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Influence of endosulfan and monocrotophos exposure on the activity of NADPH cytochrome C reductase (NCCR) of *Labeo rohita* (Ham)

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Abstract: *The response of NADPH cytochrome C reductase (NCCR) activity in liver of Labeo rohita fish exposed to the pesticides, 0.25 μgl^{-1} endosulfan and 2 mg/l monocrotophos was studied. In terms of specific enzyme activity (mU/mg protein) a significant level of NCCR was observed in the liver tissues of Labeo rohita exposed to the pesticides, when compared to the control fish (2.460 mU/mg protein). Increase of NCCR activity was more in the liver of the fish exposed to monocrotophos(4.595 mU/mg protein) than those exposed to endosulfan (2.850 mU/mg protein). The results demonstrate that the pesticides, endosulfan and monocrotophos, interfere with NADPH dependent monoxygenase mechanism and are effective inducers of NADPH cytochrome C reductase. The activity of NCCR in the liver tissue of Labeo rohita may serve as a useful tool for monitoring aquatic pollution.*

Key words: *Labeo rohita, Pesticides, Endosulfan, Monocrotophos, Liver tissue, NADPH cytochrome C reductase, Biomarker*
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Introduction

An important part of biomarker research is evaluation and validation of biomarker responses in field studies. The aquatic environment is particularly sensitive to the toxic effect of contaminants, since a considerable amount of chemicals used in industry and agriculture enter aquatic environment. Both organophosphate and organochloride insecticides represent major sources of environmental contamination. Pollutants that bioaccumulate in the organism, initially show their effect at the molecular and cellular levels. Fish as a bio-indicator species can play an important role in the monitoring of water pollution, as they respond with great sensitivity to changes in the aquatic environment. The best characterized and widely used biochemical biomarker, so far, is the cytochrome P450 1A (CYP1A) dependent mixed function oxidase (MFO) system. Organic contaminants specifically induce liver CYP 1 A in fish and other vertebrates. Monoxygenase activities are comparatively high in the liver of fish and may lead to formation of more toxic metabolites and the liver is vulnerable to injury (Lindstrom-Seppa *et al.,* 1981; Smolowitz *et al.,* 1991; Singh and Singh, 2006, 2007). The objective of the present study was to evaluate biomarker responses in the cultivable carp, *Labeo rohita* exposed to the pesticides endosulfan and monocrotophos.

Materials and Methods

Juveniles of *Labeo rohita* were purchased from a local fish farm and acclimatized to laboratory conditions for a period of two weeks. Fish were fed with rice bran and oil cake mixture during the acclimatization period. Feeding was stopped 3 days prior to the experiment. The test fish were measured 10.5-13 cm in length and 18-33 g in weight. The fish were divided into three groups, each consisting of five fish. The first group served as the control, second group as test fish for endosulfan exposure and the third group as test fish for monocrotophos exposure. The experiment was carried out in fibre tanks of 180 litre capacity. Desired concentrations of endosulfan (0.25 μgl^{-1}) and monocrotophos (2 mg/l) were prepared by adding aliquots of stock solutions dissolved in acetone. Acetone, not exceeding 0.01ml/l, was added to the control group. Static bioassay method with 24 hr renewable water medium was adopted for the study. The experiments were carried out in duplicate. The physico-chemical parameters of the water were examined by standard procedures (APHA, 2005). The condition factor of the fish was determined by Bagenal and Tesch (1978) method.

After 15 days, the fish were terminated by a blow on the head and the livers were carefully removed. To prepare the microsomes, they were washed in ice-cold 0.15M KCl, weighed and then individual livers were minced, to which 5 volumes of buffer A (0.05M Tris HCl, 0.15 M KCl, 1mM EDTA, 2 mM reduced glutathione, pH 7.4) was added and homogenized. The homogenate was centrifuged at 10,000 g for 15 min at 0-4°C. The post mitochondrial supernatant was filtered through glass wool and re-centrifuged at 45,000 g for 60 min. The microsome pellet was rinsed once with 0.1M KH_2PO_4 (pH 7.4) and suspended in the same buffer to make a final concentration of 2-5 mg protein/ml. Protein determinations were done by the method Lowry *et al.* (1951). The cytochrome dependent monoxygenase activity of NADPH cytochrome C reductase was analysed in an assay mixture containing 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM NADPH and 80 μ M of horse heart cytochrome C in a total volume of 3 ml. Reduction of cytochrome C was followed at 550 nm wave length with a UV-VIS spectrophotometer and the product was determined using an extinction coefficient of 21 $cm^{-1}\ \mu M^{-1}$ (Phillips and Langden, 1962). Data was analysed for statistical significance using one way ANOVA.

Results and Discussion

The physico-chemical parameters of the water are presented in Table 1. The morphometric data of *Labeo rohita* are given in Table 2. The NCCR activity was 2.460 mU/mg protein in the control fish, 2.850 mU/mg protein in the fish exposed to endosulfan and 4.595 mU/mg protein in fish exposed to monocrotophos. In the study it was observed that there was significant induction of NADPH cytochrome C reductase (NCCR) activity in liver of *Labeo rohita* exposed to endosulfan and monocrotophos compared to control fish (Table 3). Activity of NCCR was greater in test fish exposed to monocrotophos than in those exposed to endosulfan.

The primary biochemical effect associated with organophosphate toxicity is inhibition of acetylcholinesterase (Arnold *et al.*, 1995). Endosulfan was shown to activate lipid peroxidation (Singh and Pandey, 1991). Destruction of microsomal cytochrome P 450 due to lipid peroxidation was described by Plaa and Witschi (1976). Lipid peroxidation and inhibition of acetylcholineesterase represents a major mechanism of sub-lethal toxicity (Ernster *et al.*, 1982; Levi *et al.*, 1988). Microsomal membranes are vulnerable to lipid peroxidation due to cytochrome P450 associated NADPH cytochrome C reductase (Ernster *et al.*, 1982). Induction of microsomal monoxygenases is known to inhibit lipid peroxidation of microsomal fractions (Stepanova *et al.*, 1985). Therefore it appears from this study, that endosulfan and monocrotophos interfere with NADPH dependent monoxygenase mechanism.

From the earlier studies on biotransformation of endosulfan and monocrotophos by *Labeo rohita* (Ramaneswari and Rao, 2000), it was observed that endosulfan is metabolized to endosulfan sulfate and monocrotophos to hydroxyl methyl and des-methyl monocrotophos. As observed in the study on *Labeo rohita* by Rao and Ramaneswari (2000), enhanced toxicity of endosulfan is due to an intermediary product, endosulfan sulfate, which is as toxic as the technical material and metabolites of monocrotophos are non-cholinesterase inhibiting products of low toxicity. The enhanced toxicity of endosulfan is due to its reduced biotransformation capacity, as the induction of NCCR in *Labeo rohita* is lower, when compared to the induction of NCCR by monocrotophos.

In fish, CYP 1A is the major P450 subfamily responsive to organic compounds. CYP 1A has been used to monitor the presence, bio-availability and effects of contaminants in numerous aquatic environments (Stegeman and Hahn, 1994; Goksoyr, 1995). NCCR is regarded as a typical monoxygenase in fish liver (Braubenck and Volkl, 1991). Recent interest in CYP 1A induction is based on its reliable inducibility and response to aromatic and chlorinated contaminants. In fish expression of CYP 1A is typically high in the hepatocytes and endothelial cells.

Cytochrome P450 reductase activities in feral roach (*Rutilus rutilus*) from a polluted site did not differ from the activities in control animals (Vander Oost *et al.*, 1994). In laboratory studies, however, elevated P450 reductase activities were observed in killifish exposed to B-napthoflavonone (Kloepper-Sams and Stegemann, 1992) and in eel exposed to dinitro-O-cresol (Braunbeck and Volkl, 1991). No significant alterations were observed in P 450 reductase activities of channel catfish exposed to PCB's (Ankley *et al.*, 1986), rainbow trout exposed to beta-naptho flavonone (Celander and Forlin, 1991) and eel exposed to benzo (a) pyrene (Lemaire-Gony and Lemaire, 1992).

Table - 1: Physico-chemical parameters of the water

Parameters	Value
Temperature	27±1°C
pH	7.6
Dissolved oxygen	6.9 mgl^{-1}
Total hardness	118 mgl^{-1}

Table - 2: Morphometric data of *Labeo rohita* fish with ± SD

	Control	Endosulfan	Monocrotophos
Body length (cm)	11.82±1.087	11.61±0.971	11.34±0.890
Body weight (g)	26.75±3.835	25.38±4.714	25.49±4.235
Condition factor	1.192±1.1	1.173±1.12	1.185±1.1
Liver weight (g)	0.77±0.52	0.73±0.58	0.78±0.52

n = 5

Table - 3: Specific activity of NCCR in liver of *Labeo rohita* following exposure to endosulfan and monocrotophos

	Concentration of pesticide	NCCR activity (mU/mg protein)
Control	—	2.460±0.490
Endosulfan	0.25 (mgl^{-1})	2.850±0.692*
Monocrotophos	2.00 (mgl^{-1})	4.595±0.848*

* indicates significant difference at $p \leq 0.05$

The results of the present study reveal that endosulfan and monocrotophos are effective inducers of NADPH cytochrome C reductase (NCCR) and its activity may provide a useful tool for monitoring aquatic pollution.

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