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Research Article

# Estimation of Median Lethal Dose of Cypermethrin and *Beta-cyfluthrin*

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**ABSTRACT**

Present study was aimed to evaluate the median lethal dose (LD50) of two broadly used, type II pyrethroid pesticides Cypermethrin and *Beta-cyfluthrin* against Wistar albino rats. The albino rats corresponding to experimental sets were orally administrated different doses of selected pyrethroids for estimation of median lethal dose. LD50 has been calculated by Log-dose/probit regression line method, and came out to be 416.98 and 354.8 mg/Kg b.wt. for Cypermethrin and *Beta-cyfluthrin* respectively. Difference in the median lethal of these compounds may be a consequence of structural differences.

**Keywords**: Pyrethroids, median lethal dose, albino rats.

## INTRODUCTION

There has been a great concern throughout the world regarding the non-target toxicity evaluations of pesticides[1-4]. Pyrethroids represent about 30% of the total pesticide market by virtue of their broad spectrum specificity and comparatively safer consideration. However, their greater use also carries more chances of contaminating various ecosystems and residents therein[5-8].

Synthetic pyrethroids are modified structures, having originated from basic pyrethrins contained in the flowers of genus *Chrysanthemums*. Major modification is the absence and presence of an α-cyano group to the basic structure, by virtue of which all the pyrethroids available are broadly divided into type I and II respectively. Further among the type II pyrethroids, modifications both in the form of structural changes as well as additions of one or more halogenated groups has given rise to variety of compounds available in the market having enhanced potency to counteract genetically modified pest species and resistance development[9-12]. Activity modulation of pesticides on the basis of structural changes is the demand of the day, but it is also important on the other hand to have an idea of the toxic potential and non-target toxicity of the coming pyrethroids. It is with this reason the $LD_{50}$ evaluation of two type II pyrethroids, Cypermethrin and *Beta-cyfluthrin* (Fig. 1-2) have been condidered in the present study.

## MATERIAL AND METHODS

*Experimental animal*

Present study was conducted on seventy female albino rats, *Rattus norvegicus*, weighing 110 ± 20 gm. These albino rats were of eight weeks age, selected from an inbred colony and provided standard rat pellet feed and water *ad libitum*. The rats were divided into two main sets each of which was further sub-divided in five sub-sets comprised of seven rats corresponding to different doses of Cypermethrin and *Beta-cyfluthrin* respectively. The experimentation was approved by the Ethical committee of Dr. B. R. Ambedkar University, Agra, India.

*Experimental compounds*

Technical grade of Cypermethrin and *Beta-cyfluthrin* (purity 95%) were obtained from Bayer India Ltd., Mumbai and their $LD_{50}$ was calculated[13].

*Dose administration and determination of $LD_{50}$:*

*Cypermethrin*

Experimental albino rats were divided into five groups, each consisting of seven individuals. Standard solution of experimental test compound cypermethrin was prepared by dissolving in distilled water. Different doses 200, 400, 600 and 800 and 1000 mg/kg b.wt. were administrated orally. The mortality and survival number of rats were recorded for each dose after 96 hours. The data was analyzed statistically by log dose/probit regression line method[13]. Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for $LD_{50}$ determination (Table 1).

*Beta–Cyfluthrin*

Experimental albino rats were divided into five groups, each consisting of seven individuals. Standard solution of experimental test compound *Beta-cyfluthrin* was prepared by dissolving in distilled water. Different doses 100, 200, 400, 800 and 1600 mg/kg b.wt. were administrated orally. The mortality and survival number of rats were recorded for each dose after 96 hours. The data was analyzed statistically by log dose / probit regression line method[13]. Regression line was drawn on the basis of two variables, log dose and empirical probit on a simple graph paper and

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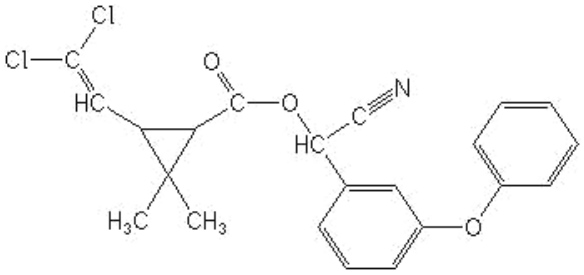

Fig. 1: Structure of Cypermethrin

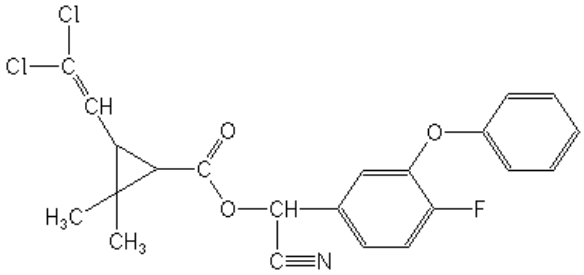

Fig. 2: Structure of *Beta-cyfluthrin*

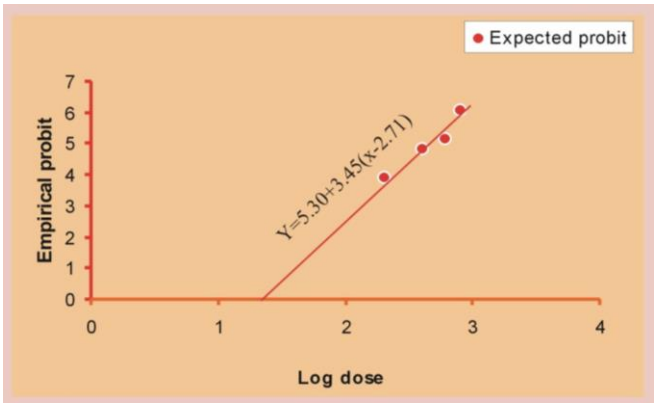


Figure 3: Regression line for calculating $LD_{50}$ of Cypermethrin

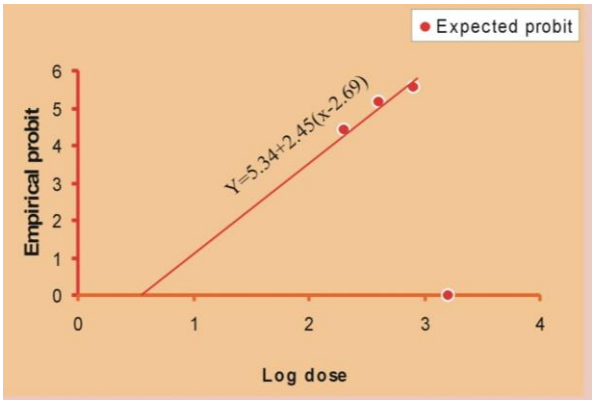


Figure 4: Regression line for calculating $LD_{50}$ of *Beta-cyfluthrin*

Table 1: Percentage survival of Rattus norvegicus after Cypermethrin and *Beta-cyfluthrin* intoxication

| S. No. | No. of rats in each set | Exposure Duration (in hours) | Dose (mg/kg b.wt.) | | Survival number | | Survival percentage | |
|---|---|---|---|---|---|---|---|---|
| | | | Cyper-methrin | *Beta-cyfluthrin* | Cyper-methrin | *Beta-cyfluthrin* | Cyper-methrin | *Beta-cyfluthrin* |
| 1. | 7 | 96 | 200 | 100 | 6 | 7 | 85.71 | 100 |
| 2. | 7 | 96 | 400 | 200 | 4 | 5 | 57.14 | 71.42 |
| 3. | 7 | 96 | 600 | 400 | 3 | 3 | 42.86 | 42.85 |
| 4. | 7 | 96 | 800 | 800 | 1 | 2 | 14.29 | 28.57 |
| 5. | 7 | 96 | 1000 | 1600 | 0 | 0 | 0 | 0 |

used to determine the expected probit necessary for $LD_{50}$ determination (Table 1).

*Statistical analysis*

Results so obtained were statistically analysed using log-dose/probit regression analysis[13].In the different experimental sets, experimental rats were treated with different doses of different concentrations of Cypermethrin and *Beta-cyfluthrin* for estimation of $LD_{50}$. Different doses of experimental pyrethroids have been selected and survival number and survival percentage for each dose have been noted after 96 hours (Table I). The survival percentage decreased with increasing dose of Cypermethrin and *Beta-cyfluthrin*. $LD_{50}$ has been calculated (Tables-II-III, Fig. 3-4) by log dose/probit regression line method. The test doses were then converted to their logarithm. The empirical probit values, equivalent to percentage mortality were then obtained from the table and plotted against log dose on graph paper. The provisional lines was drawn fitting to the expected values were read for the values of log dose (X).

Working probits

$Y = Y0 + kp$

Where, p = percentage mortality

Y0 and k are two factors.

The weighting coefficients for each point were also obtained from the table. The weight has been calculated by multiplying each coefficient by number of rats used (Table II-III). The value of 'b' is obtained by the formula

$$b = \left(\sum wxy - \overline{X}\sum wy\right)/\left(\sum wx^2 - \overline{X}\sum wx\right)$$

The values of Y can be obtained by regression equation (Fig.3-4)

$$Y = \overline{Y} + b(X - \overline{X})$$

From the equation values of X, equivalent to Y and $\overline{Y}$ were estimated, and the calculated values of $LD_{50}$ thus been obtained (Tables-II-III). Variance V was calculated followed by 95% confidence fiducial limits as below:

Table 2: Determination of $LD_{50}$ by log-dose/probit Regression analysis after oral intoxication of different doses of Cypermethrin to *Rattus norvegicus*

| S.No. | Dose in mg/kg b.wt. | No. of rats 'n' | Mortality Number | Mortality (%) | Log dose 'X' | Empirical Probit | Expected Probit 'Y' | Working Probit 'y' | Weighing coefficient 'N' | Weight W=n×N | WX | Wy | Wxy | $WX^2$ | $Wy^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 200 | 7 | 1 | 14.29 | 2.30 | 3.93 | 3.89 | 3.92 | 0.405 | 2.84 | 6.53 | 11.13 | 25.61 | 15.02 | *43.64* |
| 2. | 400 | 7 | 3 | 42.86 | 2.60 | 4.82 | 4.84 | 4.82 | 0.627 | 4.39 | 11.41 | 21.16 | 55.02 | 29.68 | *101.99* |
| 3. | 600 | 7 | 4 | 57.14 | 2.78 | 5.18 | 5.43 | 5.17 | 0.601 | 4.21 | 11.70 | 21.77 | 60.51 | 32.54 | *112.53* |
| 4. | 800 | 7 | 6 | 85.71 | 2.90 | 6.07 | 5.84 | 6.05 | 0.503 | 3.52 | 10.21 | 21.30 | 61.76 | 29.60 | *128.84* |
| 5. | 1000 | 7 | 7 | 100.00 | 3.00 | +∞ | 6.18 | 6.79 | 0.370 | 2.59 | 7.77 | 17.59 | 52.76 | 23.31 | *119.41* |
| | | | | | | | | | | *ΣW=* | *ΣWX=* | *ΣWy=* | *ΣWX²=* | *ΣWX²=* | *ΣWy²=* |
| | | | | | | | | | | *17.55* | *47.62* | *92.95* | *255.66* | *130.15* | *506.41* |

$$\text{Variance (V)} = \frac{1}{b^2}\left(\frac{1}{\sum w} + \frac{(X-\overline{X})^2}{\Sigma wx^2 - \frac{(\sum wx)^2}{\sum w}}\right)$$

By the following formula 95% confidence fiducial limits have been obtained (Table IV).

m1 = m + 196 V

m2 = m-1.696 V

## RESULTS

Applying all the calculation, $LD_{50}$ for Cypermethrin and Beta cyfluthrin came out to be 416.98 and 354.8 mg/Kg b.wt. respectively.

## DISCUSSION

Farmers all over the world with particular reference to agriculturally economised countries face a serious problem of pests who wither the annual crop yield. However, pesticides have helped the farmers to minimize this loss and simultaneously are helpful in eradication of vector borne diseases as well as pests of household. Pesticides therefore are greatly in but their widespread and excessive continuous use indicates great risk of misapplication and accidental exposure and recent non-target toxicological evaluations have revealed heart wrenching story[3, 14-17].

Cypermethrin and *Beta-cyfluthrin*, both type II pyrethroids, being commonly and extensively used and therefore reflect risk to non-target species[8].

Determination of median lethal dose is essential to determine the toxic potential of a compound and comparison in turn is very important for the better evaluation of the toxic characteristic. Hence, there are limited guidelines for toxicological analysis in pyrethroid

poisoning cases; evaluation of $LD_{50}$ becomes essential for better evaluation of toxic characteristic of pyrethroids. This evaluation also assists in measurement of acute toxicity, through food poisonings as well as accidental domestic poisonings cases of pyrethroids in subject[18-20].

*Beta-cyfluthrin* has been found to be more toxic than Cypermethin in the present study based on $LD_{50}$ evaluation. This difference can be attributed to the structural differences in their chemistry where *Beta-cyfluthrin* has been designed by introduction of fluorine group to position 4 of phenyl ring in Cypermethrin. The fluorine atom shares common characteristics with that of hydroxyl ion, alongwith has strong electronegativity, resulting in strong bond with carbon atom. The C-F bond is one of the strongest bonds known and in case of *Beta-cyfluthrin*, resonance effect provides additional strength to this bond. By virtue of these factors this compound is capable of altering normal cellular as well as body functions, which might have been a reason for enhanced toxicity of *Beta-cyfluthrin* in comparison to Cypermethrin in the present study[3,8, 21-23].

Table 3: Determination of $LD_{50}$ by log-dose/probit Regression analysis after oral intoxication of different doses of Beta-cyfluthrin to *Rattus norvegicus*

| S.No. | Dose in mg/kg b.wt. | No. of rats 'n' | Mortality Number | Mortality (%) | Log dose 'X' | Empirical Probit | Expected Probit 'Y' | Working Probit | Weighing coefficient 'N' | Weight W=n×N | WX | Wy | Wxy | $WX^2$ | $Wy^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 100 | 7 | 0 | 0 | 2.0 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 2. | 200 | 7 | 2 | 28.57 | 2.30 | 4.45 | 3.92 | 4.45 | 0.601 | 4.20 | 9.67 | 18.72 | 43.07 | 22.25 | *83.38* |
| 3. | 400 | 7 | 4 | 57.14 | 2.60 | 5.18 | 4.47 | 5.17 | 0.634 | 4.43 | 11.53 | 22.97 | 59.74 | 30.00 | *118.99* |
| 4. | 800 | 7 | 5 | 71.42 | 2.90 | 5.55 | 5.02 | 5.55 | 0.581 | 4.06 | 11.79 | 22.58 | 65.46 | 34.20 | *125.41* |
| 5. | 1600 | 7 | 7 | 100.00 | 3.20 | 0.0 | 5.57 | 6.94 | 0.104 | 1.07 | 3.44 | 7.48 | 23.93 | 11.03 | *1.90* |
| | | | | | | | | | | *ΣW= 14.52* | *ΣWX= 39.06* | *ΣWy= 77.75* | *ΣWxy= 211.99* | *$ΣWX^2$ = 97.48* | *$ΣWy^2$= 379.67* |

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Table 4: Toxicity evaluation of Cypermethrin and *Beta-cyfluthrin* for *Rattus norvegicus* specifying fiducial limits

| Experimental compound | Regression Equation | $LD_{50}$ (in mg/kg b.wt.) | Variance | Fiducial limits |
|---|---|---|---|---|
| Cypermethrin | Y=5.30+3.45(x-2.71) | 416.98 | 0.006 | $m_1$= (+)2.63<br>$m_2$= (-)2.61 |
| *Beta-cyfluthrin* | *Y=5.34+2.45(x-2.69)* | *354.8* | *0.01* | $m_1$= (+)2.7096<br>$m_2$= (-)2.6704 |


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