Teratogenic Studies of Fenitrothion on White Leghorn Chick Embryos

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With the growing awareness of the problems associated with environmental persistance of chlorinated hydrocarbon insecticides, the use of organophosphorus pesticides in agriculture, public health is on the increase. Fenitrothion (Sumithion, O,O-dimethly-O-(3-methyl-4-nitrophenyl) phosphorotioate) has recently been introduced as a crop protectant in agriculture and as an ectoparasiticide in poultry and livestock (S.C.C., 1971). The detection of thalidomide induced teratisms (congenital abnormalities) in human bodies has refocused the attention of teratogenic capabilities of drugs and other chemicals (SOMERS, 1962). DUNACHIE and FLETCHER (1966) observed marked reduction in hatchability of hen's eggs treated with a variety of chlorinated hydrocarbon and phosphorus pesticides. Recently, many reports have been published indicating the teratogenic nature of malathion, malaoxon, parathion, diazinon, trithion, ruelene, phosdrin and DDVP (GHADIRI and GREENWOOD, 1966; KHERA, 1966; GREENBERG and LeHAM, 1969, 1970; WALKER, 1971). However, there are no such data on fenitrothion, except a very recent report from Japan where non-teratogenic nature of this insecticide in rabbit has been reported (S.C.C., 1971). In the present investigations the effect of fenitrothion on embryonic development and post hatch growth rate of White Leghorn chicken was studied.

METHODS AND MATERIALS

A total of 245 fertile White Leghorn eggs procured from a local commercial hatchery were employed in this study. The technique of testing teratogenecity was followed according to MACLAUGHLEN *et al.* (1963) and WALKER (1967). Initially a preliminary trial was conducted to assess the concentrations of fenitrothion which were embryotoxic. For this, 90 eggs divided into 9 groups with 10 eggs in each were used. The eggs in the first group were left untreated and served as control. The eggs in the second and third groups also served as control where drilling of the hole in the centre of air space and inoculation of 0.1 cc distilled water from 4th to 12th day of incubation were done respectively. The eggs in the next six groups were inoculated with 0.1 ml of 0.1, 1, 5, 10, 20 and 30% fenitrothion respectively. Basing on the results of the preliminary trial, fenitrothion in concentrations of 0.1, 0.5 and 1% was selected to study its effects on the embryo during 4th to 12th day of incubation and only 0.1% concentration was used in subsequent stages of embryonic development. The details are shown in Table 1.

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TABLE 1

Experimental design and period of treatment of chick embryos*

rial roup	No. of eggs	Treatment	Period of treatment
rial I			
	20	0.1 cc of distilled water (control)	4th to 12th day of incubation
	20	0.1 cc of 0.1% fenitrothion	-do-
	20	0.1 cc of 0.5% fenitrothion	-do-
	20	0.1 cc of 1.0% fenitrothion	-do-
rial II			
	10	0.1 cc of distilled water (control)	8th to 12th day of incubation
	15	0.1 cc of 0.1% fenitrothion	-do-
rial III			
	10	0.1 cc of distilled water (control)	10th to 12th day of incubation
	15	0.1 cc of 0.1% fenitrothion	-do-
rial IV			
	10	0.1 cc of distilled water (control)	12th day alone
	15	0.1 cc of 0.1% fenitrothion	-do-

* Inoculations of test materials were made into yolk spaces.

The different concentrations of Sumithion* (100% emulsifiable oncentrate of fenitrothion) were made in distilled water. The test aterial in 0.1 ml volume was injected into the yolk space through ie hole drilled in the centre of the air space of the egg and the ole was sealed with a piece of cellophane tape. The eggs were ncubated at 37°C temper-ture and 60% humidity. Before injecting

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the test material the eggs were candled daily in order to discard any dead embryos. On 18th day they were transferred to the hatcher.

The per cent hatch and visible abnormalities, if any, in the chi were observed in each group. The chicks were maintained on a chick starter ration in a battery brooder. Post-hatch mortality and the body weight gains were also recorded. The data of body weight were analysed by student 't' test (KAPUR and SAXENA, 1970).

RESULTS

Preliminary trial

There was 100, 90 and 80% hatch in control groups of untreated having atab only and distilled water injected eggs respectively. Fenitrothion in concentrations of 5 to 30% produced lethal effect to the embryos and in concentrations of 0.1 and 1% there was 40 and 30% hatch respectively. The chicks hatched from 0.1 and 1% fenitrothion treated eggs were dwarfed and showed visible abnormalities such as curled toe, leg weakness and abnormal gait.

Detailed embryo toxic study

The effect of fenitrothion in different concentration on per cen hatch and post-hatch mortality is shown in Table 2.

TABLE 2

Effect of fenitrothion in different concentrations on hatchability and the post-hatch mortality of chicks

Trial & Treatment Group No*	Hatch		Post-hatch mortality							Mortality	
	No. of chicks	Per cent	1st	2nd	3rd	4th	5th	6th	7th	Total	Per cent
Trial I											
1	16	80	-	-	-	-	-	-	-	0	0
2	8	40	-	1	-	3	-	-	-	4	50
3	7	35	-	3	2	2	-	-	-	7	100
4	6	30	2	-	2	1	1	-	-	6	100
Trial II											
1	8	80	-	-	-	-	-	-	-	0	0
2	8	53.6	-	-	-	-	-	-	2	2	25
Trial III											
1	8	80	-	-	-	-	-	-	-	0	0
2	9	60	-	-	-	-	-	-	-	0	0
Trial IV											
1	9	90	-	-	-	-	-	-	-	0	0
2	12	80	-	-	-	-	-	-	-	0	0

* Details in Table 1

It is obvious from the table that in trial I (4-12 day incubation), the highest hatchability rate (40 per cent) and least mortality (50 per cent) were seen in group 2 where 0.1% of fenitrothion was used. The survived chicks in all the groups showed dwarf growth (Fig. 1), curled toe, leg weakness and abnormal gait. In trial II (8-12 day incubation) the hatchability rate (53.6 per cent) with 0.1% fenitrothion treatment was lower as compared to trial III (10-12 day incubation) and trial IV (12th day alone incubation) where it was of the order of 60 and 80% respectively. There was no mortality in trial III and IV as compared to 25% in trial II.

Fig. 1 : Effect of embryonic treatment of fenitrothion (0.1% conc.) on the hatched chicks after 4 weeks. The upper is control and the lower chick shows the dwarfed growth after fenitrothion treatment.

Body weight

Table 3 shows the effect of embryonic treatment of fenitrothion in different concentration on body weight of chicks.

TABLE 3

Effect of embryonic treatment of fenitrothion in different concentrations on the body weight of chicks

Trial & Treatment Group No.*	Body weight (g ± S.D.)			
	0 day (day of hatch)	14th day	28th day	42nd day
Trial I				
1	41.88 ± 3.72	186.88 ± 19.67	267.50 ± 18.68	337.50 ± 20.8
2	28.89 ± 5.64	102.00 ± 10.95***	151.00 ± 18.17***	201.00 ± 5.17
3	25.71 ± 5.56	-	-	-
4	22.50 ± 3.54**	-	-	-
Trial II				
1	40.63 ± 4.18	185.00 ± 5.98	266.25 ± 19.39	340.00 ± 16.89
2	25.63 ± 4.95	117.50 ± 8.22***	170.00 ± 10.95***	205.00 ± 10.72
Trial III				
1	41.25 ± 4.43	184.38 ± 9.50	271.88 ± 13.78	353.75 ± 12.06
2	34.44 ± 4.64	136.000± 17.85	198.89 ± 18.33**	302.22 ± 24.12
Trial IV				
1	42.78 ± 4.62	188.89 ± 6.51	265.00 ± 14.27	346.67 ± 21.01
2	36.89 ± 5.56	161.67 ± 16.00	223.17 ± 14.48	296.25 ± 26.12

* Details in Table 1

** P/0.05

*** P /0.01

It is obvious from the results that the body weight of chicks on the day of hatch was less in fenitrothion treated chicks in all the trials as compared to control and it was significant ($P<0.05$) in group 4 (1%) of trial I. The body weight was also reduced on the subsequent days with 0.1% in all the trials and it was significant ($P<0.01$) through 14th and 42nd day in trial I and II. In trial III it was significantly less ($P<0.05$) on 28th day.

DISCUSSION

In order to assess the concentration of fenitrothion likely to cause embryo toxic effects, preliminary trial was conducted. The results of these trials indicated that fenitrothion in concentration of 5% and above was lethal to all embryos during 4-12 days of incubation and the hatchability rate was 40 and 30% with 0.1 and 1.0% concentration of fenitrothion, respectively. Basing on the results of this study the detailed studies were made with 0.1, 0.5 and 1.0% concentration of fenitrothion during 4 to 12 days of incubation and the results were in confirmation where these treatments caused 40, 35 and 30% hatchability, respectively. These observations further suggested that fenitrothion treatment during 4th and 12th day of embryonic development was highly toxic and 0.1% treatment produced comparatively higher hatchability rate. Accordingly, another three trials were conducted by taking only 0.1% concentration of fenitrothion during more advanced periods of incubation viz. 8th to 12th day, 10th to 12th day and 12th day. The results indicated that with the advancement of incubation period, the hatchability rate was increased and it was maximum (80 per cent) on the 12th day as compared to 53.6% during 8th to 12th day and 60% during 10th to 12th day.

Marked reduction in hatching rate in general from the eggs treated with several chlorinated and organophosphorus pesticides has also been reported earlier by DUNACHIE and FLETCHER (1966). The highly embryotoxic effects of malathion during earlier period of incubation (0-7 days) and increased hatchability rate during later stages of embryonic development has also been reported by KHERA and LYON,(1968) and GREENBERG and LaHAM,(1969), respectively.

The chicks hatched from 0.1 to 1.0% fenitrothion treated eggs during 4th to 12th day of incubation showed dwarfism, curled toe, leg weakness and abnormal gait. Similar abnormalities have also been observed by GREENBERG and LaHAM (1969, 1970) from malathion treated eggs. However, in comparison to malathion, where the teratogenic dose was 3.99 to 6.22 mg/egg, the teratogenic dose (0.01 to 1 mg/egg) of fenitrothion used in this study is comparatively far less and it may be concluded that fenitrothion is more toxic to chick embryos as compared to malathion. Further the extent of abnormalities in the chicks hatched during the later period of incubation were far less and they only showed dwarfism.

The body weight of the chicks hatched from the eggs treated during later period of incubation also showed comparatively more weight gain as compared to those hatched from earlier period of

incubation although the body weight in general was reduced in all the chicks hatched from the eggs treated with different concentrations of fenitrothion.

The effect on growth rate may be due to adverse effects of the insecticides on the growth promoting endocrine glands as it has been reported by DYADICHEVA (1971) that pesticides produced thyroid and adrenal insufficiency in animals. It may also be due to the inhibited utilization of several metabolites such as nicotinamide, nicotinic acid, quindic acid and tryptophan during embryonic development (GREENBERG and LaHAM, 1970).

SUMMARY

Teratogenic studies of fenitrothion, an organophosphorus insecticide were conducted on White Leghorn chick embryos. Fenitrothion injections of 5 to 30% concentration in 0.1 ml volumes into the egg yolk space during 4th to 12th day of incubation were lethal to the embryos and the concentration of 0.1, 0.5 and 1.0% resulted in 40, 35 and 30% hatch, respectively. The chicks showed visible abnormalities such as dwarfism, curled toe, leg weakness and abnormal gait. Fenitrothion injections were comparatively less toxic during the later stages of embryonic development (8th to 12th day of incubation). The growth rate of chicks hatched from fenitrothion treated eggs was reduced as compared to the control chicks (distilled water treated).

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