Punjab Univ. J. Zool., Vol.14, pp.141-151, 1999

TOXICITY OF METHAMIDOPHOS IN PREGNANT MICE AND DEVELOPING FETUSES

ASMATULLAH AND TAYYBA ASLAM

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: The effects of an organophosphorus insecticide, methamidophos were evaluated in pregnant mice and developing fetuses. To calculate LD_{50} values for pregnant mothers and developing fetuses different concentrations (5 to 20 µg/g BW) of the insecticide were given orally to pregnant mothers on day 6 of gestation. The mothers were watched after the dose administration up to 24 hours. The dose of 15 and 20 µg/g BW proved 100% mother lethal. The mothers that survived for 24 hours after dose administration were found recovered from insecticide's toxic effects as the usual symptoms of eye, oral and anal secretions and limb weakness subsided. The LD_{50} values for mothers were turned out 9 µg/g BW. The fetuses from mothers survived after 24 hours of treatment were recovered on day 15 of gestation. Dead fetuses were noted and were fixed in Bouin's fixative. The LD_{50} value for fetus was calculated as 8.2 µg/g BW of mother.

Key words: Organophosphates, methamidophos, maternal toxicity, fetotoxicity.

INTRODUCTION

Eversince the time, humans came to occupy the earth they found themselves competing with other animals for existence. There was competition for food as well as space. Humans by all virtue of superior intelligence outmaneuvered their competitors and created all sources of habitats in which they lived severely. With the increase in their agricultural skills, they started growing and cultivating many new crops to supplement their food. But to their dismay, they found that many animals like their crops and took away a lot of their yield. Among these animals, insects were the main culprits. To combat this situation, an organized pest control programme had to be developed. Many poisons were used to kill these insects and were named insecticides.

The use of these insecticides has increased many folds during the last three decades. It has been estimated, that about 2.7 billion kg of insecticides were used in one year (Munnecke, 1980). 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 is 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. Lahman *et al.* (1990) studied a case of polyneuropathy with flaccid paresis of the extremities and accompanying

0079-8045/99/0001-0141 $ 03.00/0

vegetative system impairments in a 39-year old woman farmer after acute poisoning with phytosol. Another study was done on a group of 48 workers employed at production of organophosphorus (OP) pesticides. In a clinical picture, the subjective symptoms complex was predominant including pains, vertigo, feeling of fatigue after work, difficulties with attention, concentration, memorizing paresthesia and polyneuropathy (Halina, 1990).

The primary action of OP insecticides is inhibition of AChE at cholinergic nerve terminals which has been extensively reported (Aldrin *et al.*, 1947; Holmstedt, 1959; Usdin, 1970; Davis and Richardson, 1980; Ecobichon, 1983).

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 pregnant mothers and the unborn and thus plan and design ways and means for the use of these insecticides in the most appropriate way.

MATERIALS AND METHODS

Swiss Webster variety of *Mus musculus* was used in this experiment. Breeding stock was kept in steel cages in controlled environmental conditions in the form of 12 hours light/dark cycles and temperature of 28±1°C and humidity 40-55%. Each cage had wood shavings as bedding material, which was replaced every other day. Chick feed No.3 (Punjab Feeds, Lahore) was given to these mice.

For experiment, first objective was to mate male and female under controlled conditions so that exact conception time and day "0" of gestation can be determined accurately. Three estrus females were kept with one male overnight in each cage. Next morning, these females were examined for presence of vaginal plug, which was considered as confirmation of fertile mating. These females were isolated in separate cages and marked day "0" of gestation.

During this research, maternotoxic and embryotoxic effects of methamidophos were tested. Various concentrations of methamidophos were used to determine LD_{50} values. Since no such values for pregnant mice were found in literature, so it was decided to try a wide range of concentrations (5 to 20 μg/g BW) to reach LD_{50} values. These doses were prepared by dissolving the insecticide in water in such a way that 0.1 ml of solution contains desired concentration. The doses were given to pregnant mice with the help of 1 ml glass syringe to which a capillary rubber tubing was attached. The doses were pumped into the gullet, which were readily gulped by the mice. By this technique, there was minimum escape of dose. These doses were given at day 6 of gestation. The treated mothers who survived after 24 hours of dose application were kept singly in different cages till day 15 of gestation. The control groups were maintained by giving only distilled water.

On day 15 of gestation, the pregnant mothers were weighed and then anaesthesized with ether. After giving a cesarian section, the two horns of uterus were taken out of the body and weighed. The live and dead/resorbed fetuses were counted and were dissected out of the uterus and then fixed in Bouin's fixative for 48 hours. In cases, where the fetuses were resorbed, such fetuses were not taken out of the uterus.

Both control and treated fetuses were prepared for morphological and anatomical studies. The fetuses, which were preserved in Bouin's fluid, were then shifted to 70% alcohol for overnight. Then they were preserved in 80% alcohol. Morphological and morphometric studies involved wet weight as well as crown-rump length measurement of each fetus. These fetuses were closely examined by placing under binocular dissecting microscope. The photographs were taken with the help of camera fitted with close-up-lenses.

Data Analysis

LD_{50} values were calculated by probit analysis (Finny, 1964) and plotting regression line between dose concentrations and maternal as well as fetal mortality. The other data were analyzed by student "t" test.

RESULTS

Single treatment of all concentrations (5-20 μg/g BW) was given to pregnant mothers on day 6 of gestation. In order to see the effects of insecticide on mother's morphology and behaviour, some observations were made such as lordosis, heavy sweetening, oral and anal secretions and weakness. When higher doses (15-20 μg/g BW) were administered mothers died immediately after treatment.

After calculations and plotting regression line, the LD_{50} value for pregnant mothers was found to be 9.00 μg/g BW for 24 hours (Table I, Fig.1). Some recovered fetuses were found dead. In some of these cases, all fetuses were resorbed within the uterus (Fig.2). For fetuses, LD_{50} value was turned out as 8.2 μg/g BW of pregnant mothers (Table II, Fig.1).

From 20 different mice, 151 control fetuses were recovered (Table III). These fetuses were uniform in appearance and in their other anatomical details. The average weight of fetuses was 431.76±88.16 mg and average CR length was 14.02±1.50 (Table III). These fetuses were well developed (Fig.2).

After the exposure of fetuses to different concentrations of methamidophos, different morphological observations were made. It was found that low dose (5 μg/g BW) also proved to be toxic for some mothers themselves. With the administration of higher doses, rate of maternal mortality increased from 10 to 100% as a result of single dose of 5 to 20

Table I: **Calculation of log-dose/probit line for pregnant mice exposed to 6 different doses of methamidophos, administered orally on day 6 of gestation.**

	1	2	3	4	5	6	7	8	9	10	11	12	13
	Dose conc.	# of animals used	% Dead	Corrected % Dead	Log of the dose	Empirical probits	Expected probits	Weighing co-efficient	Weight				
	µg/g BW			P	x		Y	y		w	wx	wy	y^1
1	20	20	100	100	1.3	-	7.2	5.69	0.092	1.84	2.392	10.469	7.12
2	15	20	100	100	1.176	-	6.5	7.01	0.269	5.38	6.326	37.714	6.44
3	10	20	70	70	1.00	5.52	5.5	5.53	0.581	11.62	11.62	64.258	5.47
4	7.5	20	40	40	0.875	4.75	4.7	4.75	0.616	12.32	10.78	58.52	4.78
5	5.00	20	10	10	0.699	3.72	3.9	3.74	0.405	8.1	5.66	30.294	3.81
6	0.00	20	0.00	00	-	-	-	-	-	-	-	-	-

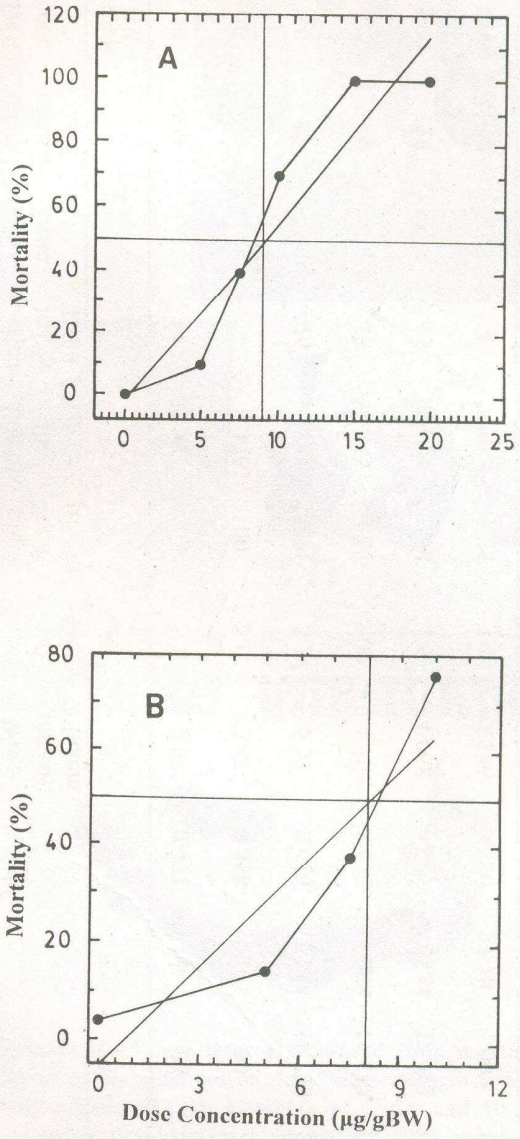

Fig.1: Regression lines of toxicity of methamidophos given on day 6 of gestation to the pregnant mothers (A) as developing fetuses (B), recovered on day 15 of gestation.

Table II: **Calculation of log-dose/probit line for mouse fetuses exposed to 6 different doses of methamidophos, administered orally on day 6 of gestation.**

	1	2	3	4	5	6	7	8	9	10	11	12	13
	Dose conc.	# of animals used	% Dead	Correc-ted % Dead	Log of the dose	Empi-rical probits	Expec-ted probits	Weig-ing co-efficient	Weight				
	µg/g BW			P	x		Y	y		w	wx	wy	y^1
1	20	00	-	-	1.3	00	5.9	2.83	0.471	00	00	00	5.71
2	15	00	-	-	1.176	00	5.6	3.42	0.558	00	00	00	5.44
3	10	83	75.9	76	1.00	5.71	5.3	5.52	0.616	51.128	51.128	282.22	5.1
4	7.5	74	37.8	38	0.875	4.69	5.00	4.70	0.637	471.38	41.245	221.548	4.8
5	5.00	124	14.51	15	0.699	3.96	4.6	4.068	0.601	74.524	52.092	303.163	4.42
6	0.00	1.51	3.97	4	00	3.25	3.1	3.246	0.180	27.18	00	88.226	2.92

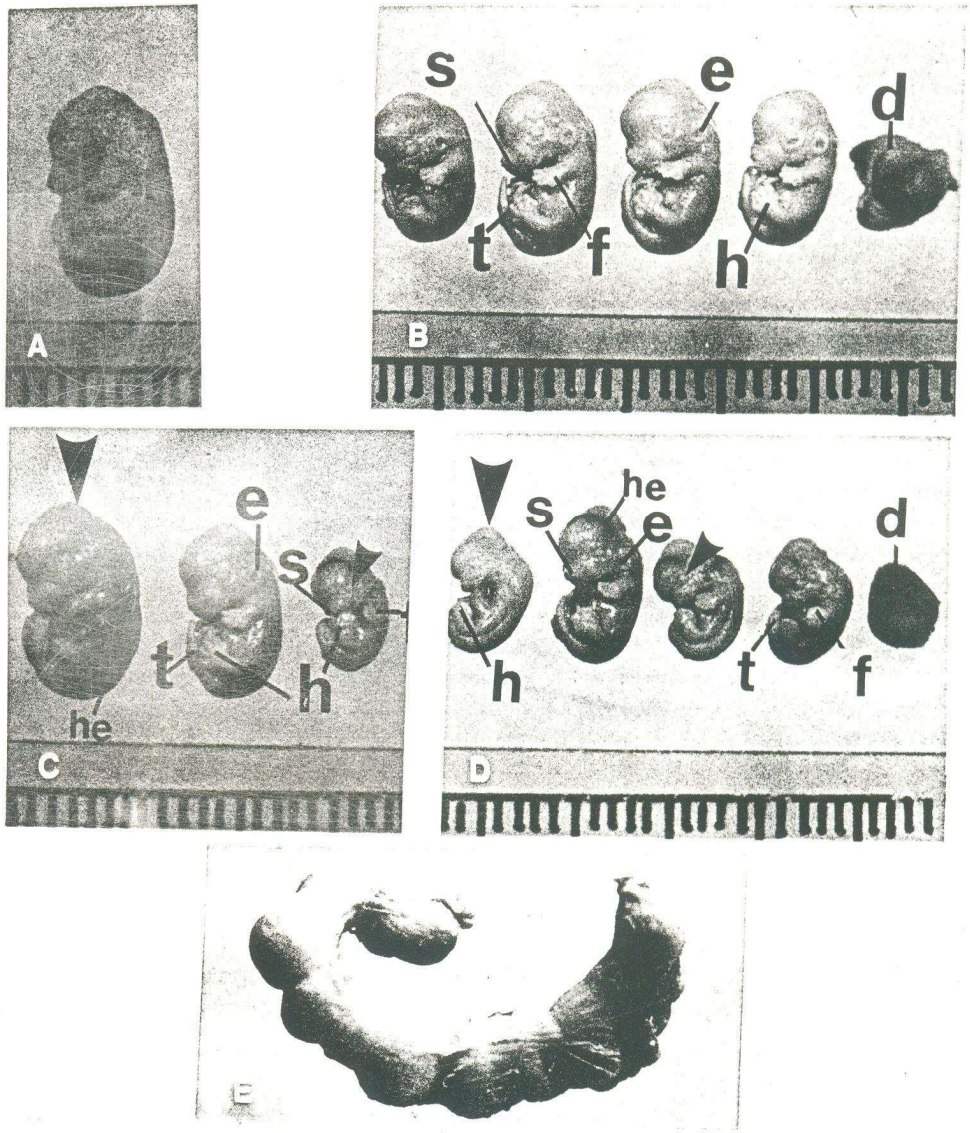

Fig.2: Macrophotographs of 15-days fetuses recovered from mothers exposed to different concentrations of methamidophos on day 6 of gestation. A, a control fetus with well developed body organs; B,C,D, members of 5, 7.5 and 10 μg/g BW dose groups, respectively showing developmental anomalies; E, a gravid uterus with resorbed fetuses, recovered from 10 μg/g BW dose group. Note: dead fetus (d), small pina (e), paddle shaped forelimb (f), hind limb (h), skin haemorrhage (he), abnormal snout (s), short tail (t), microphthalmia (small arrow head) and abnormal brain parts (large arrow head).

μg/g BW, respectively (Table I).

The fetuses recovered from survived mothers were studied and it was found that they were negatively affected. There was tendency towards dwarfism. Their body weights and CR lengths were reduced. For a dose of 5 μg/g BW to mothers, the recovered fetuses had an average body weight of 393.13±9.83 mg while CR length was 13.65±0.19 mm. Following the doses of 7.5 and 10 μg/g BW, the corresponding figures for BW and CR length were 364.58±11.74 mg and 13.38±0.17, 66.36±3.41 and 8.12±0.16 mm, respectively (Table III). In dose of 10 μg/g BW, the reduction in BW and CR length was highly significant (P<0.001) against controls.

The fetal abnormalities were also observed in recovered fetuses. In dose of 5.00 μg/g BW, out of 29 fetuses 8 were dead while 31% were microphathalmic 48% were with short pinnae, 20% had short tail, 35% had limb defects and 55% found having skin haemorrhages. Following an administration of 7.5 μg/g BW, out of 33 fetuses 5 were dead, 45% were microphathalmic, 33% were with short pinnae, 9% had short tail, 36% had limb defects, 58% showed haemorrhagic spots and 27% developed with abnormal snout.

Following a dose of 10 μg/g BW, the mortality rate was 70% (Table I), 71% had small eyes, 76% found having short pinnae, 67% had short tails, 67% had brain abnormalities. All these fetuses were developed with abnormal snout and defected limbs (Table III).

Table III: **Developmental abnormalities caused by an organophosphorus insecticide, methamidophos in mouse fetuses.**

Parameters	Dose μg/g BW			
	C	5.00	7.5	10.00
No. of fetuses observed	45	29	33	22
No. of dead/resorbed embryos	2	8	5	5
Body weight (mg±SD)	431.75± 88.16	393.13± 9.83*	364.56± 11.74**	66.36± 3.41***
C.R. length (mm±SD)	14.02± 1.5	13.65± 0.19	13.38± 0.17*	8.12± 0.16**
Microphthalmia (%)	10	31	45	71
Short pinnae (%)	00	48	33	76
Short tail (%)	00	20	9	67
Limb defects (%)	00	35	36	100
Skin haemorrhage (%)	8	55	58	67
Abnormal snout (%)	00	00	27	100
Brain abnormalities (%)	5	00	00	67

Asterisks show significant difference against controls (*P<0.05; **=P<0.01; ***=P<0.001).

DISCUSSION

The toxic effects of a commonly used OP insecticide, methamidophos were worked out in pregnant mice. LD_{50} values for the insecticide were calculated as 9 μg/g BW for pregnant mothers and 8.2 μg/g BW (mother) for developing fetuses.

It was observed that maternal mortality was greater at higher dose concentrations. After the administration of doses of 15-20 μg/g BW, there was immediate death of mothers. There was also a high mortality rate (70%), even at a dose of 10 μg/g BW (Table I). It is quite apparent from these observations that methamidophos is highly toxic to mice. This insecticide caused acute respiratory distress and restlessness, which may be due to neurotoxic effects of the insecticide.

In many previous studies as well, it has been shown that OP insecticide are toxic to the nervous system by inhibiting AchE activity. Inhibition of AchE results in an accumulation of free acetylcholine in nervous tissues (Kobayashi *et al.*, 1980) and smooth muscles (Narahashi, 1979) producing undesirable side effects including death (Murphy, 1980). The cause of death by OP poisoning was thought to be a combination of excessive bronchial secretions, pulmonary edema, bronchospasm, respiratory muscle paralysis and paralysis of respiratory medulla, in short, "Respiratory Failure" (Du Toit *et al.*, 1981; Zwiener and Ginsburg, 1988; Bardin *et al.*, 1989). It had been discovered that the cardiorespiratory system is the first to be involved during OP poisoning with death resulting from asphyxia and/or impaired circulation (Heath, 1961; O'Brien, 1967; Taylor, 1980). Bakima *et al.* (1989) described that experimental Dichlorovos toxicosis induced changes in the mechanics of breathing and pulmonary haemodynamics associated with diffused bronchoconstriction and cardiac insufficiency, respectively. This type of poisoning also has been identified in humans. Jamil (1989) reported that 1900 cases of poisoning admitted to the Department of Intensive Care at Jinnah Postgraduate Medical Centre, Karachi between 15th January 1978 to 31st December 1985. Out of which 755 cases were of OP insecticide poisoning, forming 39.7% of the total poisoning cases and mortality rate of OP poisoning was twice as high (3.8%) as the mortality rate from all other poisons excluding OPs (1.8%). In another report respiratory failure developed in 43 (40.2%) of 107 patients with acute OP poisoning out of which 22 (51.2%) died (Tsao *et al.*, 1990). The observations made on fetuses during the present study have also proved that these OPs are embryotoxic and teratogenic in mice.

As far as the effects of organophosphate, methamidophos on BW and CR length are concerned, there was significant ($P<0.001$) decrease in all treated fetuses as compared to controls (Table III). Morphological studies also supported the toxic effects of the insecticide. Some cases of hydrocephaly, fetal distortion, hydroneurocoel, deformed limbs and tail, microphthalmia and abnormal ear were found (Fig.2).

It is quite apparent from the results that methamidophos is embryotoxic. These results are not surprising because many of the previous studies or investigations have also shown that OP insecticides are highly toxic to mammalian embryonic systems, even at lower concentrations. Budreau and Singh (1973) tested two OP insecticides, Demeton and Fenthion for their teratogenic effects on mice. It was discovered that Demeton administered in the form of a single dose of 7 to 10 mg/kg or in the form of doses of 5 mg/kg between 7^{th} and 12^{th} day of gestation, proved to be only mildly teratogenic.

Karlow and Marton (1961) 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. Recently, Machin and McBride (1989) also studied the effects of a 100 mg/kg dose of malathion in rabbits, given on day 7-12 of gestation and found that although this dose produced no significant fetal abnormalities, still at least one case of fetal resorption was recorded. Lechner and Abdel Rehman (1984) also found an increased incidence of external haemorrhagic spots on rat fetuses following a treatment of 50 mg/kg of malathion. The present results are also supported by the studies of Staples and Goulding (1979) that developmental alterations are produced in mice exposed to OPs.

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

REFERENCES

ALDRIAN, E.E., FELBERG, N. AND KILBY, B.A., 1947. The cholinesterase inhibiting action of fluorophosphorates. *Br. J. Pharmacol. Chemother.*, **2**: 56-58.

BAKIMA, M., BANDET, H.M., LEKEUX, P. AND LOMBA, F., 1989. Respiratory and pulmonary haemodynamic changes during experimental organophosphate poisoning in goats. *Vet. Res. Commun.*, **13**: 127-133.

HALINA, S.W., 1990. The state of nervous system in workers employed at production of organophosphate pesticides. *Med. Pr.*, **41**: 238-245.

BARDIN, P.G., VAN EEDEN, S.F. AND JOUBERT, J.R., 1989. Intensive care management of acute organophosphate poisoning. *S. Afr. Med. J.*, **72**: 593-597.

BUDREAU, C.H. AND SINGH, R.P., 1973. Teratogenicity and embryotoxicity of Demeton and Fenthion in CF # 1 mouse embryos. *Toxicol. App. Pharmacol.*, **24**: 324-332.

DAVIS, C.S. AND RICHARDSON, R.J., 1980. Organophosphorous compounds. *Experimental and Clinical Neurotoxicology* (P.S. Spencer and H.H. Schaumburg, eds.), pp.517-544. Williams and Wilkins Col., Baltimore.

DU TOIT, P.W., MULLER, F.O., VAN TONDER, W.M. AND UNGERER, M.J., 1981. Experience with the intensive care management of organophosphate insecticide poisoning. *S. Afr. Med. J.*, **60**: 227-229.

ECOBICHON, D.J., 1983. Organophosphorous ester insecticides. *Pesticides and Neurological Diseases* (eds. D.J. Ecobichon and R.M. Joy), pp.151-203. CRC Press Ltd., Boca Raton.

FINNY, D.J., 1964. *Probit analysis*. Cambridge University Press, England.

HEATH, D.F., 1961. *Organophosphorous poisons*. Anticholinesterases and related compounds. Pergamon Press, Oxford.

HOLMSTEDT, B., 1959. Pharmacology of organophosphorous cholinesterase inhibitors. *Pharmacol. Rev.*, **11**: 567-688.

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

KARLOW, W. AND MARTON, A., 1961. Second generation toxicity of malathion in rats. *Nature (Lond.)*, **192**: 464-465.

KOBAYASHI, H., YUYAMA, A., IMAGO, S. AND MATSUSAKA, N., 1980. Effects of acute and chronic administration of dichlorovos on distribution of brain acetylcholine in rats. *J. Toxicol. Sci.*, **5**: 311-320.

LECHNER, D.M.W. AND ABDEL-REHMAN, M.S., 1984. A teratogenicity study of Carbaryl and malathion mixtures in rat. *J. Toxicol. Environ. Hlth.*, **14**: 267-278.

MACHIN, M.G. AND McBRIDE, W.G., 1989. Teratological study of malathion in the rabbit. *J. Toxicol. Environ. Hlth.*, **26**: 249-253.

MUNNECKE, D.M., 1980. Enzymatic detoxification of waste organophosphate pesticides. *J. Agric. Food Chem.*, **28**: 108-111.

MURPHY, S.D., 1980. *Pesticides Toxicology*. The basic science of poisons (eds. J. Doull *et al.*), 2nd Ed., pp.357-408. MacMillan, New York.

NARAHASHI, T., 1976. *In vitro* methods for evaluating side effects of pesticides and toxic substances. U.S. NTIS PBRep. PB-260385, 129.

NEWSOM, L.D., 1967. Consequences of insecticide use on non-target organisms. *Ann. Rev. Entomol.*, **12**: 256-286.

O'BRIEN, R.D., 1967. *Insecticides*: Action and Metabolism. Academic Press, New York, pp.33-87.

TUCKER, R.K. AND LEITZKE, J.S., 1979. Comparative toxicology of insecticides for vertebrate wildlife and fish. *Pharmac. Ther.*, **6**: 167-220.

STAPLES, R.E. AND GOULDING, E.H., 1979. Dipterex teratogenicity in the rat, hamster and mouse when given by gavages. *Environ. Hlth. Perspect.*, **30**: 105-113.

TAYLOR, P., 1980. *The Pharmacological Basis of Therapeutics* (eds. A. Goodman and T.W. Gilman), 8th Ed., pp.100-119. MacMillan, New York.

TSAO, T., JUANG, C.Y., LAN, Y.C., SHIEH, R.S., LEE, W.B. AND CHANG, C.H., 1990. Respiratory failure of acute organophosphate and carbamate poisoning. *Chest.*, **96**: 631-638.

USDIN, E., 1970. Reactions of cholinesterases with substrates, inhibitors and reactivators. *Anticholinesterase Agents* (ed. A.J. Karczmar), International Encyclopaedia of Pharmacology and Therapeutics, Section 3, Vol.1, pp.347-354. Pergamon, Oxford.

ZWIENER, R.J. AND GINSBURG, C.M., 1988. Organophosphate and carbamate poisoning in infants and children. *Pediatrics*, **81**: 121-126.

LAHMAN, BARBARA, SINCZUK-WALCZAK, H. AND SPLIT, W., 1990. Severe polyneuropathy as a result of organophosphate poisoning. *Med. Pr.*, **41**: 360-362.

(*Received: September 19, 1999*)