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DEVELOPMENTAL DEFECTS INDUCED BY DICHLORVOS IN MICE

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Abstract: The teratogenecity of an organophosphate insecticide, Dichlorvos was tested in developing fetuses of mice. Different concentrations of the insecticide i.e., 5, 10, 15 and 20 µg g^{-1} body weight were given orally on day 6 of gestation and fetuses were recovered on day 15 of gestation. The body weight and CR length decreased significantly ($P < 0.001$) in higher dose groups. Some abnormalities including hydrocephaly, gastroschisis, paddle shaped manus and pes, ectopia cordis and patent neurocoele, stenosis of cardiac valve and blood vessels, atrial septal defects, ventricular septal defects and meningomyocoel were noted. Necrobiosis was evedent in resorbed fetuses. The present study indicates that this insecticide is potentially dangerous to developing fetuses of mice, which may be equally harmful for human development too.

Keywords: Dichlorvos, insecticide teratogenic, mouse, organophosphate.

INTRODUCTION

Unfortunately, where this extensive use of insecticides had rendered great service to mankind, it also led to many problems. The most important of these problems are that these insecticides proved harmful to many non-target organisms [Newsom 1967, Tucker and Leitzke 1979] as it turned out that most vulnerable of these non target organisms were the human beings themselves. The use of organic insecticides or pesticides in agriculture has increased the production possibilities and benefited farmers, processors and consumers but the environmental and health side effects of their use has rendered difficulties for living creatures [Zilberman and Siebert 1990].

Organophosphate insecticides produce much pathology due to their cholinesterase inhibition properties in various vertebrates [Harbison 1975, Jenning *et al*. 1975, Richardson 1983, Nemcsok *et al*. 1985]. However, it has been shown that organophosphate insecticides activity extend beyond cholinesterase inhibition. Sakaguchi [1972] has shown that carps exposed to Malathion displayed elevated serum transaminase activity as well as changes in serum glucose, triglycerides and total cholesterol content. These changes imply impaired glycogen metabolism as well as excessive catabolism in muscle tissue [Tucker and Leitzke 1979]. Banerjee *et al*. [1991] have also stated that pure and commercial organophosphates are able to significantly alter the acetylcholenesterase activity. Acetylcholinesterase has been tested in *vitro* as a predictor of the toxic potential of pesticides like Organophosphate compounds in white rats [El Sebae *et al*. 1977, Chin *et al*. 1980] and goats [Guhathakurta and Bhattacharya 1989].

Organophosphate exposure increased incidences of myopia (short sightedness) and a more advanced ocular disease syndrome, Saku

disease [Dementi 1994] and depression, a major risk factor in suicides [Davies 1995].

Many organophosphate insecticides are embryotoxic at doses that are not toxic for mother, but only for few insecticides teratogenic effects have been reported [WHO 1986]. Study of birth and fetal deaths between 1987 and 1989 in California examined the effects of maternal pesticides exposure. Dichlorvos has high acute toxicity. It is classified as a class IB. "highly hazardous" [WHO 1992]. Use of pesticide in household garden appeared to be associated with increased risk for most of the congenital anomalies. Home application of pesticide was associated with an increased incidence of neural tube defects and limb anomalies. Women living within 0.25 miles of an agricultural crop showed an increased risk of offspring with neural tube defects [Shaw 1999].

Organophosphate applicators had significantly more dizziness, sleepiness, headache and higher neurological symptom scores than non applicators [London *et al*. 1998].

There has been concern about the discharge of dichlorvos and possible effects of dichlorvos on wild salmon. There was an increase in the incidence of cataracts and blindness in wild salmon in the 1980's and this has been linked with exposure to dichlorvos [Fraser *et al.* 1998].

In short term dietary studies, dichlorvos has been found to be slightly moderately toxic for birds. In study with 7 days old male chicks, there was weight loss and 50% mortality at 500mg kg^{-1} diet [Naidu *et al*. 1978].

Case studies of accidently exposed humans demonstrate the acute toxicity of dichlorvos. Children exposed to vapors of insecticides containing dichlorvos (in combination with other pesticides and chemicals) experience symptoms typical of organophosphate poisoning related to the cholinesterase inhibition caused by these chemicals [Low *et al.* 1980]. Signs of poisoning include giddiness, headache, drouziness, sweating, cold and clammy skin, abdominal pain, nausea, vomiting and constricted pupils. [London *et al.* 1998].

Oral dosing human volunteers also results in rapid depression of plasma cholinesterase activity, and in some cases, decreased erythrocyte cholinesterase activity. Groups of five men were fed 1.0, 1.5. 2.0 or 2.5 mg doses of dichlorvos daily for 28 days [Rider *et al.* 1967] Cholinesterase activity was 71% of controls at the end of the 28 days in the group receiving 2.0 mg per day and was 70% of controls at the end of 20 days in the group administered 2.5 mg per day. No significant effect on erythrocyte cholinesterase activity was observed, and there were no clinical symptoms of exposure in the men.

When 10 mg of dichlorvos or less were injected into the yolk sac of fertile eggs prior to incubation, there were no teratogenic effects [Roger *et al.* 1964]. When I mg dichlorvos was injected in eggs on day four of incubation, borderline teratogenic signs (shortened body and legs) occurred [Roger *et al.* 1969].

Keeping in view the studies cited above, it is obvious that organophosphates in spite of being degradable and non-cumulative are dangerous for non-target organisms. Thus, the present study was aimed to evaluate embryotoxic and teratogenic effects of an organophosphate insecticide, dichlorvos in developing mammals.

MATERIALS AND METHODS

Swiss Wesbster variety of Mus musculus was used during this study. Dichlorvos with trade name DDVP, 50EC (Welgreen Chemicals (Pvt.) was purchased from market. Different dose concentrations 5.0, 10.0, 15.0 and 20.0 µg g^{-1} BW of the insecticide were prepared by dissolving the insecticide in water in such a way that 0.1 ml. of the solution contains desired concentration. These doses were administered with the help of 1 ml. plastic syringe to which was attached a capillary rubber tubing. These doses were given at day 6 of gestation. The treated mice were kept singly in different cages till day 15 of gestation. In case of control the animals were given only distilled water.

On day 15 of gestation, the mothers were anaesthetized with anaesthetic Ether and fetuses were dissected out. The live and dead/resorbed fetuses were counted and fixed in Bouin's fixative for 48 hours. These fetuses were then washed with 70% alcohol for 15-30 minutes. In those cases where the fetuses were obviously resorbed within the uterus, no attempt was made to take these resorbed fetuses out of the uterus.

Morphological and morphometric studies involved the wet weight of each fetus as well as its crown rump (CR) length measurements. These fetuses were closely examined by placing under binocular dissecting microscope. Selected fetuses were macrophotographed.

Histological sections of the fetuses from each group were prepared by paraffin wax method. The sections were stained with *Heamatoxyline* and *Eosin*. These sections were, then studied under microscope and selected sections were microphotographed. Student 't' test was used for statistical analysis.

RESULTS AND DISCUSSION

In 1992, organophosphate was the most widely used group of insecticides and they maintained dominance throughout the 1990's. A number of studies have drawn attention to congenital anomalies associated with organophosphate exposures [WHO 1992]. The present study was focused on the embryotoxic and teratogenic effects of a commonly used organophoshate insecticide, Dichlorvos.

In many previous studies, it has been shown that organophosphate insecticides are toxic to the nervous system by inhibiting AchE activity. Inhibition of AchE result in an accumulation of free acetylcholine in nervous tissues [Kobayashi *et al.* 1980] and smooth muscles [Narahashi 1976] producing undesirable side effects including death [Murphy 1980].

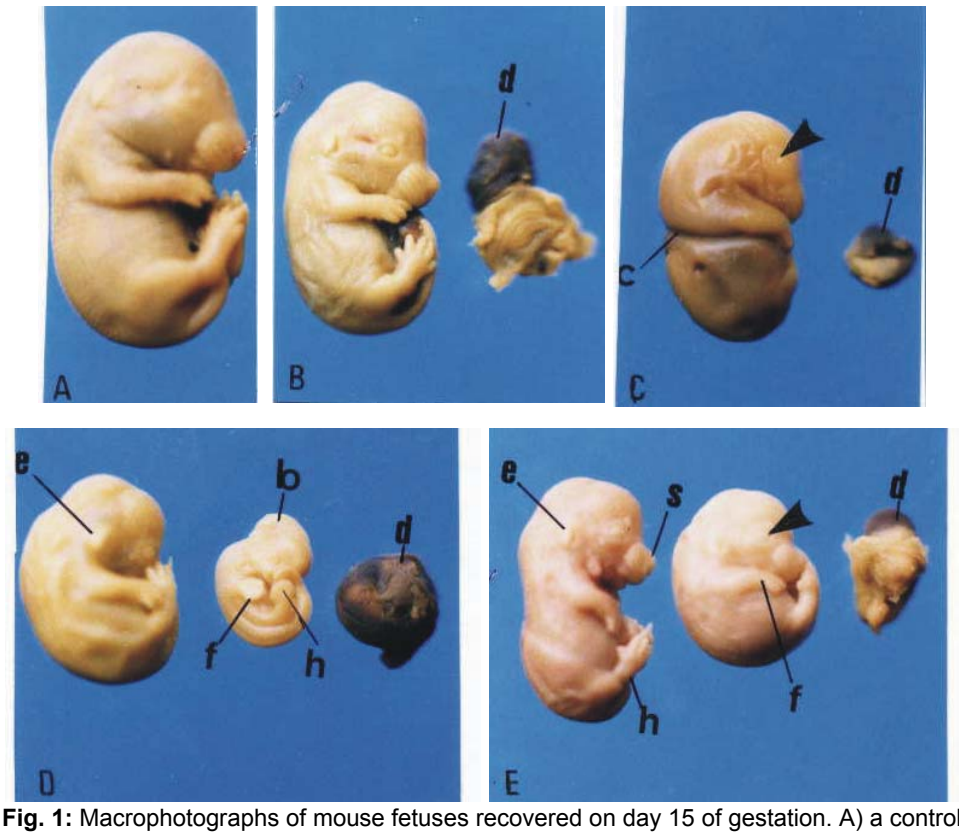

Fig. 1: Macrophotographs of mouse fetuses recovered on day 15 of gestation. A) a control group fetus with normal development; B-E) Fetuses from dose groups 5.0, 10.0, 15.0 and 20.0µg/gBW, respectively, showing different developmental anomalies. Note: microcephaly (b), amniotic constriction (C), resorbed fetus (d), microtia (e), micromelia (f and h), short snout (s) and microphthalmia (arrow head).

The observations made on fetuses have proved dichlorvos as embryotoxic and teratogenic in mice.

As far as the effects of this insecticide on body weight and CR length are concerned, there was significant ($p < 0.001$) decrease in body weight as well as CR length in treated fetuses as compared to control (Table 1).

Table 1: Effects of Dichlorvos on the CR length and body weight of 15-day old fetuses recovered from pregnant mice, administered orally on day 6 of gestation.

Dose Mg g^{-1} BW	Resorbed/dead fetuses (%)	CR Length (mm ± S.D)	Body weight (mg ± S.D)
0.00	0.0	16.3 ± 1.7 (n = 40)	439.77 ± 87.92 (n = 40)
5.00	12.5	14.8 ± 0.9 ** (n = 32)	391.00 ± 128.00* (n = 32)
10.00	17.6	14.8 ± 01.7** n = 34	354.56 ± 11.74** (n = 34)
15.00	23.I	13.4 ± 02.2*** (n = 26)	308.20 ± 144.50** (n = 26)
20.00	29.4	13.7 ± 01.9*** (n = 34)	275.23 ± 130.05*** (n = 34)

Significantly decreased against control: * = P<0.05; ** = P<0.01; *** = P<0.001.

Morphological studies also supported the toxic effects of the insecticide. Some cases of hydrocephaly, paddle shaped manus and pes, deformed limbs, under developed brain and ectopia cards were noted in treated group. A highly considerable percentage of dead / resorbed fetuses was noted in all treated groups (Fig. 1).

During present study, the histological examination of heart and spinal cord of fetuses was also done which revealed some anomalies such as slenosis of blood vessels, ventricular septal defects (Fig. 2) and patent neurocoel (Fig. 3).

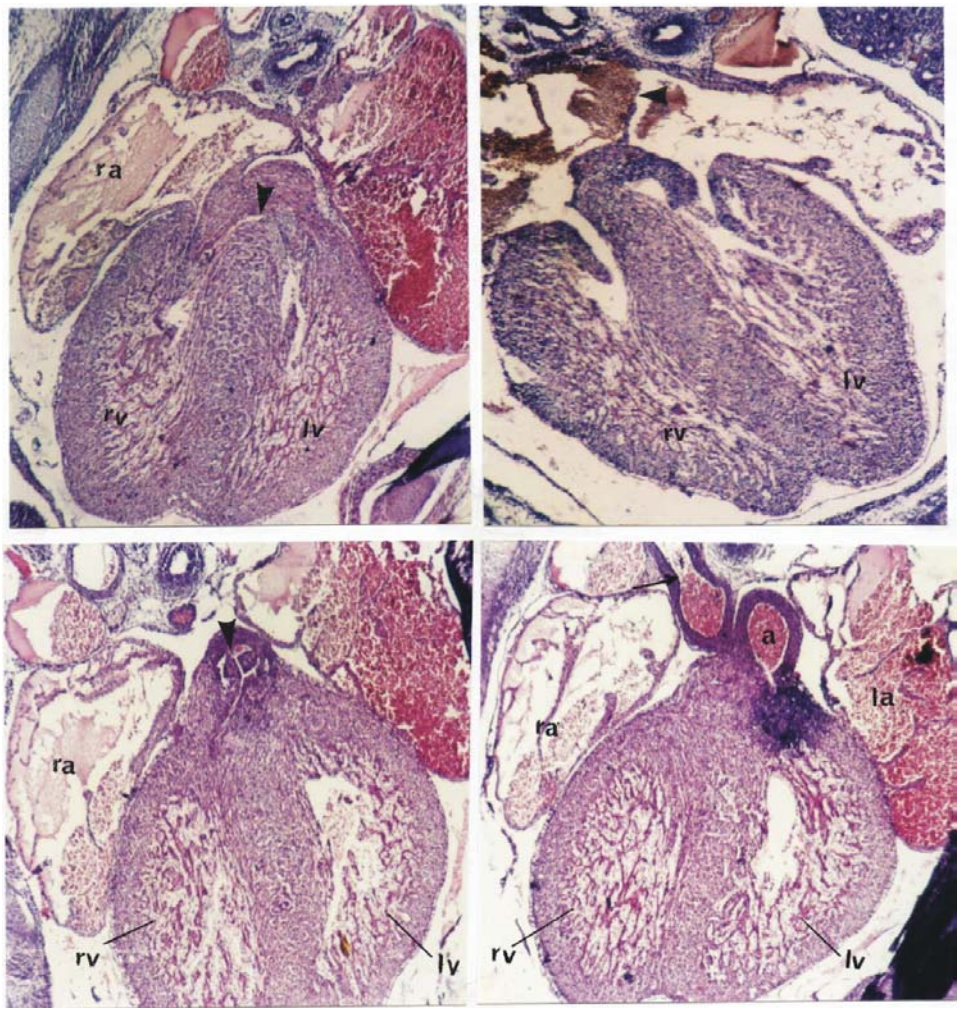

Fig. 2: Microphotographs through heart of 15-days mouse fetuses, exposed to different concentrations of Dichlorvos. (A and B) 20μg g^{-1} BW dose group. Note arterial and ventricular septal defects (arrow head). C and D) 15 μg/gBW dose group. Note Pulmonary artery stenosis (arrow) valvular stenosis (arrow head), (Right atriun, (ra) Left atrium, (la) Right ventricle, (rv) left-ventrical (lv), aorta, (a).

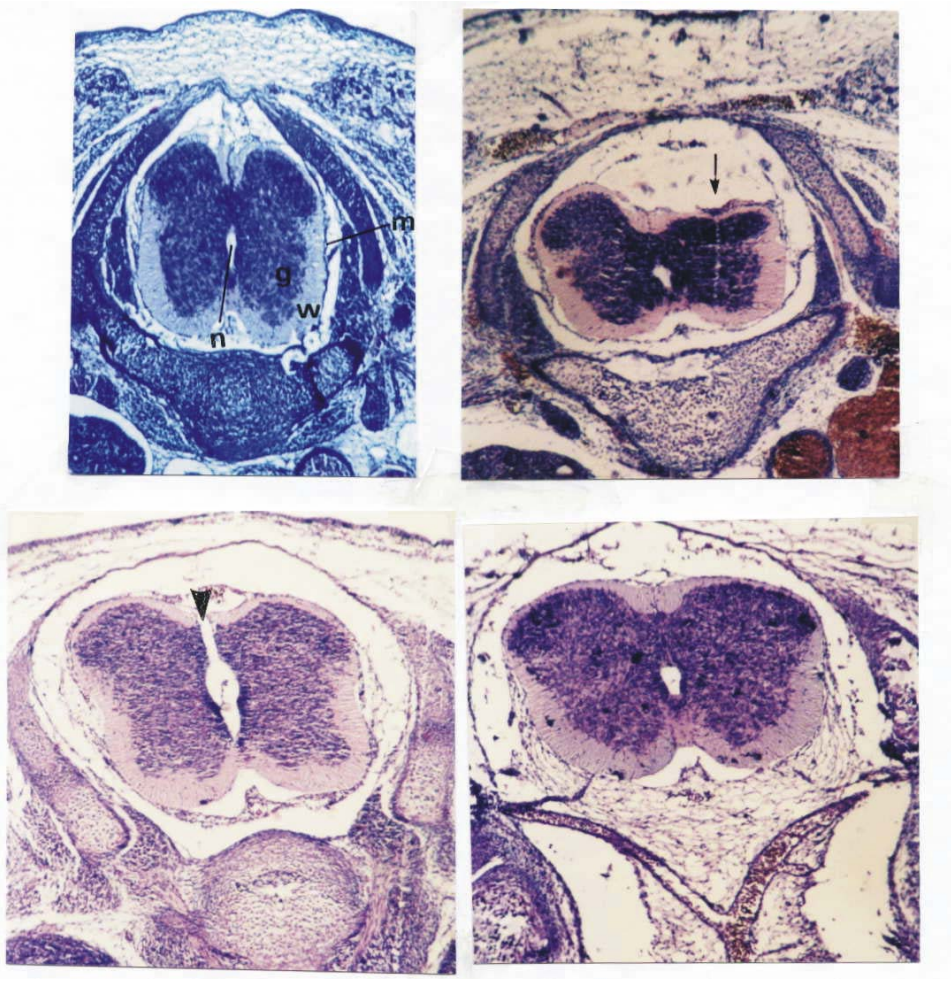

Fig. 3: Microphotographs of 15-days mouse fetuses through spinal cord. A) from control fetus with normal spinal cord. B-D) from dose groups of 20.0, 15.0 and 10. µg g^{-1} WB, respectively. Note. Neurocoel (n), gray mater (g), white mater (w), meninges (m) open neurocoel (arrow head) and meningomyocoel (arrow).

Mufti and Nasim [1987] found phosphamidon causing thinning out of myocardium in the ventricles of developing chicks. Similar defects along with ventricular septal defect have been induced by diazinon and methamidophos in mice [Mufti *et al,* 1992, Mufti and Asmatullah 1991, Asmatullah and Aslam 1999, Mufti and Asmatullah 1997, Asmatullah and Khan 2000].

Ishikawa et al. [1975] reported that acetylcholine inhibitors induced cardiac anomalies in 9 of 23 chick embryos at a dose level of 20 mg. The anomalies induced were ventricular septal defects, atrial septal defects and double aortic arch. The present results are also supported by the studies of Wyttenbach and Thompson [1985] that cardiac defects like enlargement and thinning of atrium and dorsal aorta were produced in

mice exposed to Malathion. This insecticide has also caused the reduction in the thickness of myocardial wall in the ventricles, thinning of interventricular septum and aortic valve stenosis in mice embryos [Mufti and Safdar 1991].

It has been generally assumed that liver of mother as well as that of fetus itself is capable of detoxifying the harmful effects of many harmful agents including insecticides [McEwen and Stephenson 1979]. The placenta also acts as a barrier against the transfer of the harmful agents administered to mother.

Although it is a fact that liver of mother and the fetus is capable of detoxify the harmful effect the chemical agent to a great exten [Frias and Thomas 1988] and that the placenta may filter out some more of the harmful chemical agents, but if the chemical concerned is in high quantity, it may pass through the placenta and can cause damage.

All these studies including the present study indicate, in spite of being non-accumulative and biodegradable, these insecticides are still harmful to adult animals and potentially dangerous to developing fetuses when given comparatively higher concentrations.

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