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Considerations For Supplemental Procedure To Estimate Slope And Confidence Intervals

In order to design a procedure to yield estimates of slope and confidence intervals, a great many methods were tried by means of computer simulation. Performance criteria USED were the accuracy of the median LD50 and slope calculated and the 95/5% ratios for slope. For situations with very high slopes, the ratio of 95%/median slope prediction was found to be more reliable.

Three approaches were found to yield reasonable results: (a) multiple independent Up-Down dosing sequences, with fixed dose progressions of 0.5 log units and testing stopping after the first reversal of outcome (nominal sample size 2), (b) a hybrid procedure using groups of 5 - 10 animals at each of two or three doses in the tails and the mid-point of the dose-response curve, and (c) multiple independent Up-Down sequences with nominal sample size 2 but with variable dose progression factors ranging from 2 log units to 0.125 log units. Each procedure is meant to be supplemental to the primary tier I procedure used to determine LD50. For each case, results of supplemental testing were pooled and combined with data from the tier I analysis and probit analyses were performed to estimate slope, confidence intervals, and LD50.

The hybrid procedure, case (b), could not be optimized for both high slope and low slope situations. Setting multiple doses at each of LD13, LD40, and LD70 worked best for steep slopes (slope of 8.3). Setting multiple doses at LD13, LD45 and LD87 worked best for shallow slopes (slope of 2).

Procedure (a) performed well for simulations with assumed slopes from 2 to 8 and demonstrated efficient use of animals. The optimum procedure was to use 4 modified Up-Down sequences, each starting in the region of 3 standard deviations from the approximate LD50 determined in tier I (denoted 4,3). The starting doses were offset slightly to spread out dosing as much as possible. Additional independent sequences did not provide significantly improved performance. Two variations of this "4,3" method were tried: The first was to start all dose progressions below the LD50; the second was to start two dose progressions below and two above the LD50. They were found to be roughly comparable in performance. Starting all four sequences below the LD50 is likely to lead to fewer deaths in the test animals, whereas starting two sequences above and two below is slightly more efficient in terms of overall animal usage.

The procedure in case (c) used variable dose progressions to accommodate a wide range of possible slopes. It uses somewhat more animals, but may be warranted when chemicals are anticipated to have highly variable results. For example, although laboratory rats are inbred to minimize variability in response to xenobiotic chemicals, birds and other species chosen as surrogates for wildlife are generally outbred.

The modified 4,3 Up-Down procedure described in case (a) was chosen as the supplemental procedure for the draft 425 guideline since it performs well and is reasonably efficient in animal usage. The procedure with variable dose spacing described in case (c) was inserted as an alternate supplemental method in appendix IV.