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Embryotoxic and teratogenic evaluation of cyfluthrin in Swiss albino mice

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Abstract

Pesticides are known for their toxicity on the mammalian systems including the embryonal development. Cyfluthrin (trade name: Baygon, Solfac) is used commonly in almost every household in India. It is reported to be neurotoxic, hepatotoxic and haematotoxic in rats and to cause miscarriages and resorptions in pregnant rabbits. It was therefore planned to assess its teratogenic potential in Swiss albino mice. Pregnant dams were orally administered two doses of the insecticide (16 mg/kg and 32 mg/kg body weight) during the organogenetic phase i.e. days 5-14 of gestation. There occurred a dose related decrease in the weight gained by the females during the gestation period. The higher dose affected the litter size, number of live fetuses, resorptions and the average fetal weight significantly. The fetal anomalies observed were reduced ossification of the skull bones and phalanges and widened cranial sutures.

Keywords: Cyfluthrin, embryotoxicity, mice, teratogenecity.

Introduction

Pyrethroid insecticides have achieved widespread agricultural and environmental health applications due to their strong insecticidal properties and they are one of the most frequently used classes of pesticides (Roberts and Hutson, 1999). Although synthetic pyrethroids have been modified to be more photostable, more lipophilic, and more toxic than natural pyrethrins, yet they are considerably less toxic to mammals than the other classes of insecticides, such as organochlorines, organophosphates, and carbamates. Because of their low acute human toxicity, pyrethroids are widely used to control insects in and around homes (Freeman *et al.*, 2004).

Cyfluthrin [Cyano(4-fluoro-3-phenoxy-phenyl)methyl3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], a type II synthetic pyrethroid is the active ingredient of non-systemic insecticides primarily used for the control of chewing and sucking insects and also in public health situations (Adams *et al.*, 2002). It is a commonly used household insecticide in India sold under the trade names "Baygon" and "Solfac". It has been effectively used for the control of a variety of insects like ants, silverfishes, cockroaches, grain beetles, flies, mosquitoes etc (Thompson, 1992). Cyfluthrin is a neurotoxin inducing alterations in nerve membranes, causing abnormal sodium and potassium flow. This results in the repetitive discharges from the neurons, causing convulsions and also blockage of further nerve impulses (Cremlyn, 1991). Laboratory animals exposed to high doses of cyfluthrin have shown toxic effects like convulsions, salivation, ataxia, weakness, and apathy (Schimmel, 1983). Cyfluthrin is also known to cause weight loss, changes in blood chemistry and liver weights in rats. It has been reported to cause miscarriages and resorptions in rabbits (U.S. EPA, 1989). Since much literature is not available on the developmental toxicity of cyfluthrin, the present work was therefore planned to evaluate its teratogenic potential in Swiss albino mice.

Materials and Methods

Solfac 050 EW, a formulated product containing 5% w/w cyfluthrin was used for the study. Swiss albino mice originally obtained from

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Table 1: Effect of cyfluthrin on maternal weight gain and mortality

Dose administered (mg/kg bodyweight)	Body weight gained between gestational days 6-10(g)	Body weight gained between gestational days 10-14(g)	Body weight gained between gestational days 14-18(g)	Percent Mortality
0	3.9±0.50	4.9±0.37	8±0.66	0
16	2.3±0.44*	4.4±0.96	7.3±0.96	0
32	1.1±0.40**	2.3±0.78**	2.9±0.86**	0

Student's *t*- test; *Significant Difference ($p<0.05$); **Highly Significant Difference ($p<0.01$)

the Indian Veterinary Research Institute, Bareilly (U.P.) were bred and housed in an air cooled vivarium with natural day light of 12-14 hours. The colony was maintained on standard mice feed procured from the Hindustan Lever Limited, Chandigarh, India. Tap water was provided *ad libitum*. Female and male mice were housed for mating in the ratio of 3:1. The females were checked every morning for vaginal plugs. The day a vaginal plug was seen was counted as day 0 of pregnancy. Thirty inseminated females were selected and divided into three groups; two experimental and one control. Each group contained 10 females. One of the experimental group was orally intubated with the lower dose of cyfluthrin i.e. 16 mg/kg body weight once daily from day 5 to day 14 of gestation. Second experimental group was exposed to 32 mg/kg body weight cyfluthrin in the same way and the third group i.e. control received plain tap water.

Females of all the groups were weighed on every alternate day throughout pregnancy. They were sacrificed on the 18th day of pregnancy. The corpora lutea in both the ovaries were counted. The uteri were examined for routine teratological changes. The live fetuses and placental discs were removed and their wet weights recorded. All the fetuses were sexed and inspected for external abnormalities. One third of the fetuses from each litter were fixed for skeletal examination (Dawson, 1927) and the remaining for soft tissue examination by free hand razor section technique (Wilson, 1965). The data were statistically evaluated using the student's *t*-test and Mann-Whitney U-test.

Results and Discussion

The pregnant females of the higher dose group (32 mg/kg) gained less weight than the controls (Table 1). No mortality occurred in any experimental group. The litter size and number of live fetuses were significantly reduced in this group. This decrease in maternal weight gain can be co-related to the lower litter size which seems to involve both implantation failure and resorptions. A similar dose related reduction in maternal weight gain on administration of heptachlor and chlorpyrifos during pregnancy has been reported by Purkerson-Parker *et al*. (2001) and Farag *et al*. (2003) respectively. The high dose also lowered the average fetal and placental weights. This reduction in the fetal weight may be associated with reduction in the maternal weight gain (Kavlock *et al.*, 1981; Narotsky and Kavlock, 1995). The number of resorptions was significantly increased in the group exposed to 32 mg/kg cyfluthrin. Complete resorption of all embryos also occurred in a few dams of this group (Table 2). It may be presumed that damage to cells and organ system due to pesticide treatment caused embryolethality. Similar results were also obtained by Farag *et al*. (2006) and Tian *et al*. (2005) who witnessed depressed maternal body weights accompanied by reduced fetal weights and increased number of resorptions in rats treated with other pesticides like dimethoate and chlopyrifos respectively.

The results of the present investigation showed reduced ossification of fetal skeletons which may be a probable reason for decrease in fetal weight. Similar relationship between low body weights of fetuses and retarded ossification of the skeleton has

Table 2: Influence of cyfluthrin on embryonic development of mice

Dose administered (mg/kg body weight)	Number of autopsies performed	Total implants (Mean ±S.E.)	Litter size (Mean ±S.E.)	Alive Fetuses				No. of dead fetuses (%)	No.of resorbed fetuses	Placenta weight (Mean ± S.E.)
				No. of alive fetuses	Fetal body weight (Mean ± S.E.)	Sex ratio (%) M:F	Immature or stunted fetuses			
0	10	52(5.2±0.44)	5.2±0.36	49	1.2±0.10	46.93% (M) 53.06% (F)	0	5.76%	0	0.16±0.013
16	10	56(5.33±0.55)	4.77±0.74	48	0.93±0.13	54.16%(M) 45.83%(F)	4	5.35%	8.92%	0.126±0.02
32	10	42(4.2±0.66)	2.8±0.67**	27*	0.74±0.17*	51.85%(M) 48.14%(F)	0	2.38%	33.33%**	0.107±0.02 *

Student *t*- test and Mann-Whitney U-test; *Significant Difference ($p<0.05$); **Highly Significant Difference ($p<0.01$)

Table 3: Skeletal malformations in fetuses after maternal exposure to cyfluthrin.

Dose administered (mg/kg body weight)	No. of fetuses examined	Total no. of fetuses with abnormalities	No. of fetuses with reduced skull ossification	No. of fetuses with widened cranial sutures	No. of fetuses with rib defects			Fetuses with sternal defects			Fetuses with partial/ reduced ossification	
					Ribs absent ribs	Short/ rudimentary ribs	Gap between sternebrae	Reduced no. of sternebrae	Disfigured/ fused absent	Xiphisternum cleaved/ Phalanges	Fore limb	Hind limb
0	25	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
16	28	28	28 (100)	14 (50)	3 (10.71)	8 (28.57)	0 (0)	5 (17.85)	2 (7.14)	0 (0)	8 (28.57)	9 (32.14)
32	15	14	13 (86.66)	2 (13.33)	0 (0)	2 (13.33)	0 (0)	2 (13.33)	0 (0)	2 (13.33)	0 (0)	0 (0)

Figures in parenthesis show percentages

Table 4: External and visceral anomalies in mouse fetuses after maternal exposure to cyfluthrin.

Dose administered (mg/kg body weight)	Total number of fetuses examined		External anomalies	Head Region				Thoracic region
	Externally	Internally	Open eye	Cleft palate	Hydro-cephaly	Micro-phthalmia	Ano-phthalmia	Pulmonary edema
0	49	24	0	0 (0)	3 (12.5)	4 (16.66)	0	2 (8.33)
16	48	20	0	0 (0)	5 (25)	3 (15)	6 (30)	4 (20)
32	27	12	0	0 (0)	4 (33.33)	3 (25)	3 (25)	1 (8.33)

Figures in parenthesis show percentages

been reported by Murray *et al*. (1979) due to carbaryl in rabbits and mice. No external malformation appeared in the fetuses of the treated dams. Alizarin stained skeletons of most of the fetuses showed reduced ossification of the skull bones, sternebrae and ribs (Table3). Cyfluthrin caused reduced ossification of the skull bones in all fetuses of the low dose group and in 86.66% fetuses of the high dose group. Other pesticides like phosphamidon (Bhatnagar and Soni, 1988), carbaryl (Mathur and Bhatnagar, 1991) and fenpropathrin (Dhar *et al.*, 2004) are also known to produce various skeletal abnormalities in the laboratory animals. Reduced ossification of various bones may be due to the effect of pesticide on calcium metabolism and/or bone morphometry by decreasing the supply of calcium and magnesium ions to the growing fetus thereby causing retardation in the bone development (Andrews and Gray, 1990). Further, the delay in ossification of the skeletal system may be correlated to the delay in growth of the fetus, as indicated by the lower fetal weights in the high dose group of the present study. Welsch and Morgan (1985) have also related poor ossification of the skeletal system to growth retardation of the fetuses.

Short ribs were evident in 28.57% and 13.33% fetuses of low and high dose groups respectively while ribs were missing in 10.71% fetuses in the group treated with 16 mg/kg. Khera (1974) postulated that this anomaly results from delayed development of alkaline phosphatase activity and arrested calcium deposition in ribs occurring due to pesticide treatment. Reduced number of sternebrae was seen in 17.85% fetuses of the low dose group and 13.33% fetuses of the high dose group. Similar results of reduction in the number of sternebrae have also been observed by various workers (Kavlock *et al.*, 1982; Giavini *et al.*, 1982). Examination of fetuses for limb deformities showed partial ossification of forelimb (28.57%) and hindlimb (32.14%) phalanges in the low dose group (Table 3).

Free hand razor sections through the head and trunk regions revealed a few visceral anomalies. Major anomaly noted in the head region was hydrocephaly i.e. enlargement of the cerebral

ventricles. The occurrence of hydrocephaly was 25% and 33.33% in the low and high dose groups, respectively. Fluid retention in the brain is the speculated reason for this abnormality. This is in concomitance with the results obtained by Sakata-Haga *et al*. (2004) and Sitarek (2004) with other teratogenic agents. Ophthalmic malformations including anophthalmia and microphthalmia were also observed in fetuses of both treatment groups. The percentage of microphthalmia and anophthalmia was 25% each in the group treated with 32 mg/kg, while 30% fetuses of the low dose group were anophthalmic. Hoogenboom *et al*. (1991), Ozeki and Shirai (1998) and Iyer *et al*. (1999) also reported these congenital eye abnormalities in rats and mice. The fetuses exhibited pulmonary edema as seen by enlarged spaces in lungs of both low dose (20%) and high dose (8.33%) groups (Table 4). Similar reports of edematous lungs were reported in a large number of Bhopal victims (Dave, 1985; Marwick, 1985) and in rats (Stevens *et al*., 1987). Reason for the enlarged spaces in lungs might be the accumulation of fluid in them which may in turn result in the breakdown of airways leading to wider interstitial spaces.

From the present investigation it can be concluded that the higher dose of the pesticide caused significant reduction in the litter size and number of live fetuses, and an increase in the number of resorbed fetuses. However, the animals that received the lower dose did not exhibit significant alterations in the reproductive parameters studied. Cyfluthrin also produced visceral and skeletal malformations in the fetuses. Therefore, it can be inferred that the pesticide produced embryotoxic and teratogenic effects in a dose related manner.

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