



Lambda cyhalothrin induced alterations in *Clarias batrachus*

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(Received: May 21, 2007 ; Revised received: October 28, 2007 ; Accepted: December 10, 2007)

Abstract: The present study was undertaken to find out the toxic effect of synthetic pyrethroid lambda cyhalothrin on the functioning of endocrine glands in freshwater catfish, *Clarias batrachus*. The fish were exposed to the pesticide for a period of 45 days at a sublethal concentration of 5.768 ppm. Analysis of hormone profile was carried out on the 15, 30 and 45 days of exposure to find out the alteration in hormone secretion and the response of the fish to the compound. The results obtained showed a significant decline ($p < 0.05$) in levels of thyroid hormones and testosterone while a significant increase ($p < 0.05$) was observed in cortisol levels during the different days of exposure of the fish to lambda cyhalothrin.

Key words: Lambda cyhalothrin, Thyroid hormones, Cortisol, Testosterone, *Clarias batrachus*
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Introduction

Pesticide usage is a critical concern which may have an adverse effect on the delicate ecosystem. The transport of pesticides to delicate ecosystem therefore creates a need to fully understand the effects in the resident biota. In many areas of the world these sensitive ecosystems are at a risk because of non - point source runoff of pesticides from agricultural and urban sources to aquatic ecosystems affecting aquatic biota (Austin, 1999; Srivastava *et al.*, 2008). Pesticides are carried by wind or percolate through water where it is finally washed down to rivers and streams. Pesticides not only alter the physico-chemical properties of water but also adversely affect the aquatic organisms (De Vlaming *et al.*, 2000; Parma *et al.*, 2007). Fish as a bioindicator species play an increasingly important role in monitoring water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution of aquatic ecosystem. There are also responses specific to a single pollutant or a group of contaminants (Svobodova, 1997). In aquatic organisms, the pollutants percolate upto the cellular level through the cell membrane and interact with the cellular macromolecules to inhibit the essential cellular metabolism (Siroka and Drastichova, 2004).

Environmental endocrine disruptors have been defined by the US Environmental Protection Agency as "exogenous agents that interfere with the synthesis, secretion, transport, binding action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development or behaviour". A large number of man made chemicals that have been released into the environment have the potential to disrupt the endocrine system of animals (Colborn, 2002). Much concern has recently been raised on the possibility that synthetic chemicals present

in the environment may mimic, block or modify the action of hormones, altering the pattern of synthesis and metabolism of endogenous hormones by modifying the hormone receptor level. Evidence suggest that these synthetic chemicals were detrimental to human and wild life including fish (Colborn *et al.*, 1993; Zmudzki and Szkoda, 1996).

Xenobiotics bind to specific cellular structures called receptors that are localised on the cell surface or inside the cell either in its cytoplasm, nucleus or cell organelles. The binding of a xenobiotic with its receptor may induce cellular processes that have toxic or other adverse effect on the cell and also resulting in altered gene expression (Kavlock, 1996; Danzo, 1997; Zachareswski, 1998). Some of these compounds undergo bioconcentration and biomagnification by interfering or disrupting the normal metabolic processes of organisms (Weis and Weis, 1987).

Increased use of synthetic pyrethroids as pesticides in agricultural and pest control practices has resulted in increased pyrethroid residues in freshwater and marine sediments. The offsite movement of these compounds into surface waters and water sediment is of concern (Yang and Gan, 2004). Pyrethroids have high acute toxicity to a broad spectrum of aquatic organisms and thus it is imperative to understand the ecotoxicological implication of synthetic pyrethroids in water sediments and its susceptibility to benthic dwelling fish (Laskowski, 2002). Lambda cyhalothrin is categorised as restricted use pesticide in Extension Toxicology Network for its toxicity to fish (Maud *et al.*, 1998). Effects of lambda cyhalothrin on environment depends on the amount of pesticide present, the length and frequency of exposure. Scientists have observed that cyhalothrin is extensively metabolised in mammalian species. The main routes of metabolism include ester hydrolysis, oxidation and conjugation (Anonymous,

1998). However, the metabolism of this compound in fish is oxidative. Fish tend to lack the enzymatic machinery for the metabolism of this pyrethroid which is the obvious reason for the deleterious effect of this pesticide on fish (Demonte, 1989).

Endocrine systems play an important role in the control of physiological processes, reproduction, metabolism and growth in fish (Ankley et al., 1998). Certain environmental contaminants can alter the reproductive physiology, growth and development of vertebrates by disrupting the normal functioning of the endocrine systems due to environmental stress (Colborn, 2002; Kumar et al., 2007). The internal hormonal response to stress is the endocrine status. The primary (endocrine) response to stress are more immediate than the induced secondary (metabolic) responses (Munck et al., 1984). In the present study freshwater edible catfish, *Clarias batrachus* was exposed to sublethal concentration of lambda cyhalothrin which gives an insight on the effect of this pesticide on hormonal profiles during different periods of exposure.

Materials and Methods

The technical grade synthetic pyrethroid, lambda cyhalothrin with 95% purity used in the present study was supplied by Rallis India Ltd., Bangalore, India for evaluation of its toxic effects on nutritionally important freshwater catfish, *Clarias batrachus*.

Healthy adult male catfish, *Clarias batrachus* weighing 200-225 g and 30-35 cm was used as the experimental model to evaluate the toxicity of lambda cyhalothrin, a synthetic pyrethroid widely used for agricultural applications in various sectors. The fish were procured from the local fish market at Maduravoyil, Chennai, Tamil Nadu. They were brought to the laboratory and acclimatised under laboratory conditions for a period of three weeks and fed *ad libitum*.

The fish were maintained in rectangular plastic tubs (64 cm x 44 cm x 29.5 cm) filled with 20 litres of dechlorinated tap water. The tubs were disinfected with 0.01% potassium permanganate solution and washed thoroughly prior to introduction of fish to prevent any fungal infection. Feeding was stopped 24 hr before the commencement of the toxicity test to keep the animals more or less in the same metabolic state. The water quality was determined periodically and physico-chemical characteristics such as temperature, pH, salinity, dissolved oxygen, total hardness and alkalinity were analysed following standard procedures (APHA, 2005).

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of experimental fish to synthetic pyrethroid, lambda cyhalothrin for 96 hr. To determine 96 hr LC₅₀ static renewable bioassay method was adopted (Sprague, 1971). Five groups of 10 fish each were set up in triplicates for the LC₅₀ calculation. The LC₅₀ bioassay method involved the exposure of the five groups of fish to a range of five different concentrations of lambda cyhalothrin. Each group containing 10 fish were maintained in 20 litres of tap water and the survival rate was noted for a period of 96 hr. The concentration at which around 50% survival/mortality occurred after 96 hr was taken as the median lethal concentration.

The 96 hr LC₅₀ was determined by Probit analysis method (Finney, 1971). The LC₅₀ concentration for 96 hr was found to be 28.84 ppm. One-fifth of the LC₅₀ concentration was taken as the sublethal concentration. The fish were maintained for a period of 45 days at a sublethal concentration (5.768 ppm). Group I served as control while Group II was exposed to sublethal concentration of lambda cyhalothrin for a period of 45 days.

The control and the experimental animals were fed with minced goat liver. Water was exchanged daily throughout the experimental period at 8 hr which facilitated the removal of nitrogenous waste excreted by the control and test fish and for the removal of unconsumed food. After renewal of water the required quantity of the pesticide from the stock solution was added to maintain the toxic concentration of the water medium.

At the end of every 15, 30 and 45 days six fish were sacrificed by cervical decapitation. Blood was collected from the control and experimental group animals by severing the caudal peduncle using 5.0 ml graduated hypodermic syringe fitted with 26G needle causing minimum stress to the fish. Blood was transferred into small vials containing 5% EDTA as an anticoagulant for plasma separation and further centrifuged at 2,500 rpm for 20 min to separate plasma and stored at 20°C. The plasma samples were used for study of hormone profiles.

Radioimmunoassay of T₃ and T₄: Plasma T₃ and T₄ were assayed using the RIA kits of Bhabha Atomic Research Centre, Bombay. Standard displacement curves were prepared using T₃ and T₄ standards in hormone free serum.

Estimation of plasma cortisol: Cortisol was assayed by the fluorimetric method of Mattingly (1962). The standard curve was linear over the ranges observed with the plasma samples. Known amount of cortisol (100 ng) was added to plasma samples for recovery. The percentage recovery was found to be 82.7% and the final values were adjusted accordingly.

Radioimmunoassay of testosterone: Testosterone was estimated according to the procedure of Lamba et al. (1981). Testosterone kits were purchased from New England Nuclear Corporation, Boston, MA, USA.

The sensitivity of the assay was found to be 10 pg ml⁻¹. The testosterone antiserum cross reacted with 5 α -dihydrotestosterone (3%), Δ^4 androstenedione (4.3%), 5 α -androstanediol (1.9%), progesterone (1.1%), androstane-3 α -diol (1.5%), cortisol (0.096%), androstane-3 β -diol (2.5%), estradiol (0.061%) and dehydroepiandrosterone sulphate (0.72%).

The data collected on the different parameters of the experimental study were subjected to statistical analysis (Snedecor and Cochran, 1989) by one way analysis of variance (ANOVA) followed by Duncan multiple range tests and the statistical significance was tested at 1 and 5% levels. The percentage change in experimental groups over controls was calculated to determine

Table - 1: Hormone profiles in *Clarias batrachus* exposed to lambda cyhalothrin at sublethal concentration (5.768 ppm)

Hormone profiles (ng ml ⁻¹)	Control	Experimental groups			F-value	p-value
		15 days	30 days	45 days		
Plasma T ₃	1.15±0.10 ^c	1.11±0.06 ^e (-3.47)	0.92±0.05 ^b (-20.00)	0.27±0.06 ^a (-76.52)	162.06	0.000**
Plasma T ₄	4.36±0.19 ^d	3.85±0.18 ^c (+22.93)	2.46±0.34 ^b (-43.57)	1.36±0.12 ^a (-68.80)	164.59	0.000**
Plasma cortisol	30.95±0.66 ^a	37.26±0.87 ^b (+20.38)	47.04±0.48 ^c (+51.98)	60.19±0.07 ^d (+94.47)	2269.82	0.000**
Plasma testosterone	2.42±0.04 ^d	2.08±0.06 ^c (-14.04)	1.22±0.01 ^b (-49.58)	0.92±0.01 ^a (+41.32)	1951.46	0.000**

Values are Mean ± SD (n = 5), p value < 0.01; - ** denotes significance at 1% level (highly significant). Since p value is less than 0.01 there is significant difference between the different days of exposure in higher as well as lower concentration. Different alphabets in means between days in a row denotes significance at 5% level. Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control

% elevation (+) or % reduction (-) during the different days of exposure.

Results and Discussion

The fish were maintained in dechlorinated tap water with the following physico-chemical characteristics: temperature 27 ± 2°C, pH 7.3, salinity 0.24 ± 0.02 ppt, dissolved oxygen 8 mg l⁻¹, total hardness 23.2 ± 0.5 mg l⁻¹, and the alkalinity 18.0 ± 9.0 mg l⁻¹.

The hormonal profiles of fish exposed to the pesticide along with control fish are presented in Table 1. The levels of T₃ showed a significant decrease (p<0.05). A 3.47% decrease was found on the 15th day and 76.52% on the 45th day in the fish exposed to the sublethal concentration whereas, a 22.93% and 68.80% decrease was recorded for T₄ levels on the 15 and 45 days of exposure respectively. Plasma cortisol level was found to gradually increase in experimental groups. The elevated levels was significant (p<0.05) on the 15, 30 and 45 days of exposure of the animals to the pesticide. On the 15 days a 20.38% increase was observed followed by a further increase by 94.47% on the 45 days in the animals exposed to sublethal concentration. A significant decline (p<0.05) was observed in the levels of plasma testosterone from 15 to 45 days in the fish exposed to the sublethal concentration of the pesticide.

Endocrine disruptors bind to hormone receptors and mimic the hormone or block the action of the hormone. They can stimulate or inhibit the enzymes responsible for the synthesis or clearance of a hormone and by giving rise to an increased or decreased action of hormone. The sensitivity of xenobiotics can occur at different levels of regulation including biosynthesis, transport and metabolism (Colborn *et al.*, 1993; Ankley *et al.*, 1998).

The thyroid hormones T₃ and T₄ are essential for the maintenance of metabolic homeostasis in vertebrates. In teleost fish thyroid hormones appear to stimulate virtually all aspects of lipid and protein metabolism including synthesis, mobilisation and degradation (Sheridan, 1994; Sheridan and Kao, 1998). The present study also showed a similar decrease in levels of T₃ and T₄ in experimental fish which was directly proportional to the concentration of the lambda cyhalothrin and duration of exposure. Similar results were reported

in teleost fish *Channa punctatus* exposed to mercuric chloride (Bhattacharya *et al.*, 1989). Toxic effects of Emisan-6 has been reported in thyroid function of *Clarias batrachus* (Kirubakaran and Joy, 1989). Lambda cyhalothrin may have impaired directly the hormone synthesis and its release in circulation. Environmental toxicants have been reported to affect thyroid function (Brown *et al.*, 1989; Leatherland, 1987). The decreased secretion of thyroid hormones could bring physiological alterations in their potential roles in regulating osmoregulation, growth and metabolism (Waring and Brown, 1997) which was also observed in the present study.

Cortisol is the principal and active corticosteroid in teleost fish (Van Der Boon *et al.*, 1991). Glucocorticoid like cortisol are produced in response to stress especially in metabolic adjustment to stress (Bamberger *et al.*, 1996; Mommsen *et al.*, 1999). An adaptive role of cortisol has been suggested to be linked to mobilisation of energy reserves through its catabolic functions in hepatic tissue (Bollard *et al.*, 1993). The increase in plasma cortisol levels in the fish exposed to lambda cyhalothrin may favour gluconeogenesis. There are a number of well characterised metabolic responses associated with glucocorticoids, including activation of gluconeogenic enzymes and the production of glucose via gluconeogenesis to fuel energy dependent process (Bamberger *et al.*, 1996; Mommsen *et al.*, 1999). Similar results under stress condition and the link between glucocorticoids and gluconeogenic enzymes in liver was reported in trouts (Boone and Vijayan, 2002) and salmonid fish (Barton *et al.*, 2002). The autoregulation of glucocorticoid receptor may be a crucial step in stress response and may be a mechanism to increase tissue responsiveness to glucocorticoid stimulation (Boone and Vijayan, 2002; Mommsen *et al.*, 1999). It is evident from the present study that the fish challenged by exposure of pesticide may have a high turnover of glucose and more glucose may be produced from non-carbohydrate substrates for its utilisation by various tissues (Lidman *et al.*, 1979; Murat *et al.*, 1981). The present study also showed an increase in glucose levels in blood during the period of exposure of the fish to the pesticide. The metabolic role of cortisol may have promoted pathways leading to increase in blood glucose levels of fish subjected to acute or chronic stressors (Leach and Taylor, 1980; Vijayan *et al.*, 1994). Cortisol play both direct and permissive role in

glycogenolysis (Mommensen *et al.*, 1999). Increase in cortisol inhibits protein synthesis and stimulates protein catabolism in extrahepatic tissues of teleost fish (Van Der Boon 1991; Mommensen *et al.*, 1999). In the present study the plasma concentration of cortisol has increased considerably in the fish exposed to sublethal concentration of lambda cyhalothrin which could bring about physiological and metabolic changes in the fish to combat the stress conditions at different time periods of pesticide exposure.

Alteration in the levels of testosterone can be caused by contaminants or pesticide interacting with the endocrine system at one or more sites along the hypothalamic-pituitary-testicular axis (McMaster, 1995; Colborn, 2002). The present study showed a marked decrease in the levels of plasma testosterone in fish exposed to sublethal concentration of lambda cyhalothrin. This may be due to disruption of the feed back mechanisms existing between hypothalamic-pituitary-gonadal axis. The decrease in the number of viable steroidogenic cells in the testis may have decreased sex steroid production (Kinnberg *et al.*, 2000; McMaster *et al.*, 1996) which may be due to the effect of toxicant in the testicular cells. There are substantial and consistent evidence that physiological stress has inhibitory effect on reproductive process in teleost fish and these effects are exercised through modified endocrine function (Agrahari *et al.*, 2006). Stress affect the different components of hypothalamic-pituitary-gonadal axis (Consten *et al.*, 2001). The suppressive effects of stress on reproduction are typically reflected in a rapid fall in plasma testosterone (Pottinger *et al.*, 1991; Cheek and McLachlan, 1998; Goos and Consten, 2002).

Pyrethroids are antiandrogenic in nature. These compounds may bind to, activate or inhibit androgen receptors functioning (Pesticide Action Network, 2000). Antiandrogenic pesticides that bind to the androgen receptor also include pyrethroids (Kelce and Gray, 1999). Researchers, have evaluated the potency of the following pyrethroids in terms of their interaction with androgen binding sites and in descending order, fenvalerate > phenothrin > fluvalinate > permethrin > resmethrin (Pesticide Action Network, 2000). It may affect changes in plasma androgen levels or influence bioavailability by affecting steroid synthesis or availability of androgen binding proteins. A similar mechanism could have been possible by lambda cyhalothrin in the present study. Similar studies on endosulfan as an androgenic compound and its mechanism of action in cichlids is been reported (Matthiessen and Logan, 1984).

Goos and Consten (2002) in their studies on the stress adaptation in the male carp, *Cyprinus carpio* has explained that cortisol affect the enzyme activity involved in androgen production in the testicular cells and retardation of testicular development may be mediated by cortisol (Carragher *et al.*, 1989; Consten *et al.*, 2002). Increased glucocorticoid also directly suppress

testosterone production and secretion by decreased testicular LH receptors and reduction of spermatogenesis (Mahitosh *et al.*, 2003) which is also evident from the present investigation. Xenoestrogens and antiandrogenic substances can disrupt the synthesis, transport and metabolism of androgens (Sonnenschein and Soto, 1998). Most environmental antiandrogenic agents antagonise androgen action within the target cell by competing with the androgen receptor and inducing a conformational change of the androgen receptor or by reducing transcriptional activation of target genes at crucial period of growth and development (Kelce and Gray, 1999).

From the present investigation it is clear that exposure of fish to synthetic pyrethroid lambda cyhalothrin at sublethal concentration alters the endocrine functioning and the hormone secretion which could be a condition in synchrony with toxicity induced stress and adaptability of the fish to action of synthetic compounds.

Acknowledgments

The authors are grateful to Dr.R.Kirubakaran, Scientist, Ocean Science Development, National Institute of Ocean Technology, Pallikaranai, Chennai, Tamil Nadu for his help in the assay of hormones.

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