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DEVELOPMENTAL DEFECTS INDUCED BY METHAMIDOPHOS IN CHICK EMBRYOS

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Abstract: The embryotoxic and teratogenic potential of methamidophos, an organophosphorus insecticide, was tested in chick embryos. Different concentrations of methamidophos ranging from 3 to 15 μg per egg were injected into the yolk of chick eggs before incubation. A higher embryonic mortality was observed in dose groups of 9, 12 and 15 μg per egg. Besides reduced body size the gross malformations including microcephaly, exencephaly, anophthalmia, micromelia, short neck and beak, twisted spinal cord and ectopia cardis were found in treated embryos. These malformations were more severe in higher dose levels.

Key words: Organophosphorus insecticide, methamidophos, embryotoxic, teratogenic, *Gallus domesticus*, chick, birds, aves.

INTRODUCTION

With the diminished utilization of chlorinated hydrocarbons, organophosphorus insecticides have started being used quite heavily (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, 1988). A study in Pakistan also showed that in one medical unit there were 755 cases of organophosphorus insecticide poisoning reported, which were almost 40% of the total poisoning cases (Jamil, 1989). The harmful effects of these compounds have been primarily attributed to their acetylcholinesterase (AchE) inhibition properties (Harbison, 1975; Richardson, 1983). Ishikawa *et al.* (1975) reported that acetylcholine induced cardiac anomolies in nine of 23 chick embryos at a total dose of 20 mg given for a period of 3 hours and 20 minutes. The anomolies induced ventricular septal defect, atrial defect and double aortic arch. Some other studies have also shown that many of the

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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).

All these studies indicated that organophosphorus insecticides are potentially dangerous to the avian embryos due to their potential teratogenic properties. It is quite apparent that much more research will have to be carried out before we know more specifically, about the effects of these chemicals on the unborn and thus plan and design ways and means for the use of these insecticides in the most appropriate way. The present research was designed as a step in that direction.

MATERIALS AND METHODS

One hundred and forty fertilized eggs of the white leghorn breed of *Gallus domesticus* were obtained from the Veterinary Research Institute, Lahore. These eggs were divided into 7 groups, each of 20 eggs. Five groups were administered with 0.1 ml/egg of different aquous concentrations of methamidophos (60 SL, Welgreen Chemical Ltd.) containing 3, 6, 9, 12 and 15 μg insecticide. The other two groups which were vehicle control (VC) and control (C) were treated with 0.1 ml/egg of distilled water and no treatment, respectively. The eggs were randomly selected and cleaned with a piece of cotton soaked in 70% alcohol. A small window, for the insertion of needle, was made in shell of each egg (except the C group eggs), without rupturing the shell membrane. With the help of a micro-applicator, 0.1 ml of each concentration was injected into the yolk of each egg under sterile conditions. After injection, the hole in the egg shell was immediately sealed with liquid paraffin wax. After administration of the insecticide the eggs along with VC and C groups eggs were placed in an incubator adjusted at 38 ± 0.5°C. Humidity was provided by placing a water filled beaker in each shelf of the incubator. The eggs were rotated twice a day.

The embryos were taken out of the eggs on 7th day of incubation, observed for the live/dead embryos, fixed in freshly prepared Bouin's fixative, cleared in 70% alcohol and preserved in 80% alcohol for morphological and morphometric studies. The embryos were studied macroscopically and microscopically for teratological observations. The selected embryos were macrophotographed with the help of a zoom-lens fitted camera.

RESULTS

There was 100 percent embryonic mortality at doses of 9, 12 and 15 μg per egg, while at comparatively lower doses of 3 and 6 μg per egg, embryonic mortality was 45 and 78%, respectively (Table I). All the embryos recovered from treated groups were abnormal. The rate and severeness of malformation increased with an increase in dose. The crown-rump length was decreased significantly ($P > 0.001$) in all treated embryos (Table I). The teratogenic effects of this insecticide were observed all over the body of the embryos in all dose groups. Microcephaly was observed in all treated embryos.

Table I: Developmental anomalies induced by methamidophos in embryos of *Gallus domesticus*.

Parameters	Methamidophos treatment (μg/egg)						
	C	VC	3	6	9	12	15
No. of eggs treated	20	20	20	20	20	20	20
No. of embryos recorded	18	18	20	18	18	20	20
Dead/resorbed embryos (%)	0.00	0.00	45.00	78.00	100.00	100.00	100.00
Abnormal embryos (%)	11.11	11.11	100.00	100.00	100.00	100.00	100.00
CR length (mm±S.D)	17.28 ±0.59	17.41 ±0.38	9.63*** ±1.81	10.58*** ±1.98	9.09*** ±2.35	8.94*** ±2.83	8.77*** ±2.34
Abnormal brain parts (%)	0.00	0.00	45.00	83.83	83.83	100.00 (25)*	95.00 (5)
Defective eye (%)	0.00	0.00	80.00	88.88	88.88	95.00 (5)**	100.00
Defective beak (%)	0.00	0.00	80.00	94.44	94.44	100.00	100.00
Short neck (%)	0.00	0.00	75.00	100.00	100.00	100.00	100.00
Twisted spinal cord (%)	0.00	0.00	75.00	94.44	100.00	100.00	95.00
Ectopia cardis (%)	0.00	0.00	75.00	88.88	94.44	85.00	100.00

*, Exencephaly; **, Anophthalmia; ***, Significantly decreased (P>0.001); C, Control; VC, Vehicle control; CR, Crown-rump.

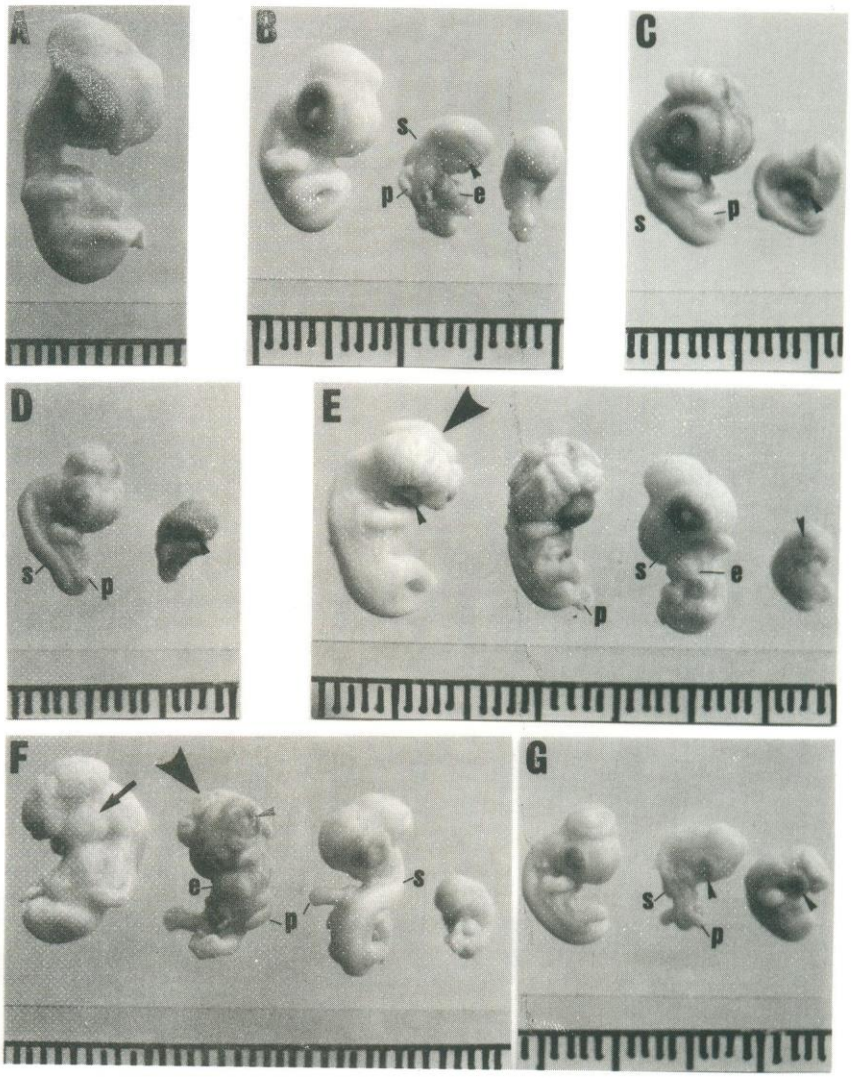

Fig. 1: Chick embryos extracted from eggs of experimental groups incubated for 7 days. (A) An embryo from control group with normally developed body organs; (B) embryos from dose group of 3 μg/egg; (C) 6 μg/egg; (D) 9 μg/egg; (E,F) 12 μg/egg; (G) 15 μg/egg. Note: abnormal development of body organs induced by methamidophos: ectopia cardis (e), peddle shaped limbs (p), twisted spinal cord (s), exencephally (large arrow head), degenerated eye (small arrow head) and anophthalmia (arrow).

Twenty five percent embryos with exencephaly were found at dose of 12 μg per egg (Table I; Fig.1E,F). Eyes were developed abnormally with a case of anophthalmia in 12 μg per egg dose group (Fig.IF). A higher percentage of gross abnormalities including short neck, micromelia, everted viscera, ectopia cardis, reduced beak size and twisted spinal cord was found in all treated embryos (Table I; Fig.1).

DISCUSSION

In all dose groups a higher embryonic mortality and severe embryonic resorptions were noted. The gross morphological and anatomical abnormalities including microcephaly, exencephaly, anophthalmia, abnormal neck, micromelia, everted viscera, ectopia cardis, short beak and twisted spinal cord were found almost in all dose levels tested during this study (Table I; Fig.1). In spite of being comparatively safe, organophosphorus insecticides are harmful and dangerous to embryonic development which has been found during the present study. This also has been supported by many studies already carried out to investigate harmful effects of this group of insecticides on embryonic development. Many 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 and Autossier-Navarro, 1980; Fishbein, 1975; Meiniel, 1976; Sternberg, 1978; Wyttenbach and Thompson, 1985). In most of these studies it has been shown that even very small quantities of organophosphates induced gross embryonic malformations which included microcephaly, eye catracts, ascites, hepatic degeneration, micromelia, ectrosyndectyly and many other musculo-skeletal abnormalities. Khera and Bedok (1967) studied teratogenicity of diazinon in ducks. They injected diazinon into yolk sac before incubation or four days after incubation at the rate of 1 mg/egg. The vertebral column was found to be twisted, shortened and composed of abnormal vertebral bodies. The teratogenic effects of diazinon with regard to skeletal development, particularly extremities and vertebrae were also examined in chick (Misawa *et al.*, 1982). Inhibited growth of femur, tibia, metatarsi and digits were observed on day 15 following injection of insecticide (0.1 mg diazinon) on day 3 after incubation.

Henderson and Kitos (1982) and Kushaba-Rugaaju and Kitos (1985) studied the effects of diazinon on nucleotide and amino acid contents in chick embryos. Teratogenic dose of diazinon was administered by the intravitelline route of chick egg at day 3 of incubation. Analysis by day 10 of development showed that the levels of free tryptophan and histidine were decreased while the levels of threonine and aspartic acid were increased. All other amino acids, however, remained unchanged in response to Diazinon. NAD+ contents of the hindlimbs of embryos also showed decrease by day 10, which further decreased by day 15 and severe type I and type II teratogenic responses were observed (Henderson and Kitos, 1982). The presence of tryptophan served to maintain the NAD+ levels of diazinon in treated embryos close to normal. A possible involvement of tryptophan in inducing micromelia, parrot beak and abnormal feathering in chick embryo is considered (Kushaba-Rugaaju and Kitos, 1985).

Many recent studies have shown that diazinon is both embryotoxic and teratogenic in mice, if it is orally administered during pregnancy. A dose level of 1 mg/g body wt.

of diazinon proved 100% lethal to pregnant mothers. A dose as low as 50 μg/g body wt. resulted into a tendency in embryos towards dwarfism and decrease in body weight. Defects in spinal cord, ventricular septum and myocardial atrophy were common observations made by Mufti and Asmatullah (1991). Malathion, Malaoxon, Parathion and Paraoxon caused dose-dependent defects, such as abnormal pigmentation, abnormal gut development, notochordal defects and reduced growth in African clawed frog (Snawder and Chambers, 1990). Greenberg and LaHam (1969) found that Malathion caused shortening of hindlimbs, shortening of plumage and beak defects in chick embryos.

The results of this study suggest that methamidophos is potentially dangerous to chick development under present experimental conditions.

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