ISSN (Print): 0972-4397
ISSN (Online): 0974-0805
**Journal of Ecophysiology and Occupational Health,** *Vol 20(3&4), DOI: 10.18311/jeoh/2020/25877, 159–164, July–December 2020*

# Evaluation of $LD_{50}$ of Fenvalerate in Male Wistar Rats by Miller and Tainter Method

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## Abstract

The principal aim of the present study was to evaluate median lethal dose ($LD_{50}$) of fenvalerate, a synthetic pyrethroid (Type II) insecticide, in male Wistar rats. Median lethal dose ($LD_{50}$) is defined as the dose which is lethal and proved to cause death in half of the experimental group of animals after specified test duration. The $LD_{50}$ is a standardized measure for expressing and comparing the toxicity of chemicals. This test is proved to be an initial screening phase for the evaluation, measurement and comparing of the acute toxicity of a chemical. Fenvalerate dissolved in groundnut oil was administered to 5 different groups of animals (10 animals in each group) as a single dose by oral intubation. Based on the pilot study the selected doses of fenvalerate were 400,425,450,475 and 500mg/kg b.wt. for male rats. The animals were kept under observation for any type of toxic symptoms and death in 96 hours. The percentage of dead animals after 96 hours was calculated, which was then transformed into probits for the estimation of median lethal dose ($LD_{50}$). In this study, the calculated median lethal dose ($LD_{50}$) of fenvalerate dissolved in groundnut oil was found to be 434.51±29.90 mg/kg b.wt. in Wistar rats. In sub-chronic and chronic studies, this data is very helpful for the establishment pf the dosage regimen.

**Keywords:** Fenvalerate, $LD_{50,}$ Pyrethroid, Wistar Rats

## 1. Introduction

Pesticides, substances or mixture of substances, is used for controlling, destroying, or preventing pests. The target pests may be insects, weeds, molluscs, birds, nematodes, microbes and plant pathogens that spread disease, cause nuisance, or destroy property, or are disease vectors. It becomes a common practice all over the world to use and handle toxic pesticides for the management of pests or vectors. The benefits/advantages of pesticides include improved economic potential in terms of amelioration of vector-borne diseases, and increased production of food and fibre. On the other hand, the disadvantages include severe health consequences to man and environment[1].

Exposure to pesticide shows a variety of serious health effects that can cause irritation of simple eyes and skin, and sometimes also cause more severe effects on the reproductive system, nervous system, brain damage and behavioural problems in young children. It therefore becomes essential to know about the toxicity of these compounds including their median lethal dose ($LD_{50}$).

The acute toxicity test is proved to be a primary screening process for the evaluation and measurement of the lethal properties of a drug or chemical. In this test only a single dose of the chemical is administered once to animals for the estimation of median lethal dose (the dose which is toxic and proved to cause death in half of the animals of a dose group) and gross behaviour. Acute toxicity tests are usually the primary tests performed to gives information on the relative toxicity likely to arise from a single or brief exposure of the chemical. It is one of the preliminary screening experiments conducted with

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all substances and is a preliminary measurement of toxic manifestations (providing information about potential hazards arising from short-term exposure to drugs)[2].

We can calculate $LD_{50}$ by various accepted methods such as those proposed by Miller and Tainter (1944)[3], Bliss (1934)[4], Thompson (1947)[5], Weil (1951)[7], Litchfield and Wilcoxon (1949)[6] and Finney (1971)[8].

A multiple of well-known techniques have been usually used for the determination of median lethal dose with the objective of reducing the figure of testing animals. The test chemical is administered orally to various groups of testing animals, single dose being chosen per group. According to the results of the first experiment, either no further tests are required or the test is to be done at higher or lower dose. If mortality happens at the initial dose, then the test is done again at the lower dose levels and if initially no signs of toxicity occur, then the test is again done at a higher dose levels. All these results are thus interpreted on the basis of the survival, death and evident toxicity of the animal.

The numerical value of the median lethal dose is affected by various factors, viz. duration of exposure, animal species, substance in which the preparation is administered, route of exposure (oral, dermal, inhalation), season, sex, diet, age, food deprivation prior to dosing, caging, temperature, experimental procedures, etc.

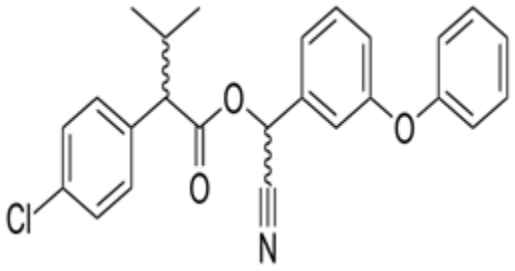
Fenvalerate [(*RS*)-_-cyano-3-phenoxybenzyl (*RS*)-2-(4-chloro-phenyl)-3-methyl-butyrate] is a third generation synthetic type II pyrethroid pesticide[9]. It is the photo stable analogue of the natural pyrethrins[10] and active against a wide range of pests[11]. This insecticide has been reported to impair motor activity by altering various neuronal processes and to induce hyperexcitability, tremors and paralysis[12]. Fenvalerate affects the nervous system by disrupting the enzyme cholinesterase[13]. Verma and Singh (2013)[14] reported *in vivo* clastogenic and spindle poisoning action of fenvalerate. This pesticide is also known to interfere with calcium homeostasis in rat ovary[15] and to inhibit production of progesterone by attenuating generation of cAMP[16].

The toxicity of any chemical to humans is generally evaluated on the basis of experiment performed on rats and other animal models[17]. Data related to median lethal dose ($LD_{50}$) of fenvalerate in rats is negligible. Thus, this study was designed for the determination of $LD_{50}$ of fenvalerate in male Wistar rats using groundnut oil as vehicle.

# 2. Materials and Methods

## 2.1 Test Chemical

Technical grade Fenvalerate (99.8% pure) PESTANAL® (chemical formula— $C_{25}H_{22}ClNO_3$; CAS registry no. 51630-58-1) was purchased from Sigma-Aldrich, Germany.




**Structure of Fenvalerate.**

## 2.2 Experimental Animals and Design

For experimental purpose, adult male Wistar rats from inbred colony, around 100 days of age were used. Animals were kept under standard laboratory conditions, with temperature 25+5°C and 12 hours light and 12 hours dark schedule, in polypropylene cages (size 43 x 27 x 15 cm). All the animals were allowed free access to standard rodent pellet diet obtained from *Ashirwad Pvt. Ltd.*, India and water was provided *ad libitum*. The research topic and protocols were approved by the Institutional Animal Ethical Committee (UDZ/IAEC/I/17). The animals were administered fenvalerate orally as a single dose in 0.5 ml of groundnut oil for the determination of $LD_{50}$.

## 2.3 Dose Preparation and Administration

The doses of fenvalerate were prepared by dissolving the pesticide in 0.5ml of groundnut oil. The animals were starved for 16 hours before administering the pesticide dose. The pesticide was given only once to the rats by oral intubation.

## 2.4 Determination of the Dose Range and % Mortality

Rats were starved for 16 hours before administering the pesticide doses. Fenvalerate was administered once to the animals. By pilot study, also called "up and down", an approximate $LD_{50}$ was determined by using two animals in each group. Gradually the doses of fenvalerate were

increased viz. 50, 150, 250, 400 and 700 mg/kg b.wt. The rats exhibited 100% mortality at 700 mg/kg b.wt. and no mortality occurred upto 400 mg/kg b.wt. Therefore we repeated the experiment with the doses- 400, 420, 440, 460 mg/kg b.wt. and so on. Now it was observed that 50% mortality occurred at 440 mg/kg b.wt. On the basis of the result of this pilot study, we determined five doses of the pesticide to perform a confirmatory experiment to find out oral $LD_{50}$ in rats, according to the procedure given by Miller and Tainter (1944)[4]. For this, five groups of male wistar rats were used consisting of ten animals each. The doses of fenvalerate selected were 400, 425, 450, 475 and 500 mg/kg b.wt.. Along with this, 0.5 ml groundnut oil is also given to the control group by the same route of administration. All the animals were under observation for 3 hrs and then at 6, 24, 32, 48, 72 and 96 hrs for any type of toxic signs and symptoms and mortality from 0 % (no death) to 100% was calculated[18]. After 96 hrs, the number of dead rats were counted in each group, and by using the graphical method given by Miller and Tainter (1944)[4] percent mortality was calculated.

# 3. Results

## 3.1 Toxicity Symptoms Observed During Experiment

The experimental animals exhibited chewing, salivation, burying the head in saw dust and lethargy at 400 mg/kg b.wt. Symptoms such as lacrimation, licking, abnormal gait, arching and rolling, incoordination, tremors and hair shedding increased at higher doses.

**Table 1.** Results of the dose ranges of fenvalerate for the calculation of $LD_{50}$ in male Wistar rats

| Groups | Dose (mg/kg b.wt.) | Log Dose | % Dead | Probits |
|---|---|---|---|---|
| 1. | 400 | 2.60 | 30 | 4.48 |
| 2. | 425 | 2.62 | 40 | 4.75 |
| 3. | 450 | 2.65 | 50 | 5.00 |
| 4. | 475 | 2.67 | 70 | 5.52 |
| 5. | 500 | 2.69 | 80 | 5.84 |

## 3.2 Transformation of % mortalities to probits and estimation of $LD_{50}$

The number of deceased rats was counted at each dose level (400, 425, 450, 475, 500 mg/kg b.wt.) and their percentage was calculated (Table 1).

By using Finney's method (Table 2), the % of died animals at each dose level was then converted to probit. The log dose values were then plotted against probit values and calculation of $LD_{50}$ was done.

In this study, for male rats Log $LD_{50}$ is 2.638 (Figure 1) and calculated median lethal dose ($LD_{50}$) is 434.51 mg/kg b.wt.

## 3.3 Estimation of Standard Error of $LD_{50}$

By using the following formula, the Standard Error (SE) of the $LD_{50}$ was estimated[19]:

$$\text{Approximate SE of } LD_{50} = \frac{(\text{Log } LD_{84} - \text{Log } LD_{16})}{\sqrt{2N}} \quad (1)$$

N = Number of animals in each group i.e. 10

**Table 2.** Conversion 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 |

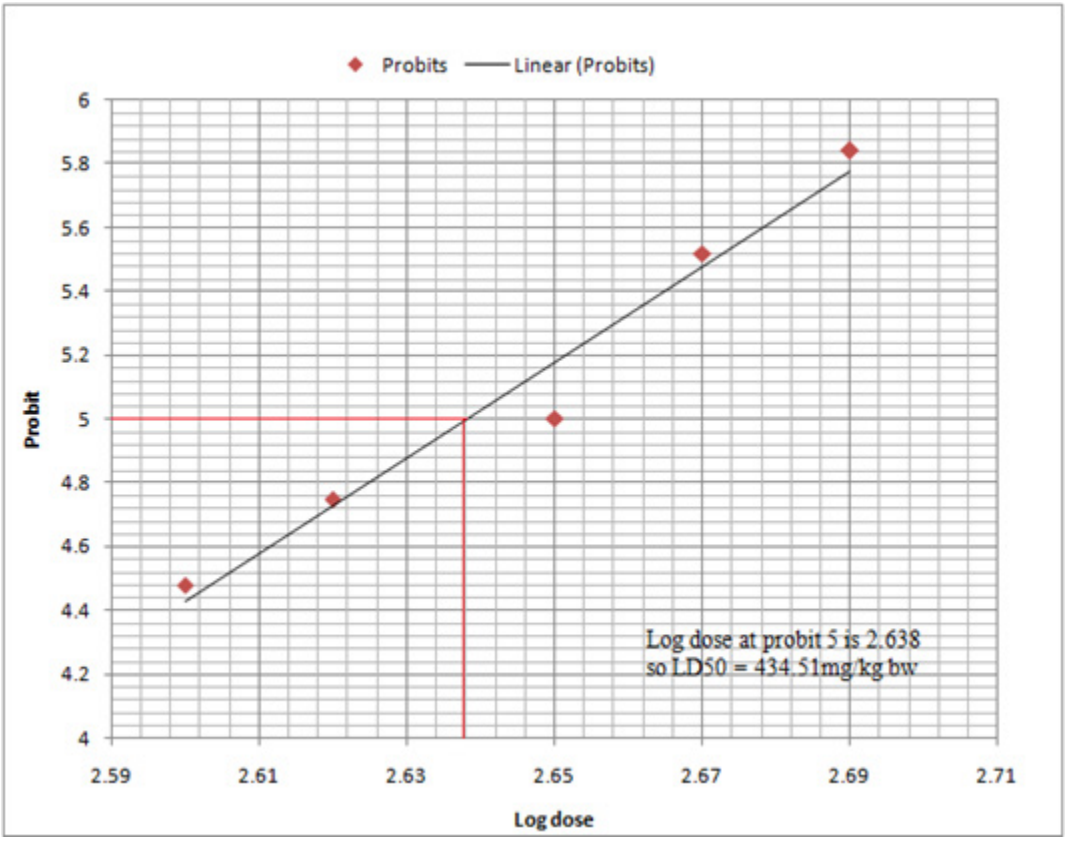



**Figure 1.** Plot of probit versus Log doses for estimation of oral $LD_{50}$ of Fenvalerate in male Wistar rats.

The calculated Probits of 16 and 84 from Table (2) are found to be 4.01 and 5.99 which is equal to approximately 4 and 6, respectively. The log LD values for the probits 4 and 6 are observed from the line on the graph in Figure 1, which, in this case are 2.572 and 2.705 and their antilogs are 373.250 and 506.990 respectively. Put these values in formula (1), the standard error of $LD_{50}$ is 29.90 for male rats. Therefore, $LD_{50}$ of fenvalerate in groundnut oil for male rats is 434.51±29.90 mg/kg b.wt. when given orally, with 95% confidence interval.

## 4. Discussion

Pesticides are effectively used all over the world to control or destroy a variety of crop pests and disease vectors so that crop yields increased tremendously, economies boosted, insect-borne diseases have decreased, and saving the lives of millions of people. However, contamination of the environment by pesticides has become a serious problem. The rampant use of pesticides in crop protection, insect pest control and food preservation has led to contamination of water, food and feed materials and thereby results in acute or chronic poisoning incidents in domestic animals, humans and wildlife. Thus, it becomes essential to know the median lethal dose ($LD_{50}$) of the pesticides as they may cause acute toxicity.

Pyrethroids are synthetic insecticidal compounds that are similar to pyrethrins, the natural toxins found in the Chrysanthemum flowers[20]. Their usage has increased in recent years due to the phase out of other insecticides such as organophosphates and organochlorines. A variety of commercial formulations of pyrethroid pesticides are available to ordinary consumers for use in the home these days.

Pyrethroids are well-known to affect the nervous system of insect by modifying the kinetics of VGSC i.e. voltage-gated sodium channels[21]. A proposed mechanism of their action is the persistence of the open state of neuronal VGSC[22] and this result in permanent depolarization of nerve cells causing neurotoxicity[21].

In the present study, we investigated the oral median lethal dose of fenvalerate, a pyrethroid pesticide in male Wistar rats. Fenvalerate solution was prepared by dissolving in groundnut oil and was administered to

each group of experimental animals at different doses. Common manifestations of pyrethroid poisoning viz. dullness, abnormal gait, burying the head in saw dust, arching, rolling, lacrimation, salivation, tremors, incoordination, imbalance, difficulty in breathing, hair shedding and lethargy were observed in the treated animals. The results obtained from this study suggest that median lethal dose ($LD_{50}$) of the fenvalerate was found to be 434.51±29.90 mg/kg b.wt. in male Wistar rats.

The mathematical value of the $LD_{50}$ is affected by many factors viz. vehicle used, route of exposure (oral, dermal, inhalation), animal species and strain, and sex. The $LD_{50}$ of fenvalerate was found to be 1785 mg/kg b.wt. in bobwhite quail *Colinus virginianus*[23], 939.9ng in microplitis croceipes[24], 349.98 mg/kg b.wt. in bullfrog *Haplobatrachus tigrinus*[25], and 312.8 mg/kg b.wt. in blue rock pigeon[26] respectively.

The $LD_{50}$ of fenvalerate in experimental mice was 100 mg/kg b.wt.[27] but it was 477.5 mg/kg b.wt. in female rats using corn oil as a vehicle[15].

However, published experimental work is limited on fenvalerate toxicity in Wistar rats, especially with groundnut oil as a vehicle. The result of the overall present study shows that oral median lethal dose ($LD_{50}$) of fenvalerate in male Wistar rats is 434.51 ± 29.90 mg/kg b.wt using groundnut oil as a vehicle.

## 5. Conclusion

Determination of $LD_{50}$ is very important for toxicologist to recognize a chemical compound and correlate its toxic effects. The $LD_{50}$ value provides statistical estimation of the acute toxicity of a chemical administered under particular conditions; it also gives a idea about the relative toxicities of different chemicals under identical or similar conditions. The present study demonstrates that the $LD_{50}$ of fenvalerate using groundnut oil as a vehicle in male Wistar rats is 434.51 mg/kg b.wt. An estimation of $LD_{50}$ and lethality is extremely essential for the better understanding of the toxicity of fenvalerate and also to evaluate its short-term poisoning effects. The results of such toxicity studies on animals may be useful in human accidental and/ or domestic poisoning cases.

## 6. Acknowledgement

Authors thank the University Grants Commission for the financial assistance.

## 7. Conflict of Interest

No conflict of interest.

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