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EMBRYOTOXICITY OF METHYLPARATHION IN DEVELOPING CHICK

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Abstract: Methylparathion, an organophosphates insecticide, was tested for its embryotoxicity in chicks. Different concentrations of the insecticide ranging from 1.00 to 10.00 µg/egg were injected into the yolk sac of chick eggs at day 4 of incubation. All treated embryos were reduced significantly ($P<0.00$) in CR length on day 7 of incubation. Besides a higher embryonic mortality, the survivors had sever gross malformations including microcephaly, anophthalmia, micromelia, twisted spinal cord and ectopia cardis. It is suggested as this insecticide is very dangerous for developing organism, it should be used very carefully.

Key words: An organophosphate insecticide, methylparathion, developing chick, embryotoxicity.

INTRODUCTION

Organophosphates are generally amongst the most acutely toxic of all pesticides to vertebrate animals. Using all relevant federal data on food consumption and pesticide residue on food, The Environment Working Group concluded that 9 of 10 American children of age 6 months to 5 years ingest organophosphate insecticide in their food each day (Wiles *et al.*, 1998). Dinham, (1993) revealed that there are in total 3 million acute sever cases of pesticide poisonings. Of the poisonings, a large proportion involves organophosphates. Organophosphate applicators had significantly more dizziness, sleepiness, headache and higher neurological symptom scores than non-applicators (London *et al.*, 1998). Wide range of neuropsychological tests, including memory, attention, problem solving and dexterity (Rosenstock *et al.*, 1991) and cardiac effects are associated with occupational exposure to organophosphates. Organophosphate insecticides exert their acute effect in both insects and mammals by inhibiting acetyl cholinesterase (AchE) in nervous system with subsequent accumulation of toxic levels of acetylcholine (Ach), which is neurotransmitter (WHO, 1986). In 1995, there were 15, 300 pesticide poisoning cases in China, 91% of which were caused by organophosphates. Of these 67% were caused by just 3 organophosphates, i.e. parathion, methamidophos and dimethoate (Shuyang and Peipei, 1996).

During a malaria eradication program in Pakistan in 1976, out of 7,500 spray men, 2800 become poisoned and 5 died due to the isomalathion present as an impurity in the malathion. (Aldridge *et al.*, 1979). Many thousands of cases of acute poisoning by organophosphorous insecticides have been recorded, the majority being due to parathion and methylparathion (WHO, 1986). When Fenitrothion was injected into the yolk of chicken eggs at doses of 0.1 ml of 0.1-30% fenitrothion, dose levels abnormalgait. At

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high dose level all the embryos died. (Paul and Vadlamudi, 1976). Caution has been advised in the use and handling of dichlorvos where birds might be exposed (Whitehead, 1971). Because Domestic fowl which has accidental access to the faeces of a horse dosed with dichlorvos pellets, picked out the pellets and more than 30 birds died during the next 24 hours (Lloyd, 1973). Administration of Malathion at low doses (10 and 20 µg/g BW) to mice produced defects like reduction in body weight, thinning of myocardium wall in ventricles, Aortic value stenosis, thinning of interventricular septum and skin hemorrhagic spots (Mufti and Safdar, 1991). In present investigation the embryotoxicity of organophosphate insecticide i.e. methylparathion was studied in chick embryo.

MATERIALS AND METHODS

Fertilized eggs of *Gallus domesticus* were purchased from Government Poultry Farm, Lahore. The eggs were divided into 6 groups. Five groups were treated with 1.0, 2.5, 5.0, 7.5, 10.0 µg per egg of methylparathion. Remaining group was control (C) without any treatment. Eggs were placed in an incubator adjusted at 38 ± 0.5°C. Humidity was maintained in the incubators by placing water filled beakers in each shelf of incubators. Eggs were rotated twice a day.

Doses were administered on day 4 of incubation. For administration of insecticide / distilled water, eggs were randomly selected and cleaned with a piece of cotton soaked in 70% alcohol. A small window was made in egg shell (except control egg) with the help of sterile needle. Using 1ml glass syringe, 0.1 ml of solution, containing various concentrations of insecticide, was injected into the yolk of each egg. After injection, the hole in the eggshell was sealed with medical tape. Eggs were incubated again by placing them in incubators. Eggs were recovered on day 7 of incubation and were fixed in Bouin's fixative for 24 hours and then shifted to 70% alcohol for over night. Then they were preserved in 80% alcohol for further studies. Student t' test was used for data analyses.

RESULTS AND DISCUSSION

Embryos recovered on day 7 of incubation were studied for embryotoxic effects of organophosphate i.e. methylparathion. The control embryos had well-developed and prominent head, neck and trunk region. The head had ell developed brain parts. The forebrain had typical divisions i.e. telencephalon and diencephalons. Mid-brain was very prominent, while the hindbrain had distinct metencephalon and myelencephalon. The eyes were well developed (Fig. 1A). The eye lens was very prominent and distinct. Beak was distinct which had been differentiated with prominent upper jaw. The neck region was well differentiated and quite elongated. The trunk region was also well developed. Trunk region had prominent forelimbs and hind limbs. The hind limbs showed more advanced stage of development as compared to fore limbs. The elbow and knee bending were quite prominent. Tail part was also distinct and curved. The embryos had normal

cardiac position. The spinal cord of control group was also normal (Fig. 1A). The development of embryos treated with distilled water was similar to control.

The eggs treated with different concentrations of methylparathion showed abnormal development. The CR length of all treated embryos was significantly ($P<0.001$) reduced as compared to the controls (Table I). Brain parts were not distinguished properly. They had small eyes, which were at early stage of development. Beak and neck were not formed. Limbs were underdeveloped. They had twisted spinal cord. There was also a case of exencephaly, anophthalmia and ectopic heart (Fig. 1 A, B C, D E F). The body parts were resorbed completely in some cases.

These results are in conformity with earlier reports in which organophosphate insecticides have been reported to induce teratogenicity in chicks.

Asmatullah *et al.* (1993) reported that when comparatively high doses of malathion, an OP insecticide were given to mice produced developmental defects in embryo body parts i.e. brain, snout, external pinnae, fore and hind limbs, tail and eye. Moscioni *et al.*(1977) have categorized that abnormalities such as micromelia, dwarfism, parrot beak and abnormal feathering, short neck and muscular hypoplasia of legs were commonly observed in chick embryos treated with malahion. Malathion caused many gross malformations in mice embryos (Mufti and Nasim, 1987). In these studies it has been discovered that even a small dose of 5µg/g BW produced gross neural defects such as microcephaly and spina bifida. Greenberg and LaHam (1969) found that malathion caused shortening of hind limbs, shortening of plumage and beak defects in chick embryos. Researchers found association between malathion exposure and increase in ear anomalies, bowing of leg bones, clubfoot and other deformities (Grether, 1987).

Injection of malathion into the yolk sac of chicken eggs caused reduced growth and weakening of leg bone (Jackson and Gibson, 1976), reduced chick weight, reduced hatch, short legs (Melaughlin, 1963), sparse plumage, limb shortening, growth reduction and beak defects (Greenberg and LaHam, 1969). In an experiment, dose of 186 mg/kg/BW cyclophosphamide showed significant increased incidence of eye defects, cleft palate and limbs defects etc. (Ujhazy *et al.*, 1993). In another case 14% cleft palate was obtained when methylparathion dissolved CMC (carbomethyl cellulose) was injected intraperitonealy in mice (Tanimura *et al.* 1967).

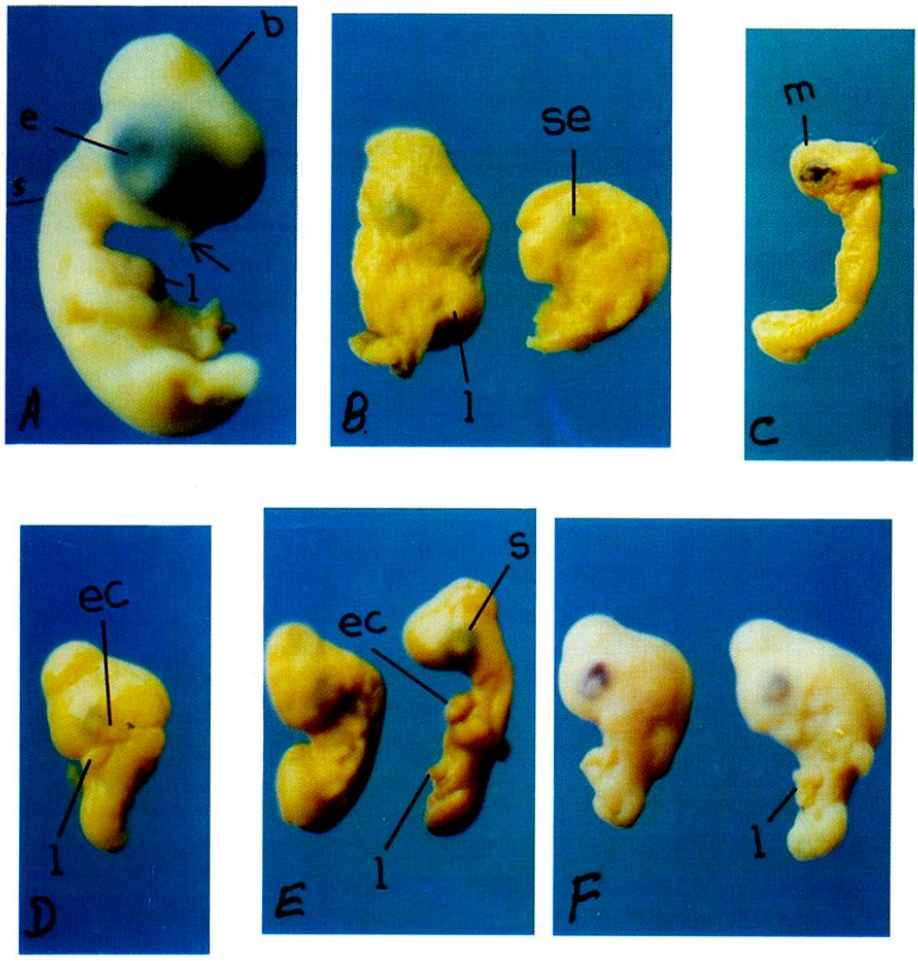

Fig I: Seven days old embryos recovered from eggs treated with different concentrations of methylparathion. **A:** a control embryo with normal development. B,C,D,E and F: embryos from different doses 1.00, 2.5, 5.00, 7.5 and 10.00 µg / egg, respectively, showing adverse effects of the insecticide development. Note: microphthalmia (se), microcephaly (m), ectopia cardis (ec) and club foot (l).

Table 1: Developmental anomalies induced by different concentrations of methyl- parathion on 7-day old chick embryos injected at day 4 of incubation.

µg / egg	CR length mm ± S.D.	Beak	Eyes	Neck	Forelimbs	Hind limbs	Cardiac Position	Spinal cord
Control	14.75 ± 2.1 (n=14)	Normal (00.0)	Normal Closed (00.0)	Normal (00.0)	Well developed (00.0)	Well developed (00.0)	Normal (00.0)	Normal (00.0)
1.00	*** 7.18 ± 2.06 (n=8)	Not formed (100)	At early stage of development (100)	Not formed (100)	Under developed (100)	Not developed (100)	Ectopic heart not formed (100)	Twisted (25)
2.5	*** 5.62 ± 2.35 (n=8)	Not formed (100)	Not developed (100)	Not formed (100)	Not developed (100)	Not developed (100)	Ectopic heart (25)	Twisted (25)
5.00	*** 6.8 ± 2.16 (n=8)	Not formed (100)	Small, Closed (100)	Not formed (100)	Not developed (100)	Not developed (100)	Ectopic heart (25)	Twisted (50)
7.5	*** 8.70 ± 2.61 (n=8)	Not formed (100)	Reduced, Closed (100)	Short (100)	Shortly developed (50) Absent (50)	Shortly developed, (50) Absent (50)	Ectopic heart (25)	Twisted (25)
10.00	*** 9.58 ± 1.11 (n=8)	Not formed (100)	At early stage of development (100)	Not formed (100)	Not prominent (100)	Not prominent (100)	Ectopic heart (50)	Twisted (50)

( ) = Percentage of abnormalities

***** = (P<0.001) significant against controls**

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