assay of glucose 6-phosphate dehydrogenase (G6-PDH) and lactate dehydrogenase (LDH). The mitochondrial pellet was washed twice in 0.1 mol/L tris-chloride buffer (pH 7.5) and each washing was followed by centrifugation at 12 100 g for 15 min. The pellet was resuspension in the above buffer and homogenized at high speed. The homogenized suspension was recentrifuged at 21 000 g for 15 min to remove particulate matter. The resulting supernatant was taken as mitochondrial fraction for the assay of citrate synthase (CS). The marker enzyme, lactate dehydrogenase (LDH), was assayed in the mitochondrial fraction to test cytoplasmic contamination, if any. The procedure employed for subcellular fractionation and extraction of the mitochondrial enzyme was based on Foster and Moon[21].

Assay Procedure

The procedures adopted for assay of citrate synthase (CS) and lactate dehydrogenase (LDH) were those of Foster and Moon[21]. The enzyme glucose 6-phosphate dehydrogenase (G6-PDH) was assayed according to the method of Mommsen *et al.*[22]. The CS activity was measured in a medium containing 100 mmol/L tris-HCI (pH 8), 0.5 mmol/L oxaloacetate, 0.3 mmol/L acetyl CoA and 0.1 mmol/L 5,5-dithiobis (2-nitrobenzoic) acid (DTNB) in 40 mmol/L sodium phosphate buffer. The total volume of the reaction mixture was 3 mL. The reaction was initiated by the addition of oxaloacetate and the extinction at 412 nm was monitored (EmM=13.6). The absorbance increase in the presence of supernatant, but without oxaloacetate, was first recorded. The complete reaction was then initiated by addition of oxaloacetate. The blank (without oxaloacetate) was subtracted from the total activity. The G6-PDH was assayed in a medium containing 100 mmol/L tris-HCl buffer (pH 7.5), 7 mmol/L $MgCl_2$, 0.4 mmol/L $NADP^+$ and 1 mmol/L glucose 6-phosphate. The total volume of the reaction mixture was 3 mL. The reaction was initiated by addition of $NADP^+$ and the increase in absorbance was monitored at 340 nm (EmM=6.22). The blank contained all chemicals except $NADP^+$. The LDH activity was determined only in brain tissue. It was assayed in a medium containing 100 mmol/L tris-HCl (pH 7.5), 5 mmol/L pyruvate and 0.15 mmol/L NADH in a total volume of 3 mL. The reaction was initiated by the addition of NADH. The decrease in absorbance was monitored at 340 nm (EmM=6.22). The blank contained all components except substrate.

Nucleic Acid and Protein Estimation

DNA and RNA contents were estimated according to the method of Schneider[23] using diphenylamine and orcinol reagents, respectively. The concentration of protein was estimated by Lowry method[24] using Folin-Ciocalteau reagent.

Statistical Analysis

The data were analyzed statistically by employing one way analysis of variance and student *t*-test. The level of significance was kept at 0.05.

RESULTS

Citrate Synthase (CS)

Concentration of CS in brain, liver and skeletal muscle of *C. batrachus* exposed to endosulfan was lower on all sampling day when compared with control (Table 1). This was found to be more pronounced in fish sampled on 21 day of exposure. The maximum reduction of CS activity was 38% in brain, 40% in liver and 43% in skeletal muscle of *C. batrachus* on 21 day of sampling (Table 1). The withdrawal of endosulfan from the medium after 21 days of exposure gradually brought back the enzyme activity to control level in brain, liver and skeletal muscle. The first significant recovery in enzyme activity was noticed after 21 days. The administration of actinomycin D for 7 day on an alternate days within 14 to 21 day of endosulfan withdrawal inhibited enzyme activity by 11%, 6% and 24% against the withdrawal-dependent increase of 16%, 17% and 30% in brain, liver and skeletal muscle, respectively. Similarly, cycloheximide partially inhibited the withdrawal-dependent recovery in enzyme activity by 6% in brain, 4% in liver and 18% in skeletal muscle (Table 2).

TABLE 1

Effect of Endosulfan (END) on the Activity (units × g wt tissue mass^{-1}) of Citrate Synthase (CS), Glucose 6-phosphate Dehydrogenase (G6-PDH) of Brain, Liver and Skeletal, Uscle and Lactate Dehydrogenase (LDH) of Brain of the Freshwater Catfish, *C. batrachus*.

Endosulfan	CS			G6-PDH			LDH
Exposure (Day)	Brain	Liver	Skeletal Muscle	Brain	Liver	Skeletal Muscle	Brain
Control	6.157	3.453	2.947	15.356	21.652	3.147	59.736
	± 0.518	± 0.469	± 0.354	± 0.396	± 0.511	± 0.414	± 2.476
4 D	5.753	3.183	2.617	14.303	19.737	2.800	57.191
	± 0.620	± 0.379	± 0.498	± 0.840	± 0.493	± 0.343	3.500
7 D	5.374	2.950	2.364	13.259	18.162	2.546	51.343
	± 0.539	± 0.322	± 0.548	± 0.769	± 0.697	± 0.465	± 3.526
14 D	4.769	2.523	2.032	11.849	16.135	2.218	44.318
	± 0.322	± 0.612	± 0.473	± 0.766	± 0.763	± 0.362	± 4.671
21 D	3.794	2.068	1.677	9.832	14.370	1.843	36.410
	± 0.503	± 0.344	± 0.374	± 0.922	± 0.667	± 0.342	± 2.616
F Value	3.272	3.289	2.782	8.050	20.441	2.791	287.804
P Value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Note. Each datum represents mean $\bar{x}\pm s$ of six individuals (n=6). Results of one way analysis of variance (ANOVA) are also given.

TABLE 2

Effect of Withdrawal of Endosulfan (END) and Treatment of Actinomycin D (ACT) or Cycloheximide (CHX) on the Withdrawal-dependent Recovery in the Activity (units × g wt tissue mass^{-1}) of Citrate Synthase (CS), Glucose 6-phosphate Dehydrogenase (G6-PDH) of Brain, Liver and Skeletal Muscle and Lactate Dehydrogenase (LDH) of Brain of the Freshwater Catfish, *C. batrachus*.

Endosulfan Exposure (Day)	CS			G6-PDH			LDH
	Brain	Liver	Skeletal Muscle	Brain	Liver	Skeletal Muscle	Brain
Control	5.843	3.588	2.831	15.666	21.203	2.977	60.132
	± 0.289	± 0.551	± 0.390	± 0.575	± 0.738	± 0.610	± 4.376
END	3.798	2.176	1.537	9.607	14.042	1.589	39.069
(21 D)	± 0.442[a]	± 0.378[a]	± 0.272[a]	± 0.471[a]	± 1.130[a]	± 0.338[a]	± 2.738[a]
END-W	4.536	2.708	1.722	11.338	15.780	2.216	45.703
(7 D)	± 0.525	± 0.425	± 0.372	± 0.559	± 0.741	± 0.516	± 4.564
END-W	5.174	3.153	2.033	13.202	18.540	2.591	53.503
(14 D)	± 0.556[b]	± 0.245[b]	± 0.353[b]	± 0.728[b]	± 0.734[b]	± 0.558[b]	± 5.506[b]
END-W	6.017	3.696	2.635	15.373	21.579	3.113	61.237
(21 D)	± 0.417[c]	± 0.442[c]	± 0.458[c]	± 0.792[c]	± 0.538[c]	± 0.556[c]	± 3.621[c]
END-W+	5.329	3.468	2.015	13.825	20.263	2.692	54.628
ACT	± 0.242[a]	± 0.287[a]	± 0.387[a]	± 0.762[a]	± 0.605[a]	± 0.484[a]	± 2.512[a]
END-W+	5.672	3.559	2.170	14.255	21.063	3.146	56.380
$\bar{x}$+*s* CHX	± 0.424[a]	± 0.465[a]	± 0.318[a]	± 0.703[a]	± 0.613[a]	± 0.661[a]	± 3.752[a]

Note. Each datum represents $\bar{x}$±*s* of six individuals (*n*=6). [a]denotes significant differences from respective controls. [b]shows non-significant differences among values of an enzyme activity in a particular tissue. [c]denotes significant differences between endosulfan treated (a) and endosulfan withdrawal (c) groups.

Glucose 6-Phosphate Dehydrogenase (G6-PDH)

Concentration of G6-PDH in brain, liver and skeletal muscle of *C. batrachus* exposed to endosulfan was lower on all sampling day when compared with control (Table 1). This was found to be more pronounced in fish sampled on 21 day of exposure. The maximum reduction of G6-PDH activity was 36% in brain, 34% in liver and 42% in skeletal muscle of *C. batrachus* on 21 day of sampling (Table 1). The withdrawal of endosulfan from the medium after 21 days of exposure recovered the enzyme activity upto the control level. The administration of actinomycin D for 7 days within 14 to 21 day of endosulfan-withdrawal inhibited enzyme activity by 10%, 6% and 14% against the withdrawal-dependent increase in G6-PDH activity of 16%, 15% and 20% in brain, liver and skeletal muscle respectively. Similarly, cycloheximide inhibited 7%, 2% and 1% the withdrawal dependent recovery in enzyme activity in all three tissues (Table 2).

Lactate Dehydrogenase (LDH)

Exposure of endosulfan to the catfish, *C. batrachus* for 21 days reduced the LDH activity when compared with control (Table 1). The decline in enzyme activity was gradual and maximum reduction was 39% in brain, on 21 day of endosulfan exposure (Table 1). The withdrawal of endosulfan from the medium after 21 days of exposure gradually recovered the enzyme activity upto the control level. The administration of actinomycin D for 7 day on

alternate days within 14 to 21 day of endosulfan-withdrawal inhibited enzyme activity by 11% against the withdrawal-dependent increase in LDH activity of 14%. Similarly, cycloheximide partially inhibited 8% the withdrawal-dependent recovery in enzyme activity (Table 2).

RNA and Protein

Treatment of endosulfan to the catfish did not produce any significant effect on DNA and RNA/DNA ratio. In contrast, endosulfan decreased the RNA and protein contents on all sampling day when compared with control values (Table 3). This was found to be more pronounced in fish sampled on 21 day of endosulfan exposure. RNA and protein contents were reduced by 30%-33% in brain, 32%-36% in liver and 30%-37% in skeletal muscle of *C. batrachus.* The withdrawal of endosulfan from the medium recovered the RNA and protein content in all three tissues of the fish (Table 4). The first significant recovery was observed after 21 days of exposure. The administration of actinomycin D for 7 days, within 14 to 21 day of endosulfan withdrawal inhibited 10%-13% RNA and protein level against the withdrawal-dependent increase of 15%-16% in brain, 13%-14% in liver and 7%-10% in skeletal muscle. Cycloheximide partially inhibited the withdrawal-dependent increase in these macromolecules contents.

TABLE 3

Effect of Endosulfan (END) on RNA, RNA/DNA Ratio and Protein in Brain, Liver and Skeletal Muscle of the Freshwater Catfish, *C. batrachus*.

Endosulfan	Brain			Liver			Skeletal Muscle		
Exposure (Day)	RNA	RNA/DNA Ratio	Protein	RNA	RNA/DNA Ratio	Protein	RNA	RNA/ DNA	Protein
Control	8.513	6.966	17.243	12.834	1.502	40.110	6.411	9.136	22.267
	± 0.556	± 1.470	± 0.820	± 0.821	± 0.193	± 0.690	± 0.584	± 2.072	± 0.845
4 D	8.366	6.916	15.993	12.274	1.366	36.421	6.560	8.355	19.590
	± 0.456	± 0.952	± 0.604	± 0.817	± 0.109	± 0.480	± 0.524	± 0.836	± 0.618
7 D	7.613	6.173	14.772	11.195	1.307	33.565	5.967	7.513	18.202
	± 0.525	± 1.129	± 0.711	± 0.502	± 0.095	± 0.958	± 0.608	± 0.889	± 0.673
14 D	6.856	5.952	13.540	10.145	1.176	30.553	5.376	7.212	16.495
	± 0.515	± 1.367	± 0.769	± 0.560	± 0.118	± 0.471	± 0.527	± 1.245	± 0.619
21 D	5.941	4.793	11.468	8.698	0.985	25.815	4.706	6.702	14.271
	± 0.480	± 0.782	± 0.609	± 0.559	± 0.060	± 0.694	± 0.358	± 0.965	± 0.676
F Value	4.476	0.574	9.907	6.219	1.544	64.218	2.115	0.563	19.242
P Value	<0.05	>0.05	<0.05	<0.05	>0.05	<0.05	<0.05	>0.05	<0.05

Note. Each datum represents $\bar{x}\pm s$ of six individuals (n=6). Results of one way analysis of variance (ANOVA) are also given. RNA and protein contents were expressed as mg×g wt. tissue mass^{-1}.

DISCUSSION

The exposure of sublethal concentration of endosulfan to the freshwater catfish, *C. batrachus*, gradually reduced the activity of CS, G6-PDH and LDH in brain, liver and skeletal muscle (Table 1). A gradual increase in endosulfan-mediated inhibition in enzyme

activity may be due to increase in endosulfan accumulation in tissues with duration of the toxicant exposure. The withdrawal of endosulfan from the medium for 21 days gradually brought back the enzyme activities upto their control levels. The recovery in enzyme activities after endosulfan-withdrawal from medium (Table 2) suggests that fish were apparently relieved from the biochemical effects of endosulfan stress within 21 days. Faster recovery may also be because of metabolic compensation. The endosulfan-mediated reductions in CS and LDH activities indicate decrease in aerobic and anaerobic capacity of fish. However, endosulfan-induced decrease in G6-PDH activity reflects reduction in glucose oxidation via pentose shunt and NADPH production for reductive syntheses in cell.

The present observation demonstrated clearly about 40% reduction in activities of CS and G6-PDH in brain, liver and skeletal muscle and LDH in brain of the fish, *C. batrachus*. The most possible explanation to endosulfan-mediated reduction in enzyme activities is that endosulfan induces stress in the exposed fish and this results in stress responses that change the production of enzymes, or redirect biochemical resources to the production of metabolic enzymes to degrade the pesticide. It may be supported from the endosulfan-mediated reduction in the activities of adenosine triphosphatase, succinate dehydrogenase, cytochrome C reductase, NADH-dehydrogenase, malate dehydrogenase, lactate dehydrogenase, acetyl-cholinesterase, alkaline and acid phosphatases, cytochrome C oxidase and monoamine oxidase in other fishes[6,8,25-31]. The withdrawal of endosulfan from the medium after 21 days of its exposure restores the activities of CS, G6-PDH and LDH. The withdrawal-dependent recovery in the activities of these enzymes suggests *de novo* synthesis of the enzymes. The administration of actinomycin D or cycloheximide between 14 to 21 days of the withdrawal

TABLE 4

Effect of Withdrawal of Endosulfan (END) and Treatement of Actinomycin D (ACT), Cycloheximide (CHX) on the Withdrawal-dependent Recovery of RNA and Protein in Brain, Liver and Skeletal Muscle of the Freshwater Catfish, *C. batrachus*.

Endosulfan	Brain		Liver		Skeletal Muscle	
Exposure (Day)	RNA	Protein	RNA	Protein	RNA	Protein
Control	8.463 ± 0.533	17.363 ± 0.581	12.733 ± 0.858	39.345 ± 1.662	6.659 ± 0.519	19.910 ± 0.666
END (21 D)	5.714 ± 0.578[a]	12.017 ± 0.689[a]	8.573 ± 0.553[a]	27.611 ± 4.598[a]	5.064 ± 0.362[a]	13.785 ± 0.926[a]
END-W (7 D)	6.447 ± 0.584	13.550 ± 0.929	9.942 ± 0.508	30.777 ± 2.859	5.463 ± 0.536	15.600 ± 1.011
END-W (14 D)	7.365 ± 0.464[c]	14.882 ± 0.887[c]	11.175 ± 0.384[c]	34.127 ± 2.504[c]	5.952 ± 0.623[c]	17.480 ± 0.588[c]
END-W (21 D)	8.511 ± 0.544[b]	17.066 ± 0.534[b]	12.683 ± 0.489[b]	38.851 ± 3.484[b]	6.773 ± 0.639[b]	19.756 ± 0.846[b]
END-W+ ACT	7.641 ± 0.534[c]	14.888 ± 0.809[c]	11.144 ± 0.699[c]	36.821 ± 2.330[c]	5.957 ± 0.511[c]	18.382 ± 0.951[c]
END-W+ CHX	7.498 ± 0.769[c]	14.473 ± 0.907[c]	9.871 ± 0.984[c]	34.000 ± 2.129[c]	6.225 ± 0.322[c]	19.644 ± 0.577[c]

Note. Each datum represents $\bar{x}\pm s$ of six individuals (n=6). RNA and protein contents were expressed as mg × g wt. tissue mass^{-1}. [a]indicates significant differences from respective controls. [b]indicates significant differences between endosulfan treated (a) and endosulfan withdrawal (b) groups. [c]hows non-significant differences among values of RNA, RNA/DNA ratio and protein of a particular tissue.

of the endosulfan inhibits either completely or partially the withdrawal-dependent recovery of enzyme activities. The degree of inhibition in response to actinomycin D was more than that of cycloheximide.

Endosulfan shows inhibitory effect on RNA and protein contents in brain, liver and skeletal muscle of the freshwater catfish, *C. batrachus* (Table 3). The endosulfan induced decrease in these contents was about 33% in all the three tissues. The reduction in RNA and protein concentrations may be due to inhibition of their syntheses and/or enhancement of degradation in the cells. It may be suggested that endosulfan effects turnover of RNA and protein thereby changing the levels of macromolecular constituents in the tissues of the fish. The present observations may be supported by the pesticide-mediated reduction in protein contents of various tissues including blood of other species of fishes[4,8,29,32]. The withdrawal of endosulfan from the medium after 21 days of its exposure restores RNA and protein content. This withdrawal-dependent recovery may be due to *de novo* synthesis of RNA and protein. The administration of actinomycin D or cycloheximide between 14 to 21 days of withdrawal of endosulfan inhibited either completely or partially the withdrawal-dependent recovery (Table 4). Since actinomycin D dependent inhibition was more pronounced than that of cycloheximide, endosulfan might be somehow affecting the transcription process. The endosulfan-mediated reduction in the some important metabolic enzymes, RNA and protein clearly suggests impairment of metabolism in fish.

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