

Median lethal dose (LD₅₀) estimation of β -cyfluthrin in male and female Swiss albino mice

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Abstract- Present study was undertaken to determine the median lethal dose (LD₅₀) of β -cyfluthrin (β -cyf), a synthetic (type II) pyrethroid pesticide in male and female Swiss albino mice. The calculation of LD₅₀ is an initial screening step in the assessment and evaluation of the toxic characteristic of a chemical substance. The selected single doses of β -cyfluthrin based on the pilot study were 200, 225, 250, 275 and 300 mg/kg b.wt. for male and 350, 375, 400, 425 and 450 mg/kg b.wt. for female animals. Pesticide was dissolved in corn oil and administered orally to different groups of male and female mice, consisting of 10 animals in each group. Signs of toxicity and possible death of animals were monitored upto 96 h to calculate the median lethal dose (LD₅₀) of β -cyf. The results obtained in this study suggest that oral LD₅₀ of the β -cyf dissolved in corn oil was found to be 260.01 \pm 30.73 and 407.38 \pm 24.68 mg/kg b.wt. in male and female Swiss albino mice respectively.

Index Terms- β -cyfluthrin, acute toxicity, LD₅₀, corn oil, mice.

I. INTRODUCTION

The toxicity of chemicals can be measured in several ways. Generally human toxicity is estimated based on the test results on rats, mice and other animal models. To quantify the effect of the toxicant in the animals is to measure death rates. Acute toxicity of a chemical can be determined by the calculation of LD₅₀, i.e., the dose that will kill 50% of the animals of a particular species in a given period under controlled and standardized laboratory conditions. LD₅₀ is an initial screening step in the assessment and evaluation of the toxic characteristic of a chemical substance. This test examines the relationship between dose and the most extreme response death. In general, the smaller the LD₅₀ value, the more toxic the substance is and vice versa.

β -cyfluthrin (β -cyf) is a relatively new insecticide and classified as Type II synthetic pyrethroid as its chemical structure is a synthetic analogue of naturally occurring pyrethrins. Its chemical name is 3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxyphenyl)-methyl ester [1]. Increasing usage of synthetic pyrethroids, both in agricultural and domestic sectors, yields an increased likelihood of human exposure. Pharmacokinetic studies indicate that pyrethroids selectively partition into nervous tissue from the blood due to their high lipophilicity [2]. It then causes hypersensitivity in the nervous system, convulsions, and death [3]. The acute oral LD₅₀ values of pyrethroids range from 22 to 5000 mg/kg in rats [4]. Although median lethal dose of β -cyf is

documented in rats but satisfactory data related to LD₅₀ in mice is not available. Moreover there is a difference in LD₅₀ of β -cyf in male and female animals and also with different vehicles. Thus an attempt was made to determine the acute oral LD₅₀ of β -cyf in male and female Swiss albino mice using corn oil as vehicle.

II. MATERIALS AND METHODS

Test chemicals

β -cyf (CAS No. 68359-37-5) was purchased from Sigma-Aldrich, Germany with 99.8% purity.

Animals and experimental design

Adult male and female Swiss albino mice of proven fertility were used for this study. The mice were initially procured from the animal facilities, IVRI Izzatnagar, Bareilly (U.P.), India and a colony was established in the animal house facility of the department. The subsequent progeny of these mice was used for experimental purpose. The mice were kept in polypropylene cages measuring 12" X 10" X 8" under standard laboratory conditions of light-dark cycle (14 -10 h) and temperature (22 \pm 3°C) and were given water and standard laboratory diet *ad-libitum*. The study has the consent of the Institutional Ethical Committee (1678/GO/a/12CPCSEA).

Estimation of the dose range and percentage mortality

Mice were fasted for 18 h prior to dosing and β -cyf was dissolved in corn oil was administered once orally to the mice. An approximate LD₅₀ was initially determined by a pilot study so called "up and down" or 'staircase method' using two animals [5] and increasing the doses of β -cyf such as 50, 100, 200, 400, 800 and 1600 mg/kg. Results of this pilot study give approximate LD₅₀ were 250 mg/kg and 410 mg/kg b.wt. for male and female mice respectively.

Based on the results of pilot study we performed confirmatory experiments according to method of Miller and Tainter, 1944 [6]. Ten groups of mice (five of male and five of female mice), containing ten animals in each group were selected. For male mice five doses of β -cyf *viz.* 200, 225, 250, 275 and 300 mg/kg b.wt. were chosen. Doses of β -cyf selected for female mice were 350, 375, 400, 425 and 450 mg/kg b.wt. Simultaneously control animals were also given corn oil by the same route of administration. Then mortality from 0% to 100% was calculated [7]. The animals were observed for 2 h and then at 4, 6, 24, 48, 72 and 96 h for any toxic signs and symptoms. After 96 h, the number of deceased mice in each group was counted,

and percentage of mortality was calculated using the graphical method given by Miller and Tainter [6].

III. RESULTS

Toxicity symptoms

Initially, at 50 mg/kg dose of β -cyf did not produce any significant toxic effects. However, at 800 and 1600 mg/kg, animal showed signs of toxicity. Common symptoms of pyrethroid poisoning such as burying the head in saw dust, abnormal gait, dullness, lethargy and hair shedding were exhibited by the treated animals of both sexes.

Conversion of percentage mortalities to probits and calculation of LD₅₀

The number of deceased mice of both sexes was recorded at each dose level of β -cyf (Table 1 and 2). The percentage of animals that died at each dose was then transformed to probit using Finney's method (Table 3).

Table-1 Results of the lethal doses of β -cyf for the determination of LD₅₀ after oral intubation in male Swiss albino mice (n=10).

Group	Dose (mg/kg)	Log Dose	% Dead	Probits
1	200	2.30	20	4.16
2	225	2.35	30	4.48
3	250	2.39	40	4.75
4	275	2.43	50	5.00
5	300	2.47	70	5.52

In the present case, for male mice Log LD₅₀ is 2.415 (Fig. 1) and calculated LD₅₀ is 260.01 mg/kg b.wt. and for female mice Log LD₅₀ is 2.61 (Fig. 2) and LD₅₀ is 407.38 mg/kg were obtained. The SE of the LD₅₀ was calculated using the following formula [8].

$$\text{Approx. SE of LD}_{50} = \frac{(\text{Log LD}_{84} - \text{Log LD}_{16})}{\sqrt{2N}} \dots(1)$$

Table-2 Results of the lethal doses of β -cyf for the determination of LD₅₀ after oral intubation in female Swiss albino mice (n=10).

Group	Dose (mg/kg)	Log Dose	% Dead	Probits
1	350	2.54	20	4.16
2	375	2.57	30	4.48
3	400	2.60	40	4.75
4	425	2.62	60	5.25
5	450	2.65	70	5.52

The Probits of 84 and 16 from Table 3 are 5.99 and 4.01 (approximately 6 and 4), respectively and N is the number of animals in each group is 10. In male mice the log LD values for the probits 6 and 4 are obtained from the line on the graph in Fig. 1, which, in the present case are 2.51 and 2.27 and their antilog are 323.59 and 186.20. In case of female mice they are 2.66 and 2.54 and their antilog are 457.08 and 346.73. Applying these values in formula (1), The SE of LD₅₀ is 30.73 and 24.68 for male and female mice respectively. Therefore, LD₅₀ of β -cyf in corn oil for male mice is 260.01±30.73 and 407.38±24.68 when given orally, with 95% confidence interval.

Table-3 Transformation of percentage mortalities to probit.

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

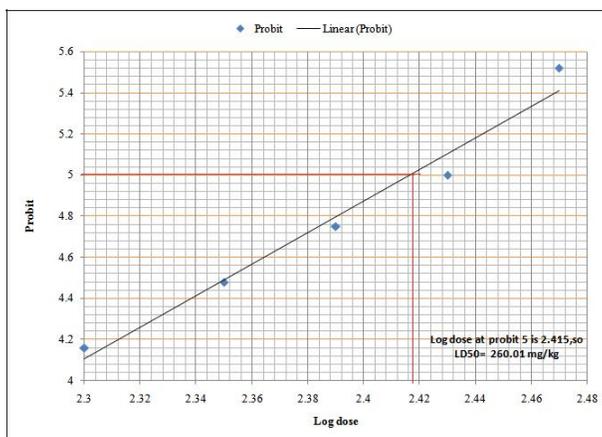


Figure 1: Graph showing Log doses versus probit from Table 3 for calculation of oral median lethal dose of β -cyf in male Swiss albino mice.

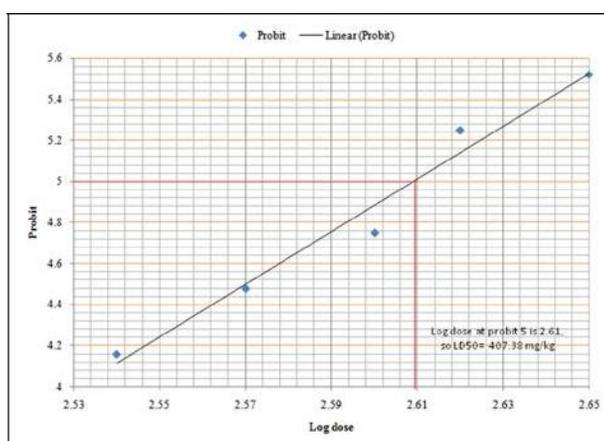


Figure 2: Graph showing Log doses versus probit from Table 3 for calculation of oral median lethal dose of β -cyf in female Swiss albino mice.

IV. DISCUSSION

Extensive use of pesticides in agriculture creates risk of environmental pollution as well as the life of the common man [9]. In this chemical era it is very important to know the LD_{50} of the pesticide before using it in the fields as excessive dose of pesticide may cause acute toxicity. In the present study, we investigated the acute toxicity of single oral dose of β -cyf, a synthetic pyrethroid in male and female mice as a part to determine median lethal dose. β -cyf was dissolved in corn oil and administered to male and female Swiss albino mice at different dose levels according to the recommendation of OECD/OCED Guidelines (2001) [10]. Common symptoms of pyrethroid poisoning such burying the head in saw dust, abnormal gait, dullness, lethargy and hair shedding were exhibited by both sexes of the treated animals at the dose levels of 800 and 1600 mg/kg b.wt. Single dose of β -cyf at 50 mg/kg did not show any toxic signs or behavioral alterations, hence it is considered as No Observed Adverse Effect Level (NOAEL). The results of this study suggest that oral LD_{50} of the β -cyf was found to be 260.01 ± 30.73 and 407.38 ± 24.68 mg/kg b.wt. in male and female Swiss albino mice respectively.

The acute oral toxicity of β -cyf is highly variable dependent on factors such as vehicle used and test animal sex. According to

EMEA reports (2000) oral LD_{50} values in male rats for cyfluthrin in polyethylene glycol, corn oil and cremophor were 600, 250 and 15 to 20 mg/kg b.wt. respectively [11]. In female rats LD_{50} value was approximately 1200 mg/kg b.wt. in polyethylene glycol. In mice oral LD_{50} of cyfluthrin in polyethylene glycol was reported 291 mg/kg b.wt. for male and 609 mg/kg b.wt. for female [11].

LD_{50} of β -cyf in water was reported in male rats after 96 h treatment to be 354.8 mg/kg b.wt. by Singh et al. (2009). They also revealed that exposure of rats to β -cyf caused extensive changes in neurosomatic, neurochemical and neurobehavioural parameters [12]. Formulated β -cyf shows wide divergence in toxicity among bird species and estimated median lethal dose were 170, 2234, 2271 and 500 mg/kg b.wt. for canaries, shiny cowbirds, eared doves [13] and quail [14] respectively.

However, published experimental work on β -cyf toxicity in mice is limited specially with corn oil as a vehicle. Moreover, we found a difference in median lethal dose of β -cyf in male and female mice, therefore it is essential to determine LD_{50} in both sexes separately. However, it is concluded that, the overall result of the present study clearly demonstrates that oral LD_{50} of the β -cyf using vehicle corn oil is as 260.01 ± 30.73 and 407.38 ± 24.68 mg/kg b.wt. in male and female Swiss albino mice respectively.

V. CONCLUSION

Determination of median lethal dose is essential for toxicologist to correlate and to identify a chemical substance. This can be useful to measure the acute toxicity of drugs, food poisonings and accidental domestic poisoning cases. The information gained from dose response studies in animals is used to set standards for human exposure. The present study demonstrated for the first time the LD₅₀ of β -cyf using corn oil as a vehicle in both sexes of Swiss albino mice. No specific guidelines are available for toxicological analysis in pyrethroid poisoning cases. Hence understanding of median LD₅₀ and toxicity is very important for the better evaluation of the toxic characteristic of β -cyf and also to measure its short term poisoning potential.

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CONFLICT OF INTEREST

None declared.

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