International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 9, Issue 3, pp: 6-10, 2024
https://doi.org/10.55126/ijzab.2024.v09.i03.002 **http://www.ijzab.com**

Research Article

MEDIAN LETHAL DOSE (LD_{50}) ESTIMATION OF CYPERMETHRIN AND PROFENOFOS IN SWISS ALBINO MICE (*MUS MUSCULUS)*

[1]Md Sarfaraz Nawaz, [1]Asif Iqbal and [2]Md Equbal Ahmad

[1]P.G Department of Biotechnology, TM Bhagalpur University, Bhagalpur
[2]University Department of Zoology, TM Bhagalpur University Bhagalpur

Article History: Received 17th February 2024; Accepted 21st April 2024; Published 1st May 2024

ABSTRACT

Present study was undertaken to determine the Oral median lethal dose (LD_{50}) of Cypermethrin, a pyrethroid and Profenofos, an organophosphate pesticide in male and female Swiss albino mice. The selected doses of Cypermethrin based on the pilot study were 200, 225, 250, 275 and 300 mg/kg B.W. for male and 350, 375, 400, 425 and 450 mg/kg B.W for female animals. Corresponding doses of Profenofos based on the pilot study was found to be 10, 15, 20, 25 and 30 mg/kg B.W for male and 20, 25, 30, 35 and 40mg/kg B.W for female. Pesticide was dissolved in corn oil and administered orally to different groups of male and female mice, consisting of 10 animals in each group. Possible death of animals was monitored up to 96 h to calculate the median lethal dose (LD_{50}) of CYP and PFF. The results obtained in this study suggest that oral LD_{50} of the CYP and PFF dissolved in corn oil was found to be (249.06 ±30.75, 406.38 ± 64.28)and (24.26 ±6.43, 39.67 ±6.2) mg/kg B.W. respectively in male and female Swiss albino mice.

Keywords: Cypermethrin, Profenofos, Acute toxicity, Oral LD50, Swiss albino mice, Probit analysis.

INTRODUCTION

Cypermethrin (CYP) and Profenofos (PFF) are agricultural pesticides that have been studied for their potential toxicity to humans and the environment. CYP is a pyrethroid insecticide that induces a persistent opening of sodium ion channels in nerve membranes, causing a continuous generation of impulses in neurons (Kirby *et al*., 1999) while PFF, an organophosphate predominantly targets the central nervous system, disrupting the normal functioning of cholinergic pathways by inhibiting the acetylcholinesterase (AChE) enzyme in the nervous synapse, which serves as the primary mechanism underlying organophosphate-induced acute toxicity (Hamadain, 2001). These sustained effects can eventually result in the death of an organism, as reported by the World Health Organization (1989). The Acute toxicity of a pesticide is often evaluated by calculating the LD_{50}, which represents the dose required to kill half of the group of experimental animals in a defined timeframe under controlled laboratory conditions. The LD_{50} comes handy as an initial screening tool in assessing and understanding the toxic characteristics of a chemical substance. This test explores the relationship between the administered dose and death. Although the median lethal dose of CYP and PFF has been documented in rats, information regarding the LD_{50} in Swiss albino mice (*Mus musculus*) is lacking. Furthermore, variations in LD_{50} values are observed based on sex of animals and vehicles used. Consequently, an effort has been made to determine the acute oral LD_{50} of CYP and PFF in male and female Swiss albino mice, utilizing corn oil as the vehicle in this investigation.

MATERIALS AND METHODS

Test Chemicals

Cypermethrin (CYP) (CAS No. 52315-07-8) and Profenofos (PFF) (CAS No.41198-08-7) was purchased from Sigma Aldrich, Germany with 99.8% and 95.4 % purity respectively.

Animals and experimental design

This study employed adult male and female Swiss albino mice with confirmed fertility. Initially sourced from animal

*Corresponding Author: Dr. Md Equbal Ahmad, University Professor, University Department of Zoology, TM Bhagalpur University Bhagalpur Email: sarfaraz.ramjas@gmail.com

facilities in India, a colony was established within the animal house facility of the department. Subsequent generations of these mice were utilized for experimental purposes. The mice were housed in polypropylene cages measuring 10" × 8" × 12" within colony rooms, maintaining a 12-hour light/dark cycle at a temperature of 22 ± 2 ℃ with humidity levels approximately ranging from 50%-55%. They had unrestricted access to water and a standard laboratory diet. Each mouse was fed at precise 10.00 am daily to avoid any variation due to circadian rhythm.Thestudy had received approval from the Institutional Animal Ethical Committee (IAEC), TM Bhagalpur University, Bhagalpur, ensuring compliance with OECD guidelines for acute toxicity in rodents.

Estimation of the dose range and percentage mortality

Prior to dosing, mice underwent a 12-hour fasting period, after which Cypermethrin (CYP), dissolved in corn oil, was administered once orally via oral gavage. To determine an approximate LD_{50}, a pilot study employing the "up and down" or 'staircase method' was conducted. (OECD, 2001). This involved using two animals and progressively increasing the doses of CYP (50, 100, 200, 400, 800, and 1600 mg/kg B.W) and PFF (10, 20, 40, 80 and 160 mg/kg B.W). The results of the pilot study indicated approximate LD_{50} values of CYP (250 mg/kg and 400 mg/kg body weight for male and female mice, respectively) and PFF(25 mg/kg and 40 mg/kg body weight for male and female mice, respectively) (Williams *et al*., 1984). The confirmatory experiments were conducted following the methodology of Miller and Tainter, 1944 taking cue from Finney's (1947) statistical data (Table 5) (Merkel, 2004). Five groups of mice were selected, consisting of six male and six female mice in each group, totalling 12 animals per group. For male mice, doses of CYP and PFF were set at CYP_M (200, 225, 250, 275, and 300 mg/kg body weight) and PFF_M (15,20,25,30 and 35 mg/kg B.W) respectively; while doses for female mice of CYP and PFF were set at CYP_F (350, 375, 400, 425, and 450 mg/kg B.W) and PFF_F (30,35,40,45 and 45 mg/kg B.W) respectively (Durondo, 2005). Mortality if any in each group of mice was recorded after 96 hours and mortality rates were calculated accordingly. Probit value for each mortality rates was recorded via Table (Finney, 1947)

Statistical analysis

The data were analysed for significancy by using Statistical 8.0 and SPSS software. ANOVA and the t-test were used to determine significant differences among groups. The results were expressed as mean ± standard error (SE). Values of $p < 0.05$ were considered significant (Figure 1-4).

RESULTS AND DISCUSSION

The empirical Probit values, corresponding to percentage mortality rates were recorded from Table 5 (Finney, 1947). A regression line graph was drawn between log of dose and probit values for each pesticide i.e. CYP and PFF. LD_{50} was calculated by Log dose/ Probit regression line method by Miller and Tainter, 1944 (Randhawa, 2009). The values were recorded taking into consideration standard error for the respective group. Standard error of LD_{50} was calculated by using following formula (Ghosh MN, 1984).

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

Where N = no of animals in each group

Table 1. Mortality Table for different dosages of CYP and PFF in Male Swiss albino mice.

S. No.	Dose per day (mg/kg B.W)		Log of Dose		No of Mice treated (Male)		Exposure time (in hours)	Mortality in Numbers (Male)		Mortality percentage %		Probits	
	CYP_M	PFF_M	CYP	PFF	CYP	PFF		CYP	PFF	CYP	PFF	CYP	PFF
1	0	0	0	0	0	0	96	0	0	0	0	0	0
1	200	15	2.477	1.477	6	6	96	1	1	15.15	15.15	3.96	3.96
2	225	20	2.544	1.544	6	6	96	2	2	33.33	33.33	4.56	4.56
3	250	25	2.602	1.602	6	6	96	3	3	50	50	5	5
4	275	30	2.653	1.653	6	6	96	4	4	66.66	50	5.41	5.41
5	300	35	2.698	1.698	6	6	96	5	5	83.33	83.33	5.92	5.92

Table 2. Mortality Table for different dosages of CYP and PFF in Female Swiss albino mice.

S. No.	Dose per day (mg/kg B.W)		LOG of DOSE		No of Mice treated (Male)		Exposure time (in hours)	Mortality in Numbers (Male)		Mortality percentage %		Probits	
	CYP_F	PFF_F	CYP	PFF	CYP	PFF		CYP	PFF	CYP	PFF	CYP	PFF
1	0	0	0	0	0	0	96	0	0	0	0	0	0
2	300	30	2.477	1.477	6	6	96	1	1	15.15	15.15	3.96	3.96
3	350	35	2.544	1.544	6	6	96	2	2	33.33	33.33	4.56	4.56
4	400	40	2.602	1.602	6	6	96	2	3	33.33	50	4.56	5
5	450	45	2.653	1.653	6	6	96	4	3	66.66	66.66	5.41	5.41
6	500	50	2.698	1.698	6	6	96	5	5	83.33	83.33	5.92	5.92

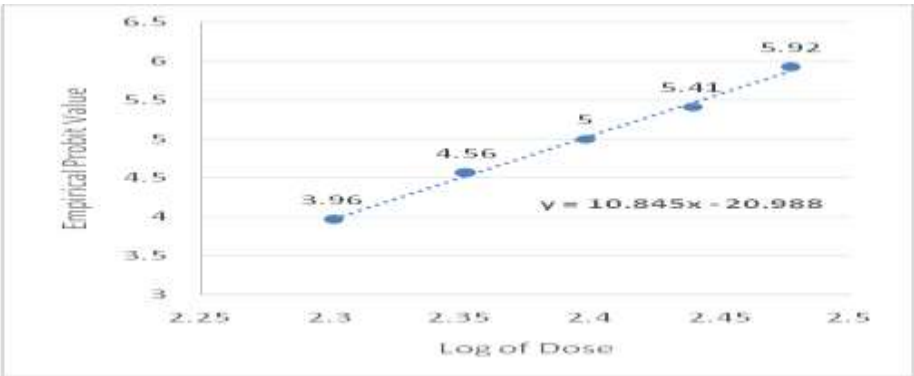

Figure 1. Regression line to determine CYP LD_{50} of Male Swiss albino mice.

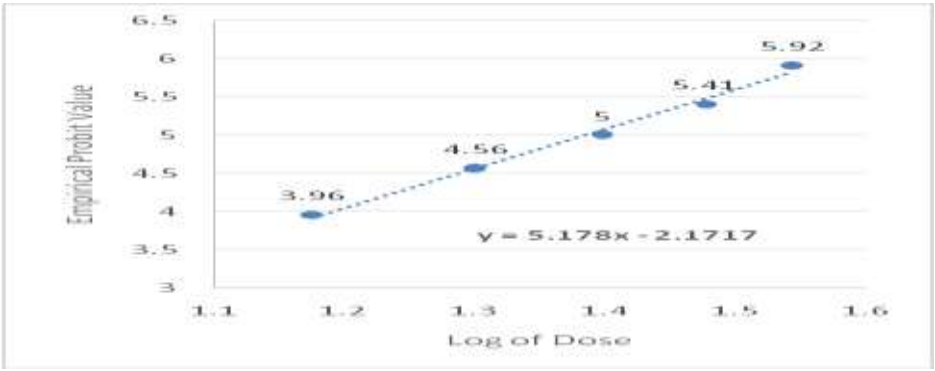

Figure 2. Regression line to determine PFF LD_{50} of Male Swiss albino mice.

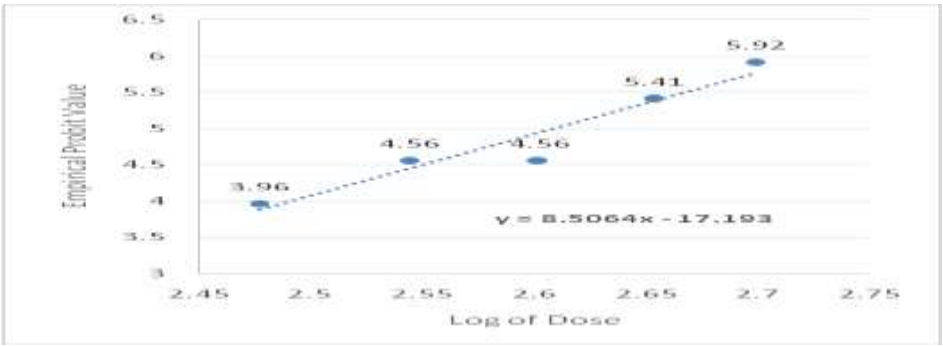

Figure 3. Regression line to determine CYP LD_{50} of Female Swiss albino mice.

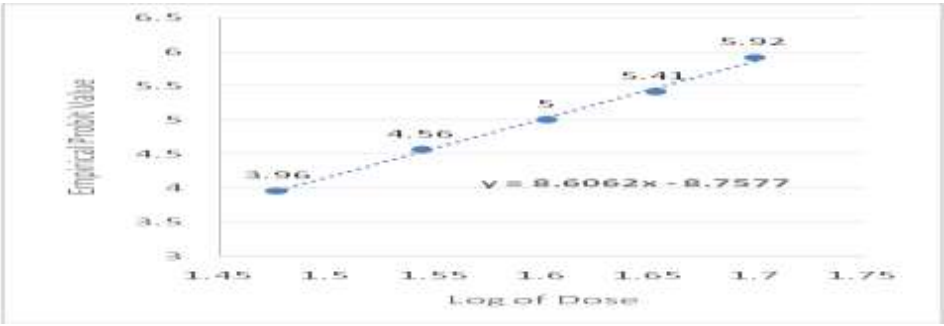

Figure 4. Regression line to determine PFF LD_{50} of Female Swiss albino mice.

The median lethal dose was calculated by taking the value of y = 5, since it represents the probit value at 50 % mortality. LD_{50} for each pesticide is calculated for male and female mice and recorded in the Table 4.

Table 3. Median Lethal Dose (LD_{50}) calculation using regression equation.

S. No	Experimental Animal	Experimental Pesticide	Regression equation	Value of x	Median Lethal Dose (LD_{50}) (Antilog of x)
1	Swiss Albino Mice Male	CYP	5 = 10.845x - 20.988	2.396312	249.06
2	Swiss Albino Mice Female	CYP	5 = 8.5064x - 17.193	2.6089768	406.38
3	Swiss Albino Mice Male	PFF	5 = 5.178x - 2.1717	1.3850328	24.26
4	Swiss Albino Mice Female	PFF	5 = 8.6062x - 8.7577	1.59858009	39.67

For similar calculation the values of y in the regression equation for separate pesticides were changed accordingly i.e. for calculating LD_{84} and LD_{16} Y was taken to be 6 and 4 respectively.

Table 4. Probit value of 84 and 16 was found to be approx. 6 and 4 respectively from Table 5 (y = 6 and 4respectively) Corresponding regression equation as mentioned in Table 3 were used for respective Pesticide.

S. No.	Experimental Animal	Experimental Pesticide	Value of x (LD_{84})	Antilog x	Value of x (LD_{16})	Antilog x	Standard error SE
1	Male mice	CYP	2.4885	307.96	2.3041	201.42	30.7554488
2	Female mice	CYP	2.7265	532.72	2.4914	310.03	64.2850657
3	Male mice	PFF	1.5781	37.85	1.1919	15.56	6.43456875
4	Female mice	PFF	1.7147	51.84	1.4823	30.36	6.20074189

Table 5. Chart to transform Mortality Rate into probit value. Finney (1947).

% Mortality rate	**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

Table 6. Calculated oral LD_{50} for CYP and PFF in Swiss Albino mice.

S. No.	Experimental Animal	Experimental Pesticide	$LD_{50} \pm SE$
1	Male mice	CYP	249.06 ± 30.755
2	Female mice	CYP	406.38 ± 64.28
3	Male mice	PFF	24.26 ± 6.43
4	Female mice	PFF	39.67 ± 6.2

The acute oral toxicity of CYP and PFF varies significantly based on factors like the vehicle used and the sex of the test animals. In both mammals and insects, pyrethroids have been found to be more toxic at lower temperatures compared to higher temperatures (Merkel, 2004). CYP causes motor deficits by prolonging the opening of voltage-gated sodium channels. This leads to hypo-polarization and hyper-excitation of neurons, contributing to impaired motor function (Ray, 2001). Based on EMEA reports from 2000, the oral LD_{50} values of CYP (a pyrethroid) in male rats were 600 mg/kg B.W. in polyethylene glycol, 250 mg/kg B.W. in corn oil, and 15 to 20 mg/kg B.W. in cremophor. For female rats, the LD_{50} value was approximately 1200 mg/kg B.W. in polyethylene glycol. Additionally, the LD_{50} of β-cyf in water was reported to be 354.8 mg/kg B.W. in male rats after 96 hours of treatment by Singh *et al*. (2009).

Insecticides like PFF, which belong to the organophosphorus category, cause phosphorylation (a chemical reaction involving the addition of a phosphate group) of several enzymes and proteins in the body (Table 6). However, the main reason for toxicity is believed to be the inhibition of the synaptic enzyme called acetylcholinesterase. The oral LD_{50} for MAG mice (male and female) was calculated to be in the range of 268–332 mg/kg B. W (Bathe, 1974).The oral LD_{50} for Russian rabbits (male and female) was calculated to be approx. 700 mg/kg BW (Sachsse, 1974).

CONCLUSION

The present study demonstrated for the 1st time oral LD_{50} via Miller and Tainter Method using Finney's data. (Table 5). Oral LD_{50} for Swiss albino mice was found to be different for both male and female (Table 6) for different pesticides. Thus doses for further experimentation with the pesticides CYP and PFF should be assessed keeping in mind the result of this experiment.

ACKNOWLEDGMENT

The authors express gratitude to the Course-Coordinator of the P.G Department of Biotechnology and the Head of the University Department of Zoology, T.M. Bhagalpur University for generously providing the necessary facilities that made this investigation possible.

REFERENCES

A.K. Singh, P.N. Saxena and Sharma HN(2009). Stress induced by beta-cyfluthrin, a type-2 pyrethroid, on brain biochemistry of Albino rat (Rattus norvegicus). *Biology and Medicine*, 1(2), 74-86.

Bathe, R. (1974) Acute oral LD_{50} of technical CGA 15324 in the rat. Unpublished report No. Siss 3647 from Ciba-Geigy Ltd, Basel, Switzerland. Submitted to WHO by Syngenta Crop Protection, Greensboro, North Carolina, USA.

Durando, J. (2005) Profenofos technical: acute oral toxicity up and down procedure in rats. Unpublished report No. T018339-04 from Product Safety Laboratories, Dayton, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection, Greensboro, North Carolina, USA.

EMEA, European agency for the evaluation of medicinal products, (2000)Veterinary medicines and information technology:EMEA/MRL/746/100-final, July. Committee for veterinary medicinal products, cyfluthrin, summary report (2).http://www.eudra.org/emea.html.

Finney, D. J. (1947). Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. London: Cambridge University Press.

Finney, D. J. (1947). The principles of biological assay. J. Roy. Statistical Society. Suppl. 9, 46-91.

Hamadain E. I. and Chambers H. W. (2001), Susceptibility and mechanismsunderlying the relative tolerance to five organophosphorousinsecticides in tobacco budworms and corn earworms. *Postaxial Biochemistry Physiology*, 69, 35-47.

Kirby, M.L., Castagnoli, K., Bloomquist, J.R. (1999). *In vivo* effects of deltamethrinon dopamine neurochemistry and the role of augmented neurotransmitterrelease. *Postaxial Biochemistry Physiology*. 65(3), 160-168. Doi:10.1006/pest..2440

L.C. Miller and M.L. Tainter (1944), "Estimation of LD_{50} and its error by means of log-probit graph paper", Proc Soc Exp Bio Med,;57:261.

M.A. Randhawa (2009), "Calculation of LD_{50}values from the method of Miller and Tainter", 1944, J Ayub Med Coll Abbottabad, , 21(3):184-185.

M.N. Ghosh. (1984) In Statistical Analysis, Fundamentals of Experimental Pharmacology, 2nd ed, Scientific Book Agency Calcutta,. Pp187–9.

Merkel, D.J. (2004) Acute oral toxicity up and down procedure in rats with profenofos technical (CGA15324). Unpublished report No. T013184-04 from Product Safety Laboratories, Dayton, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection, Greensboro, North Carolina, USA

OECD/OCDE (2001), 425: OECD Guideline for Testing of Chemicals. Acute Oral Toxicity- Up-and-Down Procedure. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl425-508.pdf

Ray, D.E. (2001). Pyrethroid insecticides: mechanisms of toxicity, systemic poisoningsyndromes, paraesthesia, and therapy. In: Handbook of Pesticide Toxicology,Krieger, R.I., Krieger, W.C. Academic Press, Cambridge, Massachusetts,United States pp. 1289-1303.

Sachsse, K. (1974) Acute oral $LD_{50}$of technical CGA 15'324 in the rabbit. Unpublished report No. Siss 3647 from Ciba-Geigy Ltd, Basel, Switzerland. Submitted to WHO by Syngenta Crop Protection, Greensboro, North Carolina, USA.

U.S. EPA, (1997). Department of pesticides regulation, medical toxicology branch: summary of toxicology data Profenofos. Washington, DC: U.S. EPA.1-10.

Williams, S.C., Marco, G.J., Simoneaux, B.J. & Ballantine, L. (1984) Percutaneous absorption of 14C-profenofos in rats. Unpublished report No. ABR-84023 from Ciba-Geigy Corporation, Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection, Greensboro, North Carolina, USA.