USDA Center for Veterinary Biologics

Statistics Section

Work Instructions

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Nonparametric Estimation of Median Effective Dose

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Contents

1 Median effective dose

In dose-response studies, the median effective dose (ED_{50}) is the estimated dose that produces a response in half the population. Particular types of dose-response studies are associated with specific forms of the ED_{50}. In accute toxicity studies, it is the median of a latent distribution known as the tolerance distribution (Section 2). The response is usually death, and it is termed the median lethal dose (LD_{50}). In vaccination-challenge studies, a median protective dose (PD_{50}) is estimated. It can be thought of as the median of an immunocompetence distribution, analagous to a tolerance distribution.[1]

Another 'dose 50' that should be mentioned for completeness is the tissue culture infective dose ($TCID_{50}$). In virus titrations, the $TCID_{50}$ is the dose that produces evidence of infection in half the wells to which it is applied. It is estimated in a similar way as the preceding measures, but unlike them the $TCID_{50}$ is not the median of an underlying latent

[1] A distribution of immunocompentences only makes sense if the response to challenge is constant in every individual. Since that is often implausible, the distribution is in fact a mixture of immunocompetences and susceptibilities.

distribution. It is simply the volume of the virus suspension that would contain an average of 0.7 infective virus particles (e.g. PFU).

In modern times, dose-response studies are usually handled by statistical modeling to estimate the dose-response curve. This has obvious advantages over the non-parametric measures discussed here. One of the most salient benefits is, of course, the estimation of the entire dose-response curve itself, rather than a single measure of one of its features, its center. Dose-response curves were not so easily estimated in the days before computers, and non-parametric estimators of ED_{50} were important historically. A glance at this document's references will show that the ones discussed here were initially published between 1908 and 1938.

Of the three, the Spearman-Karber estimator is still somewhat useful today for its valuable statistical properties. The Reed-Muench and Dragstedt-Behrens estimators are largely of historical interest. Their use is still surprisingly widespread, however, so it is worth being familiar with their mechanics. Users should be warned that they can be worse than inaccurate, they can produce meaningless results.

2 The tolerance distribution

In bioassays with binary response,[2] the probability of response is often thought to reflect an underlying latent distribution known as the tolerance distribution. An individual's tolerance is the smallest dose that produces a response. The tolerance distribution describes the distribution of tolerances in the population.

Consider for example an old fashioned 'kill-em-and-count-em' acute toxicicity assay, in which various doses of a toxin are applied to a test species. The response is death, and the probability of the response is conditional on the dose. The dose expected to produce a specified response probability is a quantile of the tolerance distribution.

For individual j let y_j denote its response, which is observed, and x_j its tolerance, which is not. Let d_i be the ith dose. Then the probability of a response is the probability that the tolerance is no greater than the dose: $\Pr(y_j = 1|d_i) = \Pr(x_j \leq d_i)$. The conditional response distribution is $y_j|d_i \sim \ BERN(\pi_i)$. The tolerance distribution is $x_j \sim \ f(\mu, \sigma^2)$, where $f(\cdot)$ is the PDF of a location-scale distribution.

The expectation of the conditional response distribution is related to the standardized tolerance distribution by

$$\pi_i = F\left(\frac{d_i - \mu}{\sigma}\right)$$

A binomial generalized linear model, $g(\pi_i) = \alpha + \beta d_i$, connects the response distribution to

[2] In the past, the term 'quantal' was often used for a binary response.

the tolerance distribution through the link function: $\pi_i = g^{-1}(\alpha + \beta d_i) = F\left((d_i - \mu)/\sigma\right)$. The estimated mean and variance of the tolerance distribution are then given by the regression parameter estimates, and $\mu = -\alpha/\beta$, $\sigma = 1/\beta$.

Logit and probit link functions correspond to logistic and normal tolerance distributions, respectively. Since they are symmetrical, their means and medians are the same: $m = \mu = -\alpha/\beta$. The complementary-log-log and log-log link functions correspond to extreme value distributions, which are asymmetrical. For those distributions, the median would be $m = \{\log(\log(2)) - \alpha\}/\beta$.

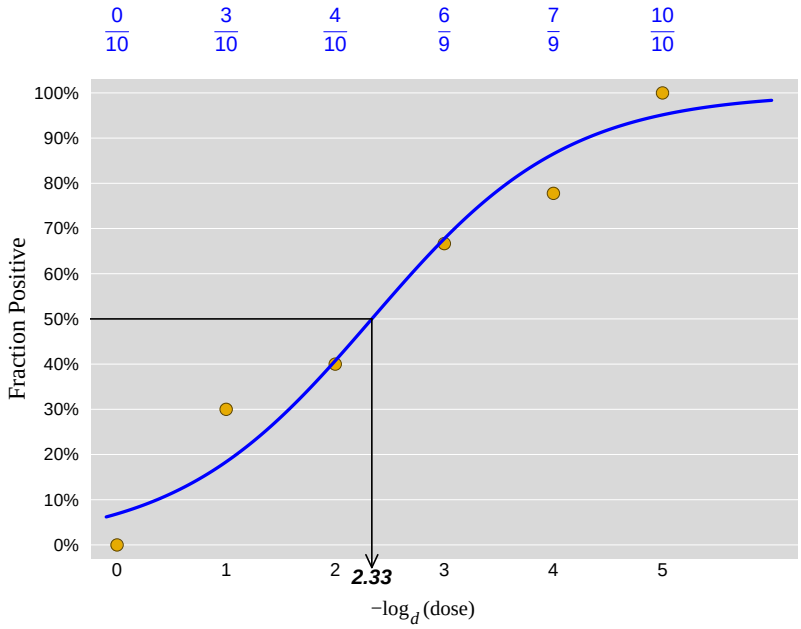


Figure 1: The tolerance distribution.

Figure 1 illustrates this relationship. The data shown at the top of the plot are the number of mice dying out of a group of mice administered a dose of toxin. Each point is a realization from a binomial response distribution that is conditional on the dose administered. The curve, estimated in this case with a logit link, represents the tolerance distribution. The ED_{50} is shown by the arrow.

3 Non-parametric estimators

Three of the non-parametric estimators most commonly used to estimate ED_{50} are described. The Spearman-Karber estimator is an explicit estimator of the mean, not the the median. The mean corresponds to the ED_{50} only for symmetrical distributions, of course,

and there is rarely enough data to evaluate this assumption in the types of experiments where it is used — it is an article of faith and hope. As an estimator of the *discretized* mean, it is uniform minimum variance unbiased and the maximum likelihood estimator.

The Reed-Muench and Dragstedt-Behrens estimators were intended as estimators of the median. Miller (1973) points out the surprising result that they are, in fact, asymptotically equivalent to Spearman-Karber and hence are estimators of the mean.

3.1 Spearman-Karber

The Spearman-Karber method (Spearman 1908; Karber 1931) gives a non-parametric estimate of the mean of a tolerance distribution from its empirical probability mass function (PMF). The observed data are thought to give an empirical estimate of the cumulative distribution function (CDF) of the tolerance distribution(Figure 2(a)).[3] The empirical probability mass function (PMF) is derived from the CDF by differencing (Figures 2(b)–(c)).

The estimator is $\sum (x \cdot f(x))$, the usual one for the mean of a discrete distribution, except that here $f(x)$ is the empirical PMF obtained from the observed data. This estimator depends on the complete distribution, which may not be available in a particular experiment. If the CDF does not cover the entire support of x, a common practice is to extend it by assuming the next lower dose would produce zero response and the next higher dose would produce complete response. Although this is not always a good idea, it is the default in the function `SpearKarb()`. While the tolerance distribution is not discrete, it is discretized by virtue of the interval censoring inherent in experiments of this type.

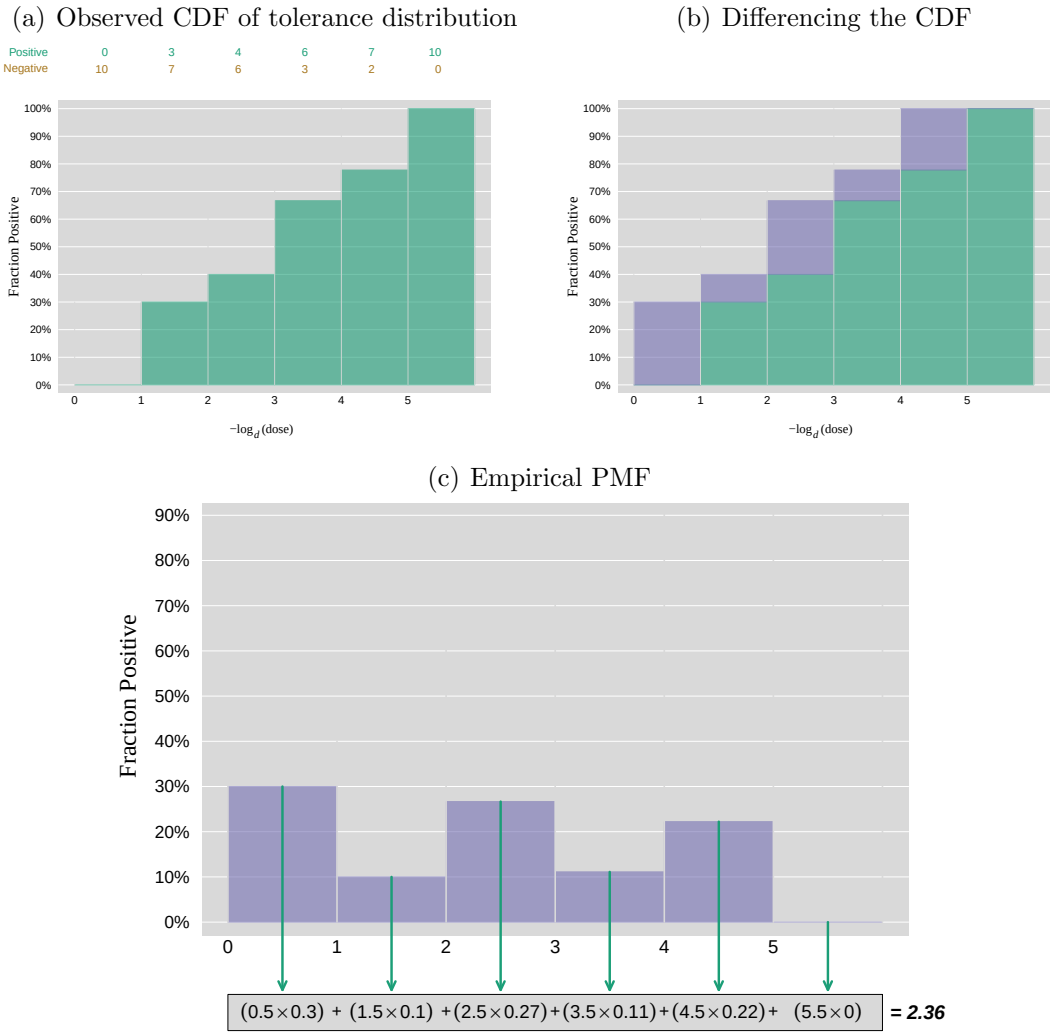
3.2 Reed-Muench

The Reed-Muench method (Reed and Muench 1938) takes a different approach. It begins with the belief that there is more information in the experiment than is given by the observed responses. Instead, we can assume that we know how some of the mice would have responded had they been given a different dose. A mouse that died at a lower dose would certainly die at a higher dose, and one that survived a higher dose would certainly survive a lower dose. This approach can only be considered quasi-statistical, since it treats observed responses as known constants rather than random variables. There is also the problem that some of the subjects contribute more information than others.

Here's how it works. Accumulate the sums in both directions that represent the hypothetical number that at each dose would have died or survived. The actual numbers of observed responses are shown in columns 1 and 2 of the table in Figure 3(a), and the hypothetical

[3]Note that the empirical CDF of the tolerance distribution estimated in a dilution experiment of this type is not the same as an empirical distribution function (EDF).

Figure 2: The Spearman-Karber method



number of known responses are shown in columns 3 and 4. (When the group sizes are unequal, as they are in this example, an adjustment is made in the cumulative sums that effectively averages the group sizes, as shown in columns 5 and 6.)

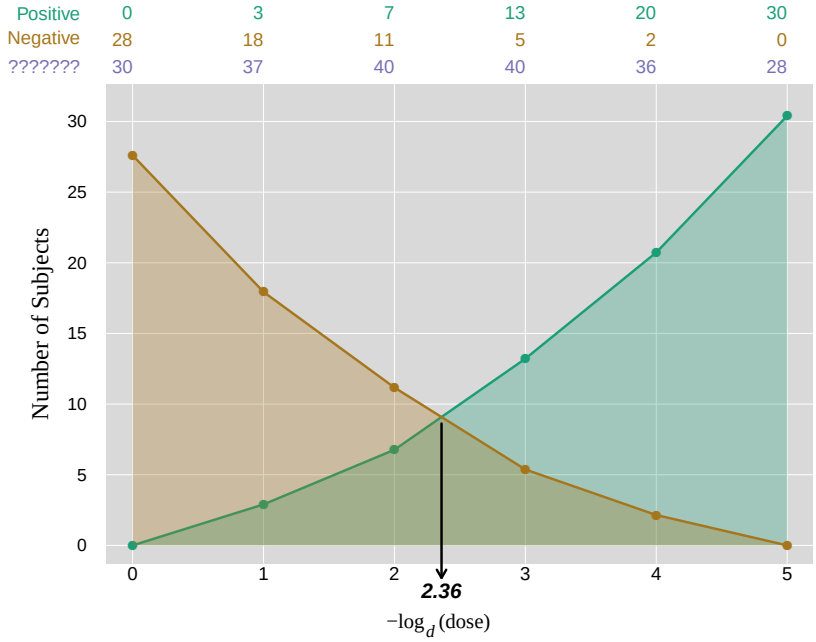
Next, find the doses that bracket the ED_{50}, i.e. the one where fewer than half of the hypothetical responses are positive and the one where more than half the hypothetical responses are positive. The ED_{50} is found by interpolation between the bracketing doses to find the dose at which the hypothetical responses would be equal. It is given by the intersection of the line that connects the hypothetical positive responses and the line that connects the hypothetical negative responses at the bracketing doses (Figure 3(b)).

Figure 3: The Reed-Muench method

(a) Observed responses (Pos, Neg), hypothetical responses (CumPos, CumNeg), adjusted hypothetical response (AdjCumPos, AdjCumNeg)

Pos	Neg	CumPos	CumNeg	AdjCumPos	AdjCumNeg
0	10	0	28	0.0	27.6
3	7	3	18	2.9	17.9
4	6	7	11	6.8	11.2
6	3	13	5	13.2	5.4
7	2	20	2	20.7	2.1
10	0	30	0	30.4	0.0

(b) Hypothetical responses



3.3 Dragstedt-Behrens

The Dragstedt-Behrens method (Dragstedt and Lang 1928; Behrens 1929) is very similar to the Reed-Muench method and is based on the same cumulative sums. For some reason, most microbiology textbooks present the Dragstedt-Behrens method under the name Reed-Muench method.[4]

Instead of working with the cumulative sums directly, the Dragstedt-Behrens method uses the fraction of the cumulative sums that are positive at each dose (Figure 4). The $ED_{50}$is estimated by interpolation on the line that connects the hypothetical fractions of the bracketing doses.

[4]For years I struggled to figure out why there were two distinct formulations of the Reed-Muench method, until Don Kolbe, a microbiologist in the CVB Bacteriology lab, pointed out that one of them was actually the Dragstedt-Behrens method.

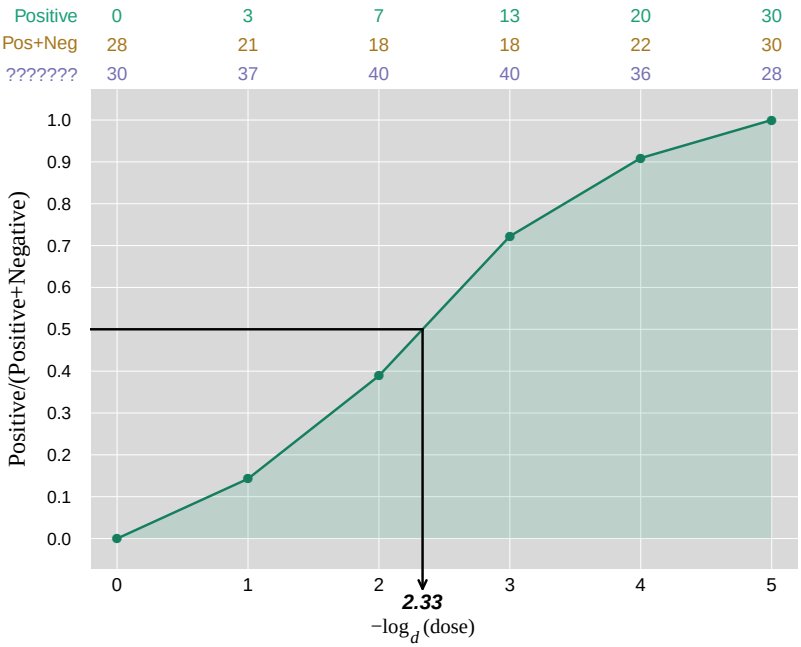


Figure 4: The Dragstedt-Behrens method

3.4 The skrmdb package

The skrmdb package provides functions for the three nonparameteric estimators described above. (See the package documentation for more details.) It is, admittedly, a bit comical to use a computer to perform simple calculations that were devised to avoid the need for difficult calculations. Nevertheless, these estimators persist, and the package is handy.

```
> require(skrmdb)
> # use data from skrmdb plots.r
> tmp <- data.frame(
+         y=c(0,3,4,6,7,10),
+         n=c(10,10,10,9,9,10),
+         x=c(0:5)
+         )
> #
> # fit the GLM
> fit <- glm(cbind(y,n-y)~x,binomial,tmp)
> ed <- -fit$coef[1]/fit$coef[2]
> #
> # Spearman-Karber estimate
> skx <- SpearKarb(cbind(y,n)~x,tmp)$ed
> #
> # Reed-Muench estimate
```

```
> rmx <- ReedMuench(cbind(y,n)~x,tmp)$ed
> #
> # Dragstedt-Behrens estimate
> dbx <- DragBehr(cbind(y,n)~x,tmp)$ed
```

The model fit gives the estimate $\hat{\mu} = -\hat{\alpha}/\hat{\beta} = 2.6066/1.1163 = 2.3350$. The table shows all the estimates.

	ED50
GLM Fit	2.335
Spearman-Karber	2.356
Reed-Muench	2.360
Dragstedt-Behrens	2.333

References

Behrens, B. (1929), "Zur Auswertung der Digitalisblatter im Froschversuch," *Arkiv fur Experimentelle Pathologie und Pharmakologie*, 140, 297–256.

Dragstedt, C. A. and Lang, V. F. (1928), "Respiratory Stimulants in acute poisoning in rabbits," *Journal of Pharmacology*, 32, 215–222.

Karber, G. (1931), "Beitrag zur kollektiven Behandlung Parmakogischer Reihenversuche," *Archiv fur Experimentelle Pathologie und Pharmakologie*, 162, 480–487.

Miller, R. G. (1973), "Nonparametric estimateors of the mean tolerance in bioassay," *Biometrika*, 60, 535–542.

Reed, L. J. and Muench, H. (1938), "A simple method of estimating fifty percent endpoints," *American Journal of Hygiene*, 27, 493–497.

Spearman, C. (1908), "The method of "right and wrong cases" ("constant stimuli") without Gauss's formulae," *British Journal of Psychology*, 2, 227–242.

Appendix

ED50 Formulas

skrmdb package

This appendix provides formulas for calculating ED50 estimates by the methods in the `skrmdb` package. See the vignette for the principles underlying the methods. Some relevant discussion may also be found in Finney (1964).

Notation

y_j	Number of positives at dilution j. Positives are those with increasing response.[1]
j	Indexes the data from 1 ... J, where 1 is the dilution with the lowest response (smallest number of positives), and J is the dilution with the greatest response.
n_j	Total number at dilution j.
$p_j = y_j/n_j$	Fraction positive at dilution j.
x_j	The 'dose' at dilution j. Most often it is the log dilution.
$d_j = x_{j+1} - x_j$	Difference of log dilution, for $j < J$.[2]
$a_j = \sum_{k=1}^{j} y_k$	Cumulative sum of the positives from the 'bottom up'
$b_j = \sum_{k=J}^{j} n_k - y_k$	Cumulative sum of the negatives from the 'top down'
$z_j = \dfrac{a_j}{a_j + b_j}$	Fraction of cumulative sums

[1] If the response is decreasing, either use the complementary response (e.g. affected rather than unaffected) or reverse the order of the data set.

[2] With a constant dilution factor, the log dilutions are evenly spaced. In that case, the subscript may be dropped and d is constant.

1 Dragstedt-Behrens

ED50 is estimated by $\tilde{x}_{DB}$, defined as the dilution at which $z = \frac{1}{2}$ The dilutions bracketing $\tilde{x}_{DB}$ are x_{low} and x_{high}. Find them by their corresponding z: $z_{low} = \max(\underset{\sim}{z} \leq 0.5)$; $z_{high} = \min(\underset{\sim}{z} > 0.5)$.

And the ED50 is found by interpolation along the z line segment connecting them.

$$\tilde{x}_{DB} = x_{low} + d_{low} \frac{\frac{1}{2} - z_{low}}{z_{high} - z_{low}}$$

2 Reed-Muench

ED50 estimated by $\tilde{x}_{RM}$, defined as the dilution at which $a = b$. Find the bracketing dilutions as for $\tilde{x}_{DB}$, but instead of interpolating on the z line segment, find the intersection of the a and b line segments.

$$\tilde{x}_{RM} = x_{low} + d_{low} \frac{b_{low} - a_{low}}{n_{low} - y_{low} + y_{high}}$$

It is easy to see that $\tilde{x}_{RM}$ and $\tilde{x}_{DB}$ are estimating the same thing, since $a = b \Leftrightarrow z = \frac{1}{2}$. However the estimates often differ slightly, since $\tilde{x}_{DB}$ is calculated by interpolation along a single line segment, while $\tilde{x}_{RM}$ is calculated by the intersection of two line segments.[3]

3 Spearman-Karber

The Spearman-Karber method requires that the p_j range from no response to complete response; i.e. $p_1 = 0\%$ and $p_J = 100\%$.

The ED50 estimate is

$$\hat{x}_{SK} = \sum_{k=1}^{J-1} (p_{k+1} - p_k) \{x_k - (x_{k+1} - x_k)/2 \}$$

In estimating the mean of the probability mass function, the first term in the summation is the estimated mass and the second term assigns it to the midpoint of the dilution interval.

Unlike the quasi-statistical Dragstedt-Behrens and Reed-Muench methods, the variance of the Spearman-Karber estimator can be calculated. It is:

$Var(\hat{x}_{SK}) = \sum_{k=1}^{J} \left\{ (x_{k+1} - x_k)^2 p_k (1 - p_k) \right\} / (n_k - 1)$

[3] See Figures 3 and 4 in the vignette

References

Finney DJ (1964). *Statistical Method in Biological Assay*, Second Edition, New York: Hafner Publishing. Chapter 20, particularly sections 20.8 (Reed-Muench) and 20.9 (Dragstedt-Behrens). (And yes, this is the penultimate 2nd edition.)