



Original article

Computational tools for fitting the Hill equation to dose–response curves

Sudhindra R. Gadagkar^{a,*}, Gerald B. Call^b^a Department of Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, AZ 85308, USA^b Department of Pharmacology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308, USA

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ABSTRACT

Introduction: Many biological response curves commonly assume a sigmoidal shape that can be approximated well by means of the 4-parameter nonlinear logistic equation, also called the Hill equation. However, estimation of the Hill equation parameters requires access to commercial software or the ability to write computer code. Here we present two user-friendly and freely available computer programs to fit the Hill equation – a Solver-based Microsoft Excel template and a stand-alone GUI-based “point and click” program, called HEPB. **Methods:** Both computer programs use the iterative method to estimate two of the Hill equation parameters (EC₅₀ and the Hill slope), while constraining the values of the other two parameters (the minimum and maximum asymptotes of the response variable) to fit the Hill equation to the data. In addition, HEPB draws the prediction band at a user-defined confidence level, and determines the EC₅₀ value for each of the limits of this band to give boundary values that help objectively delineate sensitive, normal and resistant responses to the drug being tested. **Results:** Both programs were tested by analyzing twelve datasets that varied widely in data values, sample size and slope, and were found to yield estimates of the Hill equation parameters that were essentially identical to those provided by commercial software such as GraphPad Prism and *nls*, the statistical package in the programming language R. **Discussion:** The Excel template provides a means to estimate the parameters of the Hill equation and plot the regression line in a familiar Microsoft Office environment. HEPB, in addition to providing the above results, also computes the prediction band for the data at a user-defined level of confidence, and determines objective cut-off values to distinguish among response types (sensitive, normal and resistant). Both programs are found to yield estimated values that are essentially the same as those from standard software such as GraphPad Prism and the R-based *nls*. Furthermore, HEPB also has the option to simulate 500 response values based on the range of values of the dose variable in the original data and the fit of the Hill equation to that data.

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1. Introduction

Dose–response studies typically produce data that manifest as a sigmoid curve when a response is plotted against dosage (Fig. 1). A common inference done from such a curve is the estimation of the dose at which 50% of the subjects show the desired response. This is usually done by means of the four-parameter logistic nonlinear regression

model (Eq. 1), modified from the original equation developed by A.V. Hill to quantify the binding of oxygen to hemoglobin (Hill, 1910)

$$\hat{Y} = a + \frac{(b-a)}{\left[1 + \left(\frac{c}{X}\right)^d\right]} \quad (1)$$

where \hat{Y} is the expected response at dosage X , a is the minimum asymptote or the response when dosage = 0, b = the maximum asymptote or the stabilized response for an infinite dosage, c is the dosage at which 50% of the subjects are expected to show the desired response (that is, the response halfway between the minimum response asymptote a and the maximum response asymptote b); it also denotes the point of inflection in the dosage–response curve, and is referred to by various terms (e.g., EC₅₀, ED₅₀, LD₅₀, IC₅₀), and d is the slope at the steepest part of the curve, also known as the Hill slope. The model may be written to represent an ascending sigmoid curve of the type in Fig. 1 or a descending curve, depending on the sign of d . Specifically, positive d

Abbreviations: GUI, graphic user interface; HEPB, Hill equation with prediction band; ET₅₀, EC₅₀, elution time for 50% of flies, or effective concentration for 50% response, the point on the X-axis corresponding to the point of inflection in the sigmoidal dose–response curve, this also corresponds to the dose at which 50% of the subjects being tested show the response being studied to the drug; IA, inhaled anesthetic; RSS, the residual sum of squares; VBA, visual basic for applications; R², the coefficient of determination, which reflects the proportion of variation in the response variable that is accounted for by the independent variable.

* Corresponding author. Tel.: +1 623 572 3855; fax: +1 623 572 3647.

E-mail address: sgadagkar@midwestern.edu (S.R. Gadagkar).

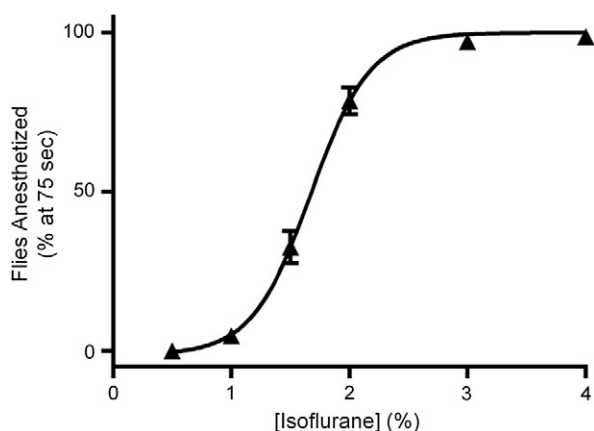


Fig. 1. The typical sigmoidal nature of a dose–response curve. This example shows the percentage of flies that are anesthetized at 75 s (mean \pm SEM; triangles) plotted versus the dose of isoflurane anesthesia given, and the Hill equation fit to the data using Graphpad Prism software (solid line).

values yield ascending curves while negative values yield descending curves.

Eq. (1) represents one of a family of Hill equations that have been used to describe specific non-linear relationships under diverse scenarios, including, but not limited to, quantitative pharmacology (Gesztelyi et al., 2012), ligand binding (Poitevin & Edelstein, 2013; Siman et al., 2012), plant growth modeling (Zub, Rambaud, Bethencourt, & Brancourt-Hulmel, 2012), and modeling patterns of urban electricity usage (To, Lai, Lo, Lam, & Chung, 2012).

Computer programs have been available since the early 1970s to estimate the parameters of different versions of the Hill equation, most of which are specific to fitting kinetic data (Atkins, 1973; Knack & Rohm, 1977; Leone, Baranauskas, Furiel, & Borin, 2005; Wieker, Johannes, & Hess, 1970). None of these uses Eq. (1) specifically, although commercial software exists that can be made to fit the four-parameter logistic curve in Eq. (1) (e.g., GraphPad Prism, www.graphpad.com; The MiraiBio Group of Hitachi Solutions at www.miraibio.com). Eq. (1) can also be fit to data using a computer program written using the open-access language, R, or the Solver Add-in in Microsoft Excel. In addition, some of these also permit the computation of confidence and prediction bands around the curve. However, the existing tools either require an investment in commercial software, which are also typically opaque to the user as to the code and algorithms used to generate the results, or require the ability of the user to write computer code in order to accomplish these tasks.

A long-term goal of the Call laboratory is to determine the mechanism of action of inhaled anesthetics (IAs), for which *Drosophila melanogaster* is used as the model system for providing in vivo responses to IAs in the presence of various genetic manipulations. *Drosophila* represents a good model for working with anesthetics as fruit flies follow the Meyer–Overton rule of anesthetics and display physiological responses to IAs similar to those in humans (Allada & Nash, 1993; Tinklenberg, Segal, Tianzhi, & Maze, 1991). Additionally, flies provide an inexpensive, yet robust model with access to a variety of genetic tools available to answer many scientific questions in vivo.

The Call laboratory has recently adapted an apparatus for the quantification of the *Drosophila* response to IAs (Dawson, Heidari, Gadagkar, Murray, & Call, 2013). Known as the inebriometer, it was originally designed to quantitatively measure the flies' response to ethanol vapors (Weber, 1988). This adaptation and modification of the inebriometer has enabled the rapid and efficient collection of simple quantitative data from large genetic screens. The inebriometer consists of a large column that is flooded with the IA. As the flies succumb to the IA, they elute out the bottom of the column and are counted. The Mean Elution Time

(MET) of the flies from the inebriometer column can then be computed, followed by standard statistical analysis (e.g., *t*-test).

In order to verify consistent inebriometer function, control flies are simultaneously assayed each day an experimental fly line is tested. In a genetic screen consisting of hundreds of experimental fly lines, this practice produces a large control dataset that presents a statistical problem: the Mean Elution Time when used with standard statistical tests is almost guaranteed to show a statistically significant difference between the experimental fly line being assayed and the control, simply due to the large numbers of flies used. Furthermore, the median test is also almost guaranteed to have low power due to the large sample sizes used; ~150 flies per assay. Therefore another approach was needed for the analysis of the genetic screen data. Since the raw fly elution data from the inebriometer was sigmoidal in nature, Eq. (1) was fit to the data, followed by the estimation of what we term the ET_{50} , which is analogous to EC_{50} , but represents the time, rather than the concentration, at which 50% of the flies elute from the inebriometer column. The ET_{50} value was then used as a measure of the flies' response to the IA. This is done by estimating the parameter c in Eq. (1), where X is the time it takes for Y percent of flies to elute through the inebriometer, a and b are the minimum and maximum asymptotes of the percentage of flies eluting through the system (0 and 100, respectively), and d is the Hill slope. Repeated assessments of the ET_{50} have shown it to be an efficient, direct and reliable indicator of the flies' response to various IAs.

Here we present two computer programs: 1) a macros-enabled, Solver-based Excel template developed in the Call laboratory, and 2) a stand-alone Windows based computer program, HEPB (Hill Equation with Prediction Band), designed and developed in the Gadagkar lab. The Microsoft Excel template with Visual Basic for Applications (VBA) macros uses the above formula and estimates the ET_{50} and the Hill slope (variables c and d in Eq. (1)) for the inebriometer data. This template utilizes the Solver tool that comes with Excel. Solver is an optimization tool that uses techniques from Operations Research and has wide applicability including regression analysis and curve fitting. However, neither the availability nor the operation of Solver is straightforward to the average researcher more familiar with the graphic user interface (GUI) of most statistical software typically used to perform this type of analysis. For this reason, and due to the large amount of data routinely collected and the large number of student users in the Call laboratory, a macro was written to automate this analysis within Excel. This made the task very easy and straightforward even for the novice user as the analysis was done simply by the press of a button after data entry (Fig. 2). Furthermore, the macro ensured consistency in the output for easy and accurate export of the data and results to the relational database (Microsoft Access) being maintained in the laboratory. The Excel macros proved to be very useful and convenient, and have become a staple in the Call laboratory.

However, while the Hill equation was easily fit to the data and the ET_{50} and Hill slope were determined quickly by the macros, the problem of meaningfully comparing an experimental line with the control still remained. In addition, an important goal of these assays was also to classify a given fly line as having a sensitive, normal or resistant phenotype to the IA. To help resolve both problems, that is, comparing an experimental line to the control and classifying the experimental line as one of the above three types, the stand-alone computer program, HEPB, was developed. HEPB has an easy-to-use GUI that, in addition to estimating the parameters c and d in Eq. (1), also computes the prediction band (at a given level of confidence) for the control fly data and solves for the X value when $Y = 50\%$ for each of the upper and lower limits of the prediction band. These form the cut-off values to objectively discriminate among sensitive, normal and resistant responses to a given anesthetic. These two limits each give the boundary value between sensitive and normal responses, and normal and resistant responses, respectively (Fig. 3). This is similar to standard statistical practice for a two-tailed test where the distribution under the null hypothesis is constructed, the critical regions delineated on either side of the curve, and

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