Punjab Univ. J. Zool., Vol.19, pp.9-16, 2004

TERATOLOGICAL EFFECTS OF CHLORPYRIFOS ON CHICK DEVELOPMENT

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Abstract: Chlorpyrifos, an organophosphate insecticide, was tested for embryotoxic and teratogenic effects in chick. Different aqueous concentrations of chlorpyrifos (2.0, 1.0, 0.5, 0.25 and 0.025 µg/egg) were injected in yolk sac of eggs at day 4 of incubation. Embryo recoveries were made at day 7 of incubation.

At day 7, morphological, anatomical and morphometric studies revealed concentration dependent adverse effects of the insecticide. The developmental defects were reduced in CR length, microcephaly, under-developed eyes, agenesis of beak, micromelia, Amelia, excencephaly and ectopia cordis.

The present study indicates that Chlorpyrifos is potentially dangerous to avian development at every very low doses.

Key words: Chlorpyrifos, embryotoxicity, teratogenicity, avian development.

INTRODUCTION

By controlling agricultural pests, pesticides have contributed to dramatic increase in crop-yields and in the quantity and variety of diet. Organophosphates and carbamates are however toxic to nervous system and caused many cases of acute poisoning (Blondell, 1977).

The pesticides may be rapidly absorbed through skin, lungs or gut producing acute toxic effects, e.g., headaches, dizziness, sweating, nausea, vomiting and difficulty in breathing. In severe cases of over-exposure, coma, respiratory depression and death may occur. Pesticides are responsible for birth defects, genetic mutation, damage to the immune system, reproductive system, deformations, neurological damage, mild cognitive dysfunction, lung damage, dysfunction of the endocrine system, cancer and other health effects (Schwartz *et al.*, 1990; Jeyaratnum, 1990; Arm *et al.*, 1993; Faustman *et al.*, 2000).

Each year, over 1 million people are poisoned by pesticides, with 20,000 deaths (Jeyaratnum, 1990). Distribution of toxic waste dump is associated with highest breast cancer mortality and birth defects. Approximately 250,000 U.S. children are born each

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year with birth-defects diagnosed at or shortly after birth. Birth defects are the leading cause of infant mortality (Croen *et al.*, 1997).

Women exposed to pesticides through agricultural or floricultural work, have been documented to have significantly higher risk of children born with musculoskeletal defects, limb defects, growth retardation and learning behavioral disorders (Faustman *et al.*, 2000; Schwartz *et al.*, 1990).

People are also exposed to organophosphates (e.g., diazinon, chlorpyrifos, malathion etc.) through food residues (e.g., fruits, vegetables, grains, fish and milk) and from a survey made by Wiles and Compbell (1993), it becomes clear that all the fruit, vegetables of daily use e.g., apple, banana, grapes, orange, potato, carrot etc., contain significant amount of pesticides. Malathion (75%), Chlorpyrifos (38%) and p,p, DDE (21%) were detected in at least 20% of all food samples. This is because organophosphorous insecticides are rapidly absorbed by the plants through roots or directly through leaves and fruits (Ware, 1986).

Chlorpyrifos CPF) is used to control mosquitoes and house-hold insects. In man, it has toxic effects on the central nervous system, the cardiovascular system and the respiratory system. A study investigated the toxicity of CPF on nervous system development in rat embryo using the mid-brain micromass culture system. All demonstrated toxicity in mid-brain micromass cells with IC sub (50) values below 30 ng/mL indicating a potent teratogen (Cosenza and Biodanset, 1995).

The use of chlorpyrifos to area of fish breeding causes toxicological hazards especially in view of its highest toxicity to fish. Several investigations have reported the toxicity and histopathological changes in the gills and kidneys of fish after chlorpyrifos treatment (Srivastava *et al.*, 1997).

Chlorpyrifos which is most numerous in home's atmosphere and children are its definite and ultimate target, because of their behavior, diet etc. Thus, the present study was planned to evaluate the embryotoxic and teratogenic potential of CPF in developing chicks.

MATERIALS AND METHODS

Fresh eggs (of White leghorn breed) were purchased from Veterinary Research Institute, Lahore. The eggs were divided randomly (irrespective of their size, shape and colour) into 6 groups. Of them, 5 groups were treated with different concentrations (0.025, 0.25, 0.5, 1.0 and 2.0 µg/egg). The other group was control without any treatment.

Chlorpyrifos was available in market in liquid form with trade name, Terminus-T.C.L.O. (Jia Ji Co. Ltd., Pakistan).

The eggs were cleaned with a piece of cotton soaked in alcohol. A small window was made in the shell of each egg except 'C' group eggs, with the help of a sterilized scalpel, provided shell membrane was not ruptured. The aqueous solution of Chlorpyrifos (0.1 ml) was injected into the yolk sac to eggs of respective groups with micro-applicator (glass syringe). All concentrations were applied before incubation. The eggs were incubated at standard conditions of chick egg incubation.

Embryonic recoveries were made at 7^{th} day of incubation. Embryos were fixed in Bouin's fixative for 48 hours. After that embryos were washed in 70% alcohol till they became clear and were finally preserved in 80% alcohol for morphological studies.

Morphological observations involved measurements of crown-rump length as well as gross anatomical observations. These observations included the studies of development conditions of brain, eyes, ear, heart, limbs and beak.

These organs were studied with the help of magnifying lens and with naked eye depending upon the size of the embryo. The observation data were tabulated and analyzed for comparison of development.

RESULTS AND DISCUSSION

The observations made during this study clearly showed the embryotoxic and teratogenic potential of chlorpyrifos. A significant ($P<0.001$) decrease in CR length was noted in all dose groups (Table I). In all above groups a high percentage of developmental abnormalities was studied. Developmental anomalies include agenesis of beak, microcephaly, microphthalmia, anophthalmia, micromelia and twisted spinal cord (Fig.1b-g).

Chlorpyrifos is a cholinesterase inhibiting organophosphate pesticide used extensively to treat crops and domestic animals. Cohn and Macphail (1997) determined the effects of acute and repeated CPF exposure on the acquisition and performance of response sequence. Radiometric analysis of serum cholinesterase activity showed CPF produced 90% inhibition at 3 hr and 85% inhibition at 24 hr post exposure.

In a set of experiments, Bagchi *et al.* (1995) determined the *in vitro* effects of CPF on DNA-SSB (Single Stand Break) and enhanced lactate dehydrogenase leakage (LDH) from neuroactive DC-12 cells in culture. In treatment of rats with chlorpyrifos increase of 4.3 folds was observed in hepatic lipid peroxidation and increase of 3.0 fold in

hepatic DNA-SSB, increase of 1.4 fold in brain nuclear DNA-SSB. Increase of 4.9 fold was observed in chemiluminescence, following *in vitro* incubation of the liver homogenates with chlorpyrifos and 3.1 fold in LDH leakage.

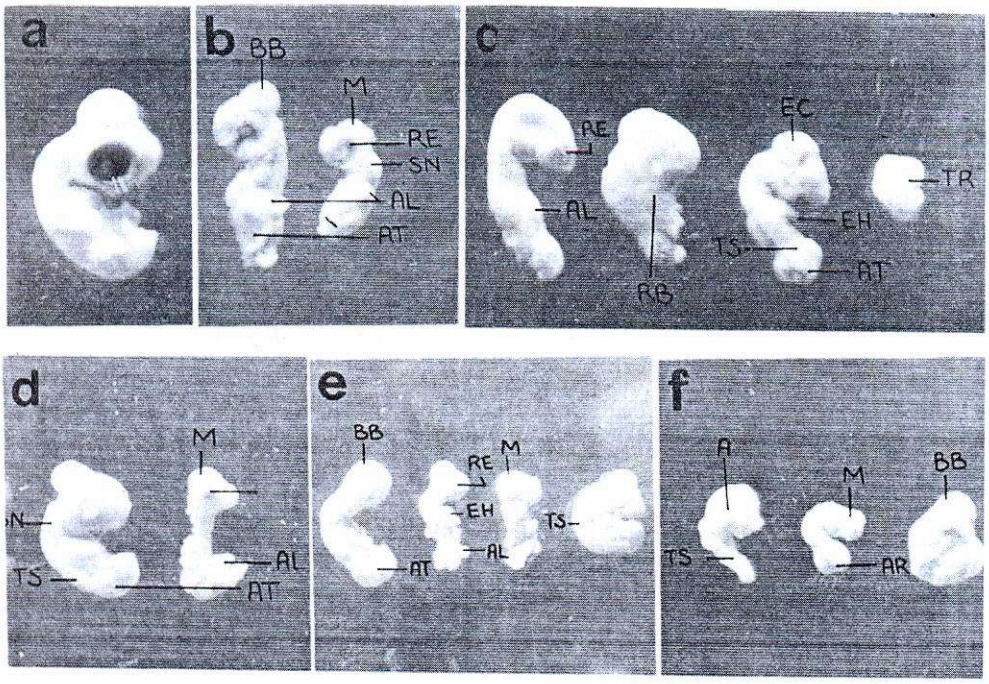

Fig. 1: Macrophotographs of embryos recovered on day 7 of incubation a) control, b) 0.025 µg/egg, c) 0.25 µg/egg, d) 0.5 µg/egg, e) 1.0 µg/egg and f) 2.0 µg/egg. Note, developmental defects caused by the insecticide in treated groups of eggs: Anophthalmia (A), micromelia (AL), abnormal rump (AR), abnormal trunk (AT), abnormal brain bulges (BB), exencephaly (EC), ectopia cordis (EH), microcephaly (M), short neck (N), resorption of limb (RB), microphthalmia (RE), twisted neck (SN), total resorption (TR) and twisted spinal cord (TS).

So, it became obvious that chlorpyrifos induces production of reactive oxygen species and oxidative tissue damage which may contribute to the toxic manifestation of programmed cell death (apoptosis) in response to many toxicants including chlorpyrifos.

Chlorpyrifos was evaluated for potential to produce developmental and reproductive toxicity in rats following oral exposure. Maternal effects noted at the two higher dose levels including decreased cholinesterase levels at 3.0 mg/kg per day and cholinergic signs (extensive salivation and tremors), decreased cholinesterase levels and decreased body weights.

Table I: Developmental anomalies induced by different concentrations of chlorpyrifos in chick embryos recovered on day 7 of incubation.

Doses (mg/egg)	CR length (mm±SD)	Beak	Eyes	Neck	Fore-limbs	Hind-limbs	Head	Spinal Cord
0.00	16.7±1.20 (20)	Normal	Normal closed	Well developed	Well developed	Well developed	Normal	Normal
0.025	12.8±4.37** (30)	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia (100)	Micromelia (100)	Abnormal brain bulges (50) Microcephaly (50)	Twisted (100)
0.25	12.3±1.30** (30)	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia	Micromelia	Abnormal brain bulges (100)	Twisted (100)
0.5	11.06±0.94*** (30	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia	Amelia (50) Reduced (50)	Abnormal brain bulges (50) Microcephaly (50)	Twisted (100)
1.00	9.59±2.24** (30)	Agenesis (100)	Anophthalmia (50)	Small (100)	Micromelia	Micromelia (100)	Microcephaly (100)	Twisted (100)
2.0	7.17±1.26***	Agenesis (100)	Anophthalmia (100)	Not different-tiated (100)	Amelia (100)	Amelia (100)	Anencephaly (50) Microcephaly (50)	Twisted (100)

Significantly reduced against the controls; ** = P<0.01; *** = P<0.001.

Parental effects included decreased plasma and erythrocyte cholinesterase at 1.0 mg/kg/day and decreased plasma, erythrocyte and brain cholinesterase and histopathological alteration of the adrenal zone fasiculate at 5.0 mg/kg/day. Effects on neonatal growth and survival were observed at a maternally toxic dose level in one generation. However, this effect was not observed in the subsequent generation (Breslin *et al.*, 1996).

The mechanism of chlorpyrifos induced neurotoxicity was studied by Song *et al.* (1997) who found that chlorpyrifos evoked deficits in multiple components of the adenylcyclase cascade in brain cells, a system that mediate cholinergic as well as adrenergic signals.

Slotkin (1999) reported that the chlorpyrifos inhibits DNA synthesis *in vitro* in cultures of fetal rat neurons, additionally, cell replication is inhibited, cell acquisition is arrested and neurotoxic apoptosis is accelerated.

Developing mammals are also markedly more sensitive to acute toxicity from exposure to a variety of organophosphorous pesticides. Brain and plasma cholinesterase activity in neonatal and adult rats exposed to sublethal doses of chlorpyrifos showed that the correlation between rats was high but lower in adults (Pope and Chakraborti, 1992; Chanda and Pope, 1996; Chanda *et al.*, 1997).

Chlorpyrifos may exhibit developmental toxicity to the fetal nervous system at relatively low doses. A study by Whitney *et al.* (1995) revealed that administration of chlorpyrifos to neonatal rats at 1 day of age (approximately equivalent to human fetal exposure at 7 months of gestation) produced significant inhibition of DNA and protein synthesis throughout brain. The author interpreted these results as indicating that low doses of chlorpyrifos target the developing brain during the critical period in which cell division is occurring, effects which may produce eventual cellular, synaptic and behavioural aberrations after repeated or prolonged sub-toxic exposure.

Similarly, another recent study found that repeated exposure of pregnant rats to low doses of chlorpyrifos result in long-term neurochemical and behavioural deficits in the offspring (Slotkin, 1999).

These results and some earlier studies have indicated that chlorpyrifos is toxic to embryonic and fatal tissues and can induce teratogenicity in chick. So, it may be used under extreme necessity and great care.

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(Received: December, 2003)