The UDP Primary Test: Proposed Revision of the Guideline 425 "Primary Procedure" for Point Estimation of the LD50: Rationale for Design, Statistical Analysis, and Simulation Studies

Prepared for Review of Proposed Guideline 425 Revisions by the
Interagency Committee for Validation of Alternative Methods (ICCVAM)

David Farrar (USEPA), March 10, 2000

A Guideline 425 is being proposed for evaluation of mammalian acute toxicity to satisfy OECD member requirements. A previous version was examined together with several other OECD guidelines in March 1999. Revisions were undertaken as part of a general effort to address statistical issues and improve performance of the procedure. Elements of the Guideline 425 include a dose progression factor, the number of animals tested at each time and dose, and a formula and procedure for toxicity estimation. Proposed revisions as included in the proposal before the Panel include an increased dose progression factor, an increased slope value assumed in the estimation procedure (but a slope is still assumed), use of a likelihood-based stopping rule, and explicit language to ensure that test doses do not progress beyond a specific experimental range.

The following text develops a number of issues for consideration by ICCVAM. In addition, we we refer to ICCVAM the following overarching question: Is the most appropriate course of action to (1) use the guideline without the modifications proposed; (2) use the guideline with the revisions proposed; or (3) delay further use of the guideline until critical issues (to be identified by ICCVAM) can be resolved?

Contents.

1. Statistical Rationale for the Primary Procedure

1.1 Design

1.1.1 The Dixon-Mood procedure as modified for a restricted range of test doses.

The basic procedure of Dixon and Mood is adequately described in the Guideline so the description will not be repeated here. Appendix I of the Guideline defines some terms used here, in particular *reversal*, and *nominal sample size*. We follow the Guideline in using the term *progression factor* to denote the ratio of successive test doses.

We propose to restrict the test doses to values not exceeding 2000 mg/kg or 5000 mg/kg, depending on the regulatory context. In addition, in practice it will be appropriate to establish a lower bound, which may depend on the test substance: "Setting of lower bounds may need to include consideration of the ability to accurately dilute the test material." It is important that modifications of the procedure associated with bounding the range of test doses not "clash" with other features of the procedure, such as stopping rules or procedures for statistical analysis. We think this has been reasonably well confirmed by Monte Carlo simulations in which the true LD50 was varied, including LD50 values beyond bounds of 1 and 5000, and removed to various degrees above or below those bounds.

The essential procedure for restricting the range of test doses was suggested in discussions with Procter and Gamble. The stepping rule is similar to the rule for the unrestricted procedure, except that steps are among a finite set of permitted doses. Here we use the term *dose progression* (or just *progression*) to denote the set of permitted test doses ranked from smallest to largest. Also, let L (for lower) denote the lowest permitted dose and let U (for upper) denote the highest permitted dose. (Thus U=2000 mg/kg or 5000 mg/kg.)

It is proposed that the dose progression will comprise doses that could be tested with the basic, unrestricted procedure, except that (1) doses below L or above U are excluded; (2) L and U are included in the progression, although this may result in a progression for which some successive doses differ by a factor not equal to the progression factor; and (3) doses can be excluded if they are permitted by the unrestricted procedure and strictly within the bounds, but considered too close to L or U, relative to the progression factor.

The proposed "default" set of test doses (to be used at least when there is little prior information about the LD50) is to be "1.75, 5.5, 17.5, 55, 175, 550, 1750, 2000, or, for specific regulatory needs, 5000 instead of 2000." The default initial test dose is to be 175 units. Note that while the progression factor for this sequence is 3.2 (equal to 0.5 in the $\log_{10}$ scale), the two highest doses may differ by a factor of 2.86 (=5000/1750) or 1.14 (=2000/1750).

When some prior estimate is available for the LD50, it is proposed that the initial test dose should equal the prior estimate, divided by the progression factor. That approach is justified on the grounds of reducing suffering (because then testing tends to be concentrated below the LD50). Also, when the dose response curve is shallow there is some tendency for the estimate of the LD50 to be biased in the direction of the initial test dose. If a bias of this type occurs, and if

the initial test dose is selected below the LD50, the bias will be in the direction of a lower LD50 estimate.

Also, the stepping rule (the rule for determining the next dose, given results for the current dose), must be modified to accommodate restriction on the range of test doses. We have proposed that if the current test dose is strictly within the range of permitted doses (greater than L and less than U), the stepping rule is as for the unrestricted Dixon-Mood procedure except that steps are to adjacent doses within the progression, so that the ratio of successive test doses does not necessarily equal the progression factor.

If the current dose is U and the subject does not respond, we propose that the next dose tested will also be U, else the next dose tested will be the dose just below U in the progression (e.g., 3200 in a default progression with U=5000). Similarly, when the current dose is L and there is an adverse response, the next dose tested will also be L, otherwise the next dose tested will be the dose immediately above L in the progression.

1.1.2 Rule for stopping testing at a bounding dose.

According to the procedure just described, if the response probability is low at U (which occurs if the LD50 is much larger than U relative to the slope) or if the response probability is high at L (the LD50 much smaller than L's relative to slope) the bound value may be tested many times, unless this is prevented by a special rule. We propose that if the dose U is tested three times in sequence without a response then testing is stopped. Similarly, three tests in a row at dose L, with each of the three animals responding, results in the study being stopped.

There has been some discussion of how the LD50 should be estimated when testing is stopped based on this rule. One option is to decide in these cases that the LD50 is beyond the bound (<L or >U). This approach has been adopted in simulations. An estimate based on the probit model might or might not generate an estimate outside the bounds.

1.1.3 Use of a progression factor of 3.2.

The relatively large progression factor (3.2) was adopted based on discussions with Proctor and Gambel. It is thought that a relatively large factor is advantageous in situations involving little prior information, because that allows for the range of test doses to traversed in a relatively small number of steps. We also believe that a relatively large factor is appropriate when the dose-response curve is shallow, a type of situation of particular concern.

However it seems that, when there is actually a good prior estimate of the LD50, the use of a relatively coarse grid of test doses will result in some loss of accuracy. We believe that, in general, the up-and-down procedure cannot distinguish between LD50 values that differ by a factor lower than the progression factor. In particular, when the dose-response relationship is steep, most individuals may have tolerances between two test doses. In those cases testing may alternate between a dose with low response probability and a higher dose with high response probability. We have observed in simulations that as the probit slope is made more steep, the

estimates tend to converge on a set of values separated by a factor equal to the progression factor.

It appears that the selection of a dose progression factor involves striking some balance between different types of statistical effects. Noordwijk and Noordwijk (1988) provide an analysis of different types of bias in up-and-down testing, which appears to be useful in this context.

1.1.4 Variants of Up-and-Down testing.

We mention two variants of the up-and-down procedure which may be advocated but which have not been made the principal focus of the evaluation: (1) The dose progression factor may be varied within a single study. (Most likely, the initial step size in a study would be doubled or halved.) (2) More than one animal may be tested per step (e.g., Hsi, 1969). Both of these options have been investigated in some preliminary simulations, which were not organized into reports and distributed.

Neither of these approaches is dismissed. Increasing the number of animals tested per step can beneficial, by decreasing the number of steps and thus decreasing the duration of the study. If the study is carried out over too long a period in time, maintenance of experimental control may be difficult. For example the animals age and experimental conditions may drift. In particular, more animals may be needed for designs to estimate the probit slope, so such designs may need to involve multiple animals per step. It has also been pointed out that a design with multiple animals per step may be helpful in the event of an "outlier," as discussed in the section below on outliers.

However, if the initial test dose is poorly chosen, the result may be an initial series of results of the same type (either all response or all nonresponse). Then, if more than one animal is tested per step, the result can easily be an increase of the numbers tested by 3 or 4, with little information added. That increase would be a substantial percentage increase relative to a baseline of 6 animals (or a few more) per test. It may be desirable to increase the number per step only after a reversal has occurred.

In principle, it seems that the step size can be decreased when there is some indication that the up-down sequence has converged to the vicinity of the LD50 (e.g., after a reversal). Options that involve a variable progression factor were not a significant focus of the evaluation, because the primary concern has been the poor performance of the procedures when the dose-response curve is shallow. With a shallow dose-response, we think it is generally better for the dose-progression factor to be relatively large. Some early simulations (not developed into a report) considered the possibility of changing the progression from 0.5 to 0.25 (in the log scale). The results of those simulations actually suggested worse performance, relative to use of the same number of animals and a uniform progression factor of 0.5. In view of the concern for shallow-slope situations, more promising may be an approach in which the progression factor ranges up to 1.0.

1.2 Analysis

1.2.1 Use of the probit dose-response model.

The statistical procedures proposed are based on the probit model, for which the parameters are the LD50 and the slope. The probit model is customarily described in terms of a "tolerance distribution." It is supposed that each individual has a "tolerance" dose, which is the lowest dose that will affect that individual adversely. For the probit model, the tolerances are assumed to have a log-normal distribution. For some purposes it is more convenient to choose as parameters ***m***=$\log_{10}$LD50 and ***sigma*** = 1/ slope. Then, in the log scale (base 10), the mean of the tolerance distribution is ***m*** and the standard deviation is ***sigma***.

Some scientists will advocate consideration of alternatives to the probit model. In particular, the logit model, like the probit model, assumes a tolerance distribution that is symmetric in the log scale. The logit model would assume a higher proportion of individuals with relatively extreme sensitivity, and also more animals with relatively extreme lack of sensitivity, relative to the probit model. We do not hold that the probit model is the only possible dose-response model for analysis of acute test data, but exploration of alternatives was not considered the highest priority in the context of review of Guideline 425. Therefore we have relied on the probit model, which is conventional in toxicology.

1.2.2 Use of an assumed value for the probit slope.

In standard probit analysis, the two parameters of the probit model (the slope and the LD50) are both estimated from the data. The current guideline indicates that the LD50 will be estimated, with a value of 2 assumed for the slope. The review by Dixon Associates emphasizes that the same feature of up-and-down testing which makes the procedure work well for estimation of the LD50, namely that the approach concentrates the test doses close to the LD50, will tend to make the approach work poorly for estimating the slope.

Actually, in standard probit situations, it is sometimes not possible to estimate the slope. In particular, we do not have information on how well Guideline 401 performs for estimating the slope.

When evaluating variants of the up-and-down procedure, we have usually assumed the same value for ***sigma*** as used (in the log scale) for the step size. In particular, we use a step size of 0.5 in the log scale, and we use the same value for ***sigma*** when estimating the LD50 by maximum likelihood. It is known that the optimal choice of a step size for estimation of the LD50 is approximately sigma (see Dixon Stat. Assoc. 1991). However, application of that principle involves using information on slopes to select a step size. Here the choice of step size is not based primarily on information on the slope. Simulations suggest that in some situations results may be sensitive to the value assumed for slopes.

The use of an assumed slope is a feature of the study by Lipnick et al. (1995). That study is significant in the development of Guideline 425. In analyses with up-and-down data for specific chemicals, Lipnick et al. found little sensitivity of the LD50 estimate to the assumed value of

sigma, for ***sigma*** as high as 0.25 (slope as low as 4). Such comparisons with real data are highly desirable; however, the question always arises whether the data used will adequately cover the range of situations encountered in practice.

At present, no strong case can be made that default statistical calculations should assume some value for ***sigma***, or that they should assume the value 0.5 in particular. The strongest case that can be made is that such an approach may result in acceptable accuracy for estimating the LD50. We have not conducted a review of alternative approaches, except that limited evaluation has been conducted for a simple dose-averaging estimator.

1.2.3 Lack of a confidence interval for the LD50.

The traditional "fiducial" interval in probit analysis requires, as an intermediate computation, the fitting of the 2-parameter probit model, including estimation of the slope. We suppose that the standard interval can be adapted to the situation where the a value is assumed for the slope. That approach was not pursued because it was decided that the uncertainty in the LD50 depends on uncertainty in the slope, and may be underestimated when a slope value is assumed. At present no confidence interval is proposed for the LD50. Some consideration may be given to intervals based on likelihood (see Meeker and Escobar, 1995), a Bayesian approach, or some other approach to be identified.

1.2.4 Viability of a Bayesian approach to uncertainty in the slope.

In the long run, the possibility of handling the slope parameter based on Bayesian procedures should not be dismissed. For the slope parameter, this approach would combine the limited slope information from a specific study with external information, in the form of a prior distribution for the slope based on historical information. For the LD50, the prior would most likely be chosen to be relatively flat so that the estimate would be determined primarily by the data from the study, and little affected by the prior.

A Bayesian procedure may be particularly viable in this situation because (1) the data from an up-and-down study will often contain little information on the slope, for which an inference is nevertheless required if a parametric estimator is used; (2) a good basis (historical information) may exist for choosing a particular prior for the slope; and (3) external information would be used primarily for the slope, which for the primary procedure is a nuisance parameter rather than a parameter of direct interest. These features of the situation may allay objections to the introduction of external information. The approach would yield the Bayesian version of a confidence interval for the LD50.

1.2.5 Use of maximum likelihood, and measurement of statistical information.

Within the context of an assumed probit model, the proposed statistical procedures are based on *likelihood* (in the technical meaning of that term in statistics). In particular, the point estimate of the LD50 is taken to be the maximum-likelihood estimate (MLE), which is the dose value for which the likelihood is highest. Maximum-likelihood is usually viewed as the basis for estimating the LD50 parametrically, for conventional probit analysis as well as for up-and-down

testing. The likelihood we use is identical to that for conventional probit analysis for the 2-parameter probit model, except that the slope is fixed at 2 (***sigma*** is fixed at 0.5), so that the likelihood is a function of the LD50 only.

Somewhat less widely known than maximum-likelihood estimation is the closely related concept of statistical *information*, which we invoke to justify a particular type of stopping rule. This concept can be explained as follows. Note that the MLE exists when the likelihood function has a peak. Conversely, in the extreme case where the data is completely uninformative regarding a parameter of interest, the likelihood is flat. More generally, the curvature of the likelihood in the vicinity of the MLE is regarded as measuring the information the data contain, regarding a parameter of interest. The text by Edwards (1972) may be helpful with regard to these concepts.

In statistics, information is usually quantified using second order partials of the log-likelihood. We have used a simple ratio of likelihoods comparing the likelihood at an estimate of the LD50 to values fixed factors above and below that estimate. The resulting computations are easily carried out in a spreadsheet.

1.2.6 How test performance depends on the probit slope.

Simulations suggest that the most important influence on test performance is the steepness of the dose-response curve (e.g., magnitude of the probit slope). Steeper dose-response curves are generally associated with better performance. This can be seen as a case of a general statistical principle, which is that when the data are more variable, more data are needed to achieve a given statistical precision or power. In this context it is useful to note that the slope is inversely related to ***sigma***, which is the standard deviation of log tolerances. Of somewhat less importance than the slope is the choice of an initial test dose. The choice of an initial test dose is more important when the slope is shallow.

In analyses conducted for OECD, it has become customary to consider ***sigma*** values of 2, 1.25, 0.5, and 0.12 (or slope values of 0.5, 0.8, 2, and 8.33). (It can be helpful to consider some additional slope values in order to characterize the relationship between the slope and test performance.) In simulations we find that, despite considerable efforts to improve test performance, this range of slopes includes values for which the primary procedure will perform poorly. We suggest that as a rule the performance of the primary procedure will tend to break down when the slope is lower than some value in the range 2-3.

Given the spacing of category boundaries in the acute oral classification, it seems reasonable to be able to estimate the LD50 within a factor of 2. In simulations with LD50=600 units, initial test dose of 60 units, and our proposed likelihood-ratio stopping rule, it was found that there would be a 90% chance of an estimate within a factor of 2 of the true values, only if the slope is 2.6 or higher (Table 2 in the Feb. 24 simulation report). If the number of test animals is kept at 15 (the Guideline 401 requirement) or lower, it is probably not possible to reliably estimate the LD50 within a factor of 2, for the full range of slope values 0.5-8. If the up-and-down procedure is used with a fixed nominal sample size of 15, a slope of 2 or higher is required for a 90% chance of an estimate accurate within a factor of 2, for the scenario described above.

1.2.7 Rationale for a stopping rule with a variable nominal sample size.

Simple versions of up-and down testing called for termination of the experiment after a fixed number of animals have been tested, counting from the reversal. (Thus, the nominal sample size is fixed while the actual number tested may vary somewhat.) At the start of our evaluation, our "working" version of up-and-down testing involved a fixed nominal sample size of 6 and a step size of 0.5. Here, denote this approach SUDP/6/0.5, SUDP stands for simple up-and-down procedure.

SUDP/6/0.5 performs poorly in some situations, in terms of the bias and/or variability of estimates. Specifically, situations involving low slopes are problematic, particularly if the initial test dose is far from the true LD50. Use of this procedure therefore assumes that such situations are relatively uncommon in practice. To obtain reliable results in these situations would require testing of more animals. Unfortunately, it is difficult if not impossible to know when one is actually in this type of situation. A possibility would be simply to increase the nominal *n* "across the board." However, that would be wasteful for the situations where the procedure already performs well.

SUDP/6/0.5 keeps the number of animals tested fairly constant, while performance is variable (depending on the slope and starting dose). The purpose of an alternative stopping rule would be to reverse this situation: We would hope for the performance to be uniformly comparable to performance of SUDP/6/0.5, and somewhat better in the problematic situations. In situations where SUDP/6/0.5 performs well, an alternative should also perform well, without substantial increase in the numbers of animals tested. However, it is reasonable that the number of animals tested should go up where SUDP/6/0.5 performs poorly (situations which, we hope, are relatively uncommon).

We have developed a specific, simple stopping rule that appears to have the characteristics suggested. According to the approach proposed, the nominal sample size may vary from study to study, subject to a requirement that the maximum number of animals tested will not exceed 15 in a given study. (This constraint refers to the actual number tested, not to the nominal sample size.) In effect, testing is stopped based on a measure of statistical information, rather than based on a count of test units, as explained in more detail in the section following. The approach is simple enough to be easily implemented in a spreadsheet program, as indicated in a Guideline appendix. We have prepared a spreadsheet program using Microsoft ©Excel. To use the program, the user should need to do little more than enter the dose-response information as it accumulates.

With the approach proposed, performance is still poor in situations involving very low slopes, although much better in those situations than SUDP/6/0.5. However, it is probably unrealistic to hold that any up-down procedure will work well with such low slopes and at the same time keep the numbers tested at the low levels which give good performance in more "ordinary" situations. (What is really needed to address the possibility of very low slopes may be some crude information on the slope, e.g., a bound.)

In principle, it is better to design a study to achieve a fixed statistical error, rather than based on a fixed number of experimental units. If a confidence interval were available for the LD50, a reasonable approach might be to stop when the upper bound and lower bound differ by some factor (e.g., if the lower bound is not more than the lower bound times 4). However, in the context of simple up-and-down testing a confidence interval is not currently available.

In cases where 15 animals have been tested and the proposed stopping rule is not satisfied, it is proposed that testing will stop. Such an outcome may indicate an estimate of low reliability, because of a shallow slope and/or a poor choice of initial test dose. However, in simulations we find that in those situations, the stopping rules are often satisfied when fewer than 15 animals have been tested.

As a matter of policy we seek an approach that will work uniformly well for a wide range of slopes. We suggest that it is preferable *not* to depend on an argument such as "the test will probably work well in practice because situations where the procedure works poorly are expected to be infrequent." While any statistical procedure will have some frequency of false positives and false negatives, it is preferable for the error rates are to be kept uniformly low for a wide range of situations.

1.2.8 The proposed likelihood-ratio stopping rule.

Based on likelihood theory we expect that as data accumulates, the likelihood will display a more clearly defined peak. The maximum-likelihood estimate (MLE) of the LD50 or other parameter is the value where the likelihood is highest. As discussed, it is recognized in likelihood theory that the information available from the data can be measured based on the curvature of the likelihood function, close to the MLE.

We measure curvature using likelihood ratios, which compare the likelihood at an estimate of the LD50 to likelihoods above and below the LD50, by factors of 2.5. Higher likelihood ratios are taken to indicate that the LD50 estimate is more strongly supported by the data, relative to values distant from the estimate. (It is recognized in likelihood theory that likelihoods are compared via ratios, i.e., log-likelihoods are compared by differences.) Testing stops when both likelihood ratios achieve a critical value of 2.5. The stopping rule is not evaluated until the nominal sample size is 6.

This approach suggests that the estimate of the LD50 should be the MLE. However, the MLE requires iterative computations. In order to achieve more simple computations, we have substituted an alternative estimator, which can be termed a "dose-averaging estimator." This is simply the geometric mean test dose, calculated over the nominal sample (*cf*. Brownlee et al., 1953). (The number of dose values averaged is the nominal sample size.)

Close analogies can be drawn between the approach and other approaches:

1. The possibility of using a stopping rule based on some measure of information has been suggested previously for sequential designs, if not for the up-and-down procedure (Armitage, 1991).

2. The possibility was mentioned above of a convergence criterion based on the width of a confidence interval. A certain type of confidence interval is based on likelihood ratios of the type suggested (see Meeker and Escobar, 1995). That approach would be very computationally intensive, as it would require a line search for parameter values above and below the MLE for which a critical likelihood ratio is attained precisely. The approach can be simplified by noting that (at least if the likelihood is unimodal), requiring that the confidence bounds fall within a given factor of the MLE is equivalent to requiring that the critical likelihood ratio is exceeded, for values separated from the MLE by that factor. The latter is the approach proposed here.

In practice likelihood-based tests and bounds usually rely on asymptotic results. Those results might be questionable in our situation because of (1) the use of an assumed slope value; and (2) small sample sizes. Therefore if asymptotic results are used, it may be desirable to confirm their accuracy using simulations. However, it seems more straightforward to use simulations to justify a critical likelihood ratio directly.

1.2.9 Stopping based on "perfect alternation" of response and non-response.

We propose that testing can be stopped when the nominal sample size reaches 6, without evaluation of the likelihood-ratio rule, provided that there have been 5 reversals between response and non-response, with the nonresponses at a dose lower than the responses. We believe that in practice such an outcome will most often represent a situation where testing alternates between a dose with low response probability and a dose with high response probability, so that the LD50 is between the two doses. Also, the criterion will sometimes simplify the conduct of the study because the likelihood-based rule will not need to be evaluated in some cases.

We have not evaluated the frequencies of such perfect alternations when slope values are very low. Also, it is possible that the procedure will work well if, say, testing can be terminated if 4 reversals occur in a nominal sample size of 5, or 4 or more reversals occur in a nominal sample size of 6, and so on. These possibilities have not been evaluated.

1.2.10 Justification for numerical parameters in the stopping criteria.

The stopping criteria that we suggest involve several numerical parameters, which can potentially be adjusted to improve the performance of the procedures, in terms of better precision and/or fewer animals tested. These parameters include the maximum number tested (15), two parameters of the likelihood-ratio rule (both currently set at 2.5), the assumed slope (2), the rule for stopping at a boundary (3 of same response type at L or U). No strong justification can be provided at this time for the specific values we have proposed: We believe that simulations indicate that, taken as a whole, our procedures will result in improved performance. However, we cannot say at this time that other choices would not result in equivalent performance or better performance.

Before setting the maximum number tested at 15, we used a maximum of 25. Use of a maximum of 25 was felt to substantially increase in the numbers tested in some situations, with marginal improvement in accuracy.

A formal approach for optimizing the parameters of the stopping criterion would require assumptions regarding the relative value of increasing precision, versus reducing numbers tested. There would be no strong basis for any specific numerical weights for these two types of criteria. However, it could happen that some choices of parameters may simultaneously increase precision and lower the numbers tested. Therefore there may be some value in conducting a formal optimization in which equal weights are assumed (in some scale) for precision and numbers tested, despite the fact that the approach would involve some arbitrariness.

The following may be considered. First develop response surfaces that relate measures of precision, and also relate the numbers tested, to the probit slope and to the parameters that can be manipulated. For example, let $f(\text{slope},\theta)$ denote the probability that the estimated LD50 will be within a factor of 2 of the true value, where θ denotes parameters that can be manipulated. Let $g(\text{slope},\theta)$ denote the expected number of animals tested. Formulae for f and g can be obtained by fitting curves to output of Monte Carlo simulations, involving various combinations of the slope and θ. Having developed the surfaces f and g, determine the value of θ that minimizes an objective function such as

$$w_1 \mid f(1,\theta) - 0.9 \mid + w_2 \mid g(4,\theta) - 6 \mid$$

where w_1 and w_2 denote relative weights for precision and numbers tested. This expression says that the target precision is an LD50 estimate that is accurate within a factor of 2, with 90% probability, when the slope is 1 (a low value) and that the target for animal testing is an average of 6 animals when the slope is 4 (a moderately low value). The minimization of the objective function would probably involve a numerical approach. If the θ that minimizes the objective function results in better precision as well as fewer numbers tested relative to the current proposal, that choice would represents an unambiguous improvement.

1.2.11 Outliers.

There has been some concern among scientists regarding whether the simulation models adequately characterize how the performance of the procedure may be affected for the range of events that may occur in actual lab situations, when the numbers tested are drastically reduced.

To address this kind of concern, an "outlier scenario" has been simulated: The initial test was assumed to be below the true LD50 (here 750 units) by a factor of 10 or 100, and the first animal tested was assumed to respond, regardless of the probability of response calculated from the probit model. The idea is that such an event could result from background mortality, mishandling, or administration of an incorrect dose. (We hope these kinds of events are rare, but even so we would like the procedures to be robust if they occur.) The question is whether the simple up-down procedure can recover in this type of situation to give an accurate estimate, with appreciable probability.

It appeared that with the scenarios simulated there was practically no chance of a reasonable estimate using the up-and-down procedure with a fixed nominal sample size of 6. Performance was substantially improved by adoption of either of two stopping rules that allow a variable nominal sample size, the rule proposed and a rule based on the number of reversals.

It could be desirable to consider some additional outlier scenarios. It could be argued that the possibility for outliers is limited because the up-and-down converges rapidly to the LD50: A test cannot be an outlier unless the dose is far from the LD50.

While the use of the new stopping rules appeared to be helpful in this situation, other solutions may also be considered. In particular, it has been suggested that use of more than one animal per step may be helpful. An outlier resistant version of the dose averaging estimator could be developed by using medians instead of averages. One might use the following estimator: (A+B)/2 where A is the median dose for responding animals and B the median dose for non-responding animals. Finally, the stopping criteria could include a requirement that the average dose for responding animals must exceed the average dose for non-responding animals (geometric averaging would be used).

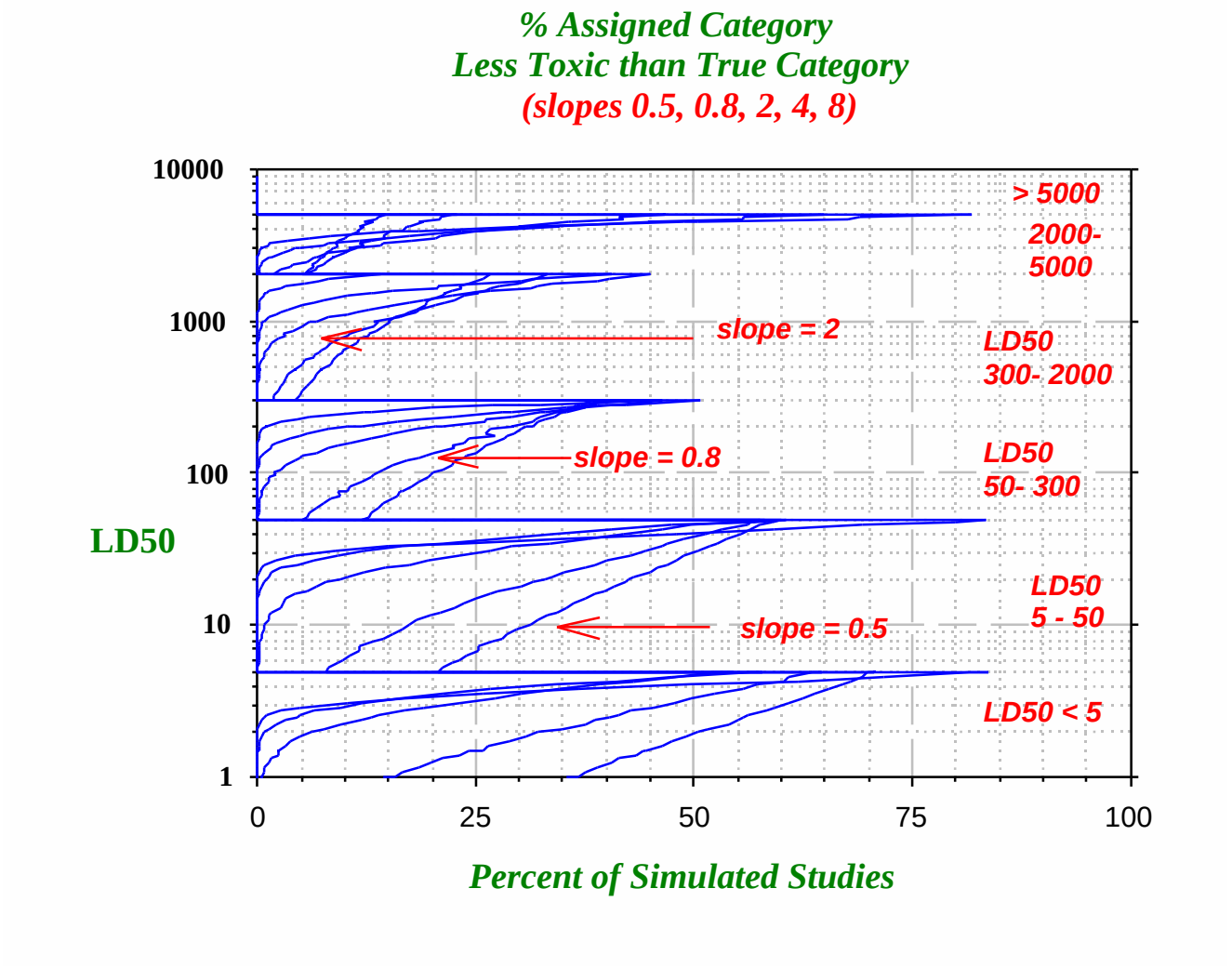
2. Simulation Results

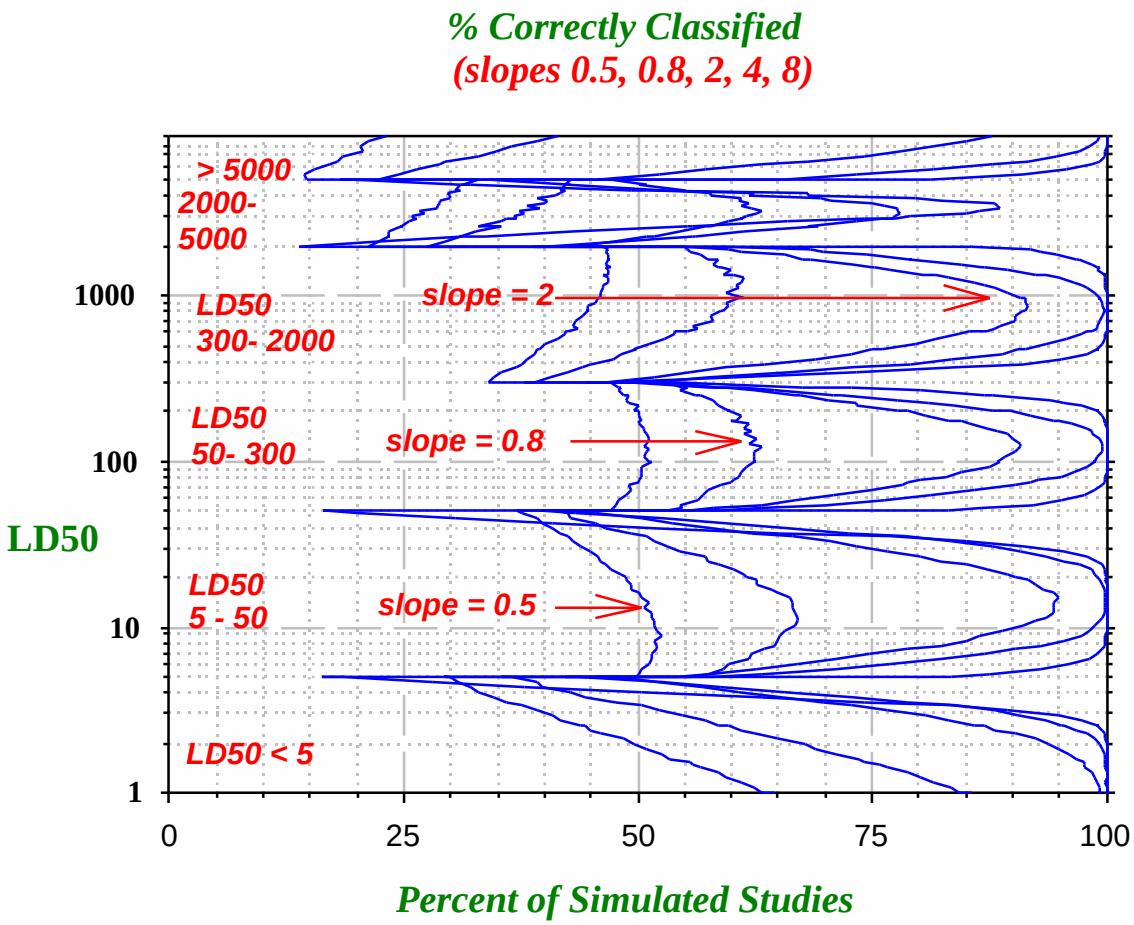
2.1 Classification probabilities plotted against LD50 and slope

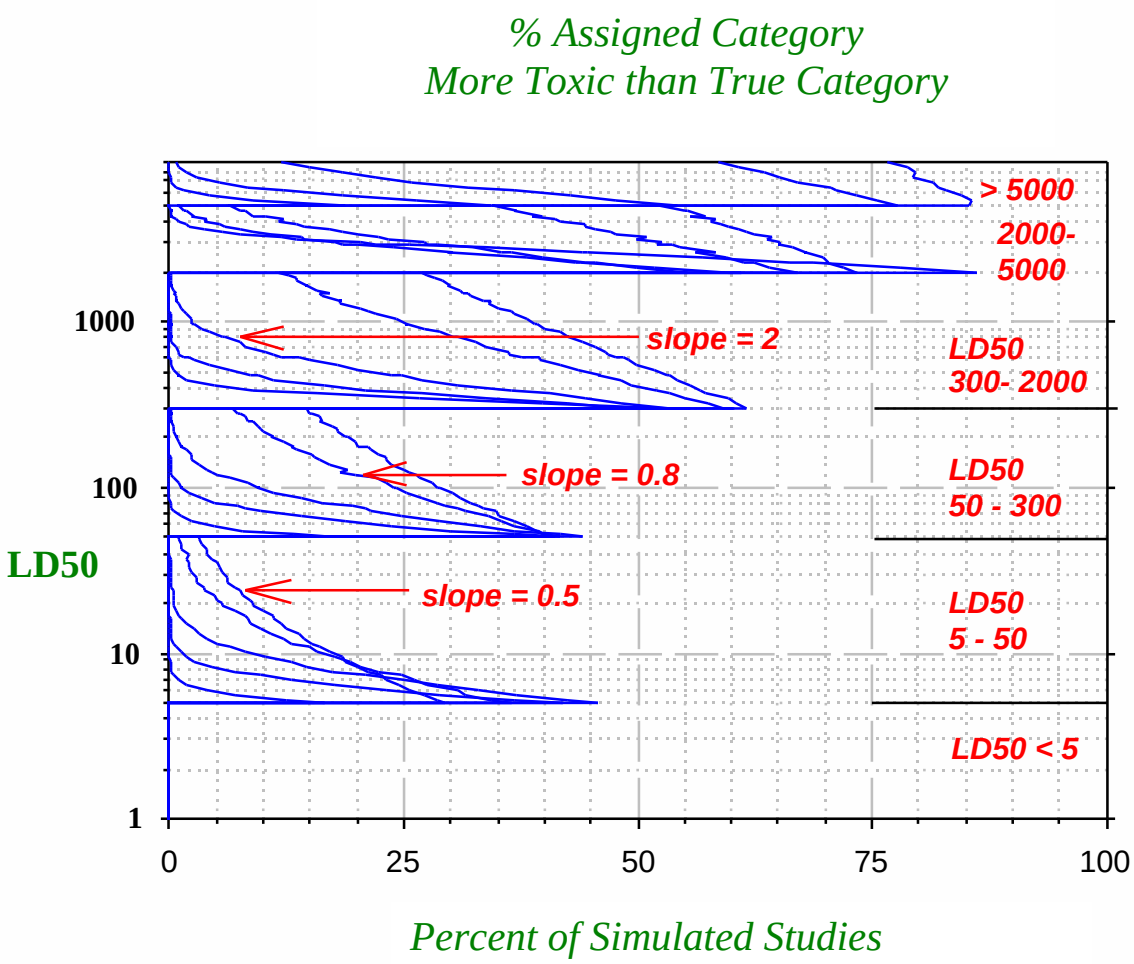
The following is abbreviated from a document distributed on March 6, 2000. The graphs attached display the probability of correct classification, as well as the probability of each kind of miss-classification (under protective or over protective classification), as a function of the LD50. A separate line is used for each of the standard slopes. The simulations follow the default procedure indicated in the Guidelines, with an initial test dose of 175 units, a minimum test dose of 1 unit, a maximum test dose of 5000 units, and use of a likelihood-ratio stopping rule. As with all the simulations conducted for this report, a probit model is assumed.

Unfortunately, it appears that when a chemical is miss-classified, it will be more often assigned to a less-toxic category than to a more-toxic category. The only explanation that comes to mind is that this is bad luck having to do with the relationship between the initial test dose and the category boundaries. It should be noted that the precision of the up-down procedure is limited by the dose progression factor (here 3.2). In particular, in steep-slope situations, the MLE may be the geometric average of two test doses which differ by a factor of 3.2 and may straddle a category boundary. Therefore, chemicals with LD50s within certain intervals may be consistently over classified or consistently under classified.

There would be some justification for additional simulations in which the initial test dose varies from 175 units. Such a simulation will be undertaken, tentatively with doses shifted by 0.25 log units, specifically 1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000 units.


% Assigned Category
Less Toxic than True Category
(slopes 0.5, 0.8, 2, 4, 8)
10000
1000
100
10
1
LD50
0
25
50
75
100
Percent of Simulated Studies
> 5000
2000-
5000
LD50
300- 2000
LD50
50- 300
LD50
5 - 50
LD50 < 5
slope = 2
slope = 0.8
slope = 0.5


% Correctly Classified
(slopes 0.5, 0.8, 2, 4, 8)
> 5000
2000-
5000
LD50
300- 2000
LD50
50- 300
LD50
5 - 50
LD50 < 5
slope = 2
slope = 0.8
slope = 0.5
1000
100
10
1
LD50
0
25
50
75
100
Percent of Simulated Studies


% Assigned Category
More Toxic than True Category
> 5000
2000-
5000
1000
slope = 2
LD50
300- 2000
100
slope = 0.8
LD50
50 - 300
LD50
slope = 0.5
LD50
5 - 50
10
LD50 < 5
1
0
25
50
75
100
Percent of Simulated Studies

2.2 Monte Carlo comparison of three stopping rules and two LD50 estimators for the primary procedure

The following is abbreviated from a report distributed on February 14, 2000.

The scenarios assumed for these simulations (starting dose, slope, and LD50) are not the standard scenarios used in recent OECD work, or the current default guideline approach. The LD50 is assumed to equal 600 units and three choices of initial test dose are considered (6, 60, and 600 units). This differs from the OECD practice, which is to use the LD10, LD50, and LD80 as the initial test doses. The slopes evaluated include the standard OECD selections as a subset. Performance is evaluated based on several "performance indices" which are calculated from Monte Carlo output. In particular, we focus on the probability of an estimate that is within a factor of 2 of the true LD50 value.

In addition to an initial test dose of 600 units, the simulations deviate from the Guideline default scenario in that the dose of 3200 was not included in the dose progression.

2.2.1 Estimators of the LD50

Estimates of the LD50 were calculated using two procedures: (1) The maximum likelihood estimate was calculated assuming a probit slope of 2 (denoted MLE(2)). (2) A "dose averaging"estimator (DAE) somewhat similar to the proposal of Brownlee et al. (1953): The LD50 estimate is the geometric average dose, for animals tested at the reversal and subsequently. (The number of values averaged is the "nominal sample size.")

While the DAE uses only the animals in the nominal sample, the MLE uses results for all animals tested. For the DAE, it seemed sensible to allow for a string of responses or non-responses before the reversal, in case of a poor choice of initial test dose. For the MLE, there is no apparent harm from including such observations: They contribute some (but probably relatively little) information.on the LD50.

Where the MLE(2) is outside the permitted range of test doses (below 1 or above 5000), it is assumed that the point estimate is not used and that the experimenter only concludes that the LD50 is below 1 or above 5000.

2.2.2 Stopping Criteria Evaluated.

Three stopping criteria have been evaluated. These are denoted #1, #2, and #5. The gap in numbering is a result of dropping two criteria considered in a previous document.

The following features are common to each of the criteria. (1) There is a maximum number of animals that can be tested, here set at 15. (2) Testing always stops if there is a "perfect alternation" of response and non-response for the first 6 animals in the nominal. (3) Testing is stopped if 3 consecutive tests at a dose of 1 unit (or another lower bound) all yield responses, or 3 consecutive tests at 5000 units (or another upper bound) result in no responses.

The stopping criteria are evaluated after each test, provided that the nominal sample is 6 or more. Therefore the number tested is always 6 or more.

Criterion 1 (Based on fixed "nominal" sample size). After the reversal, 4 additional animals are tested. The "nominal sample size" is 6.

Criterion 2 (Based on number of reversals). A stopping rule based on number of reversals was considered because the approach is simple, and has been proposed previously. For the version implemented here, testing stops after 5 reversals. The basis for the value of 5 is that in the most favorable situations, 6 test animals will tend to represent 5 reversals, i.e., there is "perfect alternation" between response and nonresponse.

Criterion 5 (LR rule with default slope of 2). This is the rule described in the current guideline.

2.2.3 Performance Statistics

Having simulated a large number of studies (here 5000) for a given scenario, and estimated the LD50 for each simulated study, statistics are calculated that characterize the performance of the procedure in terms of (1) whether or not the LD50 estimates tend to be close to the true value of the LD50; (2) whether or not the procedure tends to correctly classify a chemical with a given LD50; and (3) the number of animals tested. This section describes the statistics calculated and documents notation used in output.

Statistics calculated for numbers tested. For numbers tested I report mean number, the 95th percentile (denoted *P95*), and the percent of studies for which the number tested is the maximum (here 15).

Statistics calculated for estimates of the LD50. The following are calculated for each scenario, and separately for two estimators of the LD50 (MLE(2) and DAE). These results are reported only for "My" scenarios.

P5, *P50*, *P95.* These denote the 5th percentile, 50th percentile (median) and 95th percentile of the distribution of LD50 estimates for a given scenario. These provide a characterization of the distribution of LD50 estimates.

% in range. This is the percent of simulated studies that resulted in a point estimate of the LD50 in the range 1 unit to 5000 unit. "Out of bound" estimates resulted from either (1) stopping the experiment after repeated nonresponse at the upper bound, or repeated response at the lower bound; or (2) an MLE(2) outside the range 1-5000 units.

P50 / LD50 (index of bias) Bias represents a tendency of estimates to fall below the true value with some degree of consistency, or else above with some consistency. If this ratio equals 1, then exactly 50% of estimates fall below the true value and exactly 50% fall above. Thus values close to 1 are desirable, indicating unbiasedness. A value below 50% indicates that most estimates fall below the true value, etc.

In the log scale, the statistic is approximately equal to the bias in the strict sense of the term in statistics (the difference between the mean estimate and the true value), for a tolerance distribution that is symmetric in the log scale.

P95 / P5 (index of spread). As an index of the spread of the distribution I use the ratio of the 95th percentile to the 5th percentile. Small values are desirable provided they are not combined with too high bias.

For a lognormal distribution, and perhaps for some other distributions, this index has a simple relationship to the log-scale standard error.

These indices of bias and spread are not scaled to be comparable, *e.g.,* do not allow one to directly assess whether bias or variance contributes more importantly to the error of estimation.

PF2. This is the percent of estimates that fall within a factor of 2 of the true LD50, i.e., the percent of estimates that satisfy LD50/2 œ estimate œLD50*2. (PF2 stands for *P*ercent within *F*actor of *2* of true value.) Note that this index combines bias and precision. The index ranges between 0 and 100%, values close to 100% indicating better performance.

A value of 90% for *PF2* would be obtained for an unbiased estimator with a spread index value (P95/P5) of about 4. That would permit most of estimates to fall within a single category of the acute oral toxicity classifications, provided that the estimate is close to the geometric center of the category, and the upper and lower bounds for the category are separated by a factor greater than 4. In the acute toxicity classification, the bounds are separated by a factors as low as 6 (the 50-300 range) and 2.5 (the 2000-5000) range. On this basis a PF2 of 90% or larger is suggested as a criterion for good performance.

2.2.4 Results and Discussion

Results for Estimation of the LD50. Based on the performance statistics described in the previous section with my scenarios, a marked improvement in performance is obtained by using Criteria 2 or 5, under conditions involving relatively extreme slopes and starting values (Table 2). Under other conditions, the improvement is relatively modest. More complete output of the simulations is given in Appendices 1.1 to 1.3.

In the previous section it was suggested that a criterion for good performance could be values 90% and higher for the index PF2. It is observed that the value of this index increases with the slope. Therefore a compact table of output is obtained by interpolating in the Monte Carlo results the slope that corresponds to PF2=90%, for a given choice of initial test dose. Then the interpolated slope can be used as a bound on the range of slopes for which the procedure works well.

Results of this type of calculation are displayed below. Row 2 of the table gives, for purposes of comparison, the results from applying the procedure with a fixed nominal sample size of 15, the number used in Guideline 401. A modification of the stopping rule cannot achieve the performance indicated in Row 1, if the numbers tested are generally kept below 15.

The application of flexible-*n* stopping rules (Criteria 2-5) appears to significantly extend the range of slopes for which the procedure will work well, relative to the fixed-*n* criterion (Criterion 1), and the former should therefore be preferred if they do not result in an unacceptable increase in numbers tested. However the range of slopes that are acceptable according to this criterion does not include the complete range of slopes that we think are possible.

Table 2.2.1. Comparison of Stopping Criteria in situations involving extreme slopes and starting values: examples with low slope and poor choice of initial test dose.

Stopping Criterion	**slope**	**Method of Estimating LD50**					
		Dose Averaging			**MLE**		
		P50/LD50	**P95/P5**	**PF2**	**P50/LD50**	**P95/P5**	**PF2**
1. fixed nominal *n*= 6	0.5	0.08	209	14	0.17	212	12
	0.8	0.26	97	25	0.42	96	32
2. number of reversals = 5	0.5	0.18	125	20	0.28	157	27
	0.8	0.37	50	35	0.56	47	42
5. LR > 2.5	0.5	0.25	142	23	0.36	194	31
	0.8	0.44	33	37	0.59	39	43

Explanation: Calculations are based on an LD50 of 600 units and an initial test dose of 6 units. The table gives values of performance statistics.
P50 / LD50 = ratio of median estimated LD50 to true LD50 (closer to 1 is better)
P95 / P5 = ratio of 95th percentile estimated LD50 to 5th percentile (smaller is better)
PF2 = percent of estimates that satisfy LD50/2 < estimate < LD50*2 (larger is better)

For example (row 1) if the slope is 0.5, the initial test dose is 6 units, the true LD50 is 600 units, and the LD50 is estimated by the dose averaging method, then there is a 14% chance of an estimate within a factor of 2 of the correct value, when using Criterion 1 (column5). There would be a 23% chance of such an outcome using Criterion 5 (row 5).

Table 2.2.2. Minimal slope for at least 90% of estimates to be within a factor of 2 of the true LD50.

Stopping Criterion	**Initial Test dose**		
	LD50/100	**LD50/10**	**LD50**
1. fixed nominal $n = 6$	3.4	3.4	2.5
$n = 15$ †	2.1	2.0	1.6
2. number of reversals = 5	2.9	2.9	2.5
5. LR > 2.5	2.8	2.6	2.7

Explanation. For example (see 1st row of slopes) if the initial test dose is LD50/100 then the index PF2 will be at least 90%, provided the slope is 3.44 or larger, when stopping is based on Criterion 1. In this sense 3.4 is the lower bound for the range of slopes where Criterion 1 works well, when starting at LD50/l00.

The true LD50 was assumed to be 600 units for this calculation. Results are based on the DA estimator. Linear interpolation has been used. Based on 5000 simulated studies per scenario, except row 2 based on 3000 simulated studies.

† Given for purposes of comparison (see text).

Results for Numbers Tested. Estimated mean numbers tested per study are displayed below for each Stopping Criterion. Comparing Criteria #2 and #5 it appears that more or tested with Criterion #5 at low slopes, but more or tested with #2 at high slopes. We believe that in practice slopes will be distributed so that in the long run Criterion #5 will use somewhat fewer animals. Furthermore Criterion #5 has somewhat better statistical performance.

Table 3. Mean numbers tested

Dose0 = LD50 / 100			
slope	**Crit. #1**	**Crit. #2**	**Crit. #5**
0.5	7.6	11.1	12.4
0.8	8.2	11.4	12.7
1.5	9.1	11.5	12.1
2.0	9.3	11.4	11.8
2.5	9.4	11.2	11.5
3.0	9.4	11.1	11.4
3.5	9.4	11.0	11.2
4.0	9.5	10.9	11.2
8.3	9.5	10.8	11.0
Dose0 = LD50 / 10			
0.5	6.8	10.1	10.0
0.8	6.9	10.0	10.3
1.5	7.2	9.7	10.1
2.0	7.3	9.4	9.9
2.5	7.4	9.3	9.6
3.0	7.4	9.0	9.4
3.5	7.5	9.0	9.3
4.0	7.5	8.9	9.2
8.3	7.5	8.8	9.0
Dose0 = LD50			
0.5	6.6	9.6	8.7
0.8	6.4	9.3	8.1
1.5	6.3	8.7	7.2
2.0	6.2	8.4	6.8
2.5	6.1	8.1	6.5
3.0	6.1	7.9	6.3
3.5	6.0	7.7	6.2
4.0	6.0	7.6	6.1
8.3	6.0	7.4	6.0

Based on 5000 simulated studies per combination of LD50 and slope

2.2.4 Conclusions

Criterion 5 is simple to apply and gives relatively good performance, considering precision in the estimation of the LD50 as well as numbers of animals tested. In particular, the numbers tested are appreciably increased only for combinations of slope and initial test dose that we think are unusual.

2.2.5 Tables of Monte Carlo results: percentiles of the distribution of LD50 estimates

Convergence criterion #1 [fixed nominal N]

Critical nominal N	= 6
slope assumed in probit calculations	= 2.00
step size (dose progression) log10	= 0.50
max num. animals to test	= 15
doses restricted to range	1.0,5000.0(min,max)
Num. simulated studies per scenario	= 5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	7.3	49.5	1519.2	99.9	9.4	101.1	1986.4	99.1
2	600.0	0.80	6.0	15.7	156.6	1519.2	99.8	24.9	252.3	2404.1	99.2
3	600.0	1.50	6.0	72.7	337.4	1519.2	100.0	112.6	509.4	1764.9	99.9
4	600.0	2.00	6.0	156.6	495.2	1519.2	100.0	198.6	569.0	1579.4	99.9
5	600.0	2.50	6.0	156.6	495.2	1067.0	100.0	252.3	628.2	1401.5	100.0
6	600.0	3.00	6.0	229.9	495.2	1067.0	100.0	294.2	628.2	1397.0	100.0
7	600.0	3.50	6.0	229.9	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
8	600.0	4.00	6.0	337.4	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
9	600.0	8.33	6.0	337.4	495.2	1067.0	100.0	356.2	628.2	1126.3	100.0
10	600.0	0.50	60.0	23.0	156.6	1785.5	99.8	23.0	199.4	2404.1	98.8
11	600.0	0.80	60.0	49.5	229.9	1519.2	99.9	49.4	299.5	2404.1	99.4
12	600.0	1.50	60.0	106.7	337.4	1519.2	100.0	135.0	508.1	1764.9	99.9
13	600.0	2.00	60.0	156.6	495.2	1519.2	100.0	194.5	568.0	1579.2	100.0
14	600.0	2.50	60.0	156.6	495.2	1067.0	100.0	249.4	627.2	1401.3	100.0
15	600.0	3.00	60.0	229.9	495.2	1067.0	100.0	291.2	627.2	1395.2	100.0
16	600.0	3.50	60.0	229.9	495.2	1067.0	100.0	354.1	627.2	1126.0	100.0
17	600.0	4.00	60.0	337.4	495.2	1067.0	100.0	354.1	627.2	1126.0	100.0
18	600.0	8.33	60.0	337.4	495.2	1067.0	100.0	354.1	797.4	1126.0	100.0
19	600.0	0.50	600.0	72.7	705.2	3080.1	99.4	63.4	655.2	4345.9	96.5
20	600.0	0.80	600.0	106.7	495.2	2163.2	99.8	81.5	542.0	3230.0	98.6
21	600.0	1.50	600.0	229.9	705.2	1519.2	100.0	180.5	655.2	1945.0	99.8

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
22	600.0	2.00	600.0	229.9	705.2	1519.2	100.0	204.6	655.2	1725.3	100.0
23	600.0	2.50	600.0	229.9	495.2	1519.2	100.0	230.4	542.0	1531.0	100.0
24	600.0	3.00	600.0	337.4	495.2	1067.0	100.0	284.5	494.1	1246.1	100.0
25	600.0	3.50	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
26	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
27	600.0	8.30	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

**** Distribution of LD50 estimates ****

Convergence criterion # 2 [#reversals]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #2) Critical num reversals = 5

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	10.7	106.7	1330.4	99.9	12.8	170.1	2006.0	99.1
2	600.0	0.80	6.0	31.6	223.7	1568.2	99.8	42.6	338.9	2011.6	99.6
3	600.0	1.50	6.0	106.7	431.8	1390.8	100.0	171.6	564.3	1762.3	100.0
4	600.0	2.00	6.0	189.7	509.0	1330.4	100.0	228.5	579.8	1437.7	100.0
5	600.0	2.50	6.0	233.9	534.8	1067.0	100.0	269.9	610.0	1244.8	100.0
6	600.0	3.00	6.0	253.0	600.0	1067.0	100.0	349.2	610.0	1126.3	100.0
7	600.0	3.50	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
8	600.0	4.00	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
9	600.0	8.33	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
10	600.0	0.50	60.0	33.7	221.2	1801.1	99.6	29.9	301.7	2612.7	98.8
11	600.0	0.80	60.0	60.0	337.4	1775.7	99.9	65.7	414.2	2404.1	99.3
12	600.0	1.50	60.0	136.6	449.9	1390.8	100.0	176.0	568.0	1762.2	100.0
13	600.0	2.00	60.0	189.7	509.0	1330.4	100.0	228.5	578.9	1437.5	100.0
14	600.0	2.50	60.0	253.0	534.8	1067.0	100.0	267.8	609.3	1294.9	100.0
15	600.0	3.00	60.0	253.0	600.0	1067.0	100.0	347.9	609.3	1126.0	100.0
16	600.0	3.50	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
17	600.0	4.00	60.0	337.4	600.0	1067.0	100.0	354.1	609.3	1126.0	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
18	600.0	8.33	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
19	600.0	0.50	600.0	80.0	590.1	2568.2	99.4	63.4	600.0	3462.9	97.6
20	600.0	0.80	600.0	129.3	600.0	2123.0	99.7	110.5	600.0	3035.0	99.0
21	600.0	1.50	600.0	223.7	600.0	1568.2	100.0	204.6	600.0	1725.3	100.0
22	600.0	2.00	600.0	263.6	600.0	1390.8	100.0	253.7	600.0	1439.3	100.0
23	600.0	2.50	600.0	316.5	600.0	1114.6	100.0	281.0	600.0	1202.7	100.0
24	600.0	3.00	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
25	600.0	3.50	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
26	600.0	4.00	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0
27	600.0	8.30	600.0	337.4	600.0	1067.0	100.0	337.4	600.0	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

**** Distribution of LD50 estimates ****
Convergence criterion # 5 [LR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	600.0	0.50	6.0	10.7	148.3	1519.2	99.8	10.7	213.1	2070.6	99.2
2	600.0	0.80	6.0	47.7	263.6	1569.8	99.9	50.8	356.2	1983.0	99.7
3	600.0	1.50	6.0	148.3	495.2	1519.2	100.0	161.1	512.4	1579.4	100.0
4	600.0	2.00	6.0	206.0	509.0	1519.2	100.0	253.8	604.5	1579.4	100.0
5	600.0	2.50	6.0	253.0	586.5	1128.6	100.0	281.6	610.0	1201.2	100.0
6	600.0	3.00	6.0	337.4	600.0	1067.0	100.0	349.5	655.7	1126.3	100.0
7	600.0	3.50	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
8	600.0	4.00	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
9	600.0	8.33	6.0	337.4	600.0	1067.0	100.0	356.2	655.7	1126.3	100.0
10	600.0	0.50	60.0	25.3	268.0	1812.8	99.7	25.4	291.0	2641.1	99.0
11	600.0	0.80	60.0	49.5	366.3	1796.4	99.9	49.4	425.8	2062.1	99.7
12	600.0	1.50	60.0	156.6	495.2	1519.2	100.0	156.3	511.5	1579.2	100.0
13	600.0	2.00	60.0	189.7	509.0	1519.2	100.0	213.2	576.3	1437.5	100.0
14	600.0	2.50	60.0	288.4	600.0	1390.8	100.0	337.4	609.3	1437.5	100.0
15	600.0	3.00	60.0	337.4	600.0	1067.0	100.0	350.5	609.3	1126.0	100.0
16	600.0	3.50	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
17	600.0	4.00	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
18	600.0	8.33	60.0	337.4	600.0	1067.0	100.0	354.1	655.1	1126.0	100.0
19	600.0	0.50	600.0	72.7	584.6	2836.9	99.2	70.4	596.4	3246.3	98.1

LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
20	600.0	0.80	600.0	106.7	584.6	2220.6	99.7	102.3	596.4	2650.2	99.2
21	600.0	1.50	600.0	223.7	584.6	1568.2	100.0	226.9	596.4	1642.4	100.0
22	600.0	2.00	600.0	229.9	515.6	1519.2	100.0	230.4	494.1	1531.0	100.0
23	600.0	2.50	600.0	253.0	668.2	1390.8	100.0	253.7	673.4	1398.8	100.0
24	600.0	3.00	600.0	337.4	495.2	1128.6	100.0	337.4	494.1	1067.0	100.0
25	600.0	3.50	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
26	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
27	600.0	8.30	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0

'%in range' means % > 1.0 and <5000.0

2.2.6 Tables of Monte Carlo Results for Numbers Tested

Convergence criterion # 1 [fixed nominal N]
Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	7.61	11.00	0.00
2	600.0	0.80	6.0	8.21	11.00	0.00
3	600.0	1.50	6.0	9.07	11.00	0.00
4	600.0	2.00	6.0	9.28	11.00	0.00
5	600.0	2.50	6.0	9.37	10.00	0.00
6	600.0	3.00	6.0	9.43	10.00	0.00
7	600.0	3.50	6.0	9.44	10.00	0.00
8	600.0	4.00	6.0	9.48	10.00	0.00
9	600.0	8.33	6.0	9.50	10.00	0.00
10	600.0	0.50	60.0	6.79	9.00	0.00
11	600.0	0.80	60.0	6.91	9.00	0.00
12	600.0	1.50	60.0	7.17	9.00	0.00
13	600.0	2.00	60.0	7.29	9.00	0.00
14	600.0	2.50	60.0	7.38	8.00	0.00
15	600.0	3.00	60.0	7.42	8.00	0.00
16	600.0	3.50	60.0	7.45	8.00	0.00
17	600.0	4.00	60.0	7.47	8.00	0.00
18	600.0	8.33	60.0	7.51	8.00	0.00
19	600.0	0.50	600.0	6.55	8.00	0.00
20	600.0	0.80	600.0	6.44	8.00	0.00
21	600.0	1.50	600.0	6.25	7.00	0.00
22	600.0	2.00	600.0	6.16	7.00	0.00
23	600.0	2.50	600.0	6.11	7.00	0.00
24	600.0	3.00	600.0	6.07	7.00	0.00
25	600.0	3.50	600.0	6.04	6.00	0.00
26	600.0	4.00	600.0	6.02	6.00	0.00
27	600.0	8.30	600.0	6.00	6.00	0.00

**** Numbers Tested ****
Convergence criterion # 2 [#reversals]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #2) Critical num reversals = 5

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	11.08	15.00	10.96
2	600.0	0.80	6.0	11.40	15.00	11.70
3	600.0	1.50	6.0	11.47	15.00	8.52
4	600.0	2.00	6.0	11.37	15.00	6.04
5	600.0	2.50	6.0	11.23	14.00	3.96
6	600.0	3.00	6.0	11.09	14.00	2.44
7	600.0	3.50	6.0	10.95	14.00	1.50
8	600.0	4.00	6.0	10.89	13.00	0.72
9	600.0	8.33	6.0	10.79	13.00	0.00
10	600.0	0.50	60.0	10.10	15.00	5.62
11	600.0	0.80	60.0	9.95	14.00	4.24
12	600.0	1.50	60.0	9.68	13.00	2.02
13	600.0	2.00	60.0	9.41	13.00	1.18
14	600.0	2.50	60.0	9.31	12.00	0.54
15	600.0	3.00	60.0	9.03	12.00	0.14
16	600.0	3.50	60.0	8.98	12.00	0.04
17	600.0	4.00	60.0	8.89	11.00	0.00
18	600.0	8.33	60.0	8.79	11.00	0.00
19	600.0	0.50	600.0	9.63	14.00	4.50
20	600.0	0.80	600.0	9.33	14.00	2.54
21	600.0	1.50	600.0	8.71	12.00	0.74
22	600.0	2.00	600.0	8.36	12.00	0.16
23	600.0	2.50	600.0	8.09	11.00	0.10
24	600.0	3.00	600.0	7.86	10.00	0.00
25	600.0	3.50	600.0	7.70	10.00	0.00
26	600.0	4.00	600.0	7.56	10.00	0.00
27	600.0	8.30	600.0	7.44	10.00	0.00

**** Numbers Tested ****
Convergence criterion # 5 [LR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	600.0	0.50	6.0	12.37	15.00	44.36
2	600.0	0.80	6.0	12.68	15.00	41.04
3	600.0	1.50	6.0	12.13	15.00	22.12
4	600.0	2.00	6.0	11.78	15.00	13.60
5	600.0	2.50	6.0	11.54	15.00	8.00
6	600.0	3.00	6.0	11.44	15.00	5.86
7	600.0	3.50	6.0	11.20	14.00	3.28
8	600.0	4.00	6.0	11.16	14.00	1.88
9	600.0	8.33	6.0	11.01	14.00	0.00
10	600.0	0.50	60.0	9.98	15.00	16.42
11	600.0	0.80	60.0	10.25	15.00	16.06
12	600.0	1.50	60.0	10.13	15.00	9.42
13	600.0	2.00	60.0	9.87	15.00	6.44
14	600.0	2.50	60.0	9.64	13.00	3.70
15	600.0	3.00	60.0	9.39	13.00	2.32
16	600.0	3.50	60.0	9.26	12.00	1.30
17	600.0	4.00	60.0	9.19	12.00	0.98
18	600.0	8.33	60.0	8.99	12.00	0.00
19	600.0	0.50	600.0	8.71	15.00	5.52
20	600.0	0.80	600.0	8.13	13.00	2.76
21	600.0	1.50	600.0	7.20	10.00	0.26
22	600.0	2.00	600.0	6.78	10.00	0.02
23	600.0	2.50	600.0	6.50	8.00	0.00
24	600.0	3.00	600.0	6.32	8.00	0.00
25	600.0	3.50	600.0	6.17	8.00	0.00
26	600.0	4.00	600.0	6.10	6.00	0.00
27	600.0	8.30	600.0	6.00	6.00	0.00

2.2.7 Tables of Monte Carlo Results: Performance Statistics

Convergence criterion # 1 [fixed nominal N]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
1	600.0	0.50	6.0	0.08	209.00	13.62	0.17	211.50	19.70
2	600.0	0.80	6.0	0.26	97.01	24.68	0.42	96.41	31.98
3	600.0	1.50	6.0	0.56	20.90	51.74	0.85	15.67	58.12
4	600.0	2.00	6.0	0.83	9.70	66.34	0.95	7.95	70.80
5	600.0	2.50	6.0	0.83	6.81	77.28	1.05	5.55	80.16
6	600.0	3.00	6.0	0.83	4.64	85.04	1.05	4.75	86.70
7	600.0	3.50	6.0	0.83	4.64	91.12	1.05	3.16	92.34
8	600.0	4.00	6.0	0.83	3.16	95.30	1.05	3.16	95.48
9	600.0	8.33	6.0	0.83	3.16	100.00	1.05	3.16	100.00
10	600.0	0.50	60.0	0.26	77.67	21.06	0.33	104.34	26.82
11	600.0	0.80	60.0	0.38	30.68	30.68	0.50	48.65	35.34
12	600.0	1.50	60.0	0.56	14.24	52.34	0.85	13.08	57.40
13	600.0	2.00	60.0	0.83	9.70	64.38	0.95	8.12	69.84
14	600.0	2.50	60.0	0.83	6.81	77.16	1.05	5.62	79.50
15	600.0	3.00	60.0	0.83	4.64	86.00	1.05	4.79	87.84
16	600.0	3.50	60.0	0.83	4.64	90.62	1.05	3.18	91.40
17	600.0	4.00	60.0	0.83	3.16	95.36	1.05	3.18	95.74
18	600.0	8.33	60.0	0.83	3.16	100.00	1.33	3.18	100.00
19	600.0	0.50	600.0	1.18	42.37	53.12	1.09	68.57	41.58
20	600.0	0.80	600.0	0.83	20.27	60.90	0.90	39.63	46.98

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
21	600.0	1.50	600.0	1.18	6.61	75.98	1.09	10.77	63.98
22	600.0	2.00	600.0	1.18	6.61	84.22	1.09	8.43	75.14
23	600.0	2.50	600.0	0.83	6.61	89.62	0.90	6.64	82.44
24	600.0	3.00	600.0	0.83	3.16	93.28	0.82	4.38	88.94
25	600.0	3.50	600.0	0.83	3.16	95.78	0.82	3.16	92.72
26	600.0	4.00	600.0	0.83	3.16	97.86	0.82	3.16	95.64
27	600.0	8.30	600.0	0.83	3.16	100.00	0.82	3.16	100.00

**** Measures of performance for estimation of LD50 ****

Convergence criterion # 2 [#reversals]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #2) Critical num reversals = 5

max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
1	600.0	0.50	6.0	0.18	124.69	19.70	0.28	156.59	26.66
2	600.0	0.80	6.0	0.37	49.55	34.58	0.56	47.21	41.68
3	600.0	1.50	6.0	0.72	13.03	62.78	0.94	10.27	68.34
4	600.0	2.00	6.0	0.85	7.01	75.96	0.97	6.29	80.06
5	600.0	2.50	6.0	0.89	4.56	85.78	1.02	4.61	87.76
6	600.0	3.00	6.0	1.00	4.22	91.20	1.02	3.23	92.04
7	600.0	3.50	6.0	1.00	3.16	94.88	1.09	3.16	95.34
8	600.0	4.00	6.0	1.00	3.16	97.52	1.09	3.16	97.86
9	600.0	8.33	6.0	1.00	3.16	100.00	1.09	3.16	100.00
10	600.0	0.50	60.0	0.37	53.38	32.16	0.50	87.25	36.52
11	600.0	0.80	60.0	0.56	29.59	43.02	0.69	36.59	47.78
12	600.0	1.50	60.0	0.75	10.18	64.96	0.95	10.01	69.08
13	600.0	2.00	60.0	0.85	7.01	75.72	0.96	6.29	78.66
14	600.0	2.50	60.0	0.89	4.22	86.66	1.02	4.84	87.74
15	600.0	3.00	60.0	1.00	4.22	90.90	1.02	3.24	91.64
16	600.0	3.50	60.0	1.00	3.16	94.48	1.09	3.18	95.16
17	600.0	4.00	60.0	1.00	3.16	96.98	1.02	3.18	97.34
18	600.0	8.33	60.0	1.00	3.16	100.00	1.09	3.18	100.00
19	600.0	0.50	600.0	0.98	32.10	48.68	1.00	54.64	42.90

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
20	600.0	0.80	600.0	1.00	16.42	59.00	1.00	27.46	51.12
21	600.0	1.50	600.0	1.00	7.01	76.76	1.00	8.43	70.44
22	600.0	2.00	600.0	1.00	5.28	84.42	1.00	5.67	79.24
23	600.0	2.50	600.0	1.00	3.52	90.64	1.00	4.28	86.68
24	600.0	3.00	600.0	1.00	3.16	94.08	1.00	3.16	91.18
25	600.0	3.50	600.0	1.00	3.16	96.68	1.00	3.16	95.06
26	600.0	4.00	600.0	1.00	3.16	98.06	1.00	3.16	97.06
27	600.0	8.30	600.0	1.00	3.16	100.00	1.00	3.16	100.00

**** Measures of performance for estimation of LD50 ****

Convergence criterion # 5 [LR]

Critical nominal N = 6
slope assumed in probit calculations = 2.00
step size (dose progression) log10 = 0.50
Generate outlier (1=>yes;0=>no) = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test = 15
doses restricted to range 1.0,5000.0 (min,max)
Num. simulated studies per scenario = 5000

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
1	600.0	0.50	6.0	0.25	142.39	22.60	0.36	194.07	30.52
2	600.0	0.80	6.0	0.44	32.94	37.00	0.59	39.03	43.38
3	600.0	1.50	6.0	0.83	10.25	66.12	0.85	9.80	69.22
4	600.0	2.00	6.0	0.85	7.37	79.02	1.01	6.22	81.46
5	600.0	2.50	6.0	0.98	4.46	87.94	1.02	4.27	89.48
6	600.0	3.00	6.0	1.00	3.16	91.94	1.09	3.22	93.10
7	600.0	3.50	6.0	1.00	3.16	95.36	1.09	3.16	96.22
8	600.0	4.00	6.0	1.00	3.16	97.84	1.09	3.16	98.40
9	600.0	8.33	6.0	1.00	3.16	100.00	1.09	3.16	100.00
10	600.0	0.50	60.0	0.45	71.65	36.30	0.48	104.09	33.74
11	600.0	0.80	60.0	0.61	36.27	48.14	0.71	41.73	45.86
12	600.0	1.50	60.0	0.83	9.70	69.56	0.85	10.11	70.32
13	600.0	2.00	60.0	0.85	8.01	80.52	0.96	6.74	81.58
14	600.0	2.50	60.0	1.00	4.82	87.96	1.02	4.26	88.92
15	600.0	3.00	60.0	1.00	3.16	92.80	1.02	3.21	93.68
16	600.0	3.50	60.0	1.00	3.16	95.62	1.09	3.18	96.34
17	600.0	4.00	60.0	1.00	3.16	97.34	1.09	3.18	97.84
18	600.0	8.33	60.0	1.00	3.16	100.00	1.09	3.18	100.00

	LD50	slope	Dose0	Dose Averaging			MLE		
				P50/LD50	P95/P5	PF2	P50/LD50	P95/P5	PF2
19	600.0	0.50	600.0	0.97	39.03	44.44	0.99	46.13	43.26
20	600.0	0.80	600.0	0.97	20.81	53.64	0.99	25.90	52.26
21	600.0	1.50	600.0	0.97	7.01	72.48	0.99	7.24	71.84
22	600.0	2.00	600.0	0.86	6.61	81.96	0.82	6.64	81.66
23	600.0	2.50	600.0	1.11	5.50	87.62	1.12	5.51	87.56
24	600.0	3.00	600.0	0.83	3.35	92.90	0.82	3.16	92.88
25	600.0	3.50	600.0	0.83	3.16	95.88	0.82	3.16	95.88
26	600.0	4.00	600.0	0.83	3.16	97.72	0.82	3.16	97.72
27	600.0	8.30	600.0	1.21	3.16	100.00	1.21	3.16	100.00

2.3 Simulation of an outlier scenario

The following is an extension of the analysis described in the previous section, distributed originally on February 14, 2000. An "outlier scenario" has been simulated as follows. The initial test was assumed to be below the true LD50 (here 750 units) by a factor of 10 or 100, and the first animal tested was assumed to respond, regardless of the probability of response calculated from the probit model. Stopping Criteria 1, 2, and 5 were simulated. Results are displayed below for the index PF2 (probability of an estimate within factor of 2 of correct value). The results tabulated are based on the MLE(2) estimates of the LD50, which appeared to perform better than the dose-averaging estimator in this situation.

Table 2.3.1. Results for performance index PF2 (%) with "outlier" scenario.

Dose0 = LD50 / 100			
slope	**Crit.#1**	**Crit.#2**	**Crit.#5**
0.5	0.1%	11%	16%
1.0	0.0	19	29
1.5	0.0	24	38
2.0	0.0	24	42
2.5	0.0	22	43
3.0	0.0	23	47
3.5	0.0	19	50
4.0	0.0	20	49
8.3	0.0	19	51
Dose0 = LD50 / 10			
0.5	6.2%	22%	22%
1.0	9.1	37	36
1.5	7.8	47	49
2.0	6.5	57	55
2.5	4.1	64	59
3.0	2.9	69	62
3.5	1.7	70	68
4.0	1.1	73	71
8.3	0.0	75	73

Explanation: The index PF2 is the probability of an estimate within a factor of 2 of the true value. For example (see first row). If the slope is 0.5 and the initial test dose is 100th of the LD50 (here LD50=750), then the probability is 0.001 that the estimate will fall between 750/2 and 750*2 when stopping is based on Criterion 1 (fixed nominal *n*). In the same situation, the probability of that accuracy is 0.11 for Criterion 2 (fixed number of reversals) and 0.16 for Criterion 5 (simplified LR).

2.4 Classification probabilities for standard OECD scenarios

The following is abbreviated from an analysis distributed on February 14, 2000. For OECD evaluation of guidelines it has been customary to consider a standard set of slope and LD50 values, and to assume initial test doses equal to the LD10, LD50, and LD80. The tables below give probabilities of classification into categories of the acute oral toxicity classification, which has cut-points 5, 50, 300, 2000, and 5000 units. Based on the current guideline, initial test doses below 1 unit or above 5000 units have been excluded. The dose progression deviates from the guideline, in that a dose of 3200 was not included in the progression. Two stopping rules are simulated: a procedure with the nominal sample size fixed at 6, and the likelihood-ratio criterion recommended in the proposed guideline.

2.4.1 OECD-Type scenarios: Distribution of LD50 Estimates

Convergence criterion # 1 [fixed nominal NR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0

max num. animals to test                = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 3000
Classification cutpoints       5     50    300   2000   5000
```

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	1.5	8.33	1.1	1.5	1.9	1.9	100.0	1.5	1.9	1.9	99.0
2	1.5	8.33	1.5	1.2	1.6	2.7	100.0	1.0	1.5	2.7	94.8
3	1.5	8.33	1.9	1.4	1.4	2.5	100.0	1.0	1.4	2.4	91.5
4	1.5	4.00	1.5	1.1	1.6	2.7	99.4	1.0	1.5	2.7	80.7
5	1.5	4.00	2.4	1.3	1.6	3.1	98.9	1.0	1.6	3.0	74.5
6	1.5	2.00	1.5	1.1	1.6	3.9	98.0	1.0	1.5	3.9	74.5
7	1.5	2.00	4.0	1.3	2.0	4.6	96.3	1.0	1.6	4.7	79.5
8	1.5	0.80	1.5	1.1	2.1	8.4	95.4	1.0	1.9	10.4	71.1
9	1.5	0.80	16.9	1.3	4.5	20.5	95.2	1.0	3.1	20.5	83.4
10	1.5	0.50	1.5	1.0	2.1	12.4	94.6	1.0	2.0	14.2	72.2
11	1.5	0.50	72.3	1.3	18.9	87.6	97.7	1.0	6.9	87.8	91.7
12	2.5	8.33	1.8	2.3	3.1	3.1	100.0	2.3	3.1	3.1	100.0
13	2.5	8.33	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	100.0
14	2.5	8.33	3.1	1.8	1.8	3.8	100.0	1.8	1.8	3.8	100.0
15	2.5	4.00	1.2	1.7	2.1	4.6	100.0	1.7	2.3	5.8	99.6
16	2.5	4.00	2.5	1.6	2.2	4.4	100.0	1.5	2.2	4.4	98.4
17	2.5	4.00	4.1	2.0	2.0	4.7	100.0	1.1	2.0	4.8	99.4
18	2.5	2.00	2.5	1.6	2.7	6.5	99.6	1.0	2.2	6.5	93.0
19	2.5	2.00	6.6	1.4	3.5	8.0	99.7	1.0	2.4	8.0	95.2

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
20	2.5	0.80	2.5	1.4	3.1	14.1	96.9	1.0	2.6	14.8	86.5
21	2.5	0.80	28.2	1.4	7.5	34.1	98.6	1.0	5.0	34.2	91.9
22	2.5	0.50	2.5	1.2	3.1	20.6	96.5	1.0	3.1	21.2	83.1
23	2.5	0.50	120.5	1.6	31.5	146.0	98.8	1.0	11.5	146.4	95.0
24	20.0	8.33	14.0	17.0	24.9	24.9	100.0	17.0	24.9	24.9	100.0
25	20.0	8.33	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
26	20.0	8.33	25.2	14.2	14.2	30.6	100.0	14.2	14.2	30.6	100.0
27	20.0	4.00	9.6	11.6	17.0	36.6	100.0	11.6	17.0	39.7	100.0
28	20.0	4.00	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
29	20.0	4.00	32.5	12.4	18.3	39.3	100.0	10.0	18.3	39.4	100.0
30	20.0	2.00	4.6	5.2	17.5	55.4	100.0	6.8	19.0	60.7	100.0
31	20.0	2.00	20.0	7.7	24.2	52.2	100.0	6.8	24.3	58.7	100.0
32	20.0	2.00	52.7	8.6	29.6	63.8	100.0	6.7	20.2	64.0	100.0
33	20.0	0.80	20.0	5.0	24.2	76.6	100.0	3.4	22.0	118.0	100.0
34	20.0	0.80	225.4	5.9	58.8	273.1	100.0	4.6	38.2	273.8	99.9
35	20.0	0.50	20.0	2.6	24.2	165.1	99.9	2.2	22.0	169.4	99.4
36	20.0	0.50	964.4	8.0	171.5	1377.8	99.9	5.4	94.9	884.7	99.6
37	50.0	8.33	35.1	42.5	62.4	62.4	100.0	42.6	62.4	62.4	100.0
38	50.0	8.33	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
39	50.0	8.33	63.1	35.5	35.5	76.4	100.0	35.5	35.5	76.6	100.0
40	50.0	4.00	23.9	29.0	42.5	91.6	100.0	29.0	42.5	116.0	100.0
41	50.0	4.00	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
42	50.0	4.00	81.2	31.1	45.6	98.3	100.0	25.0	45.6	98.6	100.0
43	50.0	2.00	11.4	13.8	43.8	138.5	100.0	13.9	47.5	151.9	100.0
44	50.0	2.00	50.0	19.2	60.6	130.5	100.0	19.2	60.7	146.6	100.0
45	50.0	2.00	131.8	23.4	74.1	159.6	100.0	17.6	50.6	160.0	100.0
46	50.0	0.80	1.3	2.2	15.1	151.4	100.0	3.0	21.1	193.8	99.8

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
47	50.0	0.80	50.0	8.9	41.3	281.2	100.0	7.0	45.4	295.1	100.0
48	50.0	0.80	563.6	14.7	147.1	682.9	100.0	11.5	95.5	684.4	100.0
49	50.0	0.50	50.0	5.6	60.6	412.7	99.9	6.2	55.0	508.1	99.8
50	50.0	0.50	2411.1	19.9	629.3	2537.8	99.9	13.5	254.7	2187.0	99.4
51	150.0	8.33	105.3	127.5	187.2	187.2	100.0	127.8	187.2	187.2	100.0
52	150.0	8.33	150.0	84.4	123.8	266.7	100.0	84.4	123.5	266.7	100.0
53	150.0	8.33	189.3	106.4	106.4	229.3	100.0	106.4	106.4	229.9	100.0
54	150.0	4.00	71.7	86.9	127.6	274.8	100.0	87.1	127.6	348.1	100.0
55	150.0	4.00	150.0	84.4	181.7	266.7	100.0	84.4	165.1	266.7	100.0
56	150.0	4.00	243.5	93.3	136.9	295.0	100.0	75.1	136.9	295.7	100.0
57	150.0	2.00	34.3	41.6	131.4	415.6	100.0	41.7	142.5	455.8	100.0
58	150.0	2.00	150.0	57.5	123.8	391.5	100.0	51.1	123.5	439.9	100.0
59	150.0	2.00	395.3	70.3	222.3	478.9	100.0	52.7	151.8	480.0	100.0
60	150.0	0.80	3.8	6.5	45.4	454.3	100.0	8.4	63.2	581.4	100.0
61	150.0	0.80	150.0	39.2	123.8	579.7	100.0	25.4	136.3	885.3	99.9
62	150.0	0.80	1690.9	44.1	441.4	2003.3	100.0	34.5	286.5	2015.1	99.8
63	150.0	0.50	150.0	18.2	181.7	1040.0	100.0	17.7	165.1	1277.2	99.7
64	600.0	8.33	421.0	510.1	748.7	748.7	100.0	511.2	748.7	748.7	100.0
65	600.0	8.33	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0
66	600.0	8.33	757.2	425.8	425.8	917.3	100.0	425.8	425.8	919.4	100.0
67	600.0	4.00	286.9	347.6	510.2	1322.8	100.0	348.4	510.2	1365.3	100.0
68	600.0	4.00	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
69	600.0	4.00	974.0	373.2	547.7	1386.8	100.0	300.5	547.7	1339.8	100.0
70	600.0	2.00	137.2	166.2	525.7	1159.6	100.0	170.2	570.2	1890.9	99.9
71	600.0	2.00	600.0	229.9	726.9	1519.2	100.0	204.6	728.6	1725.3	100.0
72	600.0	2.00	1581.1	281.2	889.1	1915.6	100.0	210.9	607.1	1920.0	99.9
73	600.0	0.80	15.0	26.7	181.7	1849.5	99.7	33.7	252.7	2346.2	99.1

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
74	600.0	0.80	600.0	156.6	495.2	2163.2	99.8	106.7	535.9	3246.3	98.4
75	600.0	0.50	1.6	2.9	42.8	1345.4	99.8	4.3	80.4	1549.4	99.1
76	600.0	0.50	600.0	72.7	705.2	2542.3	99.5	63.4	655.2	4117.6	96.6
77	1500.0	8.33	1052.5	1460.4	2294.1	2294.1	100.0	1421.2	2294.1	2294.1	100.0
78	1500.0	8.33	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
79	1500.0	8.33	1892.9	1064.5	1064.5	2159.8	100.0	1064.5	1064.5	2184.1	100.0
80	1500.0	4.00	717.3	869.0	1275.6	2436.6	100.0	871.0	1275.6	3263.2	99.9
81	1500.0	4.00	1500.0	843.5	1526.6	2738.6	100.0	843.5	1848.1	2738.6	99.6
82	1500.0	4.00	2435.0	932.9	1369.3	2554.6	100.0	751.1	1369.3	2606.2	100.0
83	1500.0	2.00	343.0	415.6	953.4	2328.9	99.9	416.5	1566.9	4563.0	98.3
84	1500.0	2.00	1500.0	574.7	1249.0	2738.6	99.8	511.5	1242.1	3909.0	96.0
85	1500.0	2.00	3952.8	702.9	1908.0	3528.5	100.0	527.2	1517.8	3644.1	97.7
86	1500.0	0.80	37.5	66.7	454.4	2435.3	98.7	84.4	631.9	4709.9	95.2
87	1500.0	0.80	1500.0	266.7	1249.0	3347.2	98.3	254.2	1242.1	5000.0	89.4
88	1500.0	0.50	4.1	7.0	107.0	2546.1	99.2	12.0	173.4	3270.6	97.6
89	1500.0	0.50	1500.0	181.7	1249.0	3347.2	96.9	158.4	1242.1	5000.0	86.2
90	3000.0	8.33	2105.1	2318.3	3244.3	3244.3	100.0	2354.3	3244.3	5000.0	94.8
91	3000.0	8.33	3000.0	1687.0	2935.9	3873.0	100.0	1687.0	3008.8	3873.0	97.6
92	3000.0	8.33	3785.8	2128.9	2128.9	3428.4	100.0	2128.9	2128.9	3522.0	99.7
93	3000.0	4.00	1434.6	1795.3	2678.3	3297.8	99.5	1789.0	2678.3	5000.0	92.3
94	3000.0	4.00	3000.0	1687.0	2935.9	3873.0	99.6	1687.0	3008.8	5000.0	85.8
95	3000.0	4.00	4870.0	1865.8	2738.6	4055.2	99.9	1502.3	2738.6	5000.0	94.2
96	3000.0	2.00	686.0	831.1	1952.3	3785.2	97.9	1073.9	3146.9	5000.0	82.0
97	3000.0	2.00	3000.0	1149.4	2423.3	4217.2	98.2	1152.0	3008.8	5000.0	77.1
98	3000.0	0.80	75.0	90.9	849.5	3899.8	97.6	168.7	1263.7	5000.0	88.5
99	3000.0	0.80	3000.0	703.8	2225.5	4591.9	95.7	533.5	2502.1	5000.0	72.7
100	3000.0	0.50	8.2	14.6	214.0	3600.7	98.7	18.4	346.9	5000.0	93.5

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
101	3000.0	0.50	3000.0	363.5	2225.5	4591.9	95.7	316.9	2278.9	5000.0	73.9
102	3500.0	8.33	2455.9	2569.2	3504.2	3945.1	100.0	2621.8	3504.2	5000.0	86.2
103	3500.0	8.33	3500.0	1968.2	3253.6	4183.3	99.9	1968.2	3340.7	5000.0	91.9
104	3500.0	8.33	4416.8	2483.7	2483.7	3799.5	100.0	2483.7	2483.7	4307.3	96.7
105	3500.0	4.00	1673.7	1989.7	2892.8	3471.7	98.6	2000.3	3678.9	5000.0	63.5
106	3500.0	4.00	3500.0	1968.2	3253.6	4439.5	99.0	1968.2	3340.7	5000.0	80.5
107	3500.0	2.00	800.4	969.7	2163.6	3984.8	97.2	1252.8	3566.3	5000.0	77.7
108	3500.0	2.00	3500.0	1340.9	3253.6	4439.5	97.2	1344.0	3340.7	5000.0	71.9
109	3500.0	0.80	87.5	106.0	965.9	4105.3	97.6	196.9	1474.3	5000.0	85.6
110	3500.0	0.80	3500.0	800.2	2685.6	4711.4	96.0	593.0	3340.7	5000.0	70.6
111	3500.0	0.50	9.6	17.0	249.8	2881.5	97.4	22.2	469.2	5000.0	92.7
112	3500.0	0.50	3500.0	424.0	2530.6	5000.0	94.1	413.3	3340.7	5000.0	70.0

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0
'%in range' means % > 1.0 and <5000.0

Convergence criterion # 5 [LR]

```
Critical nominal N                      = 6
slope assumed in probit calculations    = 2.00
step size (dose progression) log10      = 0.50
Generate outlier (1=>yes;0=>no)         = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test                = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario     = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
1	1.5	8.33	1.1	1.5	1.9	1.9	100.0	1.5	1.9	1.9	99.9
2	1.5	8.33	1.5	1.2	1.6	2.7	100.0	1.2	1.5	2.7	99.1
3	1.5	8.33	1.9	1.3	1.4	2.5	100.0	1.0	1.4	2.4	99.2
4	1.5	4.00	1.5	1.2	1.6	2.7	99.4	1.0	1.5	2.7	94.0
5	1.5	4.00	2.4	1.3	1.6	3.1	98.8	1.0	1.6	3.0	91.5
6	1.5	2.00	1.5	1.1	1.7	3.9	97.8	1.0	1.5	3.9	87.6
7	1.5	2.00	4.0	1.3	2.0	3.7	96.2	1.0	1.7	3.8	80.1
8	1.5	0.80	1.5	1.1	2.0	8.4	95.5	1.0	1.7	8.9	81.7
9	1.5	0.80	16.9	1.3	3.4	14.3	95.4	1.0	2.2	14.8	84.0
10	1.5	0.50	1.5	1.0	2.0	12.4	94.9	1.0	1.7	12.7	79.6
11	1.5	0.50	72.3	1.4	6.6	59.7	98.0	1.0	4.0	59.6	91.4
12	2.5	8.33	1.8	2.3	3.1	3.1	100.0	2.3	3.1	3.1	100.0
13	2.5	8.33	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	100.0
14	2.5	8.33	3.1	1.8	2.6	3.8	100.0	1.8	2.6	3.8	100.0
15	2.5	4.00	1.2	1.7	2.4	3.8	100.0	1.7	2.3	4.1	100.0
16	2.5	4.00	2.5	1.6	2.2	4.4	100.0	1.6	2.2	4.4	99.9
17	2.5	4.00	4.1	1.9	2.0	3.8	100.0	1.6	2.0	3.9	100.0
18	2.5	2.00	2.5	1.5	2.7	6.5	99.7	1.3	2.5	6.0	98.3

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
19	2.5	2.00	6.6	1.4	2.7	8.0	99.6	1.2	2.7	8.0	98.0
20	2.5	0.80	2.5	1.4	3.1	14.1	97.2	1.0	2.5	14.6	91.8
21	2.5	0.80	28.2	1.5	4.6	34.1	98.2	1.0	3.5	34.2	93.1
22	2.5	0.50	2.5	1.3	3.1	20.6	96.4	1.0	3.1	21.3	88.4
23	2.5	0.50	120.5	1.8	9.7	120.6	98.4	1.0	6.4	120.6	95.1
24	20.0	8.33	14.0	17.0	24.9	24.9	100.0	17.0	24.9	24.9	100.0
25	20.0	8.33	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
26	20.0	8.33	25.2	14.2	14.2	30.6	100.0	14.2	14.2	30.6	100.0
27	20.0	4.00	9.6	11.6	17.0	30.2	100.0	11.6	17.0	32.6	100.0
28	20.0	4.00	20.0	11.2	16.5	35.6	100.0	11.2	16.5	35.6	100.0
29	20.0	4.00	32.5	12.1	18.3	39.3	100.0	12.5	18.3	39.4	100.0
30	20.0	2.00	4.6	7.8	19.3	45.7	100.0	8.0	20.4	49.9	100.0
31	20.0	2.00	20.0	7.7	20.0	52.2	100.0	7.7	20.0	52.1	100.0
32	20.0	2.00	52.7	8.1	20.2	63.8	100.0	8.8	22.1	64.0	100.0
33	20.0	0.80	20.0	3.8	17.8	112.5	100.0	3.5	17.7	118.0	100.0
34	20.0	0.80	225.4	5.8	30.1	273.1	100.0	4.9	27.1	273.8	100.0
35	20.0	0.50	20.0	2.8	22.7	169.7	100.0	2.7	22.8	202.1	99.8
36	20.0	0.50	964.4	6.8	68.1	799.4	100.0	5.1	51.4	776.3	99.9
37	50.0	8.33	35.1	42.5	62.4	62.4	100.0	42.6	62.4	62.4	100.0
38	50.0	8.33	50.0	28.1	60.6	88.9	100.0	28.1	60.7	88.9	100.0
39	50.0	8.33	63.1	35.5	35.5	76.4	100.0	35.5	35.5	76.6	100.0
40	50.0	4.00	23.9	29.0	42.5	75.6	100.0	29.0	42.5	81.5	100.0
41	50.0	4.00	50.0	28.1	41.3	88.9	100.0	28.1	41.2	88.9	100.0
42	50.0	4.00	81.2	30.3	45.6	98.3	100.0	31.2	45.6	98.6	100.0
43	50.0	2.00	11.4	13.8	48.2	114.3	100.0	13.9	51.0	116.1	100.0
44	50.0	2.00	50.0	19.2	60.6	130.5	100.0	19.2	60.7	130.2	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
45	50.0	2.00	131.8	22.4	50.5	159.6	100.0	22.3	55.2	160.0	100.0
46	50.0	0.80	1.3	3.4	26.9	173.7	100.0	3.5	33.6	215.6	100.0
47	50.0	0.80	50.0	9.8	50.0	281.2	100.0	8.5	50.0	289.9	100.0
48	50.0	0.80	563.6	14.3	72.8	554.1	100.0	12.0	66.6	561.5	100.0
49	50.0	0.50	50.0	7.0	56.8	418.8	100.0	6.3	56.4	443.6	99.9
50	50.0	0.50	2411.1	14.2	180.8	1855.0	100.0	9.9	130.8	1888.0	100.0
51	150.0	8.33	105.3	127.5	187.2	187.2	100.0	127.8	187.2	187.2	100.0
52	150.0	8.33	150.0	84.4	181.7	266.7	100.0	84.4	182.1	266.7	100.0
53	150.0	8.33	189.3	106.4	106.4	229.3	100.0	106.4	106.4	229.9	100.0
54	150.0	4.00	71.7	86.9	127.6	226.8	100.0	87.1	127.6	244.6	100.0
55	150.0	4.00	150.0	84.4	181.7	266.7	100.0	84.4	182.1	266.7	100.0
56	150.0	4.00	243.5	90.8	136.9	295.0	100.0	93.5	136.9	295.7	100.0
57	150.0	2.00	34.3	41.6	144.6	343.0	100.0	41.7	153.1	374.5	100.0
58	150.0	2.00	150.0	57.5	123.8	391.5	100.0	57.6	123.5	390.6	100.0
59	150.0	2.00	395.3	70.3	151.4	478.9	100.0	67.0	165.6	480.0	100.0
60	150.0	0.80	3.8	12.6	78.6	518.4	100.0	13.3	100.7	645.5	100.0
61	150.0	0.80	150.0	26.7	150.0	843.5	100.0	25.7	150.0	872.7	100.0
62	150.0	0.80	1690.9	40.1	241.0	1658.8	100.0	37.6	220.6	1775.9	100.0
63	150.0	0.50	150.0	18.2	150.7	1168.8	100.0	17.7	150.0	1277.2	99.8
64	600.0	8.33	421.0	510.1	748.7	748.7	100.0	511.2	748.7	748.7	100.0
65	600.0	8.33	600.0	337.4	495.2	1067.0	100.0	337.4	494.1	1067.0	100.0
66	600.0	8.33	757.2	425.8	425.8	917.3	100.0	425.8	425.8	919.4	100.0
67	600.0	4.00	286.9	347.6	546.9	1042.5	100.0	348.4	522.8	1067.1	100.0
68	600.0	4.00	600.0	337.4	726.9	1067.0	100.0	337.4	728.6	1067.0	100.0
69	600.0	4.00	974.0	363.1	547.7	1099.4	100.0	374.0	547.7	1054.2	100.0
70	600.0	2.00	137.2	208.5	578.6	1421.6	100.0	203.4	612.4	1444.8	100.0

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
71	600.0	2.00	600.0	229.9	495.2	1519.2	100.0	230.4	494.1	1531.0	100.0
72	600.0	2.00	1581.1	259.0	616.4	1915.6	100.0	267.9	668.7	1920.0	100.0
73	600.0	0.80	15.0	39.2	312.1	1521.7	99.8	39.1	402.7	2118.6	99.5
74	600.0	0.80	600.0	106.7	584.6	2220.6	99.8	102.7	596.4	2650.2	99.4
75	600.0	0.50	1.6	9.6	115.1	1345.4	99.8	9.7	179.9	1976.6	99.2
76	600.0	0.50	600.0	70.7	525.1	2568.2	99.5	66.7	596.4	3246.3	97.8
77	1500.0	8.33	1052.5	1165.3	2294.1	2294.1	100.0	1126.4	2294.1	2294.1	100.0
78	1500.0	8.33	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
79	1500.0	8.33	1892.9	1064.5	1064.5	2159.8	100.0	1064.5	1064.5	2184.1	100.0
80	1500.0	4.00	717.3	869.0	1275.6	2411.8	100.0	871.0	1275.6	2283.5	100.0
81	1500.0	4.00	1500.0	843.5	1849.5	2738.6	100.0	843.5	1848.1	2738.6	100.0
82	1500.0	4.00	2435.0	907.7	1369.3	2554.6	100.0	935.0	1369.3	2606.2	100.0
83	1500.0	2.00	343.0	415.6	1328.0	2403.2	99.8	416.5	1470.8	3174.5	99.2
84	1500.0	2.00	1500.0	574.7	1249.0	2738.6	99.9	629.6	1242.1	2886.1	99.5
85	1500.0	2.00	3952.8	647.4	1514.4	3528.5	100.0	669.7	1517.8	3625.5	99.8
86	1500.0	0.80	37.5	118.6	695.0	2599.9	98.7	127.9	967.2	4261.2	96.2
87	1500.0	0.80	1500.0	266.7	1249.0	3347.2	97.9	256.8	1250.1	5000.0	93.5
88	1500.0	0.50	4.1	30.7	248.3	2546.1	99.3	34.7	448.1	3805.4	96.9
89	1500.0	0.50	1500.0	181.7	1249.0	3347.2	97.0	177.1	1250.1	5000.0	90.6
90	3000.0	8.33	2105.1	2318.3	3244.3	3374.4	100.0	2354.3	3244.3	3949.0	99.9
91	3000.0	8.33	3000.0	1687.0	2754.0	3873.0	100.0	1687.0	2881.6	3873.0	99.5
92	3000.0	8.33	3785.8	2128.9	2128.9	3428.4	100.0	2128.9	2128.9	3522.0	100.0
93	3000.0	4.00	1434.6	1795.3	2678.3	3297.8	99.6	1789.0	2678.3	4965.0	95.9
94	3000.0	4.00	3000.0	1687.0	2935.9	3873.0	99.8	1687.0	3008.8	4713.0	96.4
95	3000.0	4.00	4870.0	1815.3	2738.6	4055.2	99.9	1870.0	2738.6	4167.6	98.4
96	3000.0	2.00	686.0	831.1	2356.3	3785.2	98.5	833.0	2858.2	5000.0	88.1

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
97	3000.0	2.00	3000.0	1149.4	2754.0	4128.4	98.6	1172.1	3008.8	5000.0	90.5
98	3000.0	0.80	75.0	211.4	1268.1	3812.7	97.6	228.8	1786.6	5000.0	90.0
99	3000.0	0.80	3000.0	533.5	2498.3	4272.8	96.3	513.6	2968.0	5000.0	82.6
100	3000.0	0.50	8.2	50.1	453.4	3286.1	99.1	58.9	825.4	5000.0	94.7
101	3000.0	0.50	3000.0	363.5	2225.5	4591.9	95.1	351.9	2550.0	5000.0	81.6
102	3500.0	8.33	2455.9	2569.2	3504.2	3945.1	99.8	2621.8	3504.2	4661.5	98.4
103	3500.0	8.33	3500.0	1968.2	3253.6	4183.3	99.9	1968.2	3340.7	4402.7	97.4
104	3500.0	8.33	4416.8	2483.7	2483.7	3799.5	99.9	2483.7	2483.7	3904.2	99.8
105	3500.0	4.00	1673.7	1989.7	2892.8	3471.7	98.4	2000.3	2976.3	5000.0	83.6
106	3500.0	4.00	3500.0	1968.2	3253.6	4267.0	99.1	1968.2	3340.7	5000.0	90.3
107	3500.0	2.00	800.4	1029.0	2629.7	3984.8	97.1	1033.8	3305.6	5000.0	81.0
108	3500.0	2.00	3500.0	1340.9	3052.0	4439.5	97.1	1344.0	3340.7	5000.0	83.8
109	3500.0	0.80	87.5	276.8	1440.0	4105.3	97.7	298.5	2163.6	5000.0	85.6
110	3500.0	0.80	3500.0	622.4	2530.6	4604.9	95.8	593.0	2986.7	5000.0	80.7

	LD50	slope	Dose0	Dose Averaging percentiles 5%	50%	95%	%in range	MLE (slope= 2.00) percentiles 5%	50%	95%	%in range
111	3500.0	0.50	9.6	74.1	481.5	2881.5	97.4	81.0	935.0	5000.0	92.1
112	3500.0	0.50	3500.0	412.6	2530.6	5000.0	94.9	368.8	2986.7	5000.0	77.8

Values of 1.0 indicate < 1.0 and values of 5000.0 indicate >5000.0
'%in range' means % > 1.0 and <5000.0

2.4.2 OECD-Type scenarios: Results for Numbers Tested

Convergence criterion # 1 [fixed nominal NR]

```
Critical nominal N                    = 6
slope assumed in probit calculations  = 2.00
step size (dose progression) log10    = 0.50
Generate outlier (1=>yes;0=>no)       = 0

max num. animals to test              = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario   = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	1.5	8.33	1.1	6.01	6.00	0.00
2	1.5	8.33	1.5	6.03	6.00	0.00
3	1.5	8.33	1.9	6.05	7.00	0.00
4	1.5	4.00	1.5	6.14	7.00	0.00
5	1.5	4.00	2.4	6.20	7.00	0.00
6	1.5	2.00	1.5	6.25	7.00	0.00
7	1.5	2.00	4.0	6.25	8.00	0.00
8	1.5	0.80	1.5	6.35	8.00	0.00
9	1.5	0.80	16.9	6.73	9.00	0.00
10	1.5	0.50	1.5	6.40	8.00	0.00
11	1.5	0.50	72.3	7.22	10.00	0.00
12	2.5	8.33	1.8	6.00	6.00	0.00
13	2.5	8.33	2.5	6.00	6.00	0.00
14	2.5	8.33	3.1	6.00	6.00	0.00
15	2.5	4.00	1.2	6.21	7.00	0.00
16	2.5	4.00	2.5	6.04	6.00	0.00
17	2.5	4.00	4.1	6.05	7.00	0.00
18	2.5	2.00	2.5	6.20	7.00	0.00
19	2.5	2.00	6.6	6.48	8.00	0.00
20	2.5	0.80	2.5	6.36	8.00	0.00
21	2.5	0.80	28.2	6.88	9.00	0.00
22	2.5	0.50	2.5	6.42	8.00	0.00
23	2.5	0.50	120.5	7.22	10.00	0.00
24	20.0	8.33	14.0	6.00	6.00	0.00
25	20.0	8.33	20.0	6.00	6.00	0.00
26	20.0	8.33	25.2	6.00	6.00	0.00
27	20.0	4.00	9.6	6.21	7.00	0.00
28	20.0	4.00	20.0	6.02	6.00	0.00
29	20.0	4.00	32.5	6.10	7.00	0.00
30	20.0	2.00	4.6	6.69	8.00	0.00
31	20.0	2.00	20.0	6.15	7.00	0.00
32	20.0	2.00	52.7	6.40	7.00	0.00
33	20.0	0.80	20.0	6.42	8.00	0.00
34	20.0	0.80	225.4	6.99	9.00	0.00
35	20.0	0.50	20.0	6.55	8.00	0.00
36	20.0	0.50	964.4	7.29	10.00	0.00
37	50.0	8.33	35.1	6.00	6.00	0.00
38	50.0	8.33	50.0	6.00	6.00	0.00
39	50.0	8.33	63.1	6.00	6.00	0.00
40	50.0	4.00	23.9	6.22	7.00	0.00
41	50.0	4.00	50.0	6.02	6.00	0.00
42	50.0	4.00	81.2	6.11	7.00	0.00
43	50.0	2.00	11.4	6.66	8.00	0.00
44	50.0	2.00	50.0	6.16	7.00	0.00
45	50.0	2.00	131.8	6.41	7.00	0.00
46	50.0	0.80	1.3	7.65	10.00	0.00
47	50.0	0.80	50.0	6.44	8.00	0.00

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
48	50.0	0.80	563.6	6.95	9.00	0.00
49	50.0	0.50	50.0	6.57	8.00	0.00
50	50.0	0.50	2411.1	7.28	10.00	0.00
51	150.0	8.33	105.3	6.00	6.00	0.00
52	150.0	8.33	150.0	6.00	6.00	0.00
53	150.0	8.33	189.3	6.00	6.00	0.00
54	150.0	4.00	71.7	6.22	7.00	0.00
55	150.0	4.00	150.0	6.03	6.00	0.00
56	150.0	4.00	243.5	6.09	7.00	0.00
57	150.0	2.00	34.3	6.69	8.00	0.00
58	150.0	2.00	150.0	6.17	7.00	0.00
59	150.0	2.00	395.3	6.42	7.00	0.00
60	150.0	0.80	3.8	7.64	10.00	0.00
61	150.0	0.80	150.0	6.41	8.00	0.00
62	150.0	0.80	1690.9	6.99	9.00	0.00
63	150.0	0.50	150.0	6.55	8.00	0.00
64	600.0	8.33	421.0	6.00	6.00	0.00
65	600.0	8.33	600.0	6.00	6.00	0.00
66	600.0	8.33	757.2	6.00	6.00	0.00
67	600.0	4.00	286.9	6.21	7.00	0.00
68	600.0	4.00	600.0	6.03	6.00	0.00
69	600.0	4.00	974.0	6.09	7.00	0.00
70	600.0	2.00	137.2	6.72	8.00	0.00
71	600.0	2.00	600.0	6.17	7.00	0.00
72	600.0	2.00	1581.1	6.39	7.00	0.00
73	600.0	0.80	15.0	7.58	10.00	0.00
74	600.0	0.80	600.0	6.42	8.00	0.00
75	600.0	0.50	1.6	8.31	12.00	0.00
76	600.0	0.50	600.0	6.52	8.00	0.00
77	1500.0	8.33	1052.5	6.00	6.00	0.00
78	1500.0	8.33	1500.0	6.00	6.00	0.00
79	1500.0	8.33	1892.9	6.00	6.00	0.00
80	1500.0	4.00	717.3	6.21	7.00	0.00
81	1500.0	4.00	1500.0	6.02	6.00	0.00
82	1500.0	4.00	2435.0	6.10	7.00	0.00
83	1500.0	2.00	343.0	6.61	8.00	0.00
84	1500.0	2.00	1500.0	6.17	7.00	0.00
85	1500.0	2.00	3952.8	6.43	7.00	0.00
86	1500.0	0.80	37.5	7.53	10.00	0.00
87	1500.0	0.80	1500.0	6.36	8.00	0.00
88	1500.0	0.50	4.1	8.24	11.00	0.00
89	1500.0	0.50	1500.0	6.43	8.00	0.00
90	3000.0	8.33	2105.1	6.03	6.00	0.00
91	3000.0	8.33	3000.0	6.01	6.00	0.00
92	3000.0	8.33	3785.8	6.01	6.00	0.00
93	3000.0	4.00	1434.6	6.17	7.00	0.00
94	3000.0	4.00	3000.0	6.10	7.00	0.00
95	3000.0	4.00	4870.0	6.14	7.00	0.00
96	3000.0	2.00	686.0	6.74	8.00	0.00
97	3000.0	2.00	3000.0	6.24	7.00	0.00
98	3000.0	0.80	75.0	7.60	10.00	0.00
99	3000.0	0.80	3000.0	6.34	8.00	0.00
100	3000.0	0.50	8.2	8.23	12.00	0.00
101	3000.0	0.50	3000.0	6.44	8.00	0.00
102	3500.0	8.33	2455.9	6.10	7.00	0.00
103	3500.0	8.33	3500.0	6.06	7.00	0.00
104	3500.0	8.33	4416.8	6.02	6.00	0.00
105	3500.0	4.00	1673.7	6.24	7.00	0.00
106	3500.0	4.00	3500.0	6.14	7.00	0.00
107	3500.0	2.00	800.4	6.73	9.00	0.00

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
108	3500.0	2.00	3500.0	6.22	7.00	0.00
109	3500.0	0.80	87.5	7.58	10.00	0.00
110	3500.0	0.80	3500.0	6.37	8.00	0.00
111	3500.0	0.50	9.6	8.11	11.00	0.00
112	3500.0	0.50	3500.0	6.38	8.00	0.00

**** Numbers Tested ****

Convergence criterion # 5 [LR]

```
Critical nominal N                    = 6
slope assumed in probit calculations  = 2.00
step size (dose progression) log10    = 0.50
Generate outlier (1=>yes;0=>no)       = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test              = 15
doses restricted to range      1.0   5000.0  (min,max)
Num. simulated studies per scenario  =  3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
1	1.5	8.33	1.1	6.05	6.00	0.03
2	1.5	8.33	1.5	6.29	9.00	0.03
3	1.5	8.33	1.9	6.54	9.00	0.33
4	1.5	4.00	1.5	7.07	13.00	2.47
5	1.5	4.00	2.4	8.12	15.00	8.50
6	1.5	2.00	1.5	7.77	14.00	4.70
7	1.5	2.00	4.0	9.75	15.00	23.03
8	1.5	0.80	1.5	8.47	15.00	6.40
9	1.5	0.80	16.9	10.46	15.00	24.67
10	1.5	0.50	1.5	8.69	15.00	7.10
11	1.5	0.50	72.3	11.52	15.00	34.00
12	2.5	8.33	1.8	6.01	6.00	0.00
13	2.5	8.33	2.5	6.00	6.00	0.00
14	2.5	8.33	3.1	6.00	6.00	0.00
15	2.5	4.00	1.2	6.97	9.00	0.00
16	2.5	4.00	2.5	6.28	8.00	0.10
17	2.5	4.00	4.1	7.37	11.00	0.80
18	2.5	2.00	2.5	7.39	13.00	2.33
19	2.5	2.00	6.6	8.45	15.00	6.00
20	2.5	0.80	2.5	8.39	15.00	6.10
21	2.5	0.80	28.2	10.42	15.00	22.37
22	2.5	0.50	2.5	8.61	15.00	6.27
23	2.5	0.50	120.5	11.38	15.00	31.33
24	20.0	8.33	14.0	6.01	6.00	0.00
25	20.0	8.33	20.0	6.00	6.00	0.00
26	20.0	8.33	25.2	6.00	6.00	0.00
27	20.0	4.00	9.6	6.97	9.00	0.00
28	20.0	4.00	20.0	6.10	6.00	0.00
29	20.0	4.00	32.5	6.43	8.00	0.00
30	20.0	2.00	4.6	9.04	13.00	2.07
31	20.0	2.00	20.0	6.71	9.00	0.00
32	20.0	2.00	52.7	7.77	11.00	0.03
33	20.0	0.80	20.0	8.01	12.00	1.40
34	20.0	0.80	225.4	10.47	15.00	18.07
35	20.0	0.50	20.0	8.65	14.00	4.17
36	20.0	0.50	964.4	11.97	15.00	37.80
37	50.0	8.33	35.1	6.01	6.00	0.00
38	50.0	8.33	50.0	6.00	6.00	0.00
39	50.0	8.33	63.1	6.00	6.00	0.00
40	50.0	4.00	23.9	6.94	9.00	0.00
41	50.0	4.00	50.0	6.10	6.00	0.00
42	50.0	4.00	81.2	6.47	8.00	0.00
43	50.0	2.00	11.4	8.74	12.00	1.17
44	50.0	2.00	50.0	6.74	9.00	0.00
45	50.0	2.00	131.8	7.87	11.00	0.13
46	50.0	0.80	1.3	11.86	15.00	30.03

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
47	50.0	0.80	50.0	7.98	12.00	1.17
48	50.0	0.80	563.6	10.42	15.00	15.57
49	50.0	0.50	50.0	8.70	14.00	4.23
50	50.0	0.50	2411.1	11.60	15.00	33.90
51	150.0	8.33	105.3	6.01	6.00	0.00
52	150.0	8.33	150.0	6.00	6.00	0.00
53	150.0	8.33	189.3	6.00	6.00	0.00
54	150.0	4.00	71.7	6.94	9.00	0.00
55	150.0	4.00	150.0	6.08	6.00	0.00
56	150.0	4.00	243.5	6.43	8.00	0.00
57	150.0	2.00	34.3	8.69	12.00	1.17
58	150.0	2.00	150.0	6.69	9.00	0.00
59	150.0	2.00	395.3	7.82	11.00	0.10
60	150.0	0.80	3.8	12.05	15.00	32.80
61	150.0	0.80	150.0	8.00	12.00	0.90
62	150.0	0.80	1690.9	10.30	15.00	15.80
63	150.0	0.50	150.0	8.68	14.00	4.33
64	600.0	8.33	421.0	6.01	6.00	0.00
65	600.0	8.33	600.0	6.00	6.00	0.00
66	600.0	8.33	757.2	6.00	6.00	0.00
67	600.0	4.00	286.9	7.40	10.00	0.00
68	600.0	4.00	600.0	6.10	6.00	0.00
69	600.0	4.00	974.0	7.30	10.00	0.00
70	600.0	2.00	137.2	8.79	13.00	1.67
71	600.0	2.00	600.0	6.79	10.00	0.00
72	600.0	2.00	1581.1	7.82	11.00	0.13
73	600.0	0.80	15.0	11.84	15.00	31.27
74	600.0	0.80	600.0	8.23	13.00	3.53
75	600.0	0.50	1.6	13.22	15.00	55.77
76	600.0	0.50	600.0	8.73	15.00	5.90
77	1500.0	8.33	1052.5	6.52	8.00	0.00
78	1500.0	8.33	1500.0	6.00	6.00	0.00
79	1500.0	8.33	1892.9	6.00	6.00	0.00
80	1500.0	4.00	717.3	6.97	10.00	0.03
81	1500.0	4.00	1500.0	6.11	6.00	0.10
82	1500.0	4.00	2435.0	6.49	8.00	0.00
83	1500.0	2.00	343.0	9.36	15.00	8.37
84	1500.0	2.00	1500.0	7.00	11.00	1.60
85	1500.0	2.00	3952.8	7.86	11.00	0.23
86	1500.0	0.80	37.5	11.89	15.00	34.07
87	1500.0	0.80	1500.0	8.16	15.00	5.50
88	1500.0	0.50	4.1	13.23	15.00	54.27
89	1500.0	0.50	1500.0	8.61	15.00	7.57
90	3000.0	8.33	2105.1	6.28	8.00	0.10
91	3000.0	8.33	3000.0	6.13	6.00	0.00
92	3000.0	8.33	3785.8	6.03	6.00	0.00
93	3000.0	4.00	1434.6	8.19	15.00	12.57
94	3000.0	4.00	3000.0	6.83	11.00	1.10
95	3000.0	4.00	4870.0	6.67	9.00	0.20
96	3000.0	2.00	686.0	9.89	15.00	19.07
97	3000.0	2.00	3000.0	7.73	14.00	3.93
98	3000.0	0.80	75.0	11.83	15.00	35.10
99	3000.0	0.80	3000.0	8.41	15.00	5.67
100	3000.0	0.50	8.2	13.24	15.00	56.17
101	3000.0	0.50	3000.0	8.55	15.00	6.73
102	3500.0	8.33	2455.9	6.83	11.00	1.23
103	3500.0	8.33	3500.0	6.34	9.00	0.27
104	3500.0	8.33	4416.8	6.12	6.00	0.03
105	3500.0	4.00	1673.7	8.93	15.00	15.37
106	3500.0	4.00	3500.0	7.13	13.00	2.37

	LD50	slope	Dose0	mean	95th %ile	(%)N=max (= 15)
107	3500.0	2.00	800.4	10.00	15.00	20.20
108	3500.0	2.00	3500.0	7.84	14.00	4.90
109	3500.0	0.80	87.5	12.01	15.00	37.37
110	3500.0	0.80	3500.0	8.44	15.00	6.47
111	3500.0	0.50	9.6	12.95	15.00	51.43
112	3500.0	0.50	3500.0	8.63	15.00	7.50

2.4.3 OECD-Type scenarios: Classification Probabilities

**** Classification percentages based on MLE ****

Convergence criterion # 1 [fixed nominal NR]

```
Critical nominal N                    = 6
slope assumed in probit calculations  = 2.00
step size (dose progression) log10    = 0.50
Generate outlier (1=>yes;0=>no)       = 0

max num. animals to test              = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario   = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
1	1.5	8.33	1.1	1	100.0	0.0	0.0	0.0	0.0	0.0
2	1.5	8.33	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
3	1.5	8.33	1.9	1	100.0	0.0	0.0	0.0	0.0	0.0
4	1.5	4.00	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
5	1.5	4.00	2.4	1	100.0	0.0	0.0	0.0	0.0	0.0
6	1.5	2.00	1.5	1	97.8	2.2	0.0	0.0	0.0	0.0
7	1.5	2.00	4.0	1	98.2	1.8	0.0	0.0	0.0	0.0
8	1.5	0.80	1.5	1	86.3	13.6	0.1	0.0	0.0	0.0
9	1.5	0.80	16.9	1	67.9	31.6	0.4	0.0	0.0	0.0
10	1.5	0.50	1.5	1	82.3	17.1	0.6	0.0	0.0	0.0
11	1.5	0.50	72.3	1	42.1	48.6	8.9	0.4	0.0	0.0
12	2.5	8.33	1.8	1	99.7	0.3	0.0	0.0	0.0	0.0
13	2.5	8.33	2.5	1	100.0	0.0	0.0	0.0	0.0	0.0
14	2.5	8.33	3.1	1	99.0	1.0	0.0	0.0	0.0	0.0
15	2.5	4.00	1.2	1	94.6	5.4	0.0	0.0	0.0	0.0
16	2.5	4.00	2.5	1	98.1	1.9	0.0	0.0	0.0	0.0
17	2.5	4.00	4.1	1	99.2	0.8	0.0	0.0	0.0	0.0
18	2.5	2.00	2.5	1	87.4	12.6	0.0	0.0	0.0	0.0
19	2.5	2.00	6.6	1	81.7	18.3	0.0	0.0	0.0	0.0
20	2.5	0.80	2.5	1	73.5	26.1	0.4	0.0	0.0	0.0
21	2.5	0.80	28.2	1	49.3	48.3	2.4	0.0	0.0	0.0
22	2.5	0.50	2.5	1	68.6	30.0	1.3	0.0	0.0	0.0
23	2.5	0.50	120.5	1	29.4	51.5	18.0	1.1	0.0	0.0
24	20.0	8.33	14.0	2	0.0	100.0	0.0	0.0	0.0	0.0
25	20.0	8.33	20.0	2	0.0	100.0	0.0	0.0	0.0	0.0
26	20.0	8.33	25.2	2	0.0	100.0	0.0	0.0	0.0	0.0
27	20.0	4.00	9.6	2	0.0	98.9	1.1	0.0	0.0	0.0
28	20.0	4.00	20.0	2	0.0	98.7	1.3	0.0	0.0	0.0
29	20.0	4.00	32.5	2	0.0	99.1	0.9	0.0	0.0	0.0
30	20.0	2.00	4.6	2	1.2	93.1	5.8	0.0	0.0	0.0
31	20.0	2.00	20.0	2	2.1	90.0	7.9	0.0	0.0	0.0
32	20.0	2.00	52.7	2	0.7	92.9	6.3	0.0	0.0	0.0
33	20.0	0.80	20.0	2	11.7	68.2	19.2	0.9	0.0	0.0
34	20.0	0.80	225.4	2	5.4	53.7	37.6	3.3	0.0	0.0
35	20.0	0.50	20.0	2	17.4	58.0	21.9	2.7	0.0	0.0
36	20.0	0.50	964.4	2	4.7	27.7	46.8	19.2	1.7	0.0
37	50.0	8.33	35.1	2	0.0	25.5	74.5	0.0	0.0	0.0
38	50.0	8.33	50.0	2	0.0	49.9	50.1	0.0	0.0	0.0
39	50.0	8.33	63.1	2	0.0	52.0	48.0	0.0	0.0	0.0
40	50.0	4.00	23.9	2	0.0	51.0	49.0	0.0	0.0	0.0
41	50.0	4.00	50.0	2	0.0	48.7	51.3	0.0	0.0	0.0
42	50.0	4.00	81.2	2	0.0	62.2	37.8	0.0	0.0	0.0
43	50.0	2.00	11.4	2	0.0	52.8	46.9	0.2	0.0	0.0

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
44	50.0	2.00	50.0	2	0.0	48.8	51.0	0.2	0.0	0.0
45	50.0	2.00	131.8	2	0.0	47.4	52.4	0.2	0.0	0.0
46	50.0	0.80	1.3	2	11.5	57.8	28.8	1.9	0.0	0.0
47	50.0	0.80	50.0	2	1.5	48.5	45.7	4.2	0.1	0.0
48	50.0	0.80	563.6	2	0.8	30.3	52.8	15.8	0.3	0.0
49	50.0	0.50	50.0	2	3.5	46.2	40.8	8.9	0.6	0.1
50	50.0	0.50	2411.1	2	1.8	17.0	33.8	42.0	4.7	0.6
51	150.0	8.33	105.3	3	0.0	0.0	99.6	0.4	0.0	0.0
52	150.0	8.33	150.0	3	0.0	0.0	100.0	0.0	0.0	0.0
53	150.0	8.33	189.3	3	0.0	0.0	99.3	0.7	0.0	0.0
54	150.0	4.00	71.7	3	0.0	0.1	94.6	5.4	0.0	0.0
55	150.0	4.00	150.0	3	0.0	0.3	97.8	1.9	0.0	0.0
56	150.0	4.00	243.5	3	0.0	0.2	98.7	1.0	0.0	0.0
57	150.0	2.00	34.3	3	0.0	5.5	82.1	12.4	0.0	0.0
58	150.0	2.00	150.0	3	0.0	3.9	82.8	13.3	0.0	0.0
59	150.0	2.00	395.3	3	0.0	3.6	76.7	19.7	0.0	0.0
60	150.0	0.80	3.8	3	1.6	40.3	46.8	10.9	0.4	0.0
61	150.0	0.80	150.0	3	0.0	15.3	57.8	25.8	1.0	0.1
62	150.0	0.80	1690.9	3	0.0	6.9	44.6	43.4	4.9	0.2
63	150.0	0.50	150.0	3	0.9	18.4	49.2	28.6	2.6	0.3
64	600.0	8.33	421.0	4	0.0	0.0	0.0	100.0	0.0	0.0
65	600.0	8.33	600.0	4	0.0	0.0	0.0	100.0	0.0	0.0
66	600.0	8.33	757.2	4	0.0	0.0	0.1	99.9	0.0	0.0
67	600.0	4.00	286.9	4	0.0	0.0	2.2	96.6	1.2	0.0
68	600.0	4.00	600.0	4	0.0	0.0	2.1	97.8	0.1	0.0
69	600.0	4.00	974.0	4	0.0	0.0	3.0	96.3	0.7	0.0
70	600.0	2.00	137.2	4	0.0	0.0	13.5	83.4	3.0	0.1
71	600.0	2.00	600.0	4	0.0	0.0	12.5	85.5	2.0	0.0
72	600.0	2.00	1581.1	4	0.0	0.0	12.7	85.6	1.6	0.1
73	600.0	0.80	15.0	4	0.0	12.2	43.0	37.4	6.5	0.9
74	600.0	0.80	600.0	4	0.0	1.0	26.0	62.9	8.5	1.6
75	600.0	0.50	1.6	4	5.6	37.7	32.1	20.3	3.5	0.8
76	600.0	0.50	600.0	4	0.1	3.4	27.2	53.4	12.4	3.4
77	1500.0	8.33	1052.5	4	0.0	0.0	0.0	25.7	74.3	0.0
78	1500.0	8.33	1500.0	4	0.0	0.0	0.0	86.2	13.8	0.0
79	1500.0	8.33	1892.9	4	0.0	0.0	0.0	89.8	10.2	0.0
80	1500.0	4.00	717.3	4	0.0	0.0	0.0	68.5	31.4	0.1
81	1500.0	4.00	1500.0	4	0.0	0.0	0.0	85.8	13.9	0.4
82	1500.0	4.00	2435.0	4	0.0	0.0	0.0	90.8	9.2	0.0
83	1500.0	2.00	343.0	4	0.0	0.0	1.5	68.7	28.1	1.7
84	1500.0	2.00	1500.0	4	0.0	0.0	0.2	76.1	19.8	4.0
85	1500.0	2.00	3952.8	4	0.0	0.0	0.7	63.5	33.5	2.3
86	1500.0	0.80	37.5	4	0.0	2.2	28.0	50.5	14.6	4.8
87	1500.0	0.80	1500.0	4	0.0	0.1	6.2	60.2	22.9	10.6
88	1500.0	0.50	4.1	4	1.1	24.2	34.4	29.5	8.4	2.4
89	1500.0	0.50	1500.0	4	0.0	0.4	10.5	54.0	21.3	13.8
90	3000.0	8.33	2105.1	5	0.0	0.0	0.0	2.8	92.0	5.2
91	3000.0	8.33	3000.0	5	0.0	0.0	0.0	12.4	85.2	2.4
92	3000.0	8.33	3785.8	5	0.0	0.0	0.0	0.2	99.5	0.3
93	3000.0	4.00	1434.6	5	0.0	0.0	0.0	18.5	73.7	7.7
94	3000.0	4.00	3000.0	5	0.0	0.0	0.0	15.0	70.8	14.2
95	3000.0	4.00	4870.0	5	0.0	0.0	0.0	20.8	73.5	5.8
96	3000.0	2.00	686.0	5	0.0	0.0	0.1	27.2	54.8	18.0
97	3000.0	2.00	3000.0	5	0.0	0.0	0.0	24.2	52.9	22.9
98	3000.0	0.80	75.0	5	0.0	0.3	11.1	53.3	23.7	11.5
99	3000.0	0.80	3000.0	5	0.0	0.0	1.6	34.6	36.6	27.3
100	3000.0	0.50	8.2	5	0.3	13.9	33.9	33.6	11.8	6.5
101	3000.0	0.50	3000.0	5	0.0	0.2	4.4	36.7	32.5	26.1
102	3500.0	8.33	2455.9	5	0.0	0.0	0.0	2.4	83.8	13.8

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
103	3500.0	8.33	3500.0	5	0.0	0.0	0.0	12.0	79.9	8.1
104	3500.0	8.33	4416.8	5	0.0	0.0	0.0	0.1	96.7	3.3
105	3500.0	4.00	1673.7	5	0.0	0.0	0.0	2.2	61.3	36.5
106	3500.0	4.00	3500.0	5	0.0	0.0	0.0	13.4	67.1	19.5
107	3500.0	2.00	800.4	5	0.0	0.0	0.0	20.5	57.2	22.3
108	3500.0	2.00	3500.0	5	0.0	0.0	0.0	21.6	50.3	28.1
109	3500.0	0.80	87.5	5	0.0	0.3	12.9	48.0	24.4	14.4
110	3500.0	0.80	3500.0	5	0.0	0.0	1.1	32.7	36.7	29.4
111	3500.0	0.50	9.6	5	0.2	13.4	30.6	34.7	13.7	7.3
112	3500.0	0.50	3500.0	5	0.0	0.1	3.4	32.8	33.7	30.0

** Classification percentages based on MLE **

Convergence criterion # 5 [LR]

```
Critical nominal N                    = 6
slope assumed in probit calculations  = 2.00
step size (dose progression) log10    = 0.50
Generate outlier (1=>yes;0=>no)       = 0
(if Crit #5) factor above/below g.mean = 2.50
(if Crit #5) Critical likelihood ratio = 2.50
max num. animals to test              = 15
doses restricted to range      1.0,5000.0  (min,max)
Num. simulated studies per scenario   = 3000
Classification cutpoints      5     50    300   2000   5000
```

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
1	1.5	8.33	1.1	1	100.0	0.0	0.0	0.0	0.0	0.0
2	1.5	8.33	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
3	1.5	8.33	1.9	1	100.0	0.0	0.0	0.0	0.0	0.0
4	1.5	4.00	1.5	1	100.0	0.0	0.0	0.0	0.0	0.0
5	1.5	4.00	2.4	1	99.9	0.1	0.0	0.0	0.0	0.0
6	1.5	2.00	1.5	1	98.4	1.6	0.0	0.0	0.0	0.0
7	1.5	2.00	4.0	1	96.9	3.1	0.0	0.0	0.0	0.0
8	1.5	0.80	1.5	1	87.8	12.2	0.0	0.0	0.0	0.0
9	1.5	0.80	16.9	1	76.6	23.1	0.3	0.0	0.0	0.0
10	1.5	0.50	1.5	1	81.6	17.8	0.6	0.0	0.0	0.0
11	1.5	0.50	72.3	1	55.9	36.2	7.9	0.1	0.0	0.0
12	2.5	8.33	1.8	1	100.0	0.0	0.0	0.0	0.0	0.0
13	2.5	8.33	2.5	1	100.0	0.0	0.0	0.0	0.0	0.0
14	2.5	8.33	3.1	1	99.3	0.7	0.0	0.0	0.0	0.0
15	2.5	4.00	1.2	1	96.9	3.1	0.0	0.0	0.0	0.0
16	2.5	4.00	2.5	1	99.0	1.0	0.0	0.0	0.0	0.0
17	2.5	4.00	4.1	1	97.5	2.5	0.0	0.0	0.0	0.0
18	2.5	2.00	2.5	1	91.4	8.6	0.0	0.0	0.0	0.0
19	2.5	2.00	6.6	1	79.0	21.0	0.0	0.0	0.0	0.0
20	2.5	0.80	2.5	1	77.5	22.5	0.1	0.0	0.0	0.0
21	2.5	0.80	28.2	1	63.6	34.0	2.3	0.0	0.0	0.0
22	2.5	0.50	2.5	1	71.2	27.3	1.5	0.0	0.0	0.0
23	2.5	0.50	120.5	1	42.4	44.1	12.8	0.7	0.0	0.0
24	20.0	8.33	14.0	2	0.0	100.0	0.0	0.0	0.0	0.0
25	20.0	8.33	20.0	2	0.0	100.0	0.0	0.0	0.0	0.0
26	20.0	8.33	25.2	2	0.0	100.0	0.0	0.0	0.0	0.0
27	20.0	4.00	9.6	2	0.0	98.8	1.2	0.0	0.0	0.0
28	20.0	4.00	20.0	2	0.0	99.3	0.7	0.0	0.0	0.0
29	20.0	4.00	32.5	2	0.0	99.1	0.9	0.0	0.0	0.0
30	20.0	2.00	4.6	2	1.2	96.1	2.7	0.0	0.0	0.0
31	20.0	2.00	20.0	2	0.8	93.6	5.6	0.0	0.0	0.0
32	20.0	2.00	52.7	2	0.5	92.1	7.4	0.0	0.0	0.0
33	20.0	0.80	20.0	2	8.5	72.3	18.6	0.5	0.0	0.0
34	20.0	0.80	225.4	2	5.1	64.3	28.1	2.4	0.0	0.0
35	20.0	0.50	20.0	2	15.1	60.1	22.4	2.4	0.0	0.0
36	20.0	0.50	964.4	2	4.9	44.4	35.6	13.8	1.3	0.0
37	50.0	8.33	35.1	2	0.0	26.2	73.8	0.0	0.0	0.0
38	50.0	8.33	50.0	2	0.0	49.3	50.7	0.0	0.0	0.0
39	50.0	8.33	63.1	2	0.0	51.5	48.5	0.0	0.0	0.0
40	50.0	4.00	23.9	2	0.0	55.8	44.2	0.0	0.0	0.0
41	50.0	4.00	50.0	2	0.0	50.9	49.1	0.0	0.0	0.0
42	50.0	4.00	81.2	2	0.0	60.2	39.8	0.0	0.0	0.0
43	50.0	2.00	11.4	2	0.1	45.1	54.8	0.1	0.0	0.0
44	50.0	2.00	50.0	2	0.0	49.3	50.7	0.0	0.0	0.0
45	50.0	2.00	131.8	2	0.0	41.2	58.5	0.3	0.0	0.0

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
46	50.0	0.80	1.3	2	7.5	55.5	34.6	2.4	0.0	0.0
47	50.0	0.80	50.0	2	0.7	50.3	45.6	3.5	0.0	0.0
48	50.0	0.80	563.6	2	0.4	37.2	47.9	14.4	0.1	0.0
49	50.0	0.50	50.0	2	3.4	46.0	41.8	8.7	0.2	0.0
50	50.0	0.50	2411.1	2	1.6	24.1	44.0	25.7	4.7	0.0
51	150.0	8.33	105.3	3	0.0	0.0	100.0	0.0	0.0	0.0
52	150.0	8.33	150.0	3	0.0	0.0	100.0	0.0	0.0	0.0
53	150.0	8.33	189.3	3	0.0	0.0	99.0	1.0	0.0	0.0
54	150.0	4.00	71.7	3	0.0	0.2	96.9	2.9	0.0	0.0
55	150.0	4.00	150.0	3	0.0	0.0	98.9	1.1	0.0	0.0
56	150.0	4.00	243.5	3	0.0	0.3	98.9	0.9	0.0	0.0
57	150.0	2.00	34.3	3	0.0	5.5	86.8	7.7	0.0	0.0
58	150.0	2.00	150.0	3	0.0	1.9	88.5	9.6	0.0	0.0
59	150.0	2.00	395.3	3	0.0	1.8	79.7	18.4	0.0	0.0
60	150.0	0.80	3.8	3	0.7	23.9	59.8	15.2	0.4	0.0
61	150.0	0.80	150.0	3	0.0	13.6	61.9	24.3	0.2	0.0
62	150.0	0.80	1690.9	3	0.0	8.0	55.3	31.9	4.8	0.0
63	150.0	0.50	150.0	3	0.4	19.5	51.2	27.1	1.6	0.2
64	600.0	8.33	421.0	4	0.0	0.0	0.0	100.0	0.0	0.0
65	600.0	8.33	600.0	4	0.0	0.0	0.0	100.0	0.0	0.0
66	600.0	8.33	757.2	4	0.0	0.0	0.1	99.9	0.0	0.0
67	600.0	4.00	286.9	4	0.0	0.0	1.9	97.2	1.0	0.0
68	600.0	4.00	600.0	4	0.0	0.0	1.0	99.0	0.0	0.0
69	600.0	4.00	974.0	4	0.0	0.0	2.1	97.2	0.7	0.0
70	600.0	2.00	137.2	4	0.0	0.0	12.5	85.2	2.3	0.0
71	600.0	2.00	600.0	4	0.0	0.0	10.3	88.9	0.9	0.0
72	600.0	2.00	1581.1	4	0.0	0.0	12.7	85.9	1.4	0.0
73	600.0	0.80	15.0	4	0.0	6.0	33.4	55.5	4.7	0.5
74	600.0	0.80	600.0	4	0.0	0.8	23.8	66.9	8.0	0.6
75	600.0	0.50	1.6	4	3.0	16.9	41.6	33.7	4.0	0.8
76	600.0	0.50	600.0	4	0.0	3.7	25.6	58.1	10.4	2.2
77	1500.0	8.33	1052.5	4	0.0	0.0	0.0	26.2	73.8	0.0
78	1500.0	8.33	1500.0	4	0.0	0.0	0.0	86.4	13.6	0.0
79	1500.0	8.33	1892.9	4	0.0	0.0	0.0	88.9	11.1	0.0
80	1500.0	4.00	717.3	4	0.0	0.0	0.0	83.8	16.2	0.0
81	1500.0	4.00	1500.0	4	0.0	0.0	0.0	84.4	15.6	0.0
82	1500.0	4.00	2435.0	4	0.0	0.0	0.0	89.9	10.1	0.0
83	1500.0	2.00	343.0	4	0.0	0.0	1.3	68.8	29.1	0.8
84	1500.0	2.00	1500.0	4	0.0	0.0	0.2	76.7	22.5	0.5
85	1500.0	2.00	3952.8	4	0.0	0.0	0.2	60.7	39.0	0.2
86	1500.0	0.80	37.5	4	0.0	1.6	12.9	64.0	17.6	3.8
87	1500.0	0.80	1500.0	4	0.0	0.0	6.1	63.9	23.6	6.5
88	1500.0	0.50	4.1	4	0.3	6.6	32.8	45.8	11.4	3.1
89	1500.0	0.50	1500.0	4	0.0	0.3	10.8	54.5	24.9	9.4
90	3000.0	8.33	2105.1	5	0.0	0.0	0.0	3.1	96.9	0.1
91	3000.0	8.33	3000.0	5	0.0	0.0	0.0	13.1	86.4	0.5
92	3000.0	8.33	3785.8	5	0.0	0.0	0.0	0.1	99.9	0.0
93	3000.0	4.00	1434.6	5	0.0	0.0	0.0	18.4	77.5	4.1
94	3000.0	4.00	3000.0	5	0.0	0.0	0.0	14.6	81.8	3.6
95	3000.0	4.00	4870.0	5	0.0	0.0	0.0	10.4	88.0	1.6
96	3000.0	2.00	686.0	5	0.0	0.0	0.0	26.7	61.4	11.9
97	3000.0	2.00	3000.0	5	0.0	0.0	0.0	22.2	68.3	9.5
98	3000.0	0.80	75.0	5	0.0	0.3	6.2	48.1	35.5	10.0
99	3000.0	0.80	3000.0	5	0.0	0.0	1.1	30.3	51.2	17.4
100	3000.0	0.50	8.2	5	0.2	4.5	19.7	50.7	19.5	5.3
101	3000.0	0.50	3000.0	5	0.0	0.1	3.9	32.6	44.9	18.4
102	3500.0	8.33	2455.9	5	0.0	0.0	0.0	2.5	95.8	1.6
103	3500.0	8.33	3500.0	5	0.0	0.0	0.0	13.8	83.6	2.6
104	3500.0	8.33	4416.8	5	0.0	0.0	0.0	0.1	99.7	0.2

	LD50	slope	Dose0	True Catgry	%Estimates in category, by category number 1	2	3	4	5	6
105	3500.0	4.00	1673.7	5	0.0	0.0	0.0	1.8	81.7	16.4
106	3500.0	4.00	3500.0	5	0.0	0.0	0.0	13.8	76.5	9.7
107	3500.0	2.00	800.4	5	0.0	0.0	0.0	23.0	58.0	19.0
108	3500.0	2.00	3500.0	5	0.0	0.0	0.0	21.5	62.3	16.2
109	3500.0	0.80	87.5	5	0.0	0.3	5.4	39.9	40.0	14.4
110	3500.0	0.80	3500.0	5	0.0	0.0	0.6	32.4	47.6	19.3
111	3500.0	0.50	9.6	5	0.1	3.1	17.5	50.6	20.7	7.9
112	3500.0	0.50	3500.0	5	0.0	0.1	3.5	31.6	42.5	22.2

2.5 Sensitivity to the assumed slope

The following is abbreviated from an analysis distributed on November 24, 1999. Because the guideline proposal was still under development, the up-down procedure simulated deviates from the procedure actually proposed in the guideline. In particular, test doses have not been restricted to the range 1 to 5000 units in these simulations. This difference is expected to strongly affect the results, particularly when the slopes are shallow. Therefore the results are perhaps best viewed as providing qualitative information on how the test performance may be affected by interaction of the slope, the initial test dose, and the statistical estimator.

Two estimators have been evaluated, the maximum-likelihood estimator with the slope varied, and a "nonparametric" estimator, which is simply the geometric average of doses tested at the reversal and subsequently. Elsewhere I have termed that estimator the "dose-averaging estimator."

In general it appears that in those situations where the parametric approach would give acceptable performance with an appropriate choice of slope, the performance of the nonparametric estimator is comparable. The parametric and nonparametric estimators differ in bias and variance, depending primarily on the slope. Bias is minimized by using the parametric approach with the assumed slope close to the true slope. However, that is to make use of knowledge that is not generally available. Furthermore, the parametric estimates tend to have large variance. The nonparametric estimates tend to have small variance but are subject to a strong bias of the LD50 estimate in the direction of the starting dose, particularly for shallow slopes and/or small numbers tested. An index of relative error is used to combine the bias and variance.

Indices of estimator performance. In general, indices have been used which can be interpreted as measures of relative, rather than absolute error.

- As an index of bias I use the ratio of the median of the distribution of LD50 values, to the true LD50 value. This is reported as "P50/LD50" in the tables below. In the log scale, this would be approximately the bias as usually defined in statistics, for a symmetric distribution.

- As an index of the spread of the distribution I use the ratio of the ratio of the 95th percentile to the 5th percentile, denoted "P95/P5" in the tables below. For a lognormal distribution, this index has a simple relationship to the log-scale standard deviation.

- As a measure of relative error, combining the bias and the spread, I calculate the mean square error in the log scale, take the square root to calculate the "root mean square error" (in a sense, reversing the effect of squaring the errors). Finally I transform the result back to the original scale (take the antilog) so that the result can be interpreted as a multiplicative factor. I admit that this index is less transparent than the preceding two.

Scenarios simulated.

Num. Simulated Studies per scenario: 1000

Assumed slope, true slope: 0.5, 1, 2, 4, 8 (all combinations of true and assumed);

Step size: 0.5 log10 units, or doses spaced by a factor of about 3.2

True LD50: 2500

Initial dose: Denoted "Dose0" in tables. A selection of combinations of slope and Dose0 were simulated.

Nominal n: 6, 12

Results for nominal n=6 (Explanation in text) bold lines: assumed and true slope equal

Estimator	Nom. n	slope True	slope Assumed	Dose0	P50/LD50	P95/P5	Rel. Error
param.	**6**	**0.50**	**0.50**	**2500.0**	**0.83**	**1164**	**9.72**
	6	0.50	1.00	2500.0	0.97	141	4.82
	6	0.50	2.00	2500.0	1.21	96	4.13
	6	0.50	4.00	2500.0	1.01	72	3.71
	6	0.50	8.00	2500.0	1.00	78	4.01
nonparam.	6	0.50	.	2500.0	1.21	46	3.30
param.	**6**	**0.50**	**0.50**	**50.0**	**0.73**	**2437**	**9.69**
	6	0.50	1.00	50.0	0.36	366	8.01
	6	0.50	2.00	50.0	0.21	216	8.95
	6	0.50	4.00	50.0	0.16	215	10.34
	6	0.50	8.00	50.0	0.18	201	10.64
nonparam.	6	0.50	.	50.0	0.11	215	11.58
param.	**6**	**0.50**	**0.50**	**5.0**	**0.71**	**1766**	**9.42**
	6	0.50	1.00	5.0	0.21	736	12.94
	6	0.50	2.00	5.0	0.11	478	16.88
	6	0.50	4.00	5.0	0.08	456	20.48
	6	0.50	8.00	5.0	0.11	490	19.93
nonparam.	6	0.50	.	5.0	0.05	681	32.50
param.	6	1.00	0.50	4500.0	1.24	293	5.08
	6	**1.00**	**1.00**	**4500.0**	**1.01**	**35**	**2.97**
	6	1.00	2.00	4500.0	1.01	24	2.70
	6	1.00	4.00	4500.0	1.01	22	2.48
	6	1.00	8.00	4500.0	1.01	25	2.82
nonparam.	6	1.00	.	4500.0	1.49	22	2.54
param.	6	1.00	0.50	350.0	1.96	191	5.45
	6	**1.00**	**1.00**	**350.0**	**0.99**	**44**	**3.20**
	6	1.00	2.00	350.0	0.70	33	2.99
	6	1.00	4.00	350.0	0.55	28	2.94
	6	1.00	8.00	350.0	0.50	26	3.08
nonparam.	6	1.00	.	350.0	0.54	32	3.19
param.	6	2.00	0.50	500.0	2.12	51	3.84
	6	2.00	1.00	500.0	1.42	14	2.24
	6	**2.00**	**2.00**	**500.0**	**0.97**	**8**	**1.94**
	6	2.00	4.00	500.0	0.79	10	1.93
	6	2.00	8.00	500.0	0.72	6	1.92
nonparam.	6	2.00	.	500.0	0.77	10	2.06
param.	6	4.00	0.50	4000.0	0.90	17	2.16
	6	4.00	1.00	4000.0	0.90	6	1.65
	6	4.00	2.00	4000.0	0.90	4	1.49
	6	**4.00**	**4.00**	**4000.0**	**0.90**	**3**	**1.44**
	6	4.00	8.00	4000.0	0.90	3	1.47
nonparam.	6	4.00	.	4000.0	0.90	3	1.41
param.	6	4.00	0.50	400.0	2.38	9	3.61
	6	4.00	1.00	400.0	1.13	4	1.88
	6	4.00	2.00	400.0	0.94	3	1.48
	6	**4.00**	**4.00**	**400.0**	**0.90**	**3**	**1.48**
	6	4.00	8.00	400.0	0.90	3	1.49
nonparam.	6	4.00	.	400.0	0.90	5	1.52
param.	6	8.00	0.50	3500.0	0.79	1	1.31
	6	8.00	1.00	3500.0	0.79	1	1.28
	6	8.00	2.00	3500.0	0.79	1	1.28
	6	8.00	4.00	3500.0	0.79	1	1.27
	6	**8.00**	**8.00**	**3500.0**	**0.79**	**2**	**1.29**
nonparam.	6	8.00	.	3500.0	0.79	1	1.26
param.	6	8.00	0.50	2500.0	0.83	3	1.40
	6	8.00	1.00	2500.0	0.82	3	1.39
	6	8.00	2.00	2500.0	1.21	3	1.40
	6	8.00	4.00	2500.0	1.21	3	1.40
	6	**8.00**	**8.00**	**2500.0**	**1.13**	**3**	**1.38**
nonparam.	6	8.00	.	2500.0	0.83	3	1.39

Results for nominal n=12 (Explanation in text)

Estimator	Nom. n	slope true	Assumed	Dose0	P50/LD50	P95/P5	Rel. Error
param.	12	0.50	0.50	2500	1.21	214	5.31
	12	0.50	1.00	2500	1.00	90	3.76
	12	0.50	2.00	2500	1.00	58	3.52
	12	0.50	4.00	2500	1.06	55	3.36
	12	0.50	8.00	2500	0.96	70	3.55
nonparam.	12	0.50	.	2500	1.21	38	3.15
param.	12	0.50	0.50	50	1.00	295	5.48
	12	0.50	1.00	50	0.44	115	4.90
	12	0.50	2.00	50	0.41	109	5.33
	12	0.50	4.00	50	0.34	86	5.82
	12	0.50	8.00	50	0.25	82	6.18
nonparam.	12	0.50	.	50	0.24	83	6.94
param.	12	0.50	0.50	5	0.91	206	5.11
	12	0.50	1.00	5	0.38	139	5.78
	12	0.50	2.00	5	0.28	131	7.04
	12	0.50	4.00	5	0.21	136	8.47
	12	0.50	8.00	5	0.18	199	11.06
nonparam.	12	0.50	.	5	0.14	178	12.19
param.	12	1.00	0.50	4500	0.86	30	2.90
	12	1.00	1.00	4500	1.01	16	2.35
	12	1.00	2.00	4500	1.01	13	2.19
	12	1.00	4.00	4500	1.16	12	2.12
	12	1.00	8.00	4500	1.16	13	2.16
nonparam.	12	1.00	.	4500	1.23	12	2.13
param.	12	1.00	0.50	350	1.49	28	3.00
	12	1.00	1.00	350	0.93	15	2.33
	12	1.00	2.00	350	0.90	13	2.26
	12	1.00	4.00	350	0.79	12	2.29
	12	1.00	8.00	350	0.79	16	2.35
nonparam.	12	1.00	.	350	0.65	12	2.30
param.	12	2.00	0.50	500	1.58	9	2.21
	12	2.00	1.00	500	1.09	5	1.66
	12	2.00	2.00	500	0.96	5	1.59
	12	2.00	4.00	500	0.94	5	1.60
	12	2.00	8.00	500	0.92	5	1.60
nonparam.	12	2.00	.	500	0.93	5	1.64
param.	12	4.00	0.50	4000	1.09	4	1.53
	12	4.00	1.00	4000	1.01	3	1.36
	12	4.00	2.00	4000	1.09	3	1.32
	12	4.00	4.00	4000	1.03	3	1.30
	12	4.00	8.00	4000	1.04	3	1.36
nonparam.	12	4.00	.	4000	1.09	2	1.29
param.	12	4.00	0.50	400	1.51	4	2.01
	12	4.00	1.00	400	1.22	3	1.44
	12	4.00	2.00	400	1.03	2	1.31
	12	4.00	4.00	400	0.94	3	1.30
	12	4.00	8.00	400	0.91	3	1.36
nonparam.	12	4.00	.	400	0.90	3	1.34
param.	12	8.00	0.50	3500	0.95	1	1.20
	12	8.00	1.00	3500	0.95	1	1.21
	12	8.00	2.00	3500	0.95	1	1.20
	12	8.00	4.00	3500	0.96	1	1.20
	12	8.00	8.00	3500	1.06	2	1.21
nonparam.	12	8.00	.	3500	0.95	1	1.20
param.	12	8.00	0.50	2500	1.00	2	1.28
	12	8.00	1.00	2500	1.00	2	1.27
	12	8.00	2.00	2500	1.00	2	1.27
	12	8.00	4.00	2500	1.00	2	1.26
	12	8.00	8.00	2500	1.00	2	1.20
nonparam.	12	8.00	.	2500	1.00	2	1.26

References

Armitage, P.A. 1991. Sequential Methods. Ch. 6 Hinkley, D.V., Reid, N., and Snell, E.J. *Statistical Theory and Modelling.* Chapman and Hall.

Brownlee, K.A., Hodges, J.L., and Rosenblatt, M. 1953. The up-and-down method with small samples.

Dixon Statistical Associates. 1991. Design and Analysis of Quantal Dose-Response Experiments (with Emphasis on Staircase Designs). Unpublished manuscript.

Finney, D.J. 1971. *Probit Analysis.* (3rd ed.) Cambridge U. Press.

Edwards, A.W.F. 1992. *Likelihood* (2nd ed.) Johns Hopkins.

Hsi, B. 1969. The multiple up-and-down method in bioassay. J. Amer. Statl. Assoc. V? 147-162.

Meeker, W.Q., and Escobar, L.A. 1995. Teaching about approximate confidence regains based on maximum likelihood estimation. The Amer. Statn. 49(1):48-52.