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 severe malformations including microcephaly, anophthalmia, micromelia, twisted spinal cord and ectopia cardia. It is suggested as this insecticide is very dangerous for developing organism, it should be used very carefully.

Keywords: 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). Dinh, (1993) revealed that there are in total 3 million acute severe 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 acetylcholinesterase (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
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 valve stenosis, thinning of interventricular septum and skin hemorrhagic spots (Mufi and Safdar, 1991). In present investigation the embryotoxicity
of organophosphate insecticide i.e. methyliparathion 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 methyliparathion. 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. methyliparathion. 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 diencephalon. 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 methylyparathion showed abnormal development. The CR length of all treated embryos was significantly (P<0.001) reduced as compared to the controls (Table 1). 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, dwarfishm, parrot beak and abnormal feathering, short neck and muscular hypoplasia of legs were commonly observed in chick embryos treated with malathion. 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 (carboxmethyl cellulose) was injected intraperitoneal in mice (Tanimura et al. 1967).
Fig 1: 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.

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

( ) = Percentage of abnormalities

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


