TERATOGENICITY OF DICHLORVOS IN CHICK EMBRYOS

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Abstract: Dichlorvos (DDVP), an organophosphorus insecticide, was tested for embryotoxic and teratogenic effects in chick. Different aqueous concentrations of Dichlorvos (6.25, 12.5, 25.0 and 50.0 µg/egg) were injected, in yolk sac of egg, before incubation. Embryo recoveries were made at day 3, 7 and 15 of incubation. At day 3, dose dependent developmental anomalies including undifferentiated and unfolded brain parts, patent neurocoel, incomplete somite formation, twisted spinal cord, abnormal heart position and under-developed eyes were found. At day 7, morphological, anatomical and morphometric studies revealed concentration dependent adverse effects of the insecticide. The developmental defects were reduction in CR length, microcephaly, microphthalmia, short beak, twisted spinal cord, micromelia, ectopia cardis and short and thick neck. Developmental anomalies in 15 day embryos were also found dose dependent, such as dwarfism, monopia, micromelia, amelia, turned beak, gastroschisis and ectopia cardis. The present study indicates that DDVP is potentially dangerous to avian development.

Key words: Developmental defects, organophosphates, chick embryos.

INTRODUCTION

Besides increased crop production possibilities, environmental and health side effects of the insecticides has rendered difficulties for living creatures (Wild, 1975), especially since many pesticides have been found to be mutagenic, carcinogenic and teratogenic (Axelson and Sandell, 1974; Wild, 1975; Shirasu et al., 1976; Eto et al., 1980; Gomez-Arroyo et al., 1985, 1987, 1988). Acute pesticide poisonings are an important cause of morbidity and mortality. Although data are inadequate to quantify, with certainty, the extent of the problem, recent estimates suggest that each year worldwide, there are 3 million severe pesticide poisonings with 220,000 deaths (WHO report, 1986, 1990). Much of this burden is borne by developing countries, where 99% of fatal pesticide poisonings occur and where 25 million episodes of intoxication occur annually among agricultural workers alone (Jeyaratnam, 1985, 1990).

The organophosphorus compounds represent a broad class of insecticides and are widely used for the eradication of assorted household and agricultural pests. These compounds have been considered relatively safe, especially in the sense of these being biodegradable and thus non-cumulative (Durham and Williams, 1972; Jennings et al., 1975; Harbison, 1978). Unfortunately, the effective application of these compounds is confounded by the frequent intoxication of beneficial non-target organisms, including

numerous documented cases of human fatalities. Contributing to this selectivity is the broad range of sensitivity of different organisms to acute poisoning by OP compounds, with fish being relatively resistant and rodents and birds very sensitive (Kemp and Wallace, 1990).

When these organophosphorus insecticides are used in high concentrations and over a long period of time, they can prove to be potentially teratogenic and embryotoxic in mammals (Rosenstock et al., 1991; Asmatullah, 2000). OPs are anticholinesterase agents and are among the most widely used neurotoxic pesticides. If humans or animals are exposed to OPs, their nervous system will always be affected more or less seriously (Papp and Desi, 1998). Many reports are available on OPs causing significant inhibition of the AchE in vivo. In view of the complexity of the nervous system, particularly the central nervous system (CNS), in vivo models have an important role in elucidating both the potential for, and the mechanism of, neurotoxic insults (Atterwill and Walum, 1989).

Dwivedi and Mathur (1999) tested different concentrations of fenvalerate, cypermethrin, dichlorvos and monocrotophos against the eggs of Spodoptera litura (Lepidoptera) for oviducal effect. The higher concentration (700 ppm) of fenvalerate and (500 ppm) of cypermethrin, dichlorvos and monocrotophos showed significant results and led to failed hatching and increased mortality to 100%. Nuvan mixed with piperonyl butoxide (PB) and Decis when studied, proved to be highly toxic to snail and was a strong inhibitor of AchE activity (Tripathi and Agarwal, 1998). In many other studies, the harmful effects of organophosphorus compounds, especially to avian embryos have been shown quite convincingly (Khera, 1966; Khera and Bedok, 1967; Meiniel et al., 1970; Meiniel, 1976; Sternberg, 1979; Wyttenbach and Thompson, 1985). In most of these studies, it has been shown that even very small quantities of OPs induced gross embryonic malformation which included microcephaly, eye cataracts, ascites, hepatic degeneration, micromelia, ectrosyndactyly, microphthalmia, anophthalmia and many other musculo-skeletal abnormalities.

Virtually all the known chemical agents including insecticides have at one time or another been known to cause injury or death in man. Above mentioned studies have indicated that organophosphorus insecticides are toxic for non-target organisms and are also embryotoxic and teratogenic. Thus, the present study was planned to evaluate the embryotoxic and teratogenic potential of DDVP in developing chicks.

MATERIALS AND METHODS

Fresh eggs (White leghorn breed) were purchased from Government Poultry Farm, Lahore. The eggs were divided into 6 groups. Four groups were treated with different concentrations of dichlorvos (DDVP).

All the eggs in each group were selected randomly without considering the size and colour of eggs. Eggs were cleaned with a piece of cotton soaked in alcohol, and were marked according to their respective groups. DDVP was available as emulsifiable concentrate with trade name, Neovas 50 EC (ACMES International). The concentration of DDVP used in the study ranged from 6.25 to 50 µg/egg. These concentrations were prepared by dissolving insecticide in distilled water in such a way that 0.1 ml contains desired concentration.

A small window was made in the shell of each egg except control group eggs, with the help of a scalpel, provided shell membrane was not ruptured. 0.1 ml of each concentration of aquous solution of DDVP was injected, into the yolk sac of the eggs of respective groups, with microapplicator. In case of vehicle control group, only 0.1 ml distilled water was injected in each egg. All these treatments were applied in sterilized conditions. Following injection, the hole in the egg shell was sealed with liquid paraffin wax.

The embryos recovered on day 3 of incubation were fixed in freshly prepared Bouin's fixative and whole mounts of embryos were prepared on day 7 and 15 of incubation, embryos were fixed in Bouin's fixative for 48 hours. Then washed in 70% alcohol and finally preserved in 80% alcohol for morphological studies.

Morphological observations involved measurements of crown-rump length as well as gross anatomical observations. These observations included the studies of developmental conditions of brain, eyes, ear, limbs, beak etc. These organs were studied with the help of magnifying lens and with naked eye depending upon the size of the embryo. The data were analyzed by using student's 't' test. The embryos from 3rd day recovery were microphotographed with camera fitted microscope and embryos from day 7 and day 15 of incubation were macrophotographed by using camera, equipped with telephoto lens.

RESULTS

Three days incubation

Embryos of control and vehicle control group had CR length 6.75±0.95 mm and 5.55±0.02 mm, respectively (Table I) and had well developed embryonic parts (Fig.1a). The CR length of embryos was reduced dose dependently in insecticide treated groups (Table I). The delay in differentiation of different organs was very obvious (Table I, Figs.1b, 2a).

Table 1:   Developmental anomalies induced by different concentrations of DDVP in 3 days old chick embryos.

Dose (µg/egg)	CR length (mm±S.D)	Brain (%)	Spinal cord (%)	Eyes (%)	Cardiac position (%)	Limb buds (%)	Somites (%)
Control	6.75±0.95 (n=10)	Normal & distinct	Normal	Normal with lens vesicle prominent	Normal	Prominent limb buds	Quite distinguishable somite pairs
Vehicle control	5.25±2.02 (n=10)	Under developed (50)	Under-developed (50)	Unidentifiable (50)	Unidentifiable (25)	Under-developed (50)	Undistinguishable (25)
6.25	4.02±1.50** (n=10)	Small and undifferentiated brain parts (50)	Twisted (15), Unidentifiable (15)	Small (25), Unidentifiable (25)	Unidentifiable (30)	Small & under-developed (50)	Primitive in development (70)
12.5	3.25±2.22* (n=10)	Small and under-developed (100)	Twisted (50), Unidentifiable (50)	Unidentifiable (75)	Ectopic heart (25), Unidentifiable (75)	Unidentifiable (75)	Primitive in development (75)
25	4.75±2.77 (n=10)	Unfolded brain parts (25), Unidentifiable	Twisted (60), Unidentifiable (25)	Unidentifiable (50)	Unidentifiable (50)	Unidentifiable (70)	Primitive in development (50)
50	3.57±1.86* (n=10)	Partially differentiated brain parts (30), Undifferentiated brain parts (50)	Under-developed (25), Unidentifiable (50)	Under-developed (80)	Unidentifiable (80)	Not prominent (80)	Primitive in development (80)

Significant difference against controls: * P 0.05. ** = P 0.01.
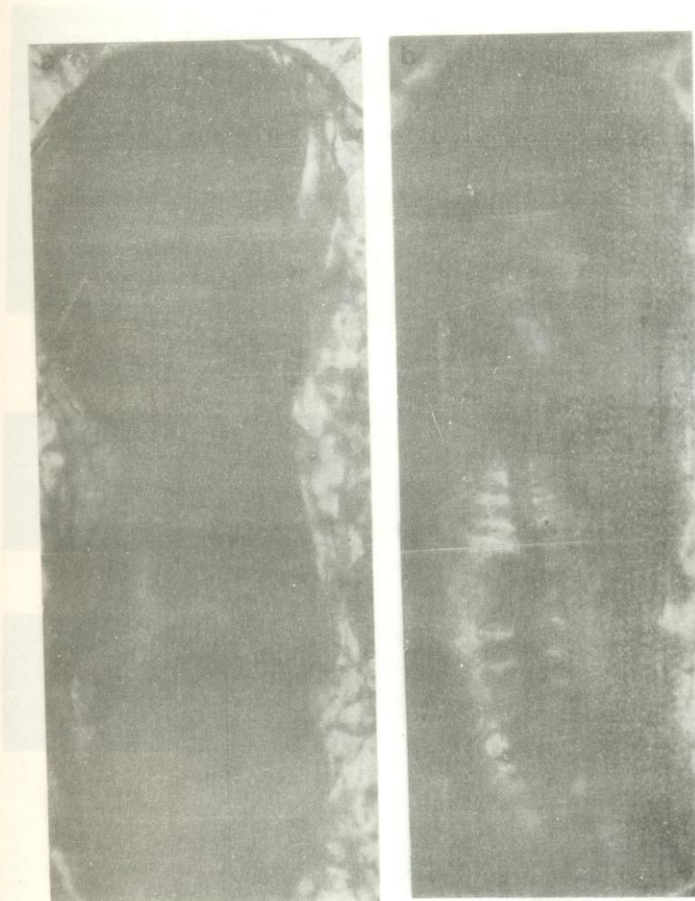
Fig. 1: Microphotographs of 3 days chick embryo: a, control embryo with normally differentiating body parts; b, an embryo from 6.25 µg/egg dose group with abnormally differentiated body parts including brain (arrow head) neural tube (p) and somites (s).
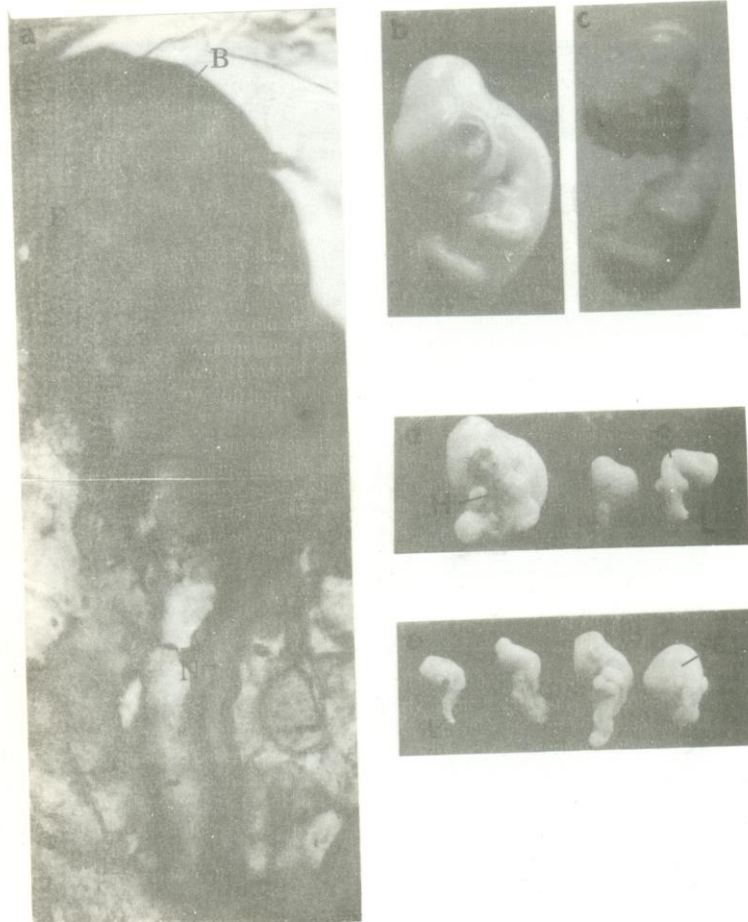
Fig. 2: a, an embryo from 50 µg/egg group showing abnormal differentiation of body parts including brain (B), eye (E) and patent neural tube (N); b-c, macrophotographs of seven days control and vehicle control chick embryos with normal development; d, embryos from 12.5 µg/egg dose group; and e, embryos from 50 µg/egg dose group with abnormally developed organs including ectopia cardis (H), hind limb (L), twisted spinal cord (S), small eye (SE).

Table II: Developmental anomalies induced by different concentrations of DDVP in 7 days chick embryos.

Dose (μg/egg)	Malformed embryos (%)	Resorbed embryos (%)	CR length (mm±S.D)	Head (%)	Eyes (%)	Beak (%)	Limbs (%)	Neck (%)	Cardiac position (%)
Control	00.0	7.69	13.3±1.13 (n=10)	All brain parts distinct	Normal	Normal	Normal	Normal	Normal
Vehicle control	00.0	60.0	12.75±0.35 (n=10)	All brain parts prominent (100)	Microphthalmia (30)	Short (50)	Normal, well developed	Normal	Normal
6.25	3.125	96.87	9.50±0.50*** (n=10)	Small, brain parts not distinct (100)	Not developed (100)	Not formed (100)	Micromelia (10)	Not prominent (100)	Not prominent (100)
12.5	12.90	87.09	8.73±0.9*** (n=10)	Brain parts distinct but small (80), Normal, well developed (20)	Microphthalmia (75)	Not formed (80)	Not prominent (75), Under developed (25)	Short & thick (75)	Ectopic heart (50)
25	18.75	81.25	6.91±2.20*** (n=10)	Distinct brain parts (40), Small, not distinct (60)	Not formed (60) Microphthalmia (20)	Not formed (100)	Not prominent (80)	Not formed (100)	Ectopic heart (50)
50	50.0	50.0	5.96±2.99*** (n=10)	Brain parts not distinct (100)	Microphthalmia (20), Undistinguishable (80)	Not formed (100)	Not prominent (80)	Short & thick (80)	Ectopic heart (20) Not formed (60)

Significant difference against controls. *** = P<0.001.

Fig. 3: Microphotographs of 7-day chick embryos: a, normally developed control; b, a member of vehicle control group with some abnormalities, including eye (E); and c, a member of 25 μg/egg dose group with turned back (TB), gross abnormalities (B), microphthalmia (M), ectopic heart (H), and

Fig. 3:   Macrophotographs of 15 days chick embryos: a, normally developed control; b, a
member of vehicle control group with some abnormalities including eye (E) and
plumage (P); c, a member of 25 µg/egg dose group with turned beak (TB),
gastroschisis (G) and anophothalmia (arrow head); d, resorbed embryos of 50 µg/egg
dose group.

Table III: Developmental anomalies induced by different concentrations of DDVP, in 15 days chick embryos, injected before incubation.

Dose (µg/egg)	Malformed embryos (%)	Resorbed embryos (%)	CR length (mm±S.D)	Head (%)	Eyes (%)	Beak (%)	Heart position (%)	Limbs (%)	Plumage (%)
Control	0.00	37.5	43.60±2.88 (n=10)	Microcephaly (20)	Normal, closed	Smaller (20)	Normal	Micromelia (20)	Partially developed (20)
Vehicle control	80.0	10.0	24.39±2.83** (n=10)	Small but distinct brain parts (100)	Opened no eyelids (100)	Short (100)	Normal	Micromelia (90)	Under-developed (90)
6.25	44.44	55.55	6.62±1.37*** (n=10)	Not distinguishable (100)	Not formed (100)	Not formed (100)	Ectopic heart (100)	Not formed (100)	Not formed (100)
12.5	11.11	88.88	7.50±1.50*** (n=10)	Microcephaly (100)	Amphthalmia (100)	Not formed (100)	Not prominent (100)	Not formed (100)	Not formed (100)
25	50.0	50.0	9.00±2.48*** (n=10)	Brain parts not distinguished (100)	Right eye is smaller than left (25), Not formed (75)	Not formed (100)	Not prominent (100)	Not formed (100)	Not formed (100)
50	37.5	62.5	8.5±2.12*** (n=10)	Microcephaly (33), Not formed (66)	Microphthaly Left side monopia (33), Under developed (66)	Turned beak (33), Not formed (66)	Not prominent (70), Ectopic heart (30)	Amelia of hind limb (33)	Patchy (33)

Significant difference against controls *= P<0.05; ** P<0.01; *** P<0.001.

Seven days incubation

The control and vehicle control groups embryos typically represented stage 31 described by Hamburger and Hamilton (1951). The CR length of the embryos was 13.3±1.13 and 12.75±0.35 mm, respectively (Table II). The insecticide treated group's CR length was significantly (P<0.001) reduced as compared to the controls (Table II). The developmental anomalies were found very severe in all insecticide treated embryos (Table II. Fig.2d,e).

Fifteen days incubation

At this stage, the control group embryos had attained the morphological features of the adult and embryos were at the stage 40, described by Hamberger and Hamilton (1951). The CR length was 43.60±2.88 mm (Table III, Fig.3a).

In case of vehicle group, the CR length was 24.39±2.83 mm which is significantly (P<0.01) less than controls (Table III). As compared to control group, embryos of this group had reduced body size and growth, with 10% resorption at the time of recovery (Table III). Head was small but with distinct parts. Eyes were smaller than controls. Beak was shorter, while neck was of normal size. Limbs and plumage were under-developed (Fig.3b).

In treated groups the fetuses were significantly (P<0.001) reduced in size (Table III). A higher rate of resorption was noted in all dose groups (Table III. Fig.3d). Some members of a higher dose groups, 25 µg/egg, showed severe developmental defects including twisted beak, patchy plumage, anophthalmia and gastroschisis (Fig.3c).

DISCUSSION

The purpose of the present study was to evaluate the developmental toxic effects of DDVP in avian systems which is comparatively more volatile than most of the organophosphorus insecticides, is highly toxic and rapid acting, having direct inhibitory effect on acetyl- and non-specific cholinesterase and is rapidly absorbed by any route (Jamil, 1989; Lindrigan *et al.*, 1999).

The main observation made during the present investigation is that dichlorvos, injected in chick eggs before incubation, even at low concentrations, produced embryotoxicity and teratogenicity. The developmental anomalies were found at all developmental stages, including 3rd, 7th and 15th day of incubation. The developmental defects observed on day 3, small, undifferentiated and unfolded brain parts, patent neurocoel, incomplete somite formation, twisting of spinal cord, ectopia cardis position and under-developed eyes; on day 7 increased embryo lethality, reduction in CR length, microcephaly, non-distinct brain parts, microphthalmia, short beak, twisting of spinal

cord, micromelia, ectopia cardis defects and short and thick neck and on day 15, dwarfism, monopia, micromelia, amelia, turned beak and ectopia cardis were increased with the increase of dose concentration.

These results are more or less in conformity with earlier reports that organophosphorus insecticides are toxic to embryonic and fetal tissues and can induce teratogenicity in chick. Miscioni et al. (1977) have categorized a whole set of abnormalities encountered in chick embryos following malathion treatment. Abnormalities such as micromelia, dwarfism, parrot beak and abnormal feathering were commonly observed and were classified as type I abnormalities. Another set of abnormalities, designated as type II, included defects such as short neck, tibiotarsal arthrogryposis and muscular hypoplasia of the legs. Many other studies have also shown embryotoxic and teratogenic effects of different organophosphorus insecticides in chick embryos (Marliac, 1964; Khera and Bedok, 1967; Greenberg and LaHam, 1969; Meiniel and Autissier-Navarro, 1980; Kitos et al., 1981; Misawa et al., 1981).

The teratogenic effects of diazinon with regard to skeletal development, particularly extremities and vertebrae were 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. In another related study, phosphamidon induced severe dwarfism as well as neural defects including complete agenesis of the eye in the chick embryo (Mufti and Dad, 1977) and abnormalities in the developing heart and kidneys (Mufti and Nasim, 1987).

Garrison and Wyttenbach (1985) showed that white leghorn chicken eggs treated with dicrotophos in a dose ranging from 250 μg – 2 mg/egg administered on day 1, 2, 3 and 4 of incubation, resulted into the production of malformed embryos showing a variety of abnormalities. These abnormalities included general growth retardation, abnormal cranial sense organs, notochordal folding with deformities of the spinal cord, deformities in the neural epiphysis and distention of major blood vessels. Jena and Bhunya (1992) studied the genotoxicity of as organophosphate, monocrotophos, upon chronic exposure in chick in vivo test system employing micronucleus bioassay. The induced frequency of micronuclei in the erythrocytes of both bone marrow and peripheral blood was significantly higher than the respective control values, which revealed the genotoxic potential of monocrotophos. Rao et al. (1992) suggested that RPR-V, an organophosphate, has teratogenic effects on chick embryos when injected on day 4 of incubation. As the dose was increased, the hatchability decreased and the incidence of deformities increased. Ghosh et al. (1998) observed that the survival rate, growth rate and size and the cholinesterase activity significantly declined, while mortality rate and the frequency of abnormalities increased in the Methyl parathion intoxication in chick embryos.

Some investigations on harmful effects of these insecticides on other developing animals have also been done. In most of these studies, it has been shown that very small

quantities of OP insecticides caused severe abnormalities or embryonic malformations. malathion, malaoxon, parathion and paraoxon caused dose dependent development, notochordal defects and reduced growth in African clawed frog (Snawder and Chambers, 1990).

Methamidophos is embryotoxic to mice and like other OPs produces different undesirable side effects including death (Murphy, 1980). Mufti and Nazir (1988) studied the effect of Malathion on mice and found that dose of 1 mg and 3 mg/g BW proved to be lethal for embryos and the viable ones were highly malformed.

Dichlorphos inhibits AChE in the embryos of Japanese quail. This enzyme inhibition leads to retardation of the development, to reduce accumulation of glucose and amino acids in the sub-embryonic liquid and finally to death of the embryo, suggesting that the developmental retardation is due to the restricted supply of glucose and amino acids (Kaltner et al., 1993).

The two principal determinants of anticholinesterase activity for various organophosphorus insecticides are steric hindrance and the electrophilic strength of phosphorus atom. Structure-activity correlation revealed that whereas steric hindrance is the principal factor governing inhibitory potency for rats and hens, the electrophilicity of the phosphorus atom is the principal determinant of anticholinesterase activity in trout (Kemp and Wallace, 1990).

In birds, the mechanisms of teratogenic action appears to be related to diminished embryonic nicotin-amide adenine dinucleotide levels as a result of Kynurenine formamidase inhibition (Seifert and Casida, 1978; Eto et al., 1980) and/or altered levels of available acetylcholine causing neuromuscular blocking effects during development (Landauer, 1975).

All studies, including present one, indicate that in spite of being non-cumulative and biodegradable, these OP insecticides are potentially dangerous to developing embryos even when given at comparatively low concentrations.

REFERENCES

ASMATULLAH, 2000. *Embryotoxic and teratogenic effects of an organophosphorus insecticide in mice*. Ph.D. Thesis, University of the Punjab, Lahore.

ATTERWILL, C.K. AND WALUM, E., 1989. Neurotoxicity *in vitro*: Model systems and practical applications. *Toxicol. in vitro*, **3**: 159-161.

AXELSON, O. AND SANDELL, L., 1974. Herbicide exposure mortality and tumor incidence. An epidemiological investigation on Swedish railroad workers. *Environ. Hlth.*, **11**: 21-28.

BARRON, M.G. AND ADELMAN, I.R., 1984. Nucleic acid, protein content and growth of larval fish sublethally exposed to various toxicants. *Can. J. Fish. Aquat. Sci.*, **41**: 141-150.

DURHAM, W.F. AND WILLIAMS, C.H., 1972. Mutagenic, teratogenic and carcinogenic properties of pesticides. *Ann. Rev. Entomol.*, **17**: 123-148.

DWIVEDI, S.C. AND MATHURS, B., 1999. Effects of synthetic pyrethroids and organophosphates on hatching of larvae, *Spodoptera litura. J. Ecotoxicol. Environ. Monitoring*, **9**: 19-22

ETO, M., SEIFERT, T., ENGEL, J.L. AND CASIDA, J.E., 1980. Organo-phosphorus and methylcarbonate teratogens: Structural requirements for inducing embryonic abnormalities in chickens and kynurenine foramamidase inhibition in mouse liver. *Toxicol. Appl. Pharmacol.*, **54**: 20-30.

GARRISON, J.C. AND WYTTENBACH, C.R., 1985. Teratogenic effects of the organophosphate insecticide Dicrotophos (Bidrin): Histological characterization of defects. *Anat. Rec.*, **213**: 466-472.

GHOSH, C., BANDYOPADHYAY, S. AND MEDDA, J.N., 1998. Protective role of thyroxine in methylparathion intoxicated chick embryos. *Drug. Chem. Toxicol.*, **21**: 495-506.

GOMEZ-ARROYO, S., BAIZA, A.M., LOPEZ, G. AND VILLALOBOS-PIETRINI, R., 1985. A comparative study of the cytogenetic effects of the insecticides heptachlor, malathion and methyl parathion in *Vicia faba. Contam. Ambient.*, **1**: 7-16.

GOMEZ-ARROYO, S., CASTELLO-RUIZ, P., CORTES-ESLAVA, J. AND VILLALOBOS-PIETRINI, R., 1988. *Vicia faba.* Sister chromatid exchanges of the organophosphorus insecticides methyl parathion, dimethoate, oxydemeton, azinphos methyl and phoxim. *Cytologia.* **53**: 627-634.

GOMEZ-ARROYO, S., NORIEGA-ALDANA, N., JUAREZ-RODRIGUEZ, D. AND VILLALOBOS-PIETRINI, R., 1987. Sister chromatid exchanges induced by organophosphorus insecticides methyl parathion, dimethoate, phoxim and methyl azinphos in cultured human lymphocytes. *Contam. Ambient.*, **3**: 63-70.

GREENBERG, J. AND LaHAM, Q.N., 1969. Malathion induced teratisms in the developing chick. *Can. J. Zool.*, **47**: 539-542.

HAMBURGER, V. AND HAMILTON, H.L., 1951. A series of normal stages in the development of the chick. *J. Morphol.*, **88**: 49-92.

HARBISON, R.D., 1985. Comparative toxicity of some selected pesticides in neonatal and adult rats. *Toxicol. Appl. Pharmacol.*, **32**: 443-446.

JAMIL, H., 1989. Organophosphorus insecticides poisoning. *J.P.M.A.*, **39**: 27-31.

JENA, G.B. AND BHUNYA, S.P., 1992. Thirty day genotoxicity study of an organophosphate insecticide, monocrotophos, in a chick *in vivo* test system. *In vivo (Athens).* **6**: 527-530.

JENNINGS, D.M., BUNYAN, P.J., BROWN, P.M., STANLEY, P. AND JONES, F.J.S., 1975. Organophosphorus poisoning, a comparative study of the toxicity of carbophenothion to the Canada goose, the pigeon, and the Japanese quail. *Pestic. Sci.*, **6**: 245-257.

JEYARATNAM, J., 1984, 1985. Occupational health in developing countries. *Scand. J. Work. Environ. Hlth.*, **11**: 229-234.

JEYARATNAM, J., 1990. Pesticide poisoning: a major global health problem. *World Health Stat. Q.*, **43**: 139-144.

KALTNER, H., ANDRAE, S. AND WITTMANN, J., 1993. Activity of cholinesterase in the Japanese quail embryo. Effects of dichlorphos on the embryonic development. *Biochem. Pharmacol.*, **45**: 87-92.

KEMP, J.R. AND WALLACE, K.B., 1990. Molecular determinants of the species-selective inhibition of brain acetylcholinesterase. *Toxicol. Appl. Pharmacol.*, **104**: 246-258.

KHERA, K.S., 1966. Toxic and teratogenic effects of insecticide in duck and chick embryos. *Toxicol. Appl. Pharmacol.*, **8**: 345.

KHERA, K.S. AND BEDOK, B., 1967. Effects of thiophosphates on mitochondrial and vertebral morphogenesis in duck and duck embryos. *Food Cosmet. Toxicol.*, **5**: 359-365.

KITOS, P.A., ANDERSON, D.S., UYEKI, E.M., MISAWA, M. AND WYTTENBACH, C.R., 1981. Teratogenic effects of cholinergic insecticides in chick embryo. II. Effects on the NAD contents of early embryos. *Biochem. Pharmacol.*, **30**: 2225-2235.

LANDAUR, W., 1975. Cholinomimetic teratogens: Studies with chicken embryos. *Teratol.*, **12**: 125-146.

LANDRIGAN, P.J., CLAUDIO, L., MARKOWITZ, S.B., BERKOWITZ, G.S., BRENNER, B.L., ROMERO, H., WETMUR, J.G., MATTE, T.D., GORE, A.C., GODBOLD, J.H. AND WOLFF, M.S., 1999. Pesticides and inner-city children: exposure, risks and prevention. *Environ. Hlth. Perspect.*, **107**: 431-437.

MARLIAC, J.P., 1964. Toxicity and teratogenic effects of 12 pesticides in the chick embryos. *Fed. Proc.*, **23**: 105.

MEINIEL, R., 1976. Pluralite dans be determinisms des effects teratogenes des composes organophosphores. *Experimentia*, **32**: 920-921.

MEINIEL, R. AND AUTISSIER-NAVARRO, C., 1970. Teratogenic activity of organophosphate pesticide in chick embryos. *Acta Embryol. Morphol. Exp.*, **1**: 33-41.

MISAWA, M., DOULL, J. AND UYEKI, E.M., 1982. Teratogenic effects of cholinergic insecticides in chick embryos: 3. Development of cartilage and bone. *J. Toxicol. Environ. Hlth.*, **10**: 551-564.

MISAWA, M., DOULL, J., KITOS, P.A. AND UYEKI, E.M., 1981. Teratogenic effects of cholinergic insecticides in chick embryos. 1. Diazinon treatment on acetylcholinesterase and choline acetyltransferase activities. *Toxicol. Appl. Pharmacol.*, **57**: 20-29.

MISCIONI, A.D., ENGEL, J.L. AND CASIDA, S.E., 1977. Kynurenine formamidase inhibition as a possible mechanism for certain teratogenic effects of organophosphorus and methylcarbamate insecticides in chick embryos. *Biochem. Pharmacol.*, **26**: 2251-2258.

MUFTI, S.A. AND DAD, K., 1977. Preliminary observations on the effects of phosphamidon on chick embryo. *Pakistan J. Zool.*, **9**: 245-246.

MUFTI, S.A. AND NASIM, R., 1987. Avian embryotoxicity of Dimicron, a commonly used insecticide. *Biologia*, **33**: 109-120.

MUFTI, S.A. AND NAZIR, N., 1988. Embryotoxic effects of Malathion in mice. *Proc. Pakistan Congr. Zool.*, **8**: 15-19.

MURPHY, S.D., 1980. *Pesticide toxicology*. The basic science of poison (J. Doull *et al.*, eds.), 2nd ed., pp.357-408. MacMillan, New York.

PAPP, A. AND DESI, I., 1998. Acute effects of different types of anticholinesterase agents on the *in vivo* hippocampal population spike in rats. *Pesticide. Biochem. Physiol.*, **61**: 21-26.

PAVLOV, D.D., CHUIKO, G.M., GERASSIMOV, Y.V. AND TONKOPIY, V.D., 1992. Feeding behavior and brain acetylcholinesterase activity in bream (*Abramis brama* L.) as affected by DDVP, an organophosphorus insecticide. *Comp. Biochem. Physiol. C. Comp. Pharmacol. Toxicol.*, **103**: 563-568.

RAO, J.V., SWAMY, A.N., YAMIN, S., RAO, S.H. AND RAHMAN, M.F., 1992. Teratism induced in the developing chick by RPR-V, an organophosphate. *Food Chem. Toxicol.*, **30**: 945-951.

ROSENSTOCK, L., KEIFER, M., DANIELL, W.E., McCONNELL, R. AND CLAYPOOLE, K., 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *The Lancet*, **338**: 223-228.

SEIFERT, J. AND CASIDA, J.E., 1978. Relation of yolk sac membrane Kynurenine formamidase inhibition to certain teratogenic effects of organophosphorus insecticides and of carbaryl and eserine in chicken embryos. *Biochem. Pharmacol.*, **27**: 2611-2615.

SHIRASU, Y., MORIYA, M., KATO, K., FURUCHASHI, A. AND KADA, T., 1976. Mutagenicity screening of pesticide in the microbial system. *Mutat. Res.*, **40**: 19-30.

SNAWDER, J.E. AND CHAMBERS, J.E., 1990. Critical time periods and the effect of Tryptophan in Malathion-induced developmental defects in xenopus embryos. *Life Sciences*, **46**: 1635-1642.

STERNBERG, S.S., 1979. The carcinogenesis, mutagenesis and teratogenesis of insecticides. Review of studies in animals and man. *Pharmac. Ther.*, **6**: 147-166.

TRIPATHI, A.M., AGARWAL, R.A., 1998. Molluscicidal and anti AChE activity of tertiary mixtures of pesticides. *Arch. Environ. Contam. Toxicol.*, **34**: 271-274.

WILD, D., 1975. Mutagenicity studies on organophosphorus insecticides. *Mutat. Res.*, **32**: 133-150.

WORLD HEALTH ORGANIZATION, 1986. Informal consultation on planning strategy for the prevention of pesticide poisoning. WHO, Geneva.

WORLD HEALTH ORGANIZATION, 1990. Public health impact of pesticides used in agriculture. WHO, Geneva.

WYTTENBACH, C.R. AND THOMPSON, S.C., 1985. The effects of the organophosphate insecticide Malathion on very young chick embryos: Malformations detected by histological examination. *Am. J. Anat.*, **174**: 187-202.

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