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Estimation of Median Lethal Concentration (LC50) Value and Its Confidence Interval for the Effect of Carbamate Pesticide (Methiocarb) on Caenorhabditis Elegans

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Estimation of Median lethal concentration (LC50) value and its confidence Interval for the effect of carbamate pesticide (methiocarb) on *Caenorhabditis elegans*

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***Abstract*— *Caenorhabditis elegans* increasingly is attractive as a toxicity test organism, particularly as a model system to study mechanisms of toxicity at a molecular level and the way that these lead to whole organism and population level effects. In this study, lethal concentration (LC_{50}) values of methiocarb on nematodes (*Caenorhabditis elegans*) were investigated. In practice, experimental setup was constituted 30 worms (a total of 300 worms with 30 control worms) to be placed in three replicates. methiocarb was added into NGM at the concentration range from 1-20 mg/l (1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 mg/l) and determined mortality of worms exposed to calculated Percentage death of worms in these concentrations, mortality was observed at all treatments. The results indicate that due to methiocarb, can be lethargy, lack of breath in media at all of the concentrations, the reason of death. The results of regression analysis indicated that the mortality rate (Y) is positively correlated the concentration (X) having a regression coefficient (R), after 24 hours LC_{50} value (with 95% confidence limits) was estimated at 4.805 mg/l.**

Keywords— *Caenorhabditis elegans*; pesticides toxicity; median lethal concentration (LC_{50}); MS Excel software 2019.

I. INTRODUCTION

The free-living nematode *Caenorhabditis elegans* increasingly is attractive as an organism for toxicological studies. It has a short life span (2–3 weeks), is easy to maintain in the laboratory[1], and can survive a wide range of pH. Its full genome sequence is available and, as a result, information rapidly is accumulating on its physiology and development, including a range of tools for examining gene expression. A number of sublethal endpoints for toxicity testing with *C. elegans* have been proposed, including population effects, development, morphology, behavior, and feeding. These endpoints are more sensitive to pesticides after 24-h acute toxicity)[2], but substantially more time consuming to measure. Thus, it will be difficult to use them in screening a large number of chemicals, multiple strains, or other assay conditions for chronic effects[3].

Methiocarb is a carbamate pesticide (an acetylcholinesterase inhibitor) which is used as an insecticide, bird repellent, acaricide and molluscicide since the 1960s[4]. Methiocarb has contact and stomach action on mites and neurotoxic effects on molluscs have been shown. Seeds treated with methiocarb also affect birds[5]. Other names for methiocarb are mesurol and mercaptodimethur. Due to its toxicity, methiocarb approval as a plant protection product has been withdrawn by the EU effective 2020[6]. Methiocarb can be taken up through different routes. The most common for humans is up take through the skin or as an aerosol, because of its use as a pesticide in agriculture. Because methiocarb is widely used as an insecticide on crops, environmental risks were also studied to establish safety risks for human health[7]. Individuals are most likely to be exposed to methiocarb dermally or by inhalation during the manufacture, formulation, and application of this insecticide. Acute (short-term) exposure of humans to methiocarb by ingestion leads to cholinesterase inhibition of red blood cells, with mild cholinergic symptoms including blurred vision, nausea, vomiting, sweating, and tachycardia; however, the effects are transient. Chronic (long-term) inhalation exposure has resulted in depressed cholinesterase levels, headaches, vomiting, and nausea in humans[8]. Chronic ingestion studies in animals have reported depressed cholinesterase levels, depressed body weight, effects to the liver and bladder, and a slight increase in neuropathy[9].

Although toxicity could be measured in several ways by observing alterations in the biochemistry, physiology, reproduction or behaviour of organisms, the most common end point chosen for toxicity studies up till now is still death[2]. Lethal concentration (LC_{50}), lethal dose (LD_{50}), effective concentration (EC_{50}) and effective dose (ED_{50}) are some of the terms frequently encountered in toxicity testing[10]. LC_{50} for liquid and LD_{50} for solid are defined as concentration or dose of a toxicant that kills 50% of test organisms within a particular period of exposure[11]. However, if the end point is not mortality, EC_{50} or ED_{50} is determined, i.e., the concentration or dose that can cause effects in 50% of test organisms[12].

In order to determine the relative toxicity of chemicals to living organisms, Probit analysis, a specialized regression model of binomial response variables comes in handy and is widely used[3]. Determination of lethal concentration (LC_{50}) of toxicants leading to 50% mortality of test samples in a toxicity test is very important and could be achieved by running Probit analysis[13]. The response is binomial (death or no death) and relationship between response and various doses or concentrations is typically sigmoid. The Probit value can either be manually calculated by hand, or automatically calculated by computer software using a higher accuracy estimation method, namely the maximum likelihood principle. When a published toxicity study failed to report the 95% confidence interval values,[14] the results can be recalculated via software[3].

In this study, we have demonstrated toxicity endpoints death percentages of methiocarb at different concentrations in

cultured *Caenorhabditis elegans* after 24 hours for median lethal concentration (LC_{50}) by MS excel 2019 software.

II. MATTERIAL AND METHODS

A. *Experimental animal and It's Culture*

The bacteria stock (*Escherichia coli*, strain OP50) and the wild-type (N2) *C. elegans* used in this experiment were obtained from The Caenorhabditis Genetic centre, University of Minnesota, (Minneapolis, MN, USA). *Caenorhabditis elegans* was propagated on Nematode growth media (3 gL−1 NaCl, 2.5 gL−1 peptone, 17 gL−1 agar, 5 mgL−1 Cholesterol, 1 mmolL−1 CaCl2, mmolL−1 MgSO4, 25 mmolL−1 potassium phosphate, pH 6.0) NGM agar plates, with bacteria (OP50) as food[1]. Standard methods were used to harvest eggs and generate age-synchronized adult worms[15]. After which a total 300 worm were transferred to 60 mm cultured petri plates accordingly experiment.

HPLC grade pesticide methiocarb was procured from Sigma-Aldrich (St. Louis, MO) used in this presence study.

B. *Dose Prepration and Experimental design*

The solution of test compound was prepared in the solvent as per its solubility. The starting dose was decided following the sighting study. A total of 300 Age-synchronized worm were used in the experiment. The worms were divided into 10 groups of 30 specimens (10 worms of each group, in 3 replicates) each as follows: Group 1 (Non-pesticide normal worms, control group) received normal commercial diet. Group 2 (the lowest concentration): pesticide-added group at the level of 1 mg/l, Group 3 (low concentration): pesticide-added group at the dose of 2.5 mg/l, Group 4 (mid concentration): pesticide-added group at the level of 5 mg/l. Group 5 (high concentration): pesticide-added group at the level of 7.5 mg/l. Group 6 (the high concentration): pesticide-added group at the level of 10mg/l. Group 7 (the high concentration): pesticide-added group at the level of 12.5mg/l. Group 8 (the high concentration): pesticide-added group at the level of 15 mg/l. Group 9 (the highest concentration): pesticide-added group at the level of 17.5 mg/l. Group 10 (the high concentration): pesticide-added group at the level of 20 mg/l[16]. All of the experimental groups received the treatments for a period of 24 hours. During the experiment, the worms of each group were examined under a dissecting microscope[17].

C. *Statistical Analysis*

All experiments were repeated three times and performed in triplicate. Data were analyzed using Probit Analysis Statistical Method[18]. The LC_{50} values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when P value was < 0.05. Also, the MS Excel 2019 was used to find regression equation (Y=mortality; X=concentrations), the $LC5_0$ was derived from the best-fit line obtained[19].

III. RESULT

The methiocarb concentration range from 1-20 mg/l (1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 mg/l) to determine mortalities on *C. elegans*. Dead specimens were removed immediately after death and their numbers registered and calculated percentage mortalities (Fig. 1). All worms died after 24 h exposure of methiocarb at the concentration of 20 mg/l, while worms' death depending on the concentration of methiocarb showed an increase mortality with increased concentrations (Table 1). These results indicate that the methiocarb had lethargy and lack of breath in NGM media at all the concentrations.

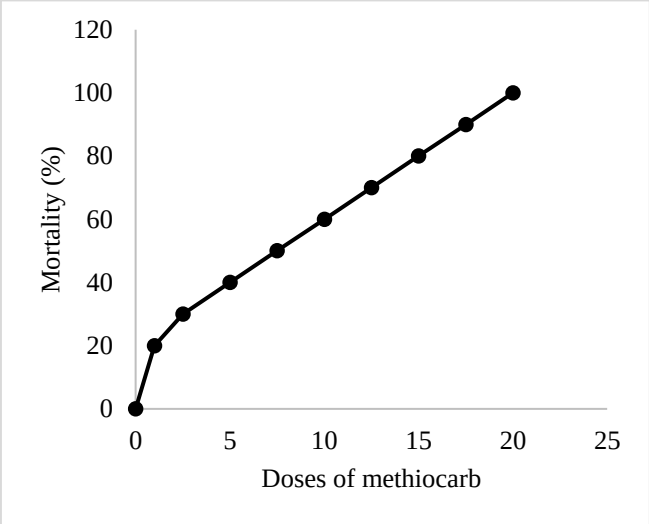

Fig. 1. Mortalities of worms exposed to different doses of methiocarb

Results indicate that the methiocarb had lethargy and lack of breath in NGM media at all the concentrations. The results of the Probit modelling exercise using MS excel 2019; regression line analysis indicated that the mortality rate (Y) is positively correlated the concentration (X) having a regression coefficient (R), after 24 hours LC50 value (with 95% confidence limits) was estimated at 4.805 mg/l (95% CI was from 3.166 to 7.929 for methiocarb (Fig. 2).

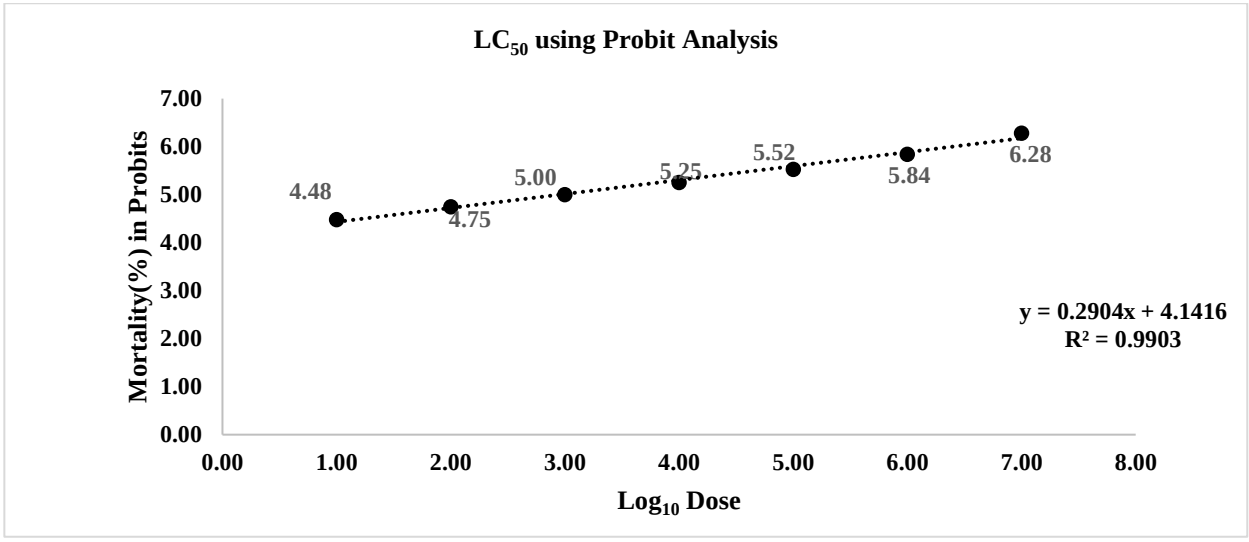

Fig. 2. LC_{50} using Probit Analysis with linear regression equation. An LC_{50} was determined for each pesticide from the linear regression of the probit transformation.

Table 1. Percentage mortality of worms exposed to different methiocarb concentrations (ND: no data because of no doses provided to control; 100 % mortality & no mortality observed)

Groups	Concentration (mg/l)	log10 (con)	No. of worms	Exposure Time	Mortality No.	Mortality (%)	Probit
Group1	0	ND	30	24 h	0	0	ND
Group2	1	0.1	30	24 h	6	20	4.16
Group3	2.5	0.4	30	24 h	9	30	4.48
Group4	5	0.7	30	24 h	12	40	4.75
Group5	7.5	0.88	30	24 h	15	50	5
Group6	10	1	30	24 h	18	60	5.25
Group7	12.5	1.1	30	24 h	21	70	5.52
Group8	15	1.18	30	24 h	24	80	5.84
Group9	17.5	1.24	30	24 h	27	90	6.28
Group10	20	1.3	30	24 h	30	100	ND

IV. DISSCUTION

The nematode, *Caenorhabditis elegans*, is one such system that has well-developed genetics, is amenable to transgenesis and has relatively low associated experimental costs with characterized neuronal network. *Caenorhabditis elegans* increasingly is attractive as an organism for toxicological studies [13].

The application of methiocarb caused toxic effects on *C. elegans*, mortality was found in all concentration, increased with respect the pesticidal concentration. In the literature, there are some studies investigating the toxic effects of other pesticides, although not methiocarb. The similar effect of methiocarb and other pesticides (oxyfluorfen, malathion, dursban 4) were reported by many researcher in field of toxicology[8], [20], [21].

Probit analysis was developed more than fifty years ago, but till today, it remains as the preferred statistical method in dose- response studies. In 1934, Chester Ittner Bliss (1934) a biologist first introduced the idea of probit analysis[22]. While conducting an experiment on the application of pesticides in controlling insects, Bliss observed that the relationship of response to dosage of insecticide was naturally sigmoidal [3]. Nonlinear regression was not an option back then as the technology was still behind and regression was only performed on linear data. Hence, Bliss proposed to transform the sigmoidal dose-response curve into a straight line instead. However, his quest to scientifically determine the effect of various pesticides to the same insect species hit another hurdle. Some statistical background was required in order to materialise his probit idea and Bliss was no expert. Fortunately, in 1952, a statistics professor at University of Edinburgh by the name of David Finney adopted and expanded Bliss' idea. This in turn led to Finney publishing a book entitled Probit Analysis[18]. Failure of numerous published toxicity studies to report the LC_{50} values and the 95% confidence intervals using well- acknowledged technique such as the Probit hampers the effort of performing a correct analysis. The confidence interval value is useful and significant as it can be utilised for comparison of another chemical or treatment to the same test organism species[3], [12], [20].

We studied lethal concentration (LC_{50}) values of methiocarb at different concentrations on *C. elegans*. Our results confirmed that mortality was observed at all treatments. Different of LC_{50} determination studies on worms may be due to the concentration differences in the methods applied or different pesticides used.

V. CONCLUDING REMAKS AND FUTURE PROSPECTIVE

Based on this study, it is proven that Probit modelling exercise via MS Excel software 2019 is very useful in estimating LC_{50} and the 95% confidence interval values of previously published toxicity studies 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 utilised in future toxicity studies at biochemical and molecular levels.

DECLARATION OF COMPETING OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

[1] S. Brenner, "The Genetics of *Caenorhabditis elegans*," *Genetlcs*, vol. 77, no. May, pp. 71–94, Dec. 1974.

[2] P. R. Hunt, "The *C. elegans* model in toxicity testing," *J. Appl. Toxicol.*, vol. 37, no. 1, pp. 50–59, 2017, doi: 10.1002/jat.3357.

[3] I. N. M. Jaafar, S. A. Ahmad, and N. A. Yasid, "Estimation of LC50 and its Confidence Interval for the Effect of NanoZero Valent Iron on the Freshwater Zooplankton Species *Daphnia magna*," *J. Environ. Microbiol. Toxicol.*, vol. 6, no. 2, pp. 25–28, 2018.

[4] H. Schmieg *et al.*, "Polystyrene microplastics do not affect juvenile brown trout (*Salmo trutta f. fario*) or modulate effects of the pesticide methiocarb," *Environ. Sci. Eur.*, vol. 32, no. 1, 2020, doi: 10.1186/s12302-020-00327-4.

[5] I. C. Yadav and N. L. Devi, "Pesticides Classification and Its Impact on Human and Environment," *Environ. Sci. Engg*, vol. 6, no. February, pp. 140–157, 2017.

[6] E. COMMISSION, "Evaluation of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market and of Regulation (EC) No 396/2005 on maximum residue levels of pesticides," vol. SWD(2020), no. 1107, pp. 1–166, Nov. 2020, doi: 10.1088/1751-8113/44/8/085201.

[7] K. B. Jadhav and P. S. Rajini, "Evaluation of sublethal effects of dichlorvos upon *caenorhabditis elegans* based on a set of end points of toxicity," *J. Biochem. Mol. Toxicol.*, vol. 23, no. 1, pp. 9–17, 2009, doi: 10.1002/jbt.20258.

[8] B. C. Leelaja and P. S. Rajini, "Biochemical and physiological responses in *Caenorhabditis elegans* exposed to sublethal concentrations of the organophosphorus insecticide, monocrotophos," *Ecotoxicol. Environ. Saf.*, vol. 94, pp. 8–13, 2013, doi: 10.1016/j.ecoenv.2013.04.015.

[9] J. A. Lewis, E. A. Gehman, C. E. Baer, and D. A. Jackson, "Alterations in gene expression in *Caenorhabditis elegans* associated with organophosphate pesticide intoxication and recovery," *BMC Genomics*, vol. 14, no. 1, p. 1, 2013, doi: 10.1186/1471-2164-14-291.

[10] R. D. Cole, G. L. Anderson, and P. L. Williams, "The nematode *Caenorhabditis elegans* as a model of organophosphate-induced mammalian neurotoxicity," *Toxicol. Appl. Pharmacol.*, vol. 194, no. 3, pp. 248–256, 2004, doi: 10.1016/j.taap.2003.09.013.

[11] A. Knight, "Animal experiments scrutinised: Systematic reviews demonstrate poor human clinical and toxicological utility," *ALTEX*, vol. 24, no. 4, pp. 320–325, 2007, doi: 10.14573/altex.2007.4.320.

[12] K. G. A, B. Altinterim, and O. Aksu, "Determination of lethal concentration (LC_{50}) values of *Cinnamomum zeylanicum* hydrosol on carp fish,"

Iran. J. Fish. Sci., vol. 12, no. 1, pp. 34–44, 2013.

[13] P. L. Williams and D. B. Dusenbery, "using nematode *C. elegans* to predict mammalian acute lethality to metallic salts," *Toxicol. Ind. Health*, vol. 4, no. 4, pp. 469–478, 1988, doi: 10.1177/074823378800400406.

[14] and J. H. F. Windy A. Boyd2, Marjolein V. Smith3, Grace E. Kissling1, "MEDIUM- AND HIGH-THROUGHPUT SCREENING OF NEUROTOXICANTS USING *C. ELEGANS*," *Neurotoxicology teratol*, vol. 32, no. 1, pp. 1514–1523, 2010, doi: 10.1038/jid.2010.15.Antagonistic.

[15] U. Ibiam and A. Grant, "RNA/DNA RATIOS AS A SUBLETHAL ENDPOINT FOR LARGE-SCALE TOXICITY TESTS WITH THE NEMATODE *CAENORHABDITIS ELEGANS*," *Environ. Toxicol. Chem.*, vol. 24, no. 5, pp. 1155–1159, 2005, doi: 10.1897/04-262R.1.

[16] J. Mills, E. McConnell, J. A. Leitão, and L. R. Lapierre, "Combined Nucleotide and Protein Extractions in *Caenorhabditis elegans*," *J. Vis. Exp.*, no. 145, pp. 1–11, 2019, doi: 10.3791/59178.

[17] S. Liang, Y. Liu, T. Fu, F. Yang, X. Chen, and G. Yan, "A water-soluble and biocompatible polymeric nanolabel based on naphthalimide grafted poly(acrylic acid) for the two-photon fluorescence imaging of living cells and *C. elegans*," *Colloids Surfaces B Biointerfaces*, vol. 148, pp. 293–298, 2016, doi: 10.1016/j.colsurfb.2016.09.001.

[18] D. J. Finney, *Probit Analysis. By D. J. Finney, M.A., Sc.D., [2nd ed. Pp. xiv + 318. Cambridge University Press, 1952. 35 s .]*, vol. 78, no. 3. 1952.

[19] D. A. Raj, "LD_{50}/LC_{50} Probit Analysis Software." p. 1, 2019.

[20] R. Dhawan, D. B. Dusenbery, and P. L. Williams, "Comparison of lethality, reproduction, and behavior as toxicological endpoints in the nematode *caenorhabditis elegans*," *J. Toxicol. Environ. Heal. - Part A*, vol. 58, no. 7, pp. 451–462, 1999, doi: 10.1080/009841099157179.

[21] P. S. Rajini, P. Melstrom, and P. L. Williams, "A comparative study on the relationship between various toxicological endpoints in *Caenorhabditis elegans* exposed to organophosphorus insecticides," *J. Toxicol. Environ. Heal. - Part A Curr. Issues*, vol. 71, no. 15, pp. 1043–1050, 2008, doi: 10.1080/15287390801989002.

[22] C. I. BLISS and W. L. Stevens, "THE CALCULATION OF THE TIME-MORTALITY CURVE," *Ann. Appl. Biol.*, vol. 24, no. 4, pp. 815–852, Feb. 2008, doi: 10.1111/j.1744-7348.1937.tb05058.x.