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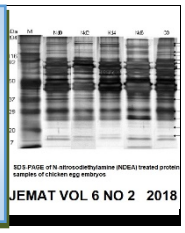

Estimation of LC_{50} and its Confidence Interval for the Effect of Nano-Zero Valent Iron on the Freshwater Zooplankton Species *Daphnia magna*

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ABSTRACT

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. 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, the results can be recalculated via software. In this study, the LC_{50} and 95% confidence interval values of the effect of nano-zero valent iron towards the freshwater zooplankton *Daphnia magna* is recalculated by employing the Probit analysis in the SPSS software. The results of the Probit modelling exercise gave an LC_{50} value of 0.405 mg/L (95% confidence interval, CI was from 0.047 to 0.953) for Nanofer 25S, 0.706 mg/L (95% CI was from 0.151 to 3.203) for Nanofer STAR, 1.020 mg/L (95% CI was from 0.683 to 1.445) for Fe^{2+} and 5.834 mg/L (95% CI was from 4.190 to 9.189) for Fe^{3+}. The wide 95% confidence interval curves for Nanofer 25S and Nanofer STAR indicate a large uncertainty meaning more data in the future should be obtained to increase the CI. The sub lethal concentration (SLC), which is one fourth of the LC_{50} value for Nanofer 25S, Nanofer STAR, Fe^{2+} and Fe^{3+} were 0.101, 0.176, 0.255 and 1.458 mg/L, respectively.

INTRODUCTION

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. 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. 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 [1]. 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 [2]

In order to determine the relative toxicity of chemicals to living organisms, Probit analysis, a specialised regression model of binomial response variables comes in handy and is widely used. The response of test organisms to different concentrations of toxicants is always binomial, resulting in two outcomes, either death or no death. An S-shaped curve is usually obtained as the relationship between the response and various concentrations is sigmoid. With Probit analysis, the sigmoid dose-response curve is transformed into a straight line before further analysed by running regression on the relationship. Important parameters previously mentioned, namely the LC_{50}, LD_{50}, EC_{50} or ED_{50} along with their confidence intervals (CI) could be identified. After regression, the resulting data from Probit analysis can be utilised to compare the quantity of toxicant required to produce similar response. Researchers can choose various end points to

compare the toxicities of different chemicals though LC_{50} and LD_{50} continue to be the most frequent end point chosen [1].

There are three possible techniques that can be applied to determine LC_{50}; manual estimation by referring to a Probit table [3], hand calculating the probits, regression coefficient and CI [4] or computer software calculations. SPSS, SAS, R or STATA are some of the frequently used software, where automatic conversion of percent responded to Probits can be attained. Nevertheless, since calculation by hand relies on the least square method, the accuracy is not as high as compared to computer software that uses the maximum likelihood method. Thus, software is considered the best and more precise technique to determine LC_{50} [3]. Confidence interval (CI) provides us with an array of probable values that might contain the true value of an unknown population parameter. More often than not, the 95% confidence interval is used [5]. In certain cases where the 95% confidence interval value failed to be reported, researchers can utilise the published results by re-evaluating them using software like SPSS that employ the more accurate maximum likelihood method, [6].

This paper intends to perform a recalculation of the LC_{50} values from the dose response effect curves of nano-zero valent iron to the freshwater zooplankton herbivore, *Daphnia magna* [7] using SPSS software. This is a published work where the LC_{50} values were not available from the publication. The 95% confidence curves of the data will also be produced and reported in this paper. An important outcome of obtaining a more accurate LC_{50} value is the ability to estimate the sub-lethal concentration, which is about one fourth of the value. The sub-lethal concentration can be utilized to study toxicological parameters at the biochemical and molecular levels.

MATERIALS AND METHODS

Data from Figure 8A to 8D from [7] were downloaded and processed using the software Webplotdigitizer 2.5 [8] which digitizes the scanned figure into a comma separated data. This method has been utilized by many researchers and acknowledged for its reliability [9,10]. The generated comma separated data were then inputted into IBM SPSS version 25.0 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) to undergo Probit regression procedure. The dose variable values along with 95% confidence interval values corresponding to an array of probabilities were calculated.

RESULTS

The results of the Probit modelling exercise using SPSS gave an LC_{50} value of 0.405 mg/L (95% CI was from 0.047 to 0.953) for Nanofer 25S (**Fig. 1**), 0.706 mg/L (95% CI was from 0.151 to 3.203) for Nanofer STAR (**Fig. 3**), 1.020 mg/L (95% CI was from 0.683 to 1.445) for Fe^{2+} (**Fig. 5**) and 5.834 mg/L (95% CI was from 4.190 to 9.189) for Fe^{3+} (**Fig. 7**). The wide 95% confidence interval curves for Nanofer 25S (**Fig. 2**) and Nanofer STAR and (**Fig. 3**) indicate a large uncertainty meaning more data in the future should be obtained to increase the CI. The sub lethal concentration (SLC), which is one fourth of the LC_{50} value for Nanofer 25S, Nanofer STAR, Fe^{2+} and Fe^{3+} were 0.101, 0.176, 0.255 and 1.458 mg/L, respectively.

A visual comparison of whether the one preparation is more sensitive to another can be done by looking at the CI in the form of a chart (**Fig. 2**).

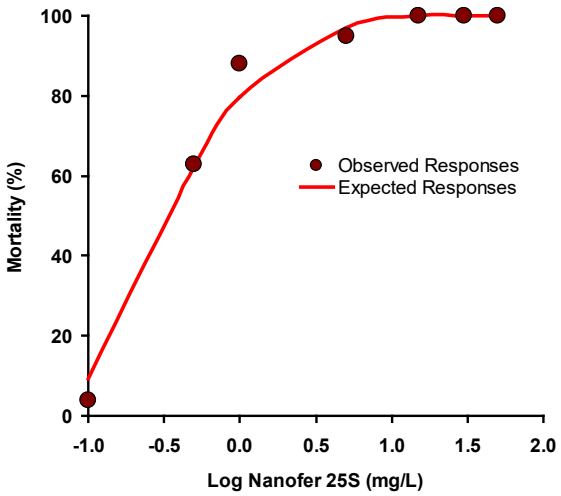

Fig. 1. Nano-zero valent iron Nanofer 25S 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

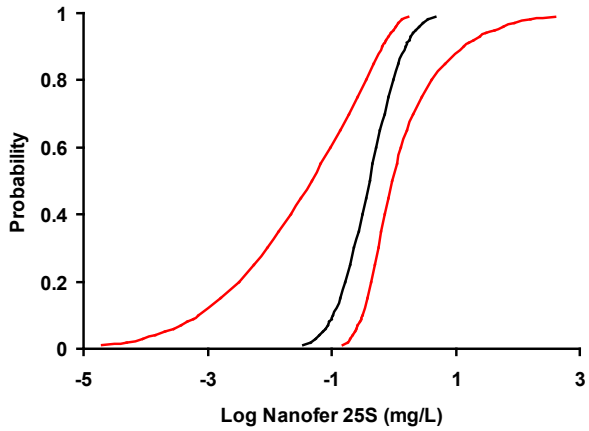

Fig. 2. 95% Confidence interval curves probability plot for the nano-zero valent iron Nanofer 25S 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

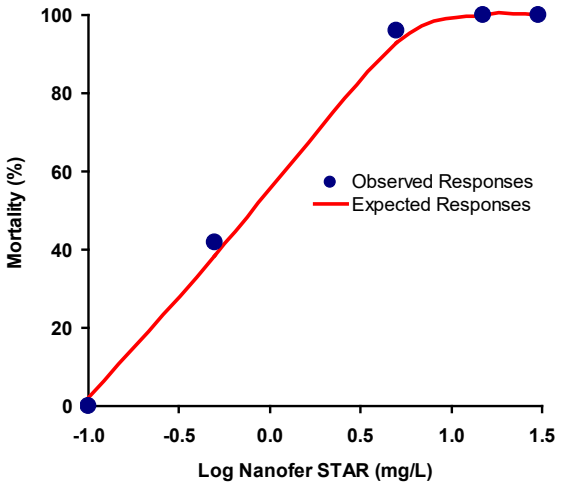

Fig. 3. Nano-zero valent iron Nanofer STAR 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

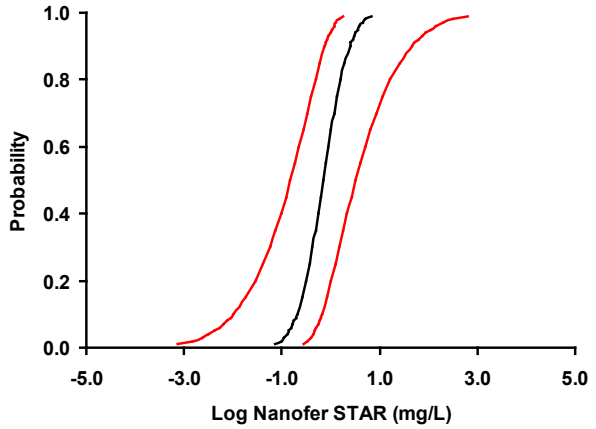

Fig. 4. 95% Confidence interval curves probability plot for the nano-zero valent iron Nanofer STAR 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

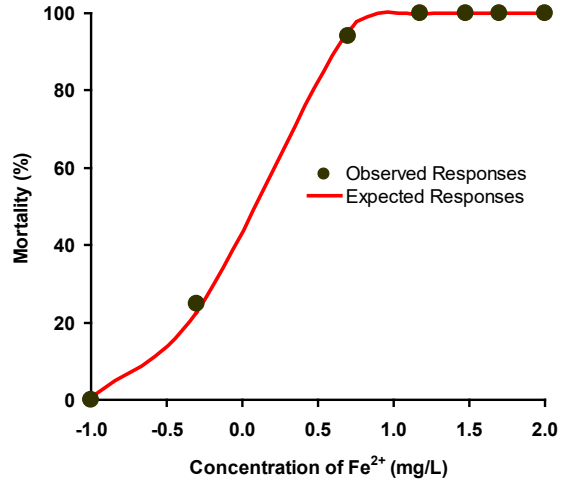

Fig. 5. Ferrous iron 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

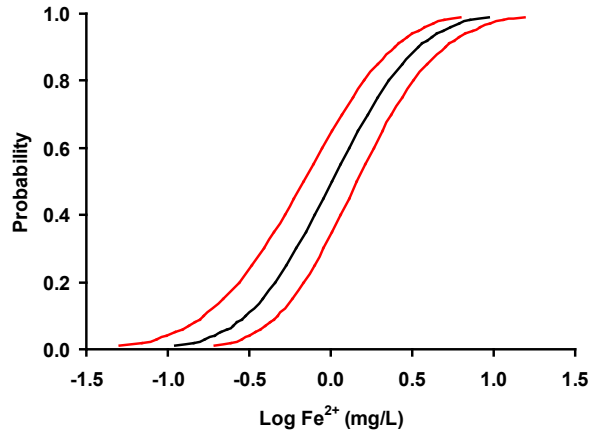

Fig. 6. 95% Confidence interval curves probability plot for ferrous iron 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

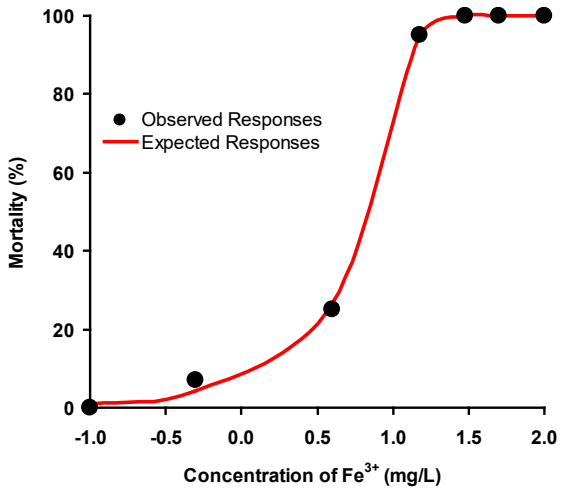

Fig. 7. Ferric iron 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

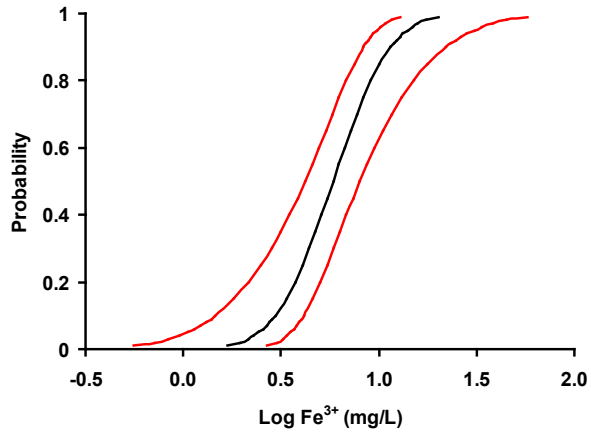

Fig. 8. 95% Confidence interval curves probability plot for ferric iron 96-h predicted mortality dose response curve for *Daphnia magna* based on parameter estimates from the Probit analysis.

A confidence interval chart for nano-zero valent iron and iron ions showed that all of the nano iron forms and ferrous ions are more toxic to *D. magna* compared to Fe^{3+} while there is an overlap in confidence interval for the rest (**Fig. 9**).

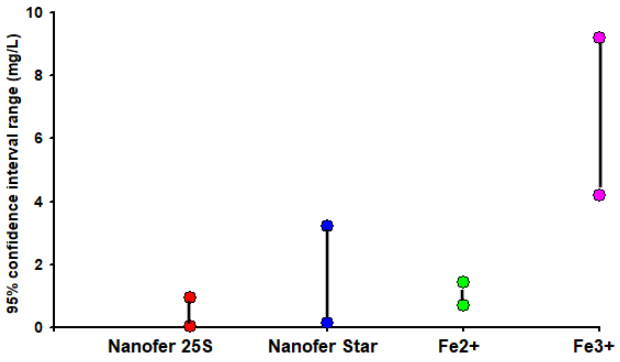

Fig. 9. Confidence interval chart for nano-zero valent iron and iron ions.

DISCUSSION

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. 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 [11]. 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 [3].

The confidence interval values obtained in this study are important statistical output that can be used to evaluate whether one is more significantly sensitive to another result. The significant difference can be looked by the overlapping or nonoverlapping of the 95% confidence interval which sets the significant value at the $p<0.05$ level. A nonoverlap CI value between two means or results indicate significant difference. On the other hand, overlapped confidence interval is more complicated since it does not necessary shows variation or not significant in differences at the $p<0.05$ level. What is needed is more data and study to assess the non-significance of overlapped confidence interval [12].

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 [13–15].

Based on this study, it is proven that Probit modelling exercise via SPSS software 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.

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