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EMBRYOTOXICITY AND TERATOGENICITY OF MALATHION IN MICE

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Abstract: An organophosphorus insecticide, malathion was tested for its embryotoxic and teratogenic properties in mice embryos. Following relatively high doses (125, 250 and 500 mg/g body wt.) of the insecticide, it was observed that alongwith a decrease in body weight and crown rump (CR) length, the embryos showed a significant lag in the development of main body parts such as brain, snout, external pinnae, fore and hind-limbs and tail while a significant increase in uncovered area of eye-ball was also noticed. It is, therefore, concluded that malathion is toxic to mouse embryos, especially in the quantities used in this study.

INTRODUCTION

In recent years, considerable interest has been generated in the studies of relationship between adverse effects on the mother caused by exposure to chemicals during pregnancy and possible indirect effects on the conceptus. A series of papers by Khera (1984, 1985, 1987a,b) has provided much of the impetus for the renewed interest in "maternal effects". It is now well established that deleterious influences resulting from maternal toxicity can indeed affect the conceptus.

There is an obvious decrease in the use of the chlorinated hydrocarbons all over the world due to their non-degradable and thus accumulative nature resulting into all kinds of general toxicities and eventually lethal effects (Niimi, 1983; Barron and Adelman, 1984). With the diminished utilization of chlorinated hydrocarbons, organophosphorus insecticides such as malathion, Parathion, Diazinon etc., have started being used quite heavily now (Davis and Richardson, 1980). These insecticides are considered relatively safe, especially in the sense of these being biodegradable and thus non-cumulative. Unfortunately, however, these compounds have also been seen to be quite harmful to the non-target organisms (Durham and Williams, 1972; Jennings *et al.*, 1975; Harbison, 1975). A survey by WHO tested about hundred organophosphorus insecticides and found "acute toxicity for (non-target) experimental animals" (WHO report, 1986). The harmful effects of these compounds have been primarily attributed to their acetylcholinesterase (AChE) inhibition properties (Harbison, 1975; Richardson, 1983). Banerjee *et al.* (1991) have also stated that pure and commercial organophosphates are able to significantly alter the acetylcholinesterase activity. Acetylcholinesterase has been tested *in vitro* as a predictor of the toxic potential of pesticides like organophosphate compounds in white rats (Chin *et al.*, 1980) and goats (Guhathakurta and Bhattacharya, 1989). Ishikawa *et al.*, (1975) reported that acetylcholine induced cardiac anomalies in nine of 23 chick embryos at a total dose of 20 mg given for a period of 3h 20 min. The anomalies induced ventricular septal defect, atrial septal defect and double aortic arch.

Many other studies have further shown that these insecticides may also be teratogenic. The harmful effects of these compounds especially to avian embryos have been shown quite convincingly (Khera, 1966; Khera and Bedok, 1967; Meiniel *et al.*, 1970; Fishbein, 1975; Meiniel, 1976; Sternberg, 1979; Wyttenbach and Thompson, 1985). In most of these studies it has been shown that even very small quantities of organophosphates induced gross embryonic malfunctions which included microcephaly, eye cataracts, ascites, hepatic degeneration, micromelia, ectrosyndactyly and many other musculo-skeletal abnormalities. Malathion, malaoxon, parathion and paraoxon caused dose-dependent development defects, such as abnormal pigmentation, abnormal gut development, notochordal defects and reduced growth (Snawder and Chambers, 1989) in African clawed frog. Greenberg and LaHam (1969) found that malathion caused shortening of hindlimbs, shortening of plumage and beak defects in chick embryos. They dubbed these defects as "malathion Syndrome". Some other studies have also shown that many of the commonly used organophosphorus insecticides are embryotoxic in birds. For example, it was discovered that Phosphamidon not only caused brain defects, dwarfism and stunted growth in chick embryos (Mufti and Dad, 1977), but many internal organs such as heart and kidneys were also adversely affected (Mufti and Nasim, 1987).

As far as the effects of organophosphorus insecticides on mammalian development are concerned, there are relatively very few studies present in literature. In most of these studies it has been generally observed that although these insecticides are relatively less toxic to mammalian fetuses than avian embryos, they can still cause many adverse effects (Tanimura *et al.*, 1967; Kimbrough and Gaines, 1968; Budreau and Singh, 1973; Lechner and Abdul-Rehman, 1984; Machin and McBride, 1989). For example, Karlow and Marton (1961) observed reduced growth rate and increased mortality of youngs following treatment of adult rat females, both prior to or during pregnancy, with malathion. Dobbins (1967) found decreased fetal weight, increased incidence in external hemorrhagic spots on the fetuses of rats following an administration of 50mg/kg of malathion. The teratogenicity was also found in rat fetuses following the treatments of dams with either diazinon or parathion (Dobbins, 1967; Kimbrough and Gaines, 1968). Budreau and Singh (1973) showed that demeton, administered as a single IP dose of 7-10 mg/kg, between days 7 and 12 of gestation in rats, proved embryotoxic with some teratogenic effects. More recently, however, Machin and McBride (1989) have shown that a dose of 100mg/kg, given on day 7 to 12 of gestation in rabbits, did not cause noticeable abnormalities in most of the fetuses, although there was seen incidence of acrania, microphthalmia and microcardia in at least one of the fetuses.

In some related studies it has been shown that malathion caused many malformations in mice embryos (Mufti and Nazir, 1988; Riaz, 1988). In these studies it has been discovered that even a small dose of 5μg/g body weight (BW) produces gross neural defects such as microcephaly and spina bifida with myeloschisis. The present study is designed as a step to evaluate embryotoxicity of malathion in mice.

MATERIALS AND METHODS

In the present series of experiments, white laboratory mice *(Mus musculus)* were used. These were maintained in standard animal room facilities with 12±1h light/dark cycle. Estrus females were caged with males for overnight mating. Vaginal plug and/or sperms in the vaginal tract in the morning confirmed mating and marked day 0 of gestation.

These impregnated females were then isolated and were administered concentrations of 125, 250 and 500μg/g BW of organophosphorus insecticide, malathion. These concentrations were prepared by dilution of malathion in corn oil in such a way that 0.1 ml of solution contained the desired concentration of the insecticide. The route of the administration was oral. The pregnant mice were force fed the solution with the help of 1ml syringe attached with a capillary rubber tubing. This tubing ensured complete ingestion of the dose by the mice. The control and vehicle control groups were also maintained by applying no treatment and only corn oil, respectively.

On day 15 of gestation the pregnant mothers were anaesthetized and uteri, bearing the fetuses were dissected out. The fetuses were taken out from these gravid uteri and were fixed in Bouin's fixative for 48 hours. These were then washed in 70% alcohol and preserved in 80% alcohol for further studies. The preserved fetuses were examined under dissecting microscope. For morphometric studies brain, snout, eye, ear, fore-limb, hind-limb and tail were considered for extent of development. These organs were measured (Fig. 2) under dissecting microscope equipped with ocular micrometer. Individual fetuses per litter were studied for each organ. Mean values obtained for every experimental group were then analyzed by applying student T-test.

RESULTS

Although a reduction in the overall size of the treated fetuses was observed which obviously signified tendency toward dwarfism, it was decided to quantify this reduction. When the fetuses were studied more closely it was discovered that the ones obtained after 500 μg/g BW did not show any distinguishable developmental difference as compared to control. On a closer examination of the fetuses, following an administration of 500 μg/g BW dose, it was noted that the three main parts of the brain showed quite distinct bulges, which is a primitive condition of brain development. Other craniofacial organs such as eyes, ears, snout and vibrissae lines were far less developed. Both fore and hind limbs and tail were also noted underdeveloped as compared to the control (Fig. 3). Decrease in brain size was significantly different ($P<0.001$) from control. A decrease in length of snout was noted following different concentrations of the insecticide, which was maximum at the dose of 500 μg/g BW (Fig. 4) and was significantly ($P<0.001$) different from controls.

Eye development was noted by examining the lens covered by eyelids. Eye lens was found mostly covered with eyelids in control group, indicating an advanced state of development. Lens was found quite uncovered by the eyelids in fetuses recovered from treated mothers. A maximum increase in eye-lens open area was noted at dose level of 500 μg/g BW which was significantly ($P<0.001$) different from controls (Fig. 5,6).

Pinna size showed decrease in the groups exposed to different concentrations of the insecticide. (Fig. 8). The decrease, however, was dose dependent and significantly different from control group.

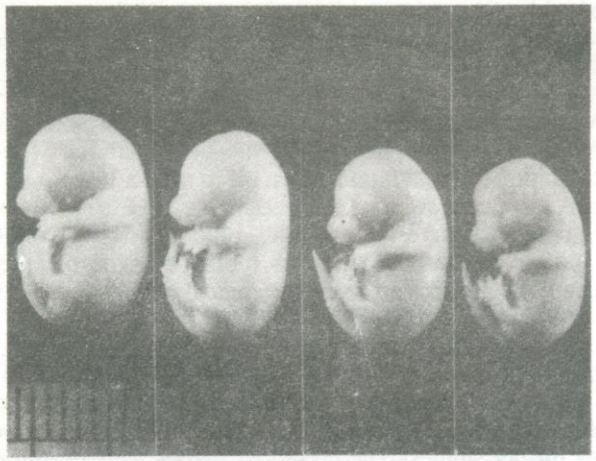

Fig. 1

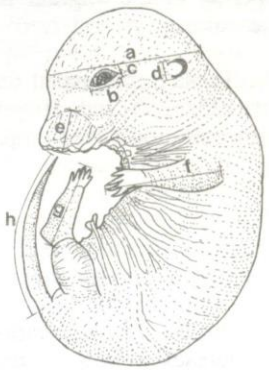

Fig. 2

Fig. 1: Composite photograph of 15-day old fetuses, recovered from pregnant mice after oral administrations of malathion at day 6 of gestation (Left to right, control, 125, 250 and 500 μg/g BW).

Fig. 2: A sketch of the 15-day old fetus showing points of morphometric studies: a, brain; b, eye length; c, eye width; d, pinna size; e, snout length; f, fore-limb length; g, hind limb length and h, tail length.

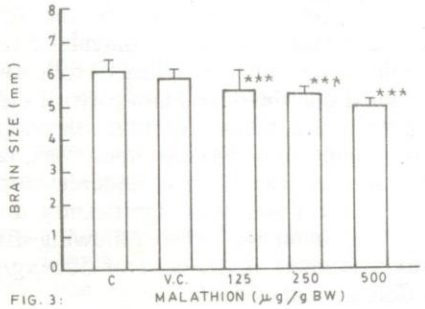

FIG. 3:

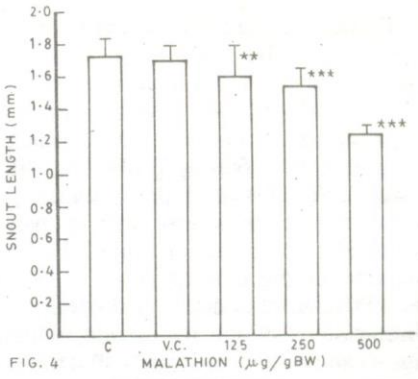

FIG. 4

Fig. 3: Histograms showing relationship of brain size of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation.. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 4: Histograms showing relationship of snout length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=Ptl<0.01; ***=P<0.001; c=control; v.c=vehicle control).

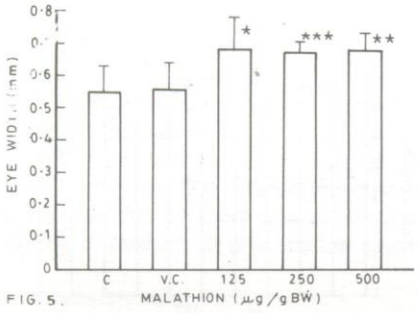

FIG. 5.

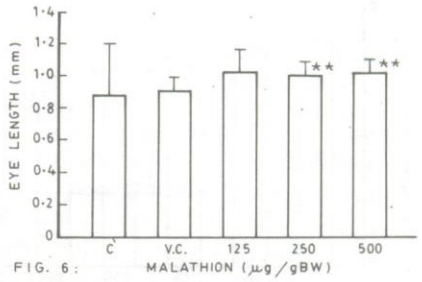

FIG. 6:

Fig. 5: Histograms showing relationship of eye width of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 6: Histograms showing relationship of eye length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

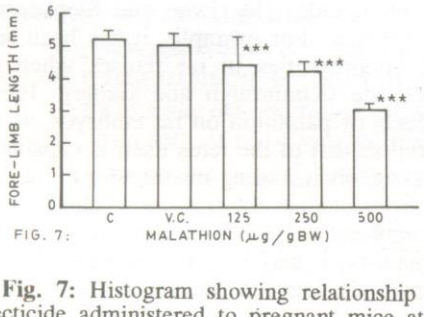

FIG. 7:

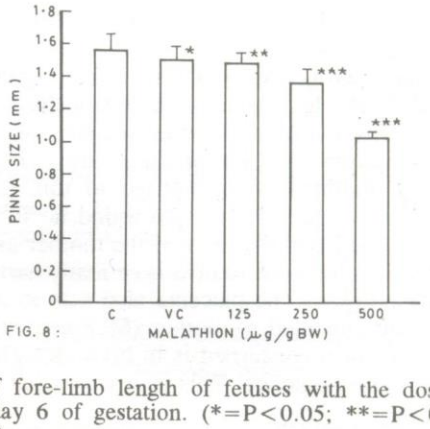

FIG. 8:

Fig. 7: Histogram showing relationship of fore-limb length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 8: Histogram showing relationship of pinna size of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

The fore-limb size was found to be decreased (Fig. 7) significantly from the controls ($P<0.001$). A significant ($P<0.001$) decrease in hind-limb size was also observed in treated group of fetuses which was dose dependent (Fig. 9). An overall decrease in tail length was observed, which was also highly significant ($P<0.001$) at high doses (250 to 500 μg/g BW when compared with control group (Fig. 10).

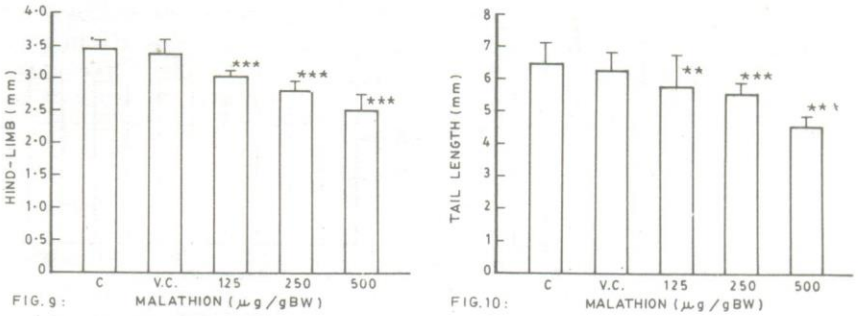

FIG. 9: FIG. 10:

Fig. 9: Histogram showing relationship of hind-limb length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 10: Histogram showing relationship of tail length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

DISCUSSION

It has been generally observed that organophosphorus insecticides such as malathion are relatively less toxic than organochlorine insecticides (McEwan and Stephenson, 1979). This is especially true of mammalian embryos. For example, it has been seen that malathion did not produce any definite abnormalities in rat fetuses when the pregnant mothers were exposed to this insecticide (Kimbrough and Gaines, 1968). Khera and Trinett (1978) also found no ill effects of parathion on rat embryos. It has been assumed that the liver of the mother as well as that of the fetus itself is capable of detoxifying the harmful effects of many harmful agents including insecticides (Frias and Thomas, 1988). The placenta also acts as a barrier against the transfer of the harmful agents administered to mother (McEwen and Stephenson, 1979). In spite of these facts if the chemical concerned is in high enough quantity, it may pass through the placenta and can cause damage. This is exactly what apparently happened during present studies.

Morphometric studies of fetuses recovered following different concentrations of malathion showed reduction in body weight and CR length, which was basically dose dependent. Detailed studies of different organs such as brain, snout, pinnae, eyes, fore-limbs, hind-limbs and tail also showed reduced development of these organs at high concentrations of the insecticide.

Our results are supported by some other studies as well. For example, it was observed that malathion caused decreased fetal weight, an increased incidence of fetal resorption and other anomalies such as hydronephrosis and hydroureter in rat embryos (Dobbins, 1967). Karlow and Martin (1961) also found that a continuous administration of malathion for a 10 week period to rats before and during pregnancy also resulted into dwarfism and increased mortality. more recently, Machin and McBrige (1989) also studied the effects of a 100mg/kg dose of malathion in rabbits, given on day 7-12 of gestation and found that although this dose produced no½

significant fetal abnormalities, still at least one case of fetal resorption and one case of fetal malformation was recorded. Some studies carried out in our laboratory also showed that malathion in a high dose of 1 to 3 mg/gBW to pregnant mice caused embryolethality and fetal resorption (Mufti and Nazir, 1988). All these studies obviously indicate that organophosphorus insecticides, which are relatively safe for adult animals due to their non-accumulative and biodegradable properties, can still be potentially hazardous to mammalian embryos.

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