A REVIEW OF STUDIES ON PYRETHROID, CYPERMETHRIN, AND REPRODUCTIVE TOXICITY IN ALBINO MICE

Dr. Vivek Kumar Singh*

Department of Zoology, Assistant Professor, Devta Mahavidyalaya Morna, U.P., India

Email ID: chaudharyv998@gmail.com

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Abstract

The objective of the present study was to find out whether or not sub lethal doses of cypermethrin (CYP), i.e.1.38, 2.76 and 5.52 mg/kg body weight corresponding to 1/476th, 1/238th and 1/119th of LD50 dose of aqueous suspension administered orally, adversely affect gametogenesis, sex steroid secretion, fertility, fertility indices, onset of puberty in progeny and accessory organs of male and female mice and if so whether effects are reversible or not. The work is divided into two chapters. First one deals with male toxicity and second with female toxicity. Three graded doses of CYP as mentioned above, were administered to adult male mice orally through intubation on alternate day for two durations, 6 weeks and12 weeks. There was a dose and duration dependent reduction in

i) weight of the body, testes and accessory reproductive organs,

ii) serum levels of testosterone,

iii) Total counts of epididymal spermatozoa whereas there was an increase in abnormal sperm counts compared to controls.

Despite these there was an increase in number of atretic follicles of all categories and weight of the uterus compared to controls. There was a significant delay in onset of puberty and reduction in number of estrous cycles of progeny of medium and high dose CYP treated mice mated with normal males. This is an interesting finding because despite female mice not treated with CYP either during mating or pregnancy period, there was reproductive effect in both male and female offspring. The effects of high dose on different parameters were not reversible within 6 weeks alter withdrawal of the treatment.

Following are the novel findings:

i) Chronic exposure to very low doses of CYP for long period irreversibly interferes with gametogenic activity via endocrine disruptive action and effects prevail even in progeny, and

ii) Fmale mice are more sensitive to reproductive toxic effects of CYP than males.

Paper Identification

**Corresponding Author*

Introduction

In mammals the male genital tract consists of the testes, the epididymis, the vas deference, the seminal vesicle and the prostate. Production of spermatozoa in mammals is enormous. Spermatogenesis is the process of germ cell proliferation and differentiation within the seminiferous tubules of the testis leading to haploid, free-swimming spermatozoa. An average human ejaculate contains about 180 million sperm (66 million/ml), but some ejaculates contain as many as 400 million sperm. Both quantity and quality of the sperm are important determinants of fertility. A man is considered clinically infertile if his sperm concentration falls below 20 million/ml semen (Sherwood, 2001). The production of healthy motile spermatozoa may be interfered at any stage of sperm development. Further total count of spermatozoa might be altered or percentage of abnormal spermatozoa might be increased leading to infertility. Exposures to pesticides adversely affect the reproduction in mammals. More than 50 pesticides are known to disrupt the production of spermatozoa or male hormones (Cox, 1996). About 200 million pounds of sperm damaging pesticides are used in agriculture every year (Cox, 1996). Cypermethrin (CYP) , a synthetic pyrethroid, is widely used to control many pests viz., moth pests of cotton, fruits, and vegetables crops (Meister, 1992); pests (lepidoptera, cockroaches, and termites) in stores, warehouses, industrial buildings, houses and apartments, greenhouses, laboratories, ships, railcars, buses, trucks , aircrafts, non-food areas in schools, nursing homes, hospitals, restaurants, hotels and food processing plants (US. EPA, 1989),ectoparasites in veterinary practice and as an insect repellent for horses (Elliot and Janes, 1978; WHO, 1989; Luty et al., 2000, Oheme and Mannala, 2001). After household treatments, CYP persists in air and on the walls and furniture for about three months (Cox, 1996).

Materials and method

Animals

Seventy adult Swiss albino male mice weighing 33-37 g obtained from inbred population of the central animal facility, University of Mysore were housed in polypropylene cages (5 animals per cage) with husk as the bedding material under 12:12 h light-dark schedule (lights on 7am to 7pm) at 27 ± 2 °C and 70 % humidity. The animals were supplied with standard mice chow pellets and water ad libitum during the period of the experimentation. The protocols were approved by Institutional Animal Ethics Committee and the guide lines of CPCSEA, Govt. of India were followed for care and maintenance of animals.

Chemical and treatment treatment treatment:

The doses of CYP used in the present study were based on mouse oral LD50 value of technical grade cypermethrin (657 mg/kg body weight; Rose, 1982; WHO 1989) when used as aqueous suspension. A commercial preparation (Superkiller- 10% EC, Dhanuka Agritech Limited, India), an emulsifiable concentrate of CYP (10%) was purchased and diluted in distilled water to get required concentrations i.e 1.38, 2.76 and 5.52 mg/kg body weight corresponding

to 1/476th, 1/238th and 1/119th of LD50 value of the aqueous suspension of CYP respectively. Further, doses were adjusted to the body weight (bw) (average bw 35g) of the mice used in the experiment. The adult male mice were randomly divided into four groups, controls (10) and three treatment groups (20 in each group). Body weight (initial bw) of each mouse was recorded before commencement of treatment. Control group received 0.1 ml distilled water per mouse, whereas each mouse in 2nd, 3rd and 4th groups received 1.38 (low dose), 2.76 (medium dose) and 5.52 (high dose) mg CYP /kg bw in 0.1 ml distilled water respectively. Animals were administered vehicle or CYP orally (intubation) through a smooth plastic tube attached to a syringe on alternate day for two durations, 6 weeks (D1) and 12 weeks (D2). Body weight of each mouse was recorded at weekly intervals throughout the experimental period i.e. 3 months.

Autopsy:

After each treatment period i.e. 6 weeks or 12 weeks five mice of each group were autopsied 24 h after last administration and the weights of the body, testes, epididymes, vas deference, seminal vesicle and prostate were recorded and later converted into organ weight per 100g body weight (relative weight). Testes were removed, washed in normal saline to free from blood and connective tissue and fixed in Zenker-formol and epididymides were fixed in Bouin's fluid for histopathological study. The blood samples were collected; serum was separated and stored at -80 C until used to determine testosterone levels. Five mice in each treated group were maintained for 6 weeks without CYP treatment and then autopsied to find out recovery of CYP effects if any.

Fertility test:

Forty adult female mice with proven fertility were used for fertility test. Fertility was determined after each treatment period. Each male in all the experimental groups (five per group) was left in a cage with the normal female (1♂ + 1♀ per cage) for 2 weeks. The females were examined for the presence of vaginal plug and spermatozoa in the vaginal smear every day after they were placed with males. The presence of vaginal plug and spermatozoa in the smear confirmed the mating. The pregnant females were allowed to deliver the pups to find out differences if any in the litter size, litter weight or gestation period

between control and treated animals. Number of females conceived, average length of gestation, litter size, and litter weight of each group were recorded. Other fertility parameters were determined according to the procedure of Kennedy et al. (1973), Adilaxmamma et al. (1994) and Narayana et al. (2005) as follows

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- Fertility index of male = $\frac{\text{Number of fertile males}}{\text{Number of males used in the test}} \times 100$,

- Fertility index of female = $\frac{\text{Number of pregnant rats}}{\text{Number of males used in the test}} \times 100$,

- Parturition index = $\frac{\text{Number of females delivered}}{\text{Number of pregnant rats}} \times 100$,

- Gestation index = $\frac{\text{Number of pups born alive}}{\text{Total number of pups born}} \times 100$,

- Viability index = $\frac{\text{Number of pups alive at day 4}}{\text{Total number of pups born alive}} \times 100$, and

- Lactation index = $\frac{\text{Number of pups alive at day 21}}{\text{Number of pups alive at day 4.}} \times 100$.

- ***Hormone Assay:***

Groups	**Mean % change in body weight after 12 weeks treatment compared to initial body weight ± SE**			**Mean % change in body weight 6 weeks after cessation of treatment compared to body weight after 12 weeks treatment ± SE**
	4th week	**8th week**	**12th week**	

Control	+ 6.64 ± 0.84[a]	+ 9.11 ± 0.68[a]	+15.5 ± 1.36[a]	+16.19 ± 1.03[a]
Low dose	+ 5.36 ± 1.3[a]	-3.95 ± 1.74[b]	-5.77 ± 1.24[b]	+3.5 ± 1.71[b]
Medium dose	+ 5.05 ± 0.81[a]	-6.65 ± 1.14[b]	-8.16 ± 0.83[c]	+2.8 ± 1.52[b]
High dose	-2.16 ± 0.83[b]	-9.31 ± 1.14[c]	-12.34 ± 1.38[d]	-2.07 ± 0.71[c]
ANOVA F value df= (3,16)	6.01 P<0.05	4.71 P<0.05	14.9 P<0.001	5.207 P<0.05

The serum concentration of testosterone was measured by enzyme immunoassay using the kits purchased from Adaltis Italia S.P.A., Italy (EIAgenTESTOSTERONE, LOT: 1674, REF: LI4011K) following the procedure of the manufacturer.

RECOVERY STUDY

To study whether the effect of CYP was reversible or not, five males of each treatment groups were maintained without treatment for 6 weeks after each CYP treatment period (i.e. after 6 or 12 weeks treatment). Body weight was recorded at weekly intervals. Animals were autopsied at the end of the recovery period. Weight of the body, testes, epididymis, vas deference, seminal vesicle and prostate was recorded and later converted into organ weight per 100g body weight (relative weight). Testes were removed, washed in normal saline to free from blood and connective tissue and fixed in Zenker-formol andepididymides were fixed in Bouin's fluid for histopathological study. Blood samples were collected and serum was separated and stored at -80 □C until used to determine serum testosterone concentration.

STATISTICAL ANALYSIS

The mean value of each parameter was computed considering data on five mice in each group. Two way ANOVA was used to determine dose and duration dependent action of CYP. The mean values of each parameter of different groups were compared using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test fixing a minimum significance level of P<0.05. Wherever only two groups were compared, Student's t-test was used to compare the mean values.

Groups	**Mean organ weight (mg/100g body weight) ± SE**	
	After 6 weeks treatment (D1)	**After 12 weeks treatment (D2)**

Effect of CYP on body weight of female mice

		Ovary	Fallopian tube	Uterus	Ovary	Fallopian tube	Uterus
Control	**TR**	40.15 ± 1.58[a] -	36.44 ± 1.67[a] -	354.55 ± 30.74[a] -	45.45 ± 5.24[a] -	38.05 ± 0.60[a] -	344.53 ± 8.72[a] -
Low dose	**TR**	37.48 ± 2.26[a]	34.03 ± 1.69[a]	362.1 ± 79.87[a]	39.57 ± 0.92[a]	33.12 ± 1.67[a,b]	348.9 ± 16.31[a]
		38.54 ± 6.37[a]	34.41 ± 1.28[a]	357.83 ± 60.52[a]	40.82 ± 1.34[a]	31.47 ± 3.05[a,b]	345.2 ± 38.01[a]
Medium dose	**TR**	35.80 ± 2.27[a,b]	31.56 ± 1.45[a,b]	367.26 ± 43.50[a]	34.45 ± 3.77[b]	31.09 ± 1.87[b]	455.5 ± 16.37[b]
		36.37 ± 2.15[a,b]	33.68 ± 0.54[a]	349.68 ± 66.74[a]	39.37 ± 2.00[a]	31.8 ± 1.08[b]	360.1 ± 35.80[a]
High dose	**TR**	29.27 ± 0.93[b]	27.54 ± 1.69[b]	390.89 ± 43.50[b]	29.70 ± 0.97[b]	28.47 ± 1.56[b]	464.1 ± 90.89[b]
		30.27 ± 0.96[b]	28.92 ± 1.98[b]	358.05 ± 30.7[a]	32.80 ± 4.99[b]	30.08 ± 2.27[b]	473.3 ± 47.06[b]
ANOVA							
F value		3.313	2.948	4.401	2.25	1.796	1.38
df=(6,28)		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Note: Mean values in each column were compared by one way ANOVA followed by Duncan's multiple range test. Values with same superscript letters are not significantly different, whereas those with different superscript letters are significantly (P<0.05) different. + denotes gain in body weight, - denotes loss in body weight. Low dose= 1.38 mg/kg bw, Medium dose= 2.76 mg/kg bw, High dose= 5.52 mg/kg bw.

Effect of cypermethrin on the weight of the ovary and accessory organs of female mice

Note: Mean values in each column were compared by one way ANOVA followed by Duncan's multiple range test. Values with same superscript letters are not significantly different whereas those with different superscript letters are significantly (P<0.05) different. Mean values of treatment (T) and after cessation of treatment (R) of each organ weight at each dose were compared with the control using student's t test and

judged significant if P<0.05, df= degrees of freedom, T= Treatment group, R= Recovery group. Low dose= 1.38 mg/kg bw, Medium dose= 2.76 mg/kg bw, High dose= 5.52 mg/kg bw.

RESULT:

For convenience of description following abbreviations are used in the text,

CYP: Cypermethrin D1: 6 weeks treatment
D2: 12 weeks treatment

R1: Recovery group of 6 weeks CYP treatment. R2: Recovery group of 12 weeks CYP treatment.

CONCLUSION:

The present study investigated the reproductive toxic effect of chronic exposure of female mice to very low doses of cypermethrin (CYP), a pyrethroid, compared to earlier studies, i.e. 1.38, 2.76 and 5.52 mg/kg body weight , corresponding to 1/476th, 1/238th and 1/119th of LD_{50} dose. CYP was administered orally (intubation) to adult female mice in 3 dosages mentioned above, for two durations, viz. 6 and 12 weeks. There was a significant reduction in the weight of the body in all doses of CYP treated mice in both durations. Administration of CYP caused a significant reduction in the weight of the ovary and fallopian tube following treatment with all the doses for 12 weeks whereas, high dose affected even after 6 weeks treatment. There was a significant decrease in i) number of estrous cycles/month, ii) serum 17β-estroidal concentration and iii) total number of healthy follicles of all categories and corpora lutea per ovary whereas a significant increase was found in the percentage of atretic follicles of different categories and weight of the uterus compared to the controls. These changes were dose and duration dependent. Female mice in all CYP treated groups showed 100% fertility following 6 weeks treatment, whereas, after 12 weeks treatment fertility was 80% in low and medium dose treated mice and 60% in high dose treated mice. The histopathological changes in the ovary of CYP treated groups did not show any marked variation or abnormal appearance other than increase in the number of atretic follicles. The age at vaginal opening and preputial separation in offspring of medium and high dose treated females was significantly higher than controls and also there was a significant reduction in the mean number of estrous cycles of offspring of CYP treated mothers. One month after cessation of treatment, the body weight did not show noticeable progress. Ovary and accessory organs weight, serum 17 β-estradiol levels, estrous periodicity and number of healthy and atretic follicles in the ovary did not restore to normalcy in high dose recovery group (i.e. 12 weeks treatment group). The results indicate that, i) exposure to CYP even at very low dose levels, i.e. less than $1/100^{th}$ of LD50 dose disrupts the ovarian gametogenic and steroidogenic activities resulting in reduced fertility by its endocrine disruptive action and ii) even a dose of $1/100^{th}$ of LD_{50} of CYP can induce irreversible effects on ovary.

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