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Response of Serum Calcium and Inorganic Phosphate of Freshwater Catfish, *Heteropneustes fossilis,* to Chlorpyrifos

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Due to the restrictions imposed on the use of organochlorine in many countries, organophosphate pesticides are now being widely used. Chlorpytifos [O,O-diethyl-O-(3,5,8-ttiohloro-2-pyridyl) phosphorothioate 3, is a member of organophosphate class of insecticides that displays broad-spectrum insecticidal activity against a number of important arthropod pests (Racke 1993). It is recommended as a most effective insecticide for the control of mustard aphid, *Liapaphis erysimi* (Srivastava and Srivastava 1988). The wide-spread use of chlorpyrifos is probably due to its relatively low mammalian toxicity. The use of chlorpyrifos to area of fish breeding causes toxicological hazards especially in view of its high toxicity to fish (Herzberg 1987).

The fish live in intimate contact with the surrounding water through their gills (Pratap *et al.* 1989) which comprises over half the body surface area (Wood and Soivio 1991). In fish, only a few microns of delicate gill epithelium separates the internal environment from a continually flowing external environment (Wood and Soivio 1991). Thus it makes the fish very susceptible to aquatic pollutants. In freshwater fish, blood electrolyte concentration is regulated by many interacting processes -- absorption of eletrolytes from surrounding medium through active mechanisms, predominantly at the gill; control of water permeability; and selective reabsorption of electrolytes from urine. Any alteration in one or more of the above mentioned processes would result in a change in the plasma electrolyte composition. Although, several investigations have reported the toxicity (Johnson and Finley 1980; Borthwick *et al,* 1985; Barron *et al.* 1991) and histopathological changes in the gill (Srivastava *et al.* 1989) and kidney (Srivastava *et al.* 1990) of fish after chlorpyrifos treatment, there exists no information on the impact of chlorpyrifos on blood electrolytes of fish. The present study is an attempt to fill this void and reports the effects of short-term (98 hr) and long-term (28 days) sublethal exposure of chlorpyrifos on serum calcium and phosphate levels of the fish, *Heteropneustes fossilis.*

MATERIALS AND METHODS

Live specimens of freshwater catfish, *Heteropneustes fossilis* (both sexes,

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body wt 42-53 g) were procured locally and acclimatized to the laboratory conditions (under natural photoperiod -- 11.58 -12.38 and temperature-- 25.8 + 1.8 C) for 15 *days* in plastic pools. They were fed daily with wheat flour pellets and ground dried shrimps, 2-3 times/day.

Four-day static acute toxicity test (APHA *et al.* 1985 *) was* performed *to* determine the LC_{50} value of chlorpynfos [trade name of pesticide is coroban, manufactured by Coromandel lndag Product (India) Pvt. Ltd., Madras]. The tapwater used in the experiment had pH 7.21 + 0.06; hardness 187.32 + 5.81 mg/l as $CaCO_3$; dissolved oxygen 7.78 + 0.30 mg/l; electrical conductivity 306.18 + 68.52 umho/cm and no free chlorine.

After determining the LC_{50} value of chlotpyrifos for 96 hr (which is 2.20 mg/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 1.76 mg/l (0.8 of 96 hr LC_{50} value) and 0.44 mg/l (0.2 of 96 hr LC_{50} value) solution of chlorpyrifos for short-term and long-term duration, respectively. The test solution was renewed after every 24 hr. Concurrently, a control group was used for comparison by using the 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 short-term experiment and after 7, 14,21 and 28 days in long-term experiment The blood was collected and determination of serum calcium (Trinder 1960) and inorganic phosphate (Fiske and Subbarow 1925) levels were performed.

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

RESULTS AND DlSCUSSlON

After short-term chlorpyrifos exposure, the serum calcium level of *H. fossilis* decreased progressively from 24 hr till 72 hr. The levels indicate a tendency to increase at 96 hr, however, it is still hypocalcemic (Figure 1). The serum phospahte levels of chlorpyrifos treated fish remain unaffected uptill 48 hr. From 72 hr onwards, the levels decrease progressively (Figure 2).

The serum calcium level of chronically-chlorpyrifos exposed fish exhibit a decrease on day 7. Thereafter, the levels continue to fall progressively till the end of the experiment (28 days, Figure 3).The serum phosphate levels of chlorpyrifos treated fish show a decrease on day 7 and 14. However, on day 21 and 28, the levels are almost normal (Figure 4).

In the present study chlorpyrifos exposure caused hypocalcemia in fish *H.* fossilis. This is in agreement with reports of earlier investigators who have observed decreased blood/plasma content of calcium of fish treated with toxicants - aldrin (Sane 1982; Singh *et al.* 1996), malaohite green (Srivastava *et al.* 1995) and cadmium (Larsson *et al.* 1981; Muramoto 1981; Glles 1984; Pratap *et al.* l989). In contrast, other investigators have observed elevation of

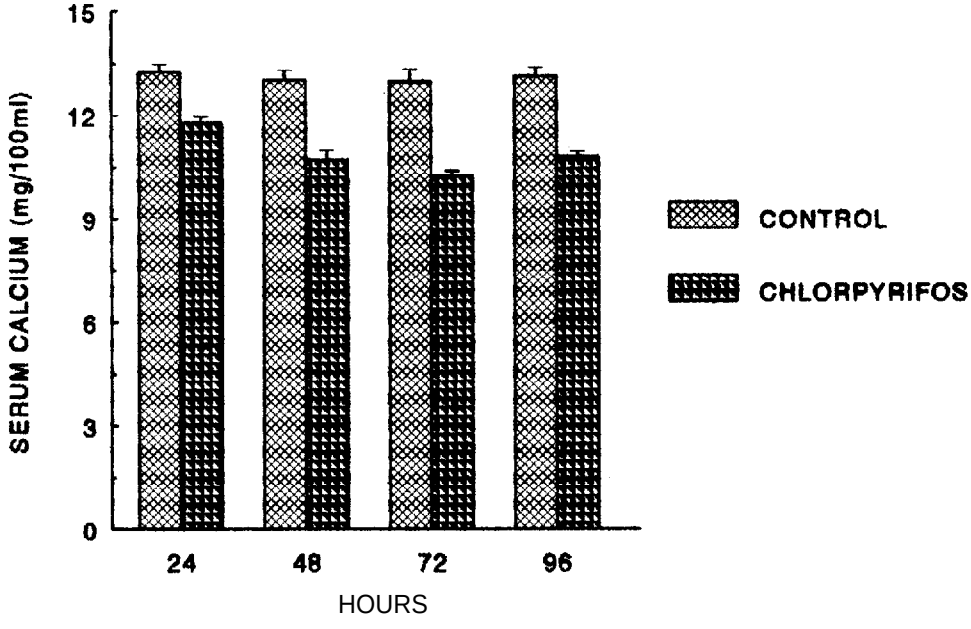

Figure 1. Serum calcium levels of short-term chlorpyrifos treated *Heteropneusts fossilis.* Values are mean ±S.E. of six specimens. 'a' indicates significant differences (P < 0.05) from control.

plasma calcium levels of fish exposed to pesticides (Sastry and Sharma 1978; Sansal *et al.* 1979; Dalela *et al.* 1981; Sharma *et al.* 1982 *).* The observed hypocalcemia in chlorpyrifos exposed *H. fossils* could be attributed to the impairment of either net electrolyte influx at the gill or renal function. Degenerative changes in gill (Srivastava et al. 1989) and kidney (Srivastava *et al.* 1990) have been reported from chlorpyrifos exposed *H. fossilis.*

Chlorpyrifos exposure to fish caused hypophosphatemia. However, Pratap *et al.* (1989) have found no effect on plasma phosphate levels of fish exposed to cadmium in water. In contrast to the present study, hyperphosphatemia has been reported in fish after exposure to various toxicants -- endrin (Colvin and Phillips 1968), endosulfan (Gill *et al.* 1991) and aldrin (Singh *et al.* 1996). Flik *et al.* (1985) have reported that phospahte is absorbed exclusively via the gut. Keeping this in view, the observed hypophosphatemia in *H. fossilis* could be linked to redistribution of electrolytes between intracellular or extracellular compartments and/or impairment of renal function.

It is concluded that although the exposure of chlorpyrifos to the fish at lower doses may not result into death of the fish but cause serious disturbances in the calcium and phosphorus homeostatsis which may affect the reproductive state of the fish as both these ions are important for the synthesis of a female-specific lipophosphoprotein, vitellogenin.

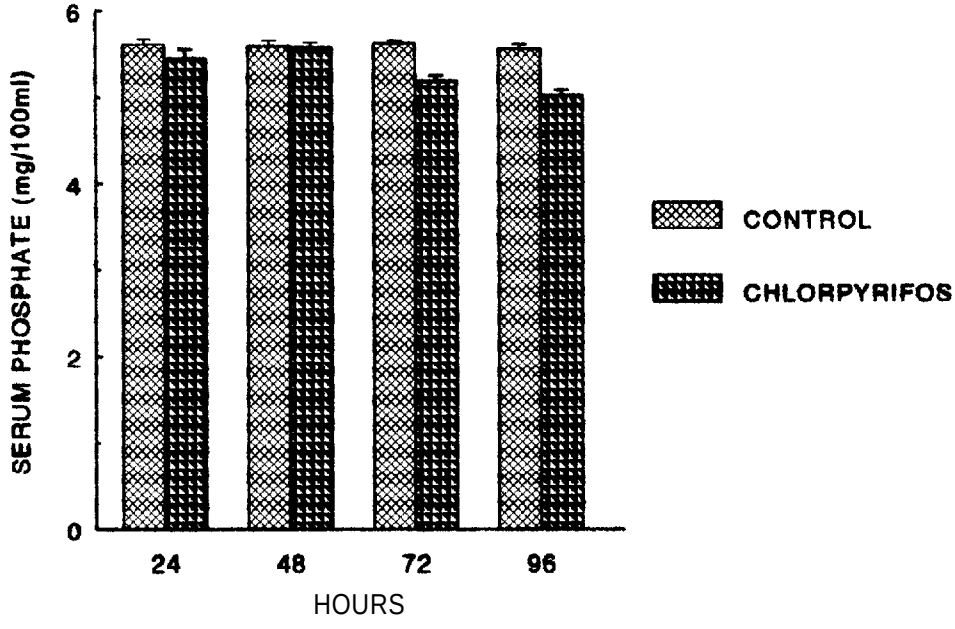

Figure 2. Serum phosphate levels of short-term chlorpyrifos exposed fish. Values are mean + S.E. of six specimens. 'a' indicates significant differences ($P < 0.05$) from control.

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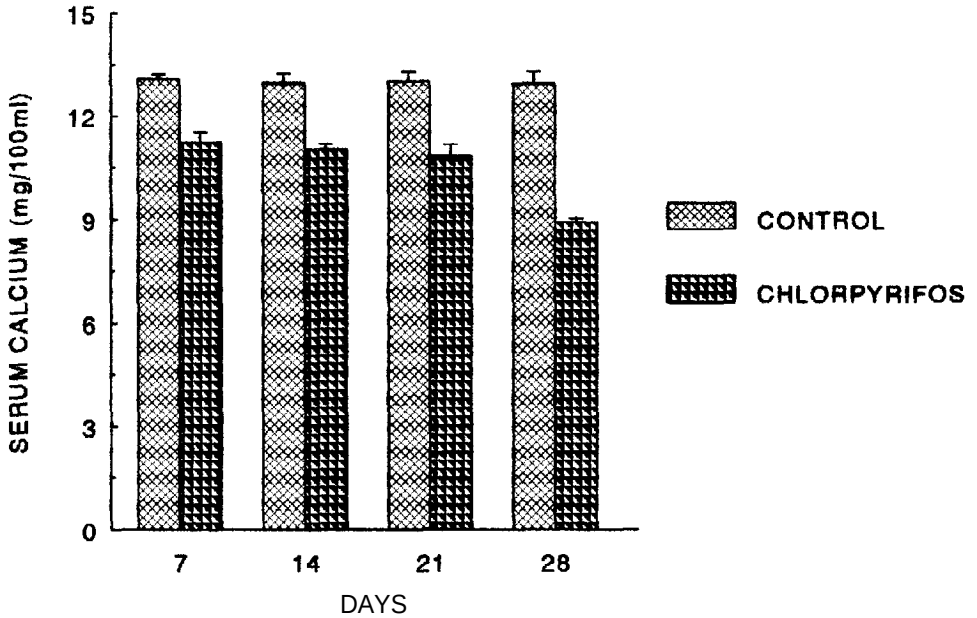

Figure 3. Serum calcium levels of long-term chlorpyrifos exposed fish. Values are mean + S.E. of six specimens. 'a' indicates significant differences ($P < 0.05$) from control.

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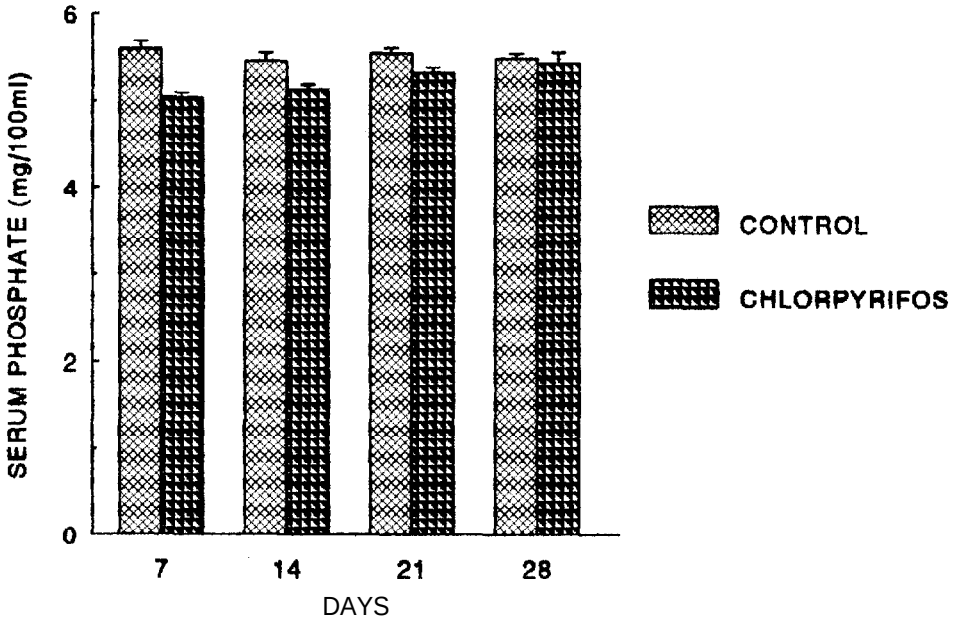

Figure 4. Serum phosphate levels of long-term chlorpyrifos exposed *Heteropneustes fossilis.* Values are mean + S.E. of six specimens. 'a' indicates significant differences (P c<0.05) from control.

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