April 6, 2000

SUPPLEMENTAL PROCEDURE TO DETERMINE SLOPE AND CI

Introduction:

The improved single sequence Up and Down Procedure (UDP) provides a reasonable estimate of the LD50. However, it does not provide an acceptable estimation of slope for the dose-response curve, or confidence intervals of LD_{50} and slope. Among others, the US needs, data on the slope of the dose response curve. At the OECD working group meeting last March the US agreed to attempt to develop a method to calculate slope and confidence intervals around the LD50 and slope. Because the original UDP procedure, which calls for several test doses after the first reversal of outcome, concentrates most of the doses near the LD50, it is not an efficient method for estimating slope.

Results were improved using two approaches involving a modified up and down testing procedure: (1) multiple sequence UDP runs, and (2) a hybrid approach, a combination of the initial up and down procedure and replicate doses at each of two or three doses, are presented in this summary document. To maximize use of already developed data, both revisions focused on a tiered approach and built on the values determined in the initial test for LD50. For this task, several approaches were tried using computer simulations.Tables summarizing all the simulations are presented in the Appendix with with arabic numbers; actual simulations are tabulated with roman numbers.

Each summary table shows, for comparison, "BEST CASE" simulations in which the correct LD50 and slope was used to assess the expected performance of two groups of 15 animals, dosed at each of LD13 and LD87. This simulation provides a standard for comparison of other simulations in the tables, although it can not be duplicated in the laboratory because It was assumed that the Investigator knew and used the correct LD50 and slope values to set the doses given. (See Best Case Simulation Table I).

All simulation trials, except the Best Case, utilized the estimated LD50 from the primary (tier I) single sequence UDP. Simulations involving one to two thousand trials each, were used to assess performance of animal populations with sigma 0.12, 0.5, 1.25, and 2, (and in some cases 0.25) corresponding to slopes of 8.3, 2.0, 0.8, and 0.5 (and 4). Tables focus on simulations that converged to estimates. In addition, actual dose and response data from the primary UDP approach were combined with additional data from the supplemental procedure (tier II) for calculation of slopes and LD50 values. Several dose selection procedures were simulated in an attempt to move toward the ideal dosing situation, but because the actual slope of the dose-response curve is not known when the doses are selected for study, it is difficult to devise selection rules that provide for the variety of possible slopes. Because this work was done simultaneously with development of the improved UDP, simulations for tier I were performed without use of the final stopping rule and with a nominal size of seven; i.e., the test was stopped when six additional animals had been dosed after the first reversal (death) occurred.

Early Trials to Determine Slope

In developing the optimized approaches, disciscussed above, preliminary simulations using the basic unmodified Up-and-Down procedure were performed and found not to provide adequate performance For completeness they are described here.

Slope Averaging From a Series of Up and Down Sequences:

Initially we attempted to use a series of UDP procedures and average the results of the individual estimates of slope (Simulation Tables VIII, IX). . This was an estimation approach developed in consultation by W. Dixon. The results of these simulations indicated that the estimate of slope depends critically upon the original assumed slope and are not accurate if the actual slope is considerably different from the assumed slope. In addition, because the basic UDP procedure concentrates most of the meaningful results near the LD50, continued work on this approach was deemed not useful for estimating slope.

Probit calculation Using Three Independent Up and Down Sequences:

Next, we used the same UDP procedure but pooled all the results from the three runs and developed an estimate of slope using a probit analysis (Simulation Table XII). This change also did not provide acceptable results because of the large number of doses administered very near the middle of the dose-response curve, in the region of the LD50, while the most efficient slope estimations are provided when dose-related partial kills are observed at doses on both ends of the dose-response curve.

Optimized Approaches

Hybrid Approach, Multiple Doses at Each of Two or Three levels Following a Single Up and Down Sequence:

The hybrid procedure uses groups of animals dosed at the tails of the dose-response curve. In these simulations we assumed a single UDP run was run first to obtain an estimate of the LD50 and then the subsequent doses (LD13, LD40, LD45, LD70 or LD87) were chosen based on that estimate together with an arbitrary assumed slope of 1. The procedure is summarized as the Hybrid approach and the results provided in Tables 1A, 2A, 3A, and 4A. Also see Simulation Tables II, III, and IV.

Various combinations of sample sizes and doses were simulated to test the performance of the hybrid approach combining information from the tier I UDP with responses from replicate groups of animals mainly dosed at the tails of the dose-response curve. After estimation of the LD50 using the tier I UDP, doses were selected from among LD13, LD40, LD45, LD70, LD87, calculated using an assumed slope of one. Data from tier I were also included in the analysis.
Multiple Independent Up and Down Sequences Using a Modified Dosing Procedure:

Finally, recognizing that even animal-efficient slope estimates require larger numbers of animals at the tails of the dose-response curve, we attempted to utilize a modified UDP-based procedure. For these simulations we assumed the dose-response curve would be symmetrical and to reduce the number of animals that would die during the test, we attempted to define only the bottom half of the curve. Additionally, to maximize the number of animals at the tails of the dose-response curve, we began each test either two or three sigmas (in this case sigma was assumed to be 0.5) below the LD50. Also, in order to make efficient use of animals, each run stopped when the first animal died; that is, a run of nominal size 2. This procedure ensures that testing is distributed along the dose-response curve and minimizes unneccessary doses near the LD50. To do otherwise would be less efficient in animal use with little or no return in information about slope. The simulations are described below (Simulation Tables V, VI) and results are presented in Tables 1B, 2B, 3B, 4B and 5.

3, 4, 5, and 6 sequences were tested with starting doses near two sigma units or three sigma units below the LD50 (as estimated by a single UDP). Starting doses were staggered or offset in order to minimize duplicate testing at any one dose level. These sequences were in addition to the UDP sequence used in tier I, however, data from tier I were included in the analysis. Starting doses at two sigmas below the estimated LD50 did not perform in an acceptable fashion and so thereafter, starting doses were set at 3 sigmas below. Results from all independent dosing sequences were pooled to estimate slope, LD50 and confidence intervals using probit analysis.

Results of Optimized Procedures

The attached Summary Tables 6, 7, 8, and 9 provide the results of these simulations, with results regarded as acceptable, based on combined evaluation of median slope value ($\leq \pm$ 5%), ratio of 95 percentile and 5 percentile (< 6, except for slope of 0.5 when < 10 was acceptable), and difference between highest and median values (difference < value of sigma for sigma of 0.12 and 0.5 and difference < twice sigma for sigma of 1.25 and 2), in light of similar results for the BEST CASE, are shown in boldface type.