Toxicology and Applied Pharmacology 314 (2016) 12–23

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Single toxin dose-response models revisited

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ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 17 October 2016
Accepted 7 November 2016
Available online 12 November 2016

Keywords:
Daphnia mortality acute tests
Hill equation
Mortality function
Logit
Probit

ABSTRACT

The goal of this paper is to offer a rigorous analysis of the sigmoid shape single toxin dose-response relationship. The toxin efficacy function is introduced and four special points, including maximum toxin efficacy and inflection points, on the dose-response curve are defined. The special points define three phases of the toxin effect on mortality: (1) toxin concentrations smaller than the first inflection point or (2) larger then the second inflection point imply low mortality rate, and (3) concentrations between the first and the second inflection points imply high mortality rate. Probabilistic interpretation and mathematical analysis for each of the four models, Hill, logit, probit, and Weibull is provided. Two general model extensions are introduced: (1) the multi-target hit model that accounts for the existence of several vital receptors affected by the toxin, and (2) model with a nonzero mortality at zero concentration to account for natural mortality. Special attention is given to statistical estimation in the framework of the generalized linear model with the binomial dependent variable as the mortality count in each experiment, contrary to the widespread nonlinear regression treating the mortality rate as continuous variable. The models are illustrated using standard EPA *Daphnia* acute (48 h) toxicity tests with mortality as a function of NiCl or $CuSO_4$ toxin.

1. Introduction

Modeling of the dose-response relationship and involved methodological issues has a long history, Fisher (1935), Berkson 1944, 1951) , and Finney (1947, 1971), to name a few. Four types of models have been developed over the years to characterize mortality rates as a function of toxin concentration: Hill, logit, probit, and Weibull. By dose-response relationship we understand the mortality probability, m among organisms exposed to a toxin with concentration, x, during a constant short period of time, typically referred to as sigmoid function. For example, in standard acute tests the number of responded (dead) organisms k_i in a beaker with toxin concentration x_i initially having n_i (typically, $n_i = \text{const}$) organisms alive is counted after 48 h of the exposure where $i = 1, 2, \ldots, N$ is the number of beakers/experiments, USEPA (2002). The dose-response relationship, or in our case the mortality function, specifies the probability that an organism dies within 48 h,

$$\Pr(\text{individual dies within 48 h}|x) = m(x; \boldsymbol{\theta}),$$

where $\boldsymbol{\theta}$ is a vector of parameters to be estimated from the mortality data. The number of death counts k_i among n_i individuals in the beaker with toxin concentration x_i follows a binomial distribution with probability $m(x_i; \boldsymbol{\theta})$. This fact gives rise to estimation of parameters $\boldsymbol{\theta}$ using the method of maximum likelihood.

Although there exists a rich literature on the dose-response relationship it is scattered across the disciplines of epidemiology, toxicology and pharmacology. It is no wonder that the terminology and emphasis varies among these disciplines sometimes leading to confusion. The goal of this paper is to systematically describe classic models for dose-response relationships using mathematical definitions and introduce some new general concepts to help discriminate between models and identify the appropriate fields of application. Although much of the focus in the current literature is on multiple toxins, we feel that the success in these fields has been obstructed due to the lack of solid and rigorous establishment of the dose-response theory for single toxins.

This paper offers a rigorous study of the sigmoid mortality functions with a single toxin and provides concrete formulas for computing special points on the mortality curve. Special attention is given to the appropriate methods of estimation using the method of maximum likelihood (ML). We show how mortality data can be analyzed with the statistical package `R` using the simple a built-in function `glm` or a ML user-contributed `R` code for more complicated mortality functions.

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http://dx.doi.org/10.1016/j.taap.2016.11.002
0041-008X/

The bulk of the work in toxicology concerns computation and analysis of LC_{50} (or in another notation EC_{50}), like in a recent paper by Jiang and Kopp-Schneider (2015). The impetus of the present paper is our belief that more insights into the mechanism of toxicity can be gained by considering other points on the mortality curve such as where the effect of the toxin concentration changes form weak to strong or reverse, referred to as inflection points.

2. Classic dose-response relationships

Since the dose-response relationship is understood as the probability $m(x)$, dying within short test period of time due to toxin exposure with concentration x, it must satisfy certain conditions, assuming that function m is continuous and differentiable.

The properties of the mortality function:

(a) $m(0) = 0$ meaning that no animals die within the short test period in the absence of the toxin;
(b) function $m(x)$ is an increasing function, i.e. higher toxin concentration lead to increased mortality rate; and
(c) $\lim_{x\to\infty} \; m(x) = 1$ meaning that all organisms die when the toxin concentration becomes indefinitely high.

All mortality functions discussed below satisfy these properties. These three conditions imply that mortality probability is bound within 0 and 1, or $0 \le m(x) \le 1$. This means that any probability cumulative distribution function (cdf) of a nonnegative random variable may serve as a mortality function. For example, the cumulative distribution function of the gamma-distribution as a mortality function was suggested by Altshuler (1981).

An important characteristic of a toxin is the ability to cause mortality. Following the rules of basic calculus, we consider the instantaneous increase of mortality, Δm, due to increase of toxin concentration from x to $x + \Delta x$. It is important to take into account the concentration level, x itself. Indeed, if $x = 1$ and $\Delta x = 1$ we are looking at the increase in mortality when concentration doubles. When $x = 10$ and $\Delta x = 1$ the concentration increases by 10% and therefore the implied rate cannot be compared with the rate at $x = 1$. When mortality is studied on the relative scale the following definition is justified.

Definition 1. The efficacy function, E, at the level concentration x is defined as

$$E = \lim_{\Delta x\to 0} \frac{\Delta m}{\Delta x/x} = \frac{dm}{d(\ln x)}. \tag{1}$$

Following this definition we compute the instantaneous mortality change due to the relative change of the concentration, $\Delta x/x$. Since the change of the toxin concentration should be computed on the relative scale the log transformation seems natural. Toxin efficacy defined by Eq. (1) allows the comparison of toxin concentrations within the mortality curve as well as across the curves. If the mortality function is viewed as cdf expressed in $\ln x$ then the efficacy function is the density function, the milestone concept of the probability theory.Geometrically, the efficacy function is the slope of the tangent line at the mortality curve plotted versus toxin concentration on the log scale.

This definition justifies expressing and plotting the mortality function and E on the log scale as $m(\ln x)$ and consequently $E(\ln x)$. The fact that it is better to plot mortality data on a log scale is well known: our definition of the efficacy just provides a theoretical justification. Since $m(0) = 0$ and $m(\infty) = 1$ elementary calculus confirms that $\lim_{x\to 0} E(\ln x) = \lim_{x\to\infty} E(\ln x) = 0$, so that E reaches its maximum at some point (see below).

2.1. Four special points of the dose-response curve

First, we discuss four special points on the general sigmoid mortality curve and then illustrate them with four popular dose-response curves.

The median lethal concentration, LC_{50} (sometimes denoted as ED_{50} or EC_{50}) is the toxin concentration x that leads to 50% of the population death, or in mathematical terms the solution of the equation $m(x) = 0.5$. This point on the mortality curve is a popular way to express the killing strength of the toxin. Dose-response relationships are often symmetric around LC_{50} on the log scale; the formal definition follows.

Definition 2. The mortality function on the log scale, $m = m(\ln x)$, is symmetric around $\ln LC_{50}$ if

$$m(\ln x - \ln LC_{50}) = 1 - m(\ln LC_{50} - \ln x). \tag{2}$$

This definition is similar to the definition of the symmetry of distribution of random variable expressed via cumulative distribution function, in our case m, given by Evans and Rosenthal (2004). We will illustrate this in more detail later.

Below we introduce three other special points in terms of the toxin efficacy using the E curve as described above; see Fig. 1 for the illustration.

Definition 3. The first inflection point on the E curve is where convexity changes to concavity, or in mathematical terms, this point is the least solution of the equation

$$\frac{d^2E(\ln x)}{d\ln x^2} = 0. \tag{3}$$

The point of the maximum efficacy is the solution of the equation

$$\frac{dE(\ln x)}{d\ln x} = 0. \tag{4}$$

The second inflection point is where concavity changes to convexity, and mathematically is the greatest solution of Eq. (3).

Inflection point Eq. (3) has two solutions: the smaller solution corresponds to the first inflection point, where the efficacy curve has maximum slope, and the larger solution corresponds to the second inflection point, where the efficacy curve has minimum slope. Eq. (4) merely tells how to find the maximum point on the E curve: take the derivative and set it to zero (the first-order condition for maximum). If the efficacy function is viewed as the density function of the normal distribution with mean μ and standard deviation σ (probit dose-response, see below) inflection points are $\mu \pm \sigma$, where the slope of the density is maximum in absolute value.

The efficacy function may be viewed as a rigorous definition of the visual perception of a sigmoid curve on the log scale: the maximum efficacy point is where the mortality function has the steepest slope; the first inflection point is where the slow mortality growth turns into a rapid growth and the second inflection point is where the growth slows down.

For mortality functions symmetric around LC_{50}, the maximum efficacy occurs at LC_{50} and the first and second inflection points are symmetric around LC_{50}. This means that four special points reduce to