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TERATOGENIC EFFECTS OF SUBLETHAL DOSES OF METHAMIDOPHOS IN MICE

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Abstract: An organophosphorous insecticide, methamidophos was tested for its embryotoxicity and teratogenecity in mice. Different sublethal doses of the insecticide i.e., 12.5, 25 and 50% of LD_{50} comprising 1.125, 2.25 and 4.5 µg/g BW were prepared by dissolving it in water in such a way that each 0.1 ml of the solution contains desired concentration. These doses were administered orally to the pregnant mice on different days of gestation. The fetuses were recovered on day 15 of gestation. Fetal body weight and crown rump length decreased in higher doses and longer exposure groups. Morphometric studies of these fetuses were also done which were comprised of the measurements of size of brain, eye, ear, snout, lower limbs and upper limbs. Sizes of these organs decreased significantly (P 0.001) in higher dose groups. Some morphological abnormalities including brain defects, dermal haemorrhagic patches, microphthalmia, limb defects and a high rate of resorptions were found in all dose groups. The present study indicates that concentrations of this insecticide used are highly dangerous for mouse development which may be equally harmful for human too.

Key words: Organophosphates, methamidophos, mouse, sublethal doses, developmental anomalies.

INTRODUCTION

Pollution is the major problem of modern age. It has created various hazards which are severely damaging the living conditions of almost all the organisms. Amongst many other factors, causing pollution, the insecticides contribute a lot. The insecticides, of course, have increased the agri-production possibilities but the environmental and health side effects of their use have rendered differences for living creatures (Zilberman and Siebert, 1990). Worldwide production of these insecticides continue to rise with a 10 fold increase in production between 1955 and 1985 (Rosenstock, 1987).

Organophosphorus insecticides have almost completely replaced chlorinated hydrocarbons due to their biodegradable and non-accumulative nature but now they are found to be relatively toxic to central nervous system of non-target organisms while inhibiting acetylcholinesterase which results in an accumulation of free acetylcholine in nervous tissue (Kobayashi *et al*., 1980).

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In a case report of suicidal attempts with organophosphates, the course of intoxication was such that OPS were absorbed into the blood and finally transferred to body tissue where they inhibited AchE at both nicotinic and muscarinic synapses (Braeckmann *et al*., 1993).

Almost all the organophosphates are involved in the inhibition of AchE in birds and mammals. Rat, mouse and human's acetylcholinesterase exhibited a 90-100% inhibition after the exposure of organophosphates (Dauberschmidt *et al*., 1997). In short we can assess that organophosphates are extremely toxic for almost all the vertebrates like fishes, aves and mammals. So there is further need of extensive studies on the effect of these chemicals on non-target organisms especially with reference to their embryotoxic and teratogenic effects. The present study was designed to investigate harmful effects of Methamidophos on the development of mice so as to extrapolate these results in man.

MATERIALS AND METHODS

Albino mice (*Mus musculus*) were used in the experiment. Breeding stock was kept in controlled condition i.e., 12 hour light/dark cycle at 28±1°C. Estrus females were caged with males for overnight mating. Females having vaginal flug on sperm in vaginal tract were separated and that day was designated as day '0' of gestation.

LD_{50} values (9 µg/g BW) of insecticide (Methamidophos) for pregnant mothers were used in this experiment (Tayyaba, 1999). Three sublethal concentrations i.e., 1.125, 2.25 and 4.5 µg/g BW were used as 12.5, 25 and 50% of LD_{50} values. The doses were prepared by dissolving the insecticide in water in such a way that each 0.1 ml contains the desired concentration. The dose was administered orally with the help of syringe having rubber tubing. In this way dose was not wasted. All the treatments were performed on gestation days 6 and 6, 9, 12 and 6-12 as acute, subchronic and chronic doses.

On day 15 the pregnant mothers were weighed and anaesthetized and uteri bearing the fetuses were dissected out. The fetuses were then taken out from these gravid uteri and placed in Bouin's fixative for 48 hours. Afterwards they were washed in 70% alcohol and preserved in 80% alcohol for morphological and morphometric studies of different body organs.

RESULTS

A higher fetal mortality was noted in all treated groups as compared to the controls (Table I). There was no fetal recovery in case of 4.5 µg chronic dose group (Table I). There was a significant ($P<0.001$) reduction in body weight and crown rump length of fetuses of treated groups as compared to control with the exception of 1.125 µg/g BW group in which only chronic group showed significant decrease ($P<0.05$; Table I).

Table I: Developmental defects induced by Methamidosphos in mice.

Parameters	Control	Doses (μg/g BW)								
		1.125			2.25			4.5		
		(s)	(d)	(c)	(s)	(d)	(c)	(s)	(d)	(c)
No. of fetuses	145	16.00	30.00	24.00	48.00	13.00	9.00	82.00	15.00	♣
Resorbed fetuses (%)	11.72	18.75	23.33	25.00	16.07	23.08	22.22	14.63	26.67	♣
Brain defects (%)	5	0.00	0.00	25.00	47.90	0.00	22.00	10.90	6.60	♣
Microphthalmia (%)	10	12.50	6.30	54.00	27.00	15.40	66.60	1.20	13.30	♣
Short pinnae (%)	0.00	0.00	6.60	0.00	2.00	23.00	66.60	0.00	40.00	♣
Limb defects (%)	0.00	12.50	3.30	0.00	20.80	23.00	33.00	12.20	13.30	♣
Skin haemorrhage (%)	8.00	81.00	9.30	66.60	89.60	53.00	0.00	93.90	86.00	♣
Short tail (%)	0.00	0.00	0.00	0.00	0.00	0.00	33.00	0.00	0.00	♣
Snout defects (%)	0.00	62.50	0.00	0.00	18.70	7.70	33.00	19.70	0.00	♣
CR length (mm±S.E)	14.02 ±1.50	14.00 ±0.18	13.75 ±0.11	13.44 ±0.07	12.23 ±0.96	12.70 ±0.51	12.09 ±0.16	12.60 ±0.16	12.40 ±0.14	♣
Body wt. (mg±S.E)	431.76 ±88.16	430.60 ±30.01	421.63 ±15.44	400.62 ±10.48	300.85 ±46.25	319.72 ±31.67	323.33 ±66.43	284.24 ±13.29	315.71 ±17.17	♣

♣ = No fetal recovery.

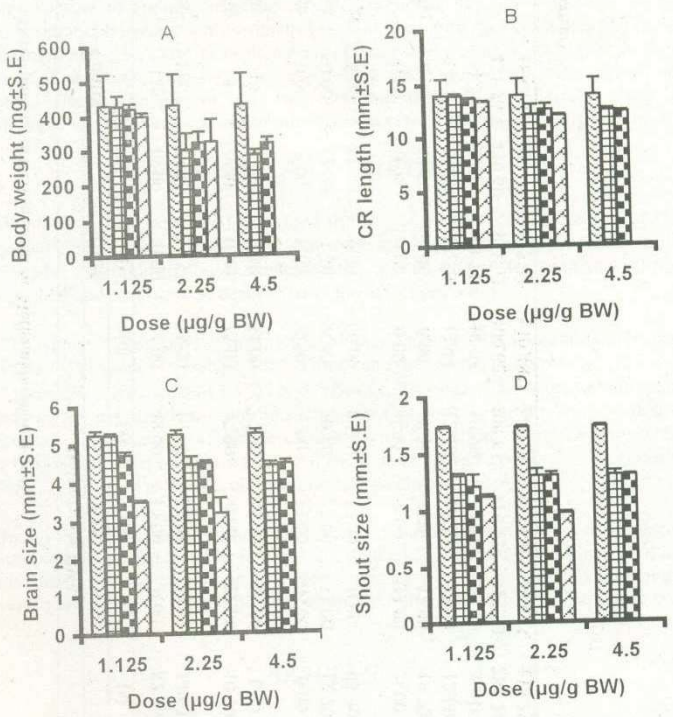

Fig. 1: Histograms showing the effects of different sublethal doses of methamidophos on: A, body weight; B, CR length; C, brain size and D, snout size of 15 days old fetuses. Asterisks show significant difference against controls (*=P<0.05; **=P<0.01; ***=P<0.001).

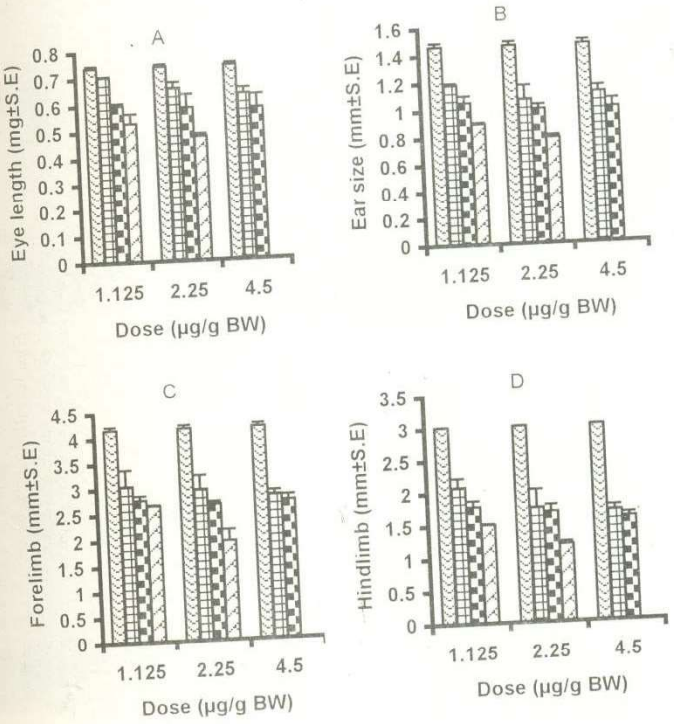

Fig. 2: Histograms showing the effects of different sublethal doses of methamidophos on: A, eye length; B, Pinnae size; C, fore limb and D, hind limb size of 15 days old fetuses. Asterisks show significant difference against controls (*=P<0.05; **=P<0.01; ***=P<0.001).

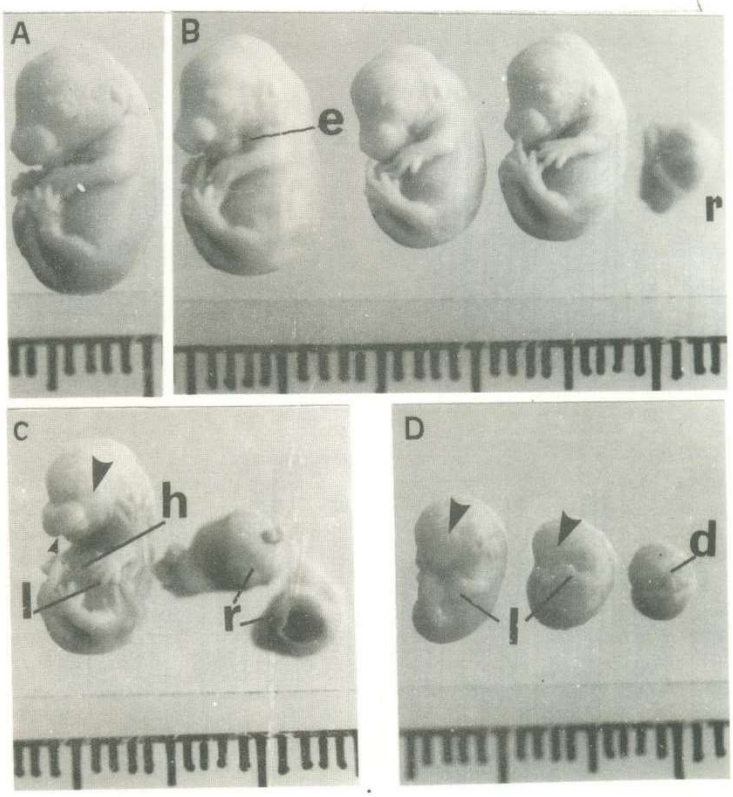

Fig. 3: Macrophotographs of 15 days old fetuses recovered from mothers exposed to different concentrations of methamidophos. A, control fetus with almost normal development; B, members of 1.125 µg/g BW dose group; C, fetuses from 2.25 µg/g BW dose group; D, fetuses from 4.5 µg/g BW dose group. *Note*: Developmental anomalies including ectopia cardis (e), a dead fetus (d), haemorrhagic spots (h), peddle like fore limbs (i), some resorbed fetuses ®, microphthalmia (large arrow head) and cleft lip (small arrow head).

During morphometric observations of different body parts like eye, brain, snout, ear and fore and hind limbs, there was also significant decrease ($P<0.001$) in the size of these organs as compared to control with the exception of brain in which 1-125 µg/g BW single dose group does not show significant decrease (Figs.1 and 2)

Different developmental defects like microphthalmia, short pinnae, skin haemorrhage, limb defects, brain defects (Table I) and cleft lip (Fig.3) were also observed.

DISCUSSION

The present investigation has clearly shown that incidences of teratogenecity and embryotoxicity were increased by the use of sublethal concentrations of Methamidophos.

Fetal mortality was 100% at 4.5 µg/g BW chronic dose level (Table I). The foetal body weight and crown rump length were significantly reduced as compared to controls. Similarly sizes of brain, eye, limbs, ear and snout decreased significantly as compared to control. Different developmental defects like microphthalmia, short pinnae, skin haemorrhage, limb defects, brain defects and cleft lip were also noted.

There are reports in the literature which clearly show that if these organophosphates are used in high concentration and over a long period of time they can found to be embryotoxic and potentially teratogenic in mammals. Vogin (1969) showed in rabbits that when dose level of 3, 12 and 24 mg/kg/day of dichlorvos were administered on day 6 of gestation. No litter was produced at 24 mg/kg dose level. Similarly Mufti and Asmatullah (1997) observed 85% fetal mortality at 50% of LD_{50} dose level in case of Diazinon while it was 61% at 50% of LD_{50} dose level in case of Malathion.

In many previous studies it has been shown that fetal body weight and CR length decreased with increase in dose concentration of Diazinon (Mufti and Asmatullah, 1991; Asmatullah, 2000). Karlow and Martin (1961) also found that a continuous administration of Malathion for 10 week to rats before and during pregnancy also resulted into dwarfism. In another study, dose of 18.6 mg/kg BW of cyclophosphomide showed significant decrease in fetal body weight and increased incidence of eye defects and cleft palate (Ujhazy *et al.*, 1993).

Dobbin (1967) and Lechner and Abdel-Rehman (1984) found decreased fetal weight and increased incidence in external haemorrhagic spots on the fetuses of rats following the administration of Malathion. In another study incidences of Acrania, microphthalmia and microcardia were observed after the administration of 100 mg/kg of Malathion in rabbits (Machine and McBride, 1989). Similarly Azodrin another organophosphate showed almost same results as it caused 100% malformations in the avian embryos at low dose levels (Abbott, 1972).

Ishikawa *et al.* (1975) reported that acetylcholine inhibitors induced cardiac anomalies in nine of 23 chick embryos at a dose level of 20 mg. The anomalies induced were interventricular septal defects, atrial septal defects and double aortic arch. The present results are also supported by the study of Wytterboch and Thompson (1985) that cardiac defects like enlargement and thinning of atrium and dorsal aorta were produced in mice exposed to Malathion.

It is thus quite apparent that the insecticide tested, if ingested by pregnant mothers can cause serious abnormalities and can even prove to be embryolethal so there is need to have extensive research work to know the dangers being caused by these insecticides in developing non-target organisms.

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