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Estimation of LC_{50} and its Confidence Interval for the Effect of Ferrous Sulphate on *Catla catla*

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ABSTRACT

Probit analysis is generally employed in the studies of toxicology, to determine the concentration of toxicant causing 50% mortality or LC_{50} value. The response of an organism is generally binomial and is typically sigmoidal in property. The Probit value can be calculated by hand or can be calculated through the use of a computer via software, the latter utilising the maximum likelihood method which is a more precise estimation of the parameters. When a published result failed to produce the 95% confidence interval, the results can be re-evaluated using software including SPSS. In this study, the LC_{50} value of the effect of ferrous sulphate to the fish *Catla catla* is re-evaluated using the Probit modelling exercise via the SPSS software, which gave an LC_{50} value of 8.271 p.p.m., with a 95% confidence interval from 7.353 to 9.189 p.p.m. The sub lethal concentration (SLC) for ferrous sulphate, which is one fourth of the LC_{50} value was 2.06 p.p.m

INTRODUCTION

In toxicology, Probit analysis is frequently utilized to ascertain the comparable toxicity of chemicals to various organisms. The response of an organism to several concentrations of toxicants is usually binomial; death or no death and this response is usually sigmoidal in property. In Probit analysis, a transformation from the sigmoidal curve to a linear curve is followed by a regression on the relationship and important parameters such as EC_{50} (concentration of a toxicant that gives half-maximal response) or LC_{50} for liquid and LD_{50} for solid (amount of toxicant that causes the death of 50% (one half) of a group of test animals) are obtained together with their confidence interval.

Once a regression is run, the researcher can use the output of the Probit analysis to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differing toxicities of chemicals, but the LC_{50} (liquids) or LD_{50} (solids) are the most widely used outcomes of the modern dose-response experiments. The LC_{50}/LD_{50} represent the concentration (LC_{50}) or dose (LD_{50}) at which 50% of the population responds.

The LC_{50} value can be estimated manually using a Probit table [1], which can also be calculated by hand [2] or can be calculated through the use of a computer via software such as SPSS, STATA, SAS or R, where the percent responded to Probits is converted automatically. The calculation by hand uses the least square method which is not accurate as the maximum likelihood method often employed by software that churn out a more precise estimation of the parameters for a more precise evaluation of the results obtained [1].

In the event where a published result failed to produce the 95% confidence interval, the results can be re-evaluated using software which take advantage of the more accurate maximum likelihood method. Software including SPSS will be able to furnish the values including the 95% confidence interval [3]. The aim of this paper is to recalculate the manually produced LC_{50} values from the dose response effect curves of copper and ferrous

sulphate to *Catla catla* [4] using the SPSS software. In addition, the 95% confidence curves for the results will also be generated.

MATERIALS AND METHODS

Date from Tables 1 from [4] were utilized for Probit regression procedure using SPSS (IBM SPSS v 25). The procedure fits a Probit sigmoidal dose-response curve and calculates the dose variable values (with fiducial confidence intervals or 95% CI) which correspond to a series of probabilities.

RESULTS

The results of the Probit modelling exercise using SPSS gave an LC50 value of 8.271 p.p.m. (95% CI was 7.353 to 9.189) (**Fig. 1**). The sub lethal concentration (SLC) for ferrous sulphate, which is one fourth of the LC_{50} value was 2.1 p.p.m. The data shows acceptable fitting with two or more points presenting as potential outliers. In addition, the 95% confidence interval curve for the resulting Probit modelling is also shown (**Fig. 2**).

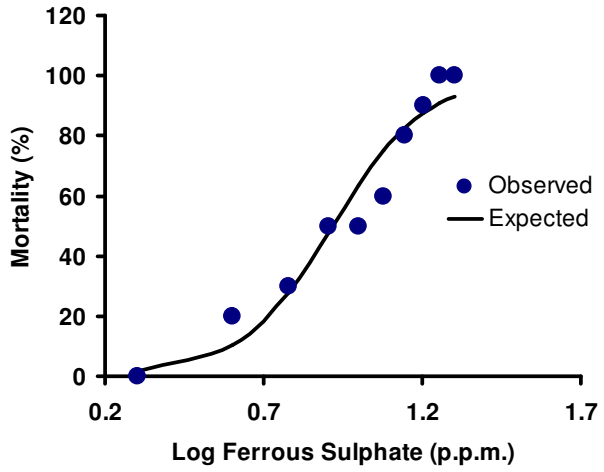

Fig. 1. Ferrous sulphate 96-h predicted mortality dose response curve for *Catla catla* based on parameter estimates from the Probit analysis.

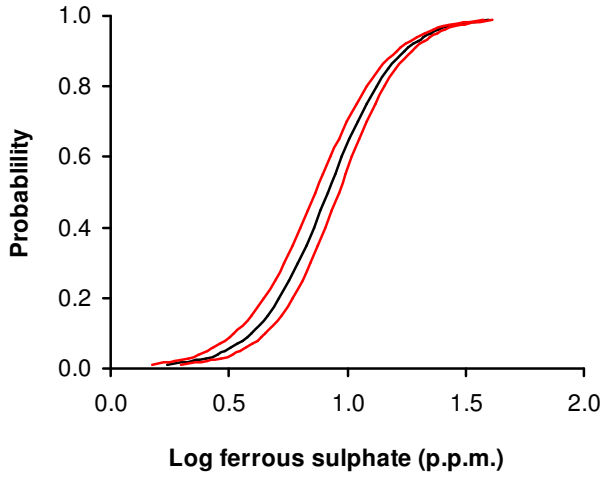

Fig. 2. 95% Confidence interval curves probability plot for the ferrous sulphate 96-h predicted mortality dose response curve for *Catla catla* based on parameter estimates from the probit analysis.

DISCUSSION

Chester Ittner Bliss an entomologist in 1934 thought of the idea of probit analysis. While studying for an effective control of insects using pesticide [5]. He discovered that the relationship of response to the insecticide dose was sigmoidal in nature. Since nonlinear regression was a prohibitive endeavour due to the absence of computing platform, he suggested that the data should be transformed into to a straight line. However, to scientifically distinguished the effect of different pesticides to the same insect some of statistical background is needed, of which he is lacking. Enter David Finney, a professor of statistics at the University of Edinburgh in 1952 expands Bliss' idea and provide statistical support for the idea and published the results in his book entitled Probit Analysis [1].

In comparison, the LC_{50} value reported by Bose et al. [4] was 8.4 p.p.m. without giving the 95% confidence interval. The sub lethal concentration (SLC) for ferrous sulphate, which is one fourth of the LC50 value was given as 2.1 p.p.m [4]. In light of the new finding of this work, the SLC should be 2.06 p.p.m, which is quite close to the published value. The importance of the confidence interval value is that it can be used to compare the effect of another metal or treatment to the same fish species by statistically analysing the confidence intervals produce and will be important in numerous studies on the effect of toxicants to the mortality of fish [6–16].

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