

Confidence limit calculation for antidotal potency ratio derived from lethal dose 50

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the method makes it easier to do the calculation using most of the programming software packages.

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Abstract

AIM: To describe confidence interval calculation for antidotal potency ratios using bootstrap method.

METHODS: We can easily adapt the nonparametric bootstrap method which was invented by Efron to construct confidence intervals in such situations like this. The bootstrap method is a resampling method in which the bootstrap samples are obtained by resampling from the original sample.

RESULTS: The described confidence interval calculation using bootstrap method does not require the sampling distribution antidotal potency ratio. This can serve as a substantial help for toxicologists, who are directed to employ the Dixon up-and-down method with the application of lower number of animals to determine lethal dose 50 values for characterizing the investigated toxic molecules and eventually for characterizing the antidotal protections by the test antidotal systems.

CONCLUSION: The described method can serve as a useful tool in various other applications. Simplicity of

INTRODUCTION

To characterize toxic effects of poisons and overdosed drugs, acute toxicity testing methods were developed in the beginning of the 19th century. Trevan^[1] first wrote up the concept of lethal dose 50 (LD₅₀) (medium lethal dose) in 1927. He also indicated that LD₅₀ is not a biological constant, its precision depends on many factors (*e.g.*, number of animals used, sex, species, strain, age, diet, general health condition, route of administration, stress, formulation, intra and inter laboratory variations, *etc.*). To express acute toxicity, LD₅₀ is a good tool, and many government agencies still rely on these data. Many methods have been developed to characterize the toxic effects (acute toxicity) of chemicals, and expressed as LD₅₀ values and its 95% confidence limit and the slope of the probit line, *e.g.*, Litchfield and Wilcoxon^[2], Bliss^[3], Holland *et al*^[4]. Up until the 90s, the Litchfield and Wilcoxon^[2] method was one of the most frequently used tool for toxicologists to characterize acute toxicity, and the *in vivo* antidotal efficacy of various antidotal systems.

An example, Pei *et al*^[5], analyzed data for LD₅₀ values of paraoxon that is an organophosphorus (OP) type nerve agent, in the presence of various antidotal systems by the method of Litchfield and Wilcoxon, as adapted to

a computer program PHARM/PCS version 4.2. by Bergol'ts *et al*^[6]. The antidotal potency ratios (APRs) derived from the dose-response curves of paraoxon were used to express the *in vivo* efficacy of various OP antidotal systems to antagonize the lethal effects of paraoxon (APR = LD₅₀ of paraoxon antagonized/LD₅₀ of paraoxon unantagonized). Tests for the parallelism of the dose-effect curves were done, and all statistical procedures were performed at the 95% confidence level. The authors used six groups of animals, 8 animals per groups (48 animals) for each LD₅₀ value.

Since the Litchfield-Wilcoxon method requires a large number (40-50) of animals, efforts were done to introduce other LD₅₀ determinations with a lesser number of animals. The up-and-down methods by Dixon^[7], Bruce^[8] can provide adequate estimation of LD₅₀ and approximation of the 95% confidence interval by using as few as 6-9 animals. When this method was compared with the traditional Litchfield-Wilcoxon method, excellent agreement was obtained for all the 10 molecules tested.

Another example: Petrikovics *et al*^[9], determined LD₅₀ values for paraoxon by the method of Dixon^[6], and 95% confidence limit was estimated by the method of Bruce^[10]. For each experiment, 6-10 animals were used. The LD₅₀ values were calculated from the equation of $\text{Log}(\text{LD}_{50}) = \log(\text{dose final}) + k \log(d)$ where *dose final* is the final dose administered, *k* is the tabular value from the table, and *d* is the interval between doses. APRs were expressed as a simple number (without confidence limit). APR = mean LD₅₀ of paraoxon antagonized/mean LD₅₀ of paraoxon unantagonized.

Another example: Petrikovics *et al*^[11], determined LD₅₀ for cyanide by the up-and-down method of Dixon^[7]. This method requires settings for the starting doses and the stage distances (dose difference between doses) for each test system. The software (based on "Implementation of Dixon and Massey UPD", Introduction to statistical Analysis, 1983, pp.434-438) provides information for the next dose for each stage, based on the mortality results for the given stage. The log dose difference of 0.1 was set up based on the earlier studies with cyanide (Petrikovics *et al*^[12], where the LD₅₀ values were determined by the classic Litchfield-Wilcoxon^[2] method. For each LD₅₀ values 10-18 were used. LD₅₀ values were expressed as average \pm 95% confidence limit by the software. APRs were expressed as a ratio of average LD₅₀ of cyanide with antidotes and average LD₅₀ of cyanide without antidotes. Again, confidence limits for APR were not expressed.

MATERIALS AND METHODS

In a situation like this where the distribution of the ratio is unknown, it is difficult to calculate the confidence intervals using classical methods. However, we can easily adapt the nonparametric bootstrap method which was invented by Efron^[13] to construct confidence intervals in such situations like this. The bootstrap method is a resampling method in which the bootstrap samples are ob-

tained by resampling from the original sample. A comprehensive coverage of the bootstrap method can be found in Efron and Tibshirani^[14], Chernick^[15], Shao and Tu^[16], Davison and Hinkley^[17], Manly^[18] and Hayden^[19] are also useful references.

There are several ways of calculating bootstrap confidence intervals. Briefly, one way of calculating the bootstrap confidence interval for APR given below:

To assume that the data set is coming from two samples which we call sample 1 and sample 2 to calculate the APR.

(1) Obtain a bootstrap sample $X^* = (X_1^*, X_2^*, \dots, X_{n1}^*)$ from the original sample 1 $X = (X_1, X_2, \dots, X_{n1})$.

(2) Calculate logLD₅₀ dose estimate using

$$LD_1^* = \frac{\sum X_i^*}{n_1} + \frac{d}{n_1} (A_1 + C_1)$$

[page 389 Dixon (1969)];

(3) Obtain a bootstrap sample $Y^* = (Y_1^*, Y_2^*, \dots, Y_{n1}^*)$ from the original sample 2 $Y = (Y_1, Y_2, \dots, Y_{n1})$;

(4) Calculate logLD₅₀ dose estimate using,

$$LD_2^* = \frac{\sum Y_i^*}{n_2} + \frac{d}{n_2} (A_2 + C_2)$$

(5) Calculate the ratio,

$$APR^* = \frac{10^{LD_1^*}}{10^{LD_2^*}}.$$

(as the values are in log base 10);

(6) Repeat step 1 through step 5, a large number of times ($B = 1000$) to get a list of values;

(7) Find the quantiles $APR_{(\alpha/2)}$ and $APR_{(1-\alpha/2)}$ for the list of *B* ratio values. $(APR_{\alpha/2}, APR_{1-\alpha/2})$ is the 100 $(1 - \alpha)\%$ confidence interval for the ratio. This confidence interval is usually called percentile bootstrap confidence interval.

RESULTS

We illustrate the method for LD₅₀ ratio for the following two experiments (Figure 1). Figure 2 shows the histogram of the bootstrap distribution of the APR. Quantiles of this distribution are used to derive the relevant confidence limits. In our illustration here we use the 95% confidence limit. LD₅₀ dose estimate for the first experiment is 7.834 and the LD₅₀ dose estimate for the second experiment is 23.812. This gives the APR to be 0.32897. Therefore, the lower confidence limit, which is the 2.5th percentile of the bootstrap distribution is 0.25821 and the upper confidence limit which is the 97.5th percentile of the bootstrap distribution is 0.41714.

DISCUSSION

We used a simple method to construct the confidence interval for calculating APR derived from two LD₅₀. The described nonparametric bootstrap method to determine confidence intervals can easily be constructed even in situations where the distribution of the ratio is un-

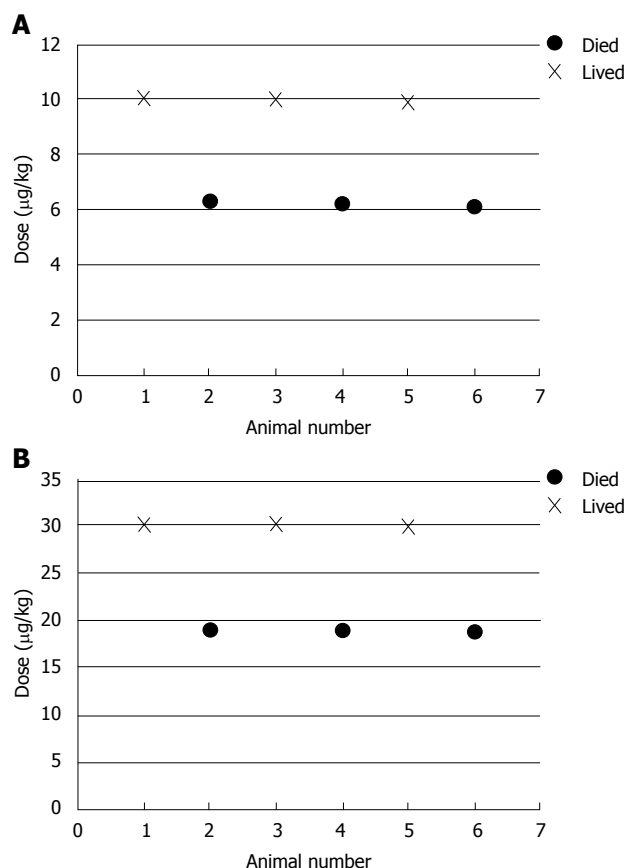


Figure 1 Graph of the dose and the outcome (lived or died) for the first (A) and second (B) experiment.

known. This presentation describes a calculation of the bootstrap confidence interval for APR. This can serve as a substantial improvement for toxicologists, who are directed to employ the Dixon up-and-down method with the application of lower number of animals to determine LD_{50} values for characterizing the investigated toxic molecules and eventually for characterizing the antidotal protections by the test antidotal systems. The described method can serve as a useful tool in various other applications. Simplicity of the method makes it easier to do the calculation using most of the programming software packages.

COMMENTS

Background

To characterize toxic effects of poisons and overdosed drugs, acute toxicity testing methods were developed in the beginning of the 19th century. To express acute toxicity, lethal dose 50 (LD_{50}) is a good tool, and many government agencies still rely on these data. Many methods have been developed to characterize the toxic effects (acute toxicity) of chemicals, and expressed as LD_{50} values and its 95% confidence limit and the slope of the probit line. To characterize antidotal efficacy of a given antidotal system, antidotal potency ratios (APRs) are calculated, that is the ratio of the LD_{50} of the toxic chemical with the test antidotal system and the LD_{50} of the toxic chemicals without any antidote(s) (control). The higher is the APR, the better is the antidotal system.

Research frontiers

When applying the classic Litchfield-Wilcoxon method for LD_{50} determination, a large number of animals (6-8 groups of animals, 6-8 animal/group = 36-64)

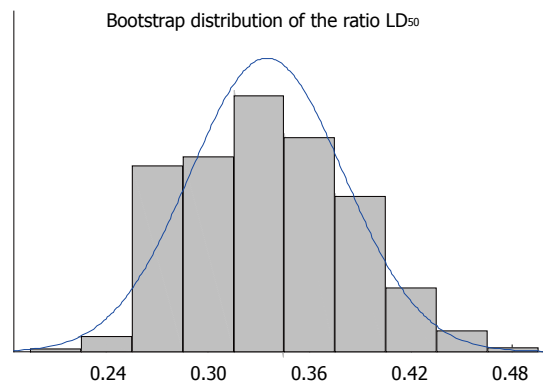


Figure 2 Bootstrap distribution of antidotal potency ratio. LD_{50} : Lethal dose 50.

are needed. To reduce the number of animals, new methods were developed, and the Dixon up-and-down method has become popular with its lower number of animal needed (8-18 animals/ LD_{50}). However, when the Litchfield-Wilcoxon method was adapted to a computer program PHARM/PCS version 4.2. by Tallarida and Murray, the APR was automatically expressed with 95% confidence limits by the software. There is a need for the 95% confidence limit determination with the Dixon up-and-down method when expressing APR values. Although Bruce provided adequate estimation for it, this article introduces a more practical tool for filling this gap.

Innovations and breakthroughs

Previous methods to characterize acute toxicity and/or determining antidotal efficacy for antidotal systems needed to be transformed in order to (1) reduce the number of animals used for LD_{50} determination (2) calculate 95% confidence limits for APR with lower number of animals used. This article can serve as a substantial help for toxicologists, who are directed to employ the Dixon up-and-down method with the application of lower number of animals to determine LD_{50} values for characterizing the investigated toxic molecules and eventually for characterizing the antidotal protections by the test antidotal systems.

Applications

The described method can serve as a useful tool in various other applications. Simplicity of the method makes it easier to do the calculation using most of the programming software packages. Authors used a simple MATLAB code to illustrate the confidence interval for the given example.

Terminology

LD_{50} is the dose that kills 50% of the tested animal population. $APR = LD_{50}$ of the toxic chemicals in the presence of the test antidotal system(s)/ LD_{50} of the toxic chemical without any antidote(s) (control). APR is used to express in vivo efficacy for antidotal systems. Bootstrap method is a standard technique in which we take simple random samples with replacement from the original sample. With this, overlapping samples is permissible in this technique. Strength of the paper is the application of the bootstrap method to calculate a confidence interval for the LD_{50} ratio. Validation of the method proven practically and theoretically in the literature.

Peer review

This is a good practical method to express 95% confidence limits for APR derived from the Dixon up-and-down method.

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