

Notes on fitting the Schild equation with CVFIT

(1) The case of full dose-response curves

When the dose-response curves all have well-defined maxima, the best procedure is to pool the curves after normalising them to the same maximum response. The normalisation can be done as follows.

- (a) Fitting the curves separately (fit mode 2) with some suitable equation (often the Hill equation will suffice).
- (b) Accept the option to normalise with respect to the fitted maxima
- (c) Re-fit the set of normalised dose-response curves with the same equation, using either fit-mode 4 to get dose-ratios, or, probably better, fit-mode 5 to fit the Schild equation directly to the set of dose-response curves. The latter procedure is illustrated in the Summer Course notes, and amounts to constraining the fitted curves to have the spacing defined by the Schild equation (as well as constraining them to be parallel). The result will, of course, correspond to an exactly straight Schild plot with a slope of 1. With this approach there is no need to do Schild plot at all, though it is probably sensible to do it anyway.

(2) The case where the dose-response curves have no well-defined maximum

If the dose-response curves have not got well defined maxima, but have an x value (agonist concentration) in common, you can use the option in CVFIT to normalise with respect to the response at a particular agonist concentration, and then proceed as above. However, good Schild data are unlikely to have such a common agonist concentration, so the following procedure will be better.

If the dose-response curves cannot safely be averaged, the Schild plot should be used.

(a) Each set of dose response curves should be fitted separately for estimation of dose-ratios, using fit mode 4. When only low agonist concentrations are used, the low-concentration limit of the Hill equation (the power function, equation 23) will often be a suitable thing to fit (see below for notes on the power function).

(b) Average the dose ratios for all experiments and calculate the standard deviation of this mean (the so-called “standard error”). Enter into CVFIT the *mean dose ratio* – 1 as the y value, antagonist concentration as the x value, and the standard deviation of the mean (smoothed if necessary) in the SD column, to be used for weighting the fit (NB do not take their logs before entering -the SD you have is for the non-logged value).

(c) Display the averaged (*dose ratio* – 1) data in log-log coordinates and fit with the power function (eq. 23). This is a straight line with a slope of n in log-log coordinates. Thus n is the slope of the Schild plot. Judge whether the line is sufficiently straight, and (from the estimate of n and its standard deviation) whether the slope is sufficiently close to 1 for you to believe that the Schild equation is valid.

(d) If you decide that the Schild equation is valid, then re-fit the (*dose ratio* – 1) with the Schild equation for estimation of the antagonist equilibrium constant, K_B . This means fitting with a straight line that has a slope fixed at 1. A new option in CVFIT allows this to

be done conveniently. If you choose eq. 1 (polynomial fit) with degree=1 (i.e. a straight line) you now get offered the option to fit the Schild equation to the original data (not in logged form). Choose this option and do a weighted fit; this fits a line to

$$y = (\text{dose ratio} - 1) = [B] / K_B.$$

There is only one free parameter, K_B , so you can get estimates of error directly for the fitted K_B value, as the usual approximate standard deviation, and/or as likelihood intervals for K_B .

Notes on the power function (eq, 23)

It is not uncommon to determine only the lower end of the dose-response curves, when doing a Schild analysis. Without maxima, it is not possible to fit Hill equations etc to the curves, and we often find that the low-concentration limit of the Hill equation is quite adequate. This is a power function which corresponds to a straight line in log-log coordinates.

There are all sorts of ways in which this could be parametrised, but the one used in CVFIT is

$$y = \bar{y} \left(\frac{x}{x_0} \right)^n .$$

The two free parameters are n and x_0 , the latter being the value of x when $y = \bar{y}$, and \bar{y} is the mean of all your y values, which is a constant determined by your data. It is not essential to include the scaling constant \bar{y} ; the reason for formulating it in this way is to make it easier to provide initial guesses for the value of x_0 , which is needed for fitting (though the current version of CVFIT does the guesses for you so you will only have to provide them manually if the automatically generated guess is bad). Since \bar{y} is roughly at the midpoint of your data, it is easy to eyeball the corresponding value of x . The value used for \bar{y} appears along with the fitted parameters, and, on the print out, the result is also expressed in its logarithmic form

$$\log_{10}(y) = a + n \log_{10}(x) ,$$

where

$$a = \log_{10}(\bar{y}) - n \log_{10}(x_0) .$$