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Ultimobranchial Gland of Freshwater Catfish, *Heteropneustes fossilis* in Response to Deltamethrin Treatment

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In fishes, apart from causing death either directly or due to starvation by destruction of food organisms (Moore *et al.* 1998), many toxicants have been shown to affect growth rate (Pratap 1999), reproduction (Ree and Payne 1997; Jyothi and Narayan 1999) and behaviour, with evidence of tissue damage (Srivastava *et al.* 1989, 1990; Lien *et al.* 1997; Poleksic and Karan 1999). While under experimental conditions, affected fish may survive but in their natural environment such influences can hardly be other than detrimental rendering the fish more vulnerable to predators, less able to compete with other fish species and less able to withstand the normal stresses such as seasonal temperature variation, reproduction or temporary starvation.

Pyrethroids have attracted our interest as they have potent insecticidal properties and are practically nontoxic to most non-target animals, especially mammals (Haya 1989). However, pyrethroids have been reported to be extremely toxic to fish and some beneficial aquatic arthropods, for example, lobster and shrimps (Bradbury and Coats 1989; Haya 1989). Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of the fish to aqueous pyrethroid exposures.

Calcium is vital for living organisms and has been implicated in controlling a wide variety of physiological and biological functions. The ultimobranchial gland (UBG) has been related to control the blood calcium level through the secretion of the hypocalcemic hormone calcitonin (CT) in non-mammalian vertebrates. Besides its calcium regulating function, CT in fishes has also been suggested to be involved either with osmotic regulation (Yamauchi *et al.* 1978), sex-related phenomenon (Deftos *et al.* 1974; Yamane and Yamada 1977) or skeletal protection during periods of high calcium demands (Wendelaar Bonga and Pang 1991). Several workers have studied the effects of environmental toxicants on fish e.g. behavioural responses (Bradbury *et al.* 1985; Edwards *et al.* 1985; Narain and Singh 1990; Singh *et al.* 1997), disturbances in carbohydrate metabolism (Sancho *et al.* 1997; Jyothi and Narayan 1999), hematological anomalies (Prasad *et al.* 1991; Van Vuren *et al.* 1994), and histopathology of vital organs (Srivastava *et al.* 1989, 1990; Chatterjee *et al.* 1997; Akram *et al.* 1999; Poleksic and Karan 1999); but there exists no detailed information regarding the impact of these

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toxicants on endocrine regulation of calcium homeostasis in fish. Hence, it has been undertaken here to study the toxic effect of a pyrethroid – deltamethrin (trade name "Decis") on the serum calcium and histological changes in the ultimobranchial gland of a freshwater catfish *Heteropneustes fossilis.*

MATERIALS AND METHODS

Freshwater catfish, *Heteropneustes fossilis* (both sexes; body wt 38-47 g) were collected locally and acclimatized for 15 days in plastic pools under laboratory conditions (natural photoperiod – 11.58-12.38 and temperature 25.8 ± 1.8 C). They were fed daily 2-3 times with wheat flour pellets and ground-dried shrimps.

Four-day static acute toxicity test (APHA *et al.* 1985) was performed to determine the LC_{50} value of deltamethrin [trade name of pesticide is decis, manufactured by Evid and Company Chemicals Ltd., Ankleshwar (India) in technical collaboration with Hoechst India Limited]. Physicochemical conditions of the tapwater used in the experiment were – pH 7.21 ± 0.06; hardness 167.31 mg/L as $CaCO_3$; dissolved oxygen 7.78 ± 0.30 mg/L; electrical conductivity 306.18 ± 68.52 μmho/cm and no free chlorine.

After determining the LC_{50} value of deltamethrin for 96 hr (which is 1.86 μg/L), the experiments were performed for short-term and long-term durations. The fish, *H. fossilis* (after 15 days acclimation to laboratory conditions) were subjected to 1.49 μg/L (0.8 of 96 hr LC_{50} value) and 0.37 μg/L (0.2 of 96 hr LC_{50} value) solution of deltamethrin for short-term and long-term, respectively. After every 24 hr the test solution was renewed. Concurrently, a control group was exposed to tapwater containing the solvent (acetone). Food was withheld 24 hr prior to the start of the experiment and during the experiment. The fish were sacrificed after 24, 48, 72 and 96 hr in the short-term experiment and after 7, 14, 21 and 28 days in the long-term experiment. Blood was collected on these intervals and serum calcium levels were determined according to the method of Trinder (1960). After collection of the blood samples, the area adjoining the heart and esophagus (as UBG has been found in this region) was extirpated and fixed in aqueous Bouin's fluid. Tissues thus fixed were routinely processed in graded series of alcohols (50%, 70%, 90% and 100%), cleared in xylene and then embedded in paraffin. Serial sections were cut at 6 μm and stained with hematoxylin-eosin (HE).

The nuclear indexes (maximal length and maximal width) of ultimobranchial cells were determined (fifty nuclei were measured per specimen, thus 300 nuclei were measured from six specimens) with the aid of an ocular micrometer and then the nuclear volume was calculated as: volume = $4/3\ \pi\ ab^2$; where 'a' is the major semiaxis and 'b' is the minor semiaxis.

Student's t test was used to analyze the statistical significance between the control and deltamethrin treated fish.

RESULTS AND DISCUSSION

After short-term deltamethrin exposure, the serum calcium levels of *H. fossilis* exhibit no change at 24 hr. The levels indicate a decrease after 48 hr exposure. This response persists till 96 hr (Figure 1). The fish chronically exposed to deltamethrin exhibit a decrease in the calcium level on day 7. This decrease persists progressively till the close of the experiment (28 days; Figure 2).

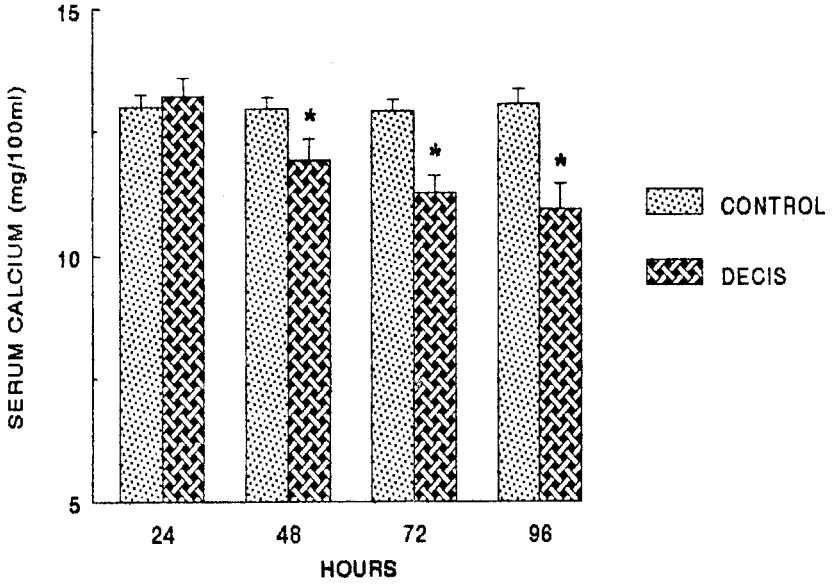

Figure 1. Serum calcium levels of short-term deltamethrin treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

The UBG of control fish usually consists of a solid parenchyma which is composed of cell cords and small follicles (Figure 3). All the cells are alike. Their cell boundaries are indistinct. When stained with HE, the cytoplasm of these cells are noticed slightly eosinophilic.

Up to 72 hr after exposure of the fish to deltamethrin, the UBG exhibit no histological change. After 96 hr a decrease in the staining response of the cytoplasm of ultimobranchial cells has been noticed (Figure 4). The nuclear volume of these cells records a slight decrease (Figure 5).

Up to 14 days following deltamethrin exposure, the UBG exhibit no change. After 21 days following the exposure, the nuclear volume of the ultimobranchial cells records a decrease (Figure 6) and these cells exhibit slight decrease in the staining response of the cytoplasm (Figure 7). Moreover, following 28 days exposure, the nuclear volume exhibits further decrease (Figure 6) and degeneration and vacuolization sets in (Figure 8).

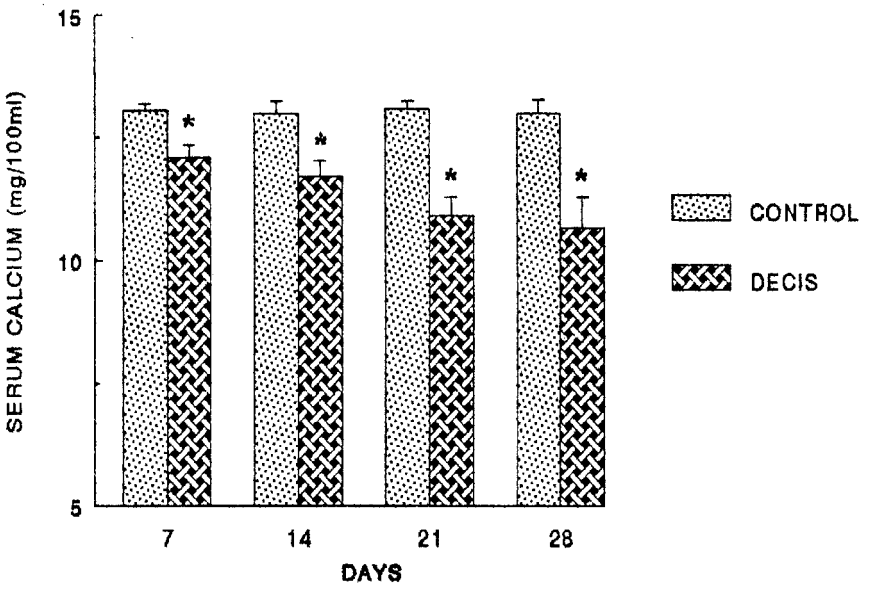

Figure 2. Serum calcium levels of long-term deltamethrin treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($P < 0.05$) from control.

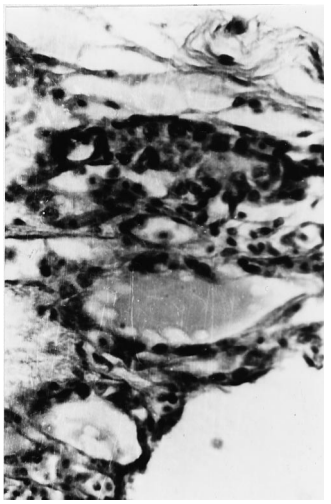

Figure 3. Ultimobranchial gland of control *Heteropneustes fossilis* exhibiting follicles and cell cords. HE x 200.

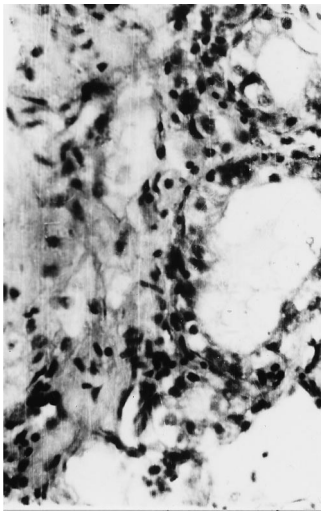

Figure 4. Ultimobranchial gland of 96 hr deltamethrin treated fish showing decreased staining response of the cytoplasm. HE x 200.

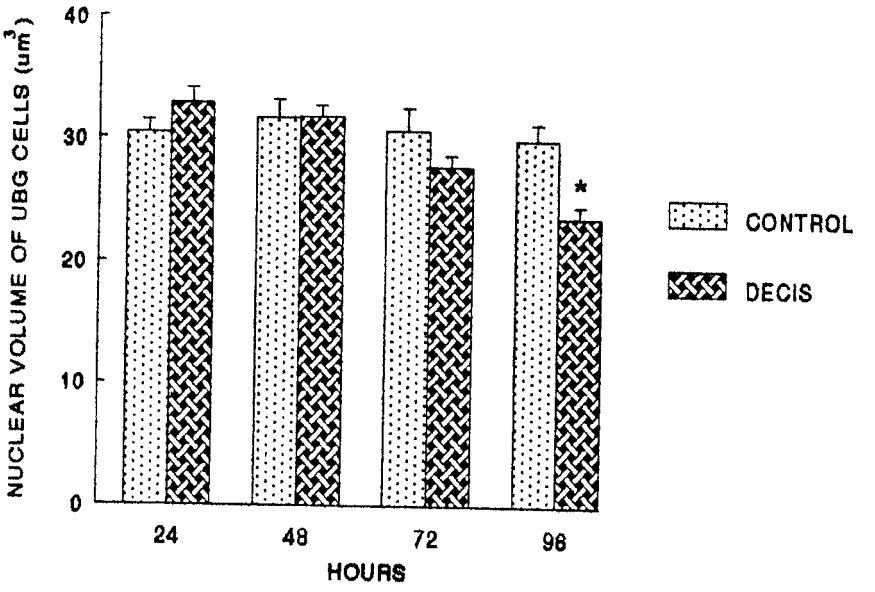

Figure 5. Nuclear volume of ultimobranchial cells of short-term deltamethrin treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P <0.05) from control.

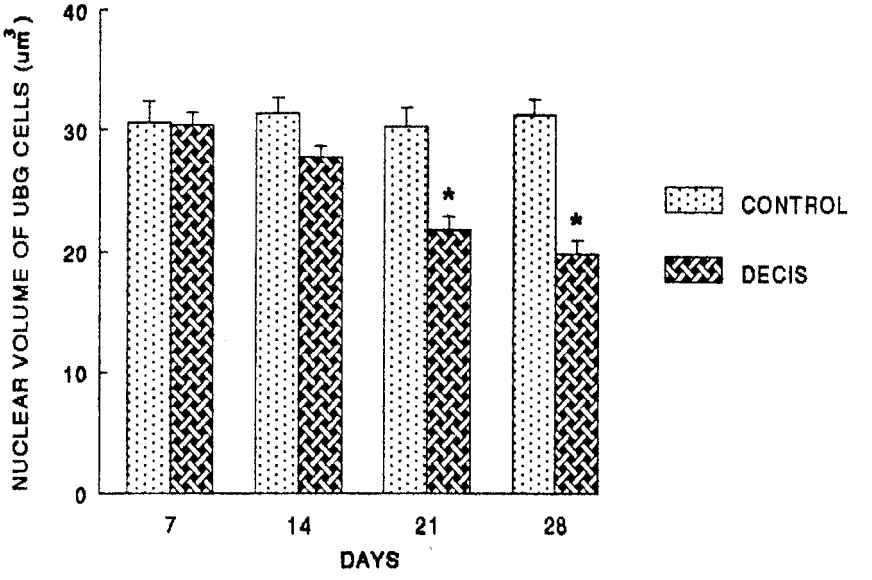

Figure 6. Nuclear volume of ultimobranchial cells of long-term deltamethrin treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P <0.05) from control.

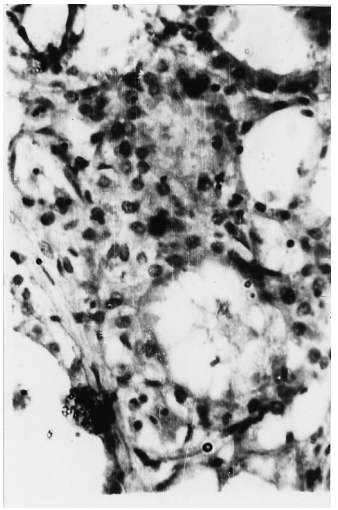

Figure 7. Ultimobranchial gland of 21 days deltamethrin exposed fish showing decreased staining response of cytoplasm. HE x 200.

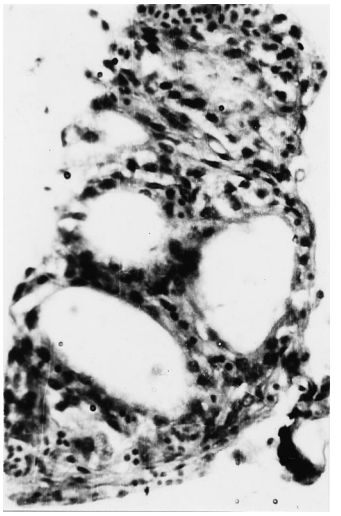

Figure 8. Ultimobranchial cells of 28 days deltamethrin treated *Heteropneustes fossilis* exhibiting degeneration and vacuolization. HE x 200.

In deltamethrin exposed fish the ultimobranchial gland displays reduced activity which is evident by the decreased nuclear volume of the ultimobranchial cells as well as poor staining response of these cells. In the present study deltamethrin exposed fish also exhibits degeneration and vacuolization in the ultimobranchial gland. The hypoactivity/inactivity of the ultimobranchial cells may be explained due to the hypocalcemia observed in the deltamethrin exposed *H. fossilis*. Except for the foregoing study there exists no other report regarding the effect of pesticides on the ultimobranchial gland of fish. The present study derives support from the reports of other investigators who have reported hypoactivity/inactivity of the ultimobranchial gland of the fish administered with a hypocalcemic hormone – calcitonin (Peignoux-Devielle *et al.* 1975; Wendelaar Bonga 1980; Srivastav *et al.* 1989; Tiwari 1993).

The observed degeneration and vacuolization in the ultimobranchial gland of the deltamethrin treated fish may be explained due to the continuous disuse of the gland in response to prolonged hypocalcemia caused by the pesticide exposure.

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