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Estimation of Median Lethal Concentration (LC_{50}) of Pyrethroid and Carbamates Pesticides using the Nematode *Caenorhabditis elegans* to Predict Mammalian Acute Lethality (LD_{50})

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Abstract

Present study was aimed to estimate the median lethal concentration of most extensively used pesticides, pyrethroid (transfluthrin and cyfluthrin) and carbamates' pesticides (methiocarb and propoxur to deter pests, using a free-living nematode, *Caenorhabditis elegans* as model organism. the Median Lethal Concentration (LC_{50}) was calculated by Log-dose/probit regression line method, and Worms showed 24-hours lethality at concentrations 37 mg/l, 61 mg/l, 63 mg/l, and 48 mg/l for transfluthrin, cyfluthrin and methiocarb, propoxur respectively on NGM. Structural and toxicidal differences may has been for Differences in the median lethal concentration. LD_{50} and LC_{50} values data was compare with mammalian oral LD_{50} and calculated LC_{50} using *C. elegans* respectively. *C. elegans* found to be the more convenient for generating LC_{50} values analogous to the mammals LD_{50} values. So, *C. elegans* has great promises in the area of toxicological research.

Keywords: Carbamates, *C. elegans,* Median Lethal Concentration, Pyrethroids, Toxicity

1. Introduction

The environment is a gift given to humans by God, but the ecological hazard arising due to the indiscriminate release of toxicants by human action into the environment is a major health concern in today's world[1]. A broad range of toxicants from inorganic substances, such as metals to many organic micropollutants, such as pesticides and herbicides are of crucial distress as they are dangerous to human health and to contribute the pathophysiology of some human diseases. Various classes of pesticides like Organophosphates, Carbamates, and Pyrethroids are widely used in the world, leading cause to progressive loss of structural as well as functional properties of neurons, leading to the dysfunction and ultimate degeneration of neurons[2]. Now these days, Pesticides are more frequently used not only in agriculture but, also in non-agricultural areas like agrochemicals and pharmaceuticals, as a

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flavoring, coloring agents and preservatives in the food industry to increase self-life and taste for a long time[3]. Pesticide is obstinate and non-biodegradable and it can be bio-accumulated through the biological chains. In the atmosphere, the extreme use of pesticides is a probable menace to human health and other living existences[4].

In recent decades, environmental dynamics and their genetic liability have been reviewed in neurodegenerative diseases like Parkinson's and Alzheimer's[5]. It has been found that pesticides are the leading neurotoxic agents, and carbamates and Pyrethroids are the most used insecticides for pest control. These pesticides are commonly used in agriculture, home, garden, and public health programs to target several pests like cockroaches, grubs, flies, termites, fire ants, lice, and mosquitoes[6,7]. Mammalian models have been increasingly used in toxicological studies in recent decades, which can be concerning for social as well as cost-effective animal handling and have engendered much interest in the use of nonmammalian species for initial screening tests. Some non-mammalian species have been proposed for toxin studies because they have proven to be reliable as well as low-cost, and easy to culture and handle[8].

To evaluate the toxic effect a reliable model organism is needed so the present study focused on *Caenorhabditis elegans* a small free-living Nematode, a natural inhabitant of the interstitial water of soil-dwelling, with a tinny, translucent body approx. 1 mm in length. Easy to culture in the laboratory under optimum conditions, develops from a unicellular embryo to a fertile adult with a short life cycle of 3-5 days at 20°C[9]. Overcrowding or lack of food converts it to the dauerlarvae stage that allows the worm to survive in a dormant stage for several months and that can further be revived as synchronized cultures. *C. elegans* has a fully (>99%) sequenced genome[10], and it is the only organism with a fully mapped nervous system and cell lineage. *Caenorhabditis elegans* is a simple animal with a centralized nervous system consisting of only 302 neurons[11], of which allows physiological and neuronal analysis simpler[12]. Of the several endpoints proposed for evaluating environmental toxicants toxicity using *C. elegans*, the data on NGM agar using LC_{50} is very limited[13]. This endpoint is also commonly used in environmental testing with other organisms[14,15]. Recent pilot data have focused that *C. elegans* might be useful in an airing trial for neurotoxicity[16-20].

In this experiment, we assessed the lethal toxicity percentages of pyrethroids and carbamates pesticides at different concentrations *of C. elegans* culture at 24 hours and investigated. The present study was conducted to determine whether a useful comparison can be made between the concentrations resulting in acute lethality in *C. elegans* and the published LD_{50} data for mammals.

2. Materials and Methods

2.1 Chemicals

HPLC grade pesticides (transfluthrin; cyfluthrin; methiocarb; propoxur) were procured from Sigma-Aldrich (St. Louis, MO) used in this presence study.

2.2 Worm Culture

Caenorhabditis elegans, wild-type strain N_2 grown on the bacterial culture of *E. coli* OP50 as food sources, both obtained from the Caenorhabditis Genetics Centre (CGC) (University of Minneapolis, MN, USA).

C. elegans was grown and maintained in the laboratory using 90 mm Petri dish culture plates filled with ~20 ml Nematode Growth Media (NGM) (3 gL^{-1} NaCl, 2.5 gL^{-1} peptone, 17 gL^{-1} agar, 5 mgL^{-1} cholesterol, 1 $mmolL^{-1}$ $CaCl_2$, $mmolL^{-1}$ $MgSO_4$, 25 $mmolL^{-1}$ potassium phosphate, pH 6.0) at 20 °C using BOD incubator[9].

2.3 Experimental Design

2.3.1 Sample Preperation

The solution of the test compound was prepared in the solvent as per its solubility. The starting dose was decided following the sighting study[15]. A total of 180 Age-synchronized worms were used and divided into 6 groups of 30 in each (10 (±1) L4 stage (3 to 4 days old) worms of each group, in 3 replicates) each as follows: Group I control group, Group II 10 mg/l, Group III 25 mg/l, Group IV 50 mg/l, Group V 75 mg/l, Group VI 100 mg/l[21]. *C. elegans* were transferred using hypodermic platinum wire to the plates containing different concentrations of pesticides ranging from 10 mg/l -100 mg/l. The cultures were maintained at 20 °C in a BOD incubator for 24 hours (~ 1 hour). After this exposure period, the plates were observed under a dissecting microscope to determine the number of dead and survivors[22]. Death was defined as a total lack of movement and lack of response to probing with a loop holder. Worms not found on the plate were assumed to be dead and decomposed beyond recognition. The testing

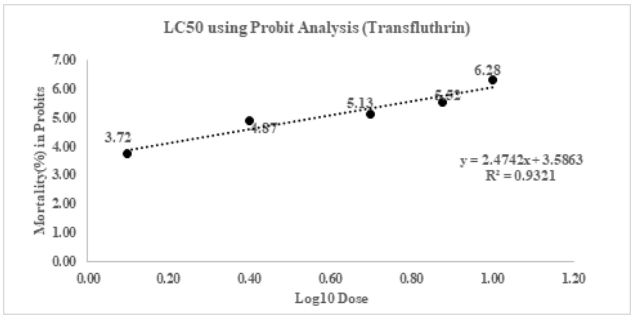
was usually repeated 10 times (≈100 individuals) for each concentration.

2.3.2 Statistical Analysis

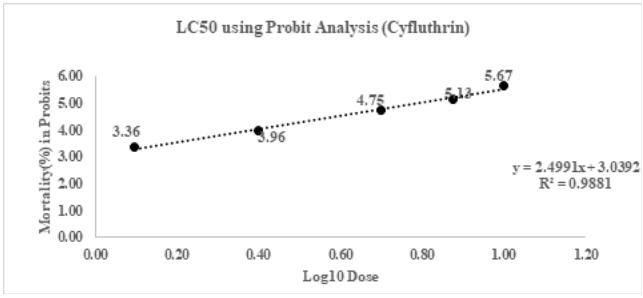
All experiments were performed in triplicate. Data was analyzed by MS Excel 2019 to find regression equation (Y = mortality; X = concentrations)[23], the LC_{50} was derived from the best-fit line obtained[22]. The LC_{50} values (with 95% confidence limits) were calculated[24]. Differences among the results were considered to be statistically significant when P value was < 0.05.

3. Results and Discussion

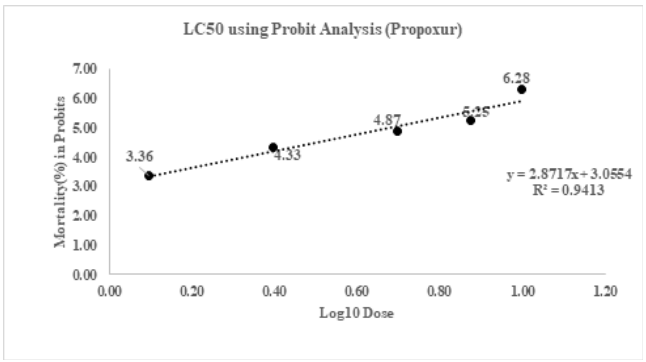
For each concentration, an overall average was derived and the dose-response relationship was plotted (Figures 1(a), 1(b), 1(c), 1(d), and 1(e)). LC_{50} has been calculated by Log-dose/probit regression line method[22], and Worms



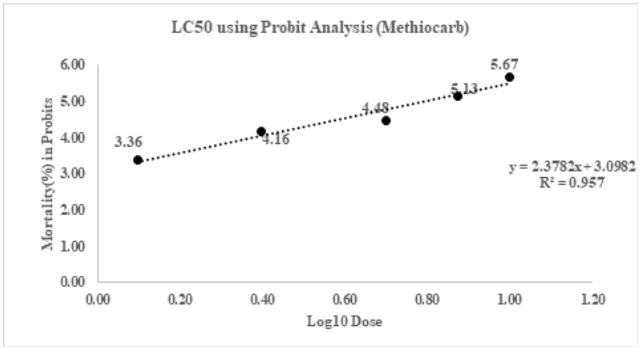
(a)



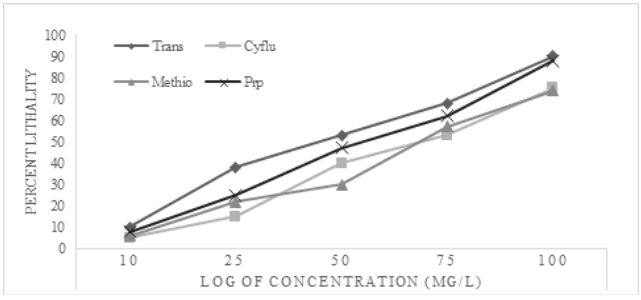
(c)



(b)



(d)



(e)

Figure 1. **(a)** LC_{50} using Probit Analysis with linear regression equation (Transfluthrin). **(b)** LC_{50} using Probit Analysis with linear regression equation (Propoxur). **(c)** LC_{50} using Probit Analysis with linear regression equation (Cyfluthrin). **(d)** LC_{50} using Probit Analysis with linear regression equation (Methiocarb). **(e)** A combined graph of the dose-response curves for the 4 pesticides exposed to *C. elegans*. An LC_{50} was determined for each pesticide from the linear regression of the probit transformation.

Table 1. Comparison of *C. elegans* LC_{50} values to mammalian LD_{50} values

Pesticides	*C. elegans* LC_{50} (mg/l)		Mammalian LD_{50} (mg/kg)				
	With *E. coli*	Without *E. coli*	Chicken	guinea pig	Rat	Mouse	Average of all mammals
Transfluthrin	37 (24-56)	34 (22-51)	50 mg/kg	-	-	583 mg/kg	316.5 mg/kg
Propoxur	48 (34-67)	51 (36-71)	46.5 mg/kg	40 mg/kg	41 mg/kg	100 mg/kg	56.87 mg/kg
Cyfluthrin	61 (43-87)	58 (41-83)	5 gm/kg	-	469 mg/kg	300 mg/kg	258 mg/kg
Methiocarb	63 (42-93)	61.5 (41-90)	179 mg/kg	40 mg/kg	20 mg/kg	20.2 mg/kg	64.8 mg/kg
Rank correlation, p value with *E. coli*			> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Rank correlation, p value without *E. coli*			> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Note: The ranges shown in the parentheses are a 95% confidence limit = $S^{2.77}/\sqrt{a}$; where S = (LC_{58}/ LC_{50} + LC_{50}/LC_{47}/2). The units of the LC_{50} values are mg/l, which are equivalent to the units of the LD_{50} values (mg/kg).

showed lethality after 24 hours at concentrations 37 mg/l, 61 mg/l, 63 mg/l, and 48 mg/l for transfluthrin, cyfluthrin, and methiocarb, propoxur respectively on NGM (Table 1). A linear regression of the probit calculations was performed to determine the LC_{50}[24].

Table 1 provides the LC_{50} values determined for each pesticide and the mammalian LD_{50} was analyzed by using data from National Pesticide Information Centre (NPIC; 2022, PubChem; 2022). The nematode data are reported as LC_{50} values. For each LC_{50} value, a 95% confidence limit was calculated[14,15] using a computer program LD50/LC50 Probit Analysis Software 2019[23,25].

The initial testing was conducted with plates having bacterial lawns. In comparison to the data from the other pesticides, the methiocarb results were found to be much higher than would have been expected. It is known that methiocarb is highly binding to a membrane protein of *E. coli* which may cause the pesticide to be less accessible to the nematode[26]. For this reason, subsequent testing was performed on plates without a bacterial lawn. Because *C. elegans* feeds almost continuously, a major concern was what effect this lack of food might have on the cultures. However, following 24-hour exposures, the vehicle control plates were observed to be normal and no lethality was found. Using this method, the cyfluthrin and methiocarb LC_{50} values were insignificantly lowered. Similar testing with all pesticides resulted in some changes in the LC_{50} values, but the overall relative ranking of the toxicity order was similar to tests using the bacterial lawn. As shown in Table 1, the nematode LC_{50} values are lower than the corresponding mammalian LD_{50} values. It may be that 24 hours is not long enough for internal concentrations in the nematode to reach equilibrium with the external concentrations.

The actual dose cannot easily be determined; however, it is assumed that the majority of the toxicant enters the worm through the gut[27]. This assumption is made because the nematode's cuticle has usually been found to be impervious to most chemicals. All the mammalian data is from oral studies using pesticides with the same valence as that used in this study.

The ability of the *C. elegans* test to predict the relative acute lethality of pesticides in mammals was analyzed by using data from National Pesticide Information Centre (NPIC; 2022, PubChem; 2022) for mammalian species and all soluble salts with the same valence as those employed in this study. Linear regression of the log of the 17 LD_{50} for all mammals' values against the log of the mouse LD_{50} (Figure 2) and the same set of 17 mammals' values against the log of *C. elegans* LC_{50} with and without a lawn (Figures 3(a) and 2(b)) was performed. This basic approach for evaluating the data has been used by other investigators in determining the usefulness of new toxicological screening tests[28].

Table 2 provides the Syx for each of the comparisons of *C. elegans* data to mammalian acute lethality data and identical comparisons using the mammals. The results for both types of tests with *C. elegans* are very close to the results for mammals. Taking the difference between the Syx with *E. coli* and without *E. coli* as an indication of what is biologically relevant, one can conclude that *C. elegans* is indistinguishable from them in its ability to predict the acute lethality of these chemicals in other mammals.

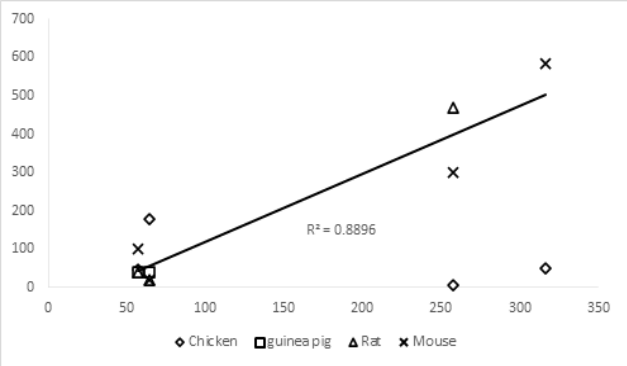
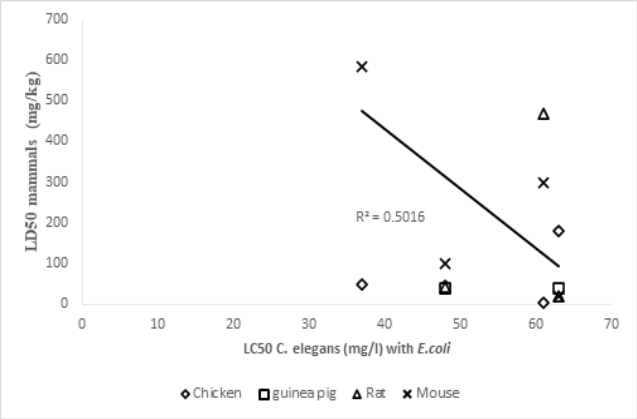
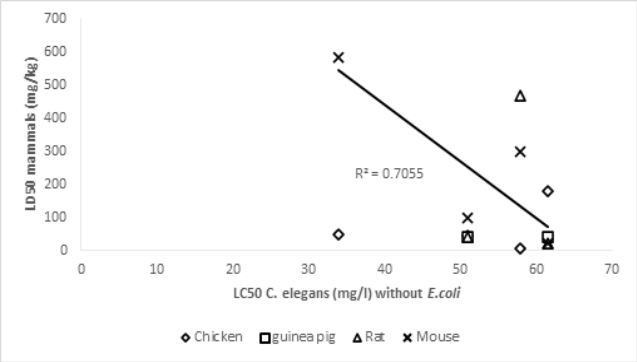


Figure 2. The log of the mouse LD_{50} (from Table 1) is plotted against the log of the LD_{50} for the same pesticides from all mammalian species. A linear regression analysis is performed, and the Syx has been calculated.



(a)



(b)

Figure 3. (a) The log of the *C. elegans* LC_{50} with a lawn of *E. coli* as a food source (from Table 1) is plotted against the mammals LD_{50} **(b)** The log of *C. elegans* LC_{50} without a lawn of *E. coli* is plotted against the mammals LD_{50}. the same set of data used in Figure 2. a linear regression analysis is performed and the Syx has been calculated.

Table 2. Standard error of the estimate for *C. elegans* with specific condition as an analyst for mammalian acute lethality

Observation	Standard Error of the Estimate (S_{yx})
Mouse versus Mammals (minus mo use)	
C. elegans versus Mammals	0.8896
With *E. coli*	0.5016
Without *E. coli*	0.7055

The primary purpose of acute lethality testing is for initial range-finding testing and to identify initial symptoms of toxicity[29]. Although the initial symptoms of toxicity cannot be found by using the test defined in this paper, it is believed that primary range-finding data can be achieved. In addition, it appears that a relative ranking of acute deadliness may be reachable.

With the present experiment planned, only a less water-soluble chemicals can be observed. Perceptibly, forthcoming assessments will include constituents that may have no solubility in water. It is predicted that DMSO, acetone, and ethyl alcohol can be used as vehicles for some of these types of substances. Initial testing has been conducted for these chemicals and the results indicate a very low level of toxicity in *C. elegans*. Explosive chemicals cannot be tested in the way described earlier for the integration of solutions of substances in poured dishes. However, it should be possible to expose the worms to known concentrations of the vapor phase of the elements under inert conditions. Air mixtures can be prepared in a closed container that can be positioned in an incubator. The cultures of *C. elegans* could be placed in a closed container for various lengths of time. A variation of this approach has been shown successfully as an exposure method[30].

4. Conclusion

Based on this study, it is proven that the Probit modeling exercise via MS excel software (2019) is very useful in estimating LC_{50} and the 95% confidence interval values that did not manage to report both parameters. A more accurate LC_{50} is achieved, leading to a better estimation of sub-lethal concentration that can be further utilized in future toxicity studies at biochemical and molecular

levels. In order to better evaluate the usefulness of this organism, additional testing is planned with other types of chemicals. Also, other types of toxicological evaluations are being performed (e.g., aquatic bioassays and neurotoxicological screening tests).

5. Acknowledgement

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