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FOREWORD

This document presents the validation report of the Avian Acute Oral Toxicity Test. TG 223 describes a sequential design in which cumulative responses at each stage are used to provide a working estimate of the LD_{50} and slope that are used to set the doses for the next stage. After the final stage all responses are combined into a single statistical analysis that determines an LD_{50}, a slope and confidence limits.

The TG 223 design is flexible in terms of the number of stages and birds. The performance of the design was evaluated through extensive computer simulations in which different designs were compared to a 1-stage 60-bird design (similar to US EPA OPPTS850.2100). The results and validation of the statistical simulations are discussed in Appendix I and Appendix II respectively.

A multi-stage design is more challenging for test laboratories than a single stage study. In order to evaluate the user-friendliness and clarity of the guideline a reading comprehension test was prepared. The results and conclusions of the reading comprehension test quizzes are presented in Appendix III. In addition, a sequential design calculator tool (SEDEC) has been developed in the form of a Microsoft® Excel spreadsheet, in order to facilitate and aid in the selection of doses at each stage and to estimate the LD_{50}, slope and confidence limits (Appendix IV). A report of the validation of this tool is included as an appendix in this validation report (Appendix V).

The United Kingdom led this project and hosted an expert group meeting in September 2007. Ring testing was carried in 2008-2009 with two chemicals tested in each of five laboratories to confirm the results of the statistical simulations. For each of these chemicals historical results were available for a standard fixed-dose design as carried out to meet US EPA requirements (OPPTS850.2100). The Validation Management Group concluded that the performance of draft TG 223 delivers regulatory endpoints of LD_{50}, slope and confidence limits to a high standard while reducing the numbers of birds tested.

The validation report was submitted to the Working Group of National Co-ordinators of the Test guidelines Programme (WNT) for comments in November 2009, and the WNT endorsed the report at its 22nd Meeting on 23-25 March 2010. The joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 1June 2010.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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EXECUTIVE SUMMARY

The acute oral toxicity of a substance to birds is core regulatory information for the registration of plant protection products. OECD requested that an acute oral toxicity guideline for birds be developed that would deliver an estimate of the LD_{50}, slope and confidence limits, using as few birds as possible. The draft TG223 guideline was developed in order to meet this request and describes a sequential design in which cumulative responses at each stage are used to provide a working estimate of the LD_{50} and slope that are used to set the doses for the next stage. After the final stage all responses are combined into a single statistical analysis that determines an LD_{50}, a slope and confidence limits.

The draft TG223 design is flexible in terms of the number of stages and birds. If available data suggest the LD_{50} may be greater than 2000 mg/kg, a single stage limit test may be performed using 5 birds at the limit concentration and 5 birds in the control group. If toxicity is expected, to be less than 2000 mg/kg a full dose response test is performed using 3 or 4 stages (24 or 34 bird, respectively – excluding controls). In the rare event the design does not result in an estimate of slope, then more stages (and birds) can be added. Alternatively, if only the LD_{50} is required without an estimate of the slope and confidence limits the numbers of birds tested may be reduced. This report addresses the validation of the full LD_{50} test design.

The performance of the design was evaluated through extensive computer simulations in which different designs were compared to a 1-stage 60-bird design (similar to OPPTS850.2100). These simulations indicated that draft TG223 would estimate the LD_{50} as well as the 60-bird design, but using substantially fewer animals, and improve the frequency with which a slope is estimated. The computer program that performed the simulations has been independently validated.

A multi-stage design is more challenging for test laboratories than a single stage study. In order to evaluate the user-friendliness and clarity of the guideline a reading comprehension test was prepared. The results and conclusions of the reading comprehension test quizzes are presented in the appendix and may be used to assist in training of future draft TG223 users and were used to further improve the clarity of the guideline. Secondly a sequential design calculator tool (SEDEC) has been developed in the form of a Microsoft® Excel spreadsheet, in order to facilitate and aid in the selection of doses at each stage and to estimate the LD_{50}, slope and confidence limits. A report of the validation of this tool is included as an appendix in this validation report. It will be made publicly available from the OECD website.

Ring testing was carried with two chemicals tested in each of five laboratories to confirm the results of the statistical simulations. For each of these chemicals historical results were available for a standard fixed-dose design as carried out to meet US EPA requirements (OPPTS850.2100). When comparing the results of the ring test studies between laboratories and with the results of the original studies, the results appeared similar. Two statistical comparisons were made. In the first, the variability in LD_{50} calculations and slope estimates was compared with the variability found in these endpoints in a selection of 15 repeat studies from the EPA data base. The LD_{50} estimates from the ring-test showed less variability than the EPA repeat studies. The variability in slopes from the ring-test data was higher than that seen in the EPA repeat studies. The latter can be explained by the higher

frequency with which slopes are determined in the draft TG223 design when the slope is steep, than in the EPA data base studies. In the second, the variability in LD_{50} and slope estimates observed in the ring-test data were compared with the variability predicted by the computer simulations and shown to be similar.

The Validation Management Group concludes that the performance of draft TG223 delivers regulatory endpoints of LD_{50}, slope and confidence limits to a high standard while reducing the numbers of birds tested. It is the Validation Management Group's recommendation that the guideline be published by OECD as a final document.

1. **Introduction**

The acute oral toxicity of a substance is core regulatory information on safe use in classification and labelling, and risk assessment in OECD member countries. OECD guidelines have a strong emphasis on animal welfare and there are incentives and opportunities to refine the design to reduce animals tested. The avian acute oral toxicity test guideline (draft TG223) has been in development since 1994 when OECD first requested an acute guideline that substantially would limit the numbers of animals used. Draft TG223 describes a sequential design where the responses at each stage are used to position the next set of doses for the purpose of identifying the LD_{50}, slope and confidence limits. The responses from all stages are combined for final estimates of these parameters. The performance of the design initially was evaluated through extensive statistical simulations comparing different designs with a 1-stage 60-bird design (similar to US EPA OPPTS850.2100) and an 'up-down procedure' (similar to TG425 for mammals) These simulations indicated that Draft TG223 would estimate the LD_{50} as well as the 60-bird design, using substantially fewer animals. The simulations also indicated that the frequency with which a slope estimate can be achieved, would be increased. It is possible to guarantee a slope estimate by adding additional stages beyond those prescribed in the design to generate reversals and partial responses. To aid the selection of doses at each stage and to estimate the LD_{50}, slope and confidence limits, a SEquential DEsign Calculator tool (SEDEC) has been developed as an Excel 2000® spreadsheet that will be publicly available from the OECD web site for use with the final TG223 guideline.

The draft TG223 of March 2007, reflected the consensus obtained after all the concerns raised in member country comments and by members of the expert group had been addressed. In 2007 a validation plan was approved by OECD of which the results are presented here. This validation report includes:

- Background information on the guideline and its development;
- Results of the statistical simulations, their validation and an account of how all the concerns raised were addressed;
- A summary of the computer programme developed to estimate the LD_{50} and provide doses for each stage of the test (Sequential Design Calculator: SEDEC);
- The results and conclusions of a reading comprehension test performed to ensure readability and clarity of the guideline;
- The results and discussion of the ring test performed with two chemicals at several different laboratories;
- Summary and evaluation of feedback received from participating laboratories.
- Examples of (regulatory) TG223 studies conducted with the draft guideline to date.

2. History

Work on the development of an OECD guideline for acute oral testing in birds started during a workshop on Avian Toxicity Testing was held in Pensacola, Florida, United States on the 4-7 December 1994. [1)] The workshop comprised 48 wildlife toxicology specialists from 10 countries including 27 from North America and 18 from Europe. This workshop was part of the OECD's Pesticide Programme and had the following objectives: i) to confirm which tests are critical for risk

assessment; ii) to evaluate the positive and negative features of existing test methods and alternatives against critical required features; iii) to revise or develop OECD test guidelines for avian toxicity testing as needed, while minimising the numbers of animals required for such tests. The workshop recommended that an acute oral toxicity guideline should describe a limit test design for chemicals of low toxicity, a definitive LD_{50} test that provided a dose response slope and confidence limits and a short version to provide an approximate LD_{50} where the toxicity to additional species was required to investigate the species sensitivity distribution (SSD). To achieve this with least numbers of animals it was agreed to focus on mortality as the primary endpoint with no attempt to determine a NOEL for other outcomes. Further it was agreed that setting doses to achieve other parameters compromise the dose response for mortality. The Workshop also identified some areas of work that were necessary to support a new acute oral toxicity guideline, including the identification of the most efficient design to estimate the LD_{50} and slope using the least number of birds.

These actions were taken forward by an OECD Expert Group at open and closed meetings at SETAC in Europe and the United States. In 1998 a draft guideline was prepared for OECD that described a limit test, an Up-and-Down test and a Dose response test. Following discussions within OECD the avian expert group was asked to re-evaluate the test design to reduce the number of animals further. The group had to balance the need for reducing the numbers of animals in the test with the requirement to fulfil the regulatory need to provide an LD_{50} and slope estimate. OECD requested that the group work with statisticians to develop a suitable design. As a consequence a team of statisticians prepared a plan to evaluate several designs with different numbers of stages and birds per stage through extensive computer simulations. The selected multi-stage design allowed a significant reduction in required individuals needed while assuring ability to estimate both an LD_{50} and a slope were estimable. The draft guideline was submitted again to OECD in 2002 and given the reference TG223. As part of the subsequent commenting round, a number of specific concerns were raised. The key issues included: i) whether or not to use controls; ii) which test species to apply the design to; iii) whether or not to randomly select birds, irrespective of their sex; iv) the impact of delayed or background mortality on the estimate of the LD_{50}.; v) how to evaluate endpoints other than mortality. All the issues raised were resolved at public (SETAC) meetings and closed (OECD) meetings. Further questions were put to the Expert Group by representatives of USEPA and these were addressed with the help of the US EPA biologists and statisticians. A third revision was prepared and submitted to OECD for further commenting in 2007. A detailed account of the concerns raised and the changes subsequently made is presented in appendix I of this report.

Following the OECD member-country commenting round it was recommended that a validation plan be prepared and conducted. A validation plan was prepared and submitted to OECD in 2007. The elements of this plan were completed in 2009 and are reported in this document. Ring testing was conducted for the LD_{50}-slope test only.

3. Rationale for the experimental design used in the draft Test Guideline 223

The Draft TG223 design is flexible in terms of number of stages, and therefore in terms of number of birds. First of all, if available data suggest the LD_{50} may be greater than 2000 mg/kg, a single stage limit test may be performed using five birds at the limit concentration and five birds in the control group. If toxicity is expected, a full dose response test (LD_{50} – slope test) is performed. If the 3 stage (24 bird) or 4 stage (34 bird) design does not result in an estimate of slope, then more stages (and birds) can be added. Alternatively, if the slope and confidence limits are not required the numbers of birds tested may be reduced by using the LD_{50}-only test. This validation report only addresses the validation of the full LD_{50} test design.

The LD_{50} – slope test is a sequential design using 24-34 individuals (excluding controls) to estimate the LD_{50}, slope and confidence limits. This is achieved by the calculated placement of doses for single or small groups of birds/dose to provide a working estimate of the LD_{50} and slope. This is used

in turn to define the placement of doses and the number of animals per dose in the next stage until a final estimate of the LD_{50} and slope are determined by combining all stages. Sequential designs can be used where there is low expectation of drift (the responses changing in time) and none to extremely low background mortality. Both these factors have been shown to be low over short intervals (days) using robust species under controlled experimental conditions. Under these conditions the responses may be combined across stages and controls limited to a single stage [1].

A stopping rule, linked to the number of reversals or partial mortalities at a single dose, is incorporated into the design. This prevents the unnecessary use of animals once an adequate estimate of the LD_{50}, slope with confidence limits are met. A reversal is an instance when percent mortality at a given dose is higher than the percent mortality at the next higher dose. Partial mortality is an instance where only a proportion of birds die at a given dose. The range over which reversals and partial mortalities occur provides information about the LD_{50} and slope.

Doses are not selected for determining NOELs and there is no requirement to estimate NOELs through statistical analysis. Information on non-lethal endpoints like food consumption and bodyweight are collected for qualitative evaluation.

A control group is five birds for both the limit test and the sequential (LD_{50}-slope) test. For laboratory-reared avian species, historical data have demonstrated that background mortality is very low.

4. Test description

The test is divided into a number of discrete stages. At each stage in either the LD_{50}-slope or the LD_{50}-only test designs, one or more birds are given a single oral dose (mg/kg body weight) of the test substance using doses that are expected to bracket the evolving working estimate of the LD_{50}. Birds are observed for 14 days, but selection of doses for subsequent stages is typically based on observed mortality and toxicity symptoms after three days. This interval may be reduced early in the test if mortality or signs of recovery occurs quickly, or the interval may be extended if delayed mortality is expected or observed.

The sequential test can be initiated using information gained from a failed limit test (one or more mortalities at the limit dose – see Figure 1 of the draft TG223). For compounds of suspected high toxicity, testing may be initiated in Stage 1 where each of four birds is given a different dose, so that doses cover the best available estimate of the LD_{50} (*e.g.,* based on the rodent or other bird species' LD_{50}). Using the outcome of Stage 1, the doses that bracket the working estimate of the LD_{50} are determined for Stage 2, and at each of ten doses, one bird is dosed. If there is a working estimate of the LD_{50} available from a failed limit test, the sequential test may start with Stage 2. The process continues to Stage 3 and possibly to Stage 4 (detailed stopping rules are given in the test guideline).

Individually-caged young adult birds are acclimated to experimental conditions before being randomly allocated to receive an oral gavage dose. Dosing procedures, test conditions and animal husbandry methods are described and are consistent with US EPA OPPTS850.2100. The recommended strategy for testing materials that are unlikely to present a significant hazard is to perform a test with multiple birds (5) dosed at the limit dose. If toxicity is expected, the recommended strategy is to use a sequential (staged) design (see Figure 2 of the draft TG223). Stages 1 and 2 require non-replicated doses, while Stages 3 and 4 require replicated doses. In Stage 1,

[1] An evaluation of background mortality recorded in 4 different laboratories is taken up into the simulations report in Appendix I of this report.

the range of doses is based on the best available estimate of the LD_{50} (*e.g.*, the rodent LD_{50}). Doses for subsequent stages are determined based on the mortalities observed in all previous stages.

After dosing, the birds are observed for a 14-day period in order to record mortality and sublethal effects. It may be necessary to extend the observation period if there is evidence of delayed effects. The data collected in the first three days of a stage usually supply sufficient information to determine whether birds are likely to recover from effects encountered, or whether additional mortality will occur. If day 3 indicates that further mortality may occur in a test stages, the calculation of the working estimate may be delayed until recovery of the remaining test birds is evident. In some cases it may be necessary to wait for up to 14 days before moving to the next stage. Calculation of the working estimate on day 3 of a test stages allows the full test and all dosing to be completed over a relative short time frame. For example, the total length of the study would be 21 days if (1) three stages were required to estimate and LD_{50} and slope, (2) 14-day observation period were sufficient and (3) three days was a sufficient time period for determination of doses for the subsequent stage.

The guideline provides information for selecting doses at each stage, that bracket the evolving working estimate of the LD_{50}. In addition a SEquential DEsign Calculator (SEDEC) will be made available on the OECD website that estimates the working LD_{50} at each stage, identifies the doses for the next stage and calculates the final estimate of the LD_{50}, slope and confidence limits.

Mortality is the primary endpoint in this study and background mortality is presumed to be negligible. Controls are required to monitor the health and husbandry of test birds to ensure the study results provide reliable results. Five untreated control birds from the same hatch will be included in the test. Control birds will be sham dosed with the same carrier (or capsule) used with the test substance, and will be maintained under the same conditions as treated birds. Control birds will be weighed prior to dosing as well as 3, 7, and 14 days after dosing. Sham dosing will be performed on the same day as the first dosing with test substance (either with the limit test if it is performed, or with Stage 1 of the sequential test). If circumstances require the use of birds that are from a hatch different from the one used to start the test, an additional control group of five birds from the second hatch must be included on the day that these birds are used.

Clinical observations, body weights and food consumption are to be recorded throughout the study. During the test, animals obviously in pain or showing signs of severe distress should be euthanised. The design aims to minimise the use of test animals as shown in the table below:

Table 1: Number of animals required in the limit test and at each stages of the sequential design.

Test stage	Treatment	Control[1]
Limit test	5	5
1st stage if necessary	4	5
2nd stage if necessary	10	
3rd stage if necessary	10	
4th stage if necessary	10	

[1] 5 birds in a limit test or 5 birds in a dose response test

The Limit Test , LD_{50}-only Test and LD_{50}-slope use 5 or 10, 24 and 34 birds, respectively, excluding controls. If the dose response curve is steep (fewer than two reversals or partial kills) at stage 3 and the slope cannot be determined it may be necessary to go to a 4th stage (34 birds ex controls). The frequency with which the investigator needs to progress to stage 4 is expected to be low.

Clinical observations, body weights and food consumption are to be recorded during the study.

5. Statistical simulations supporting the development and validation of the Draft OECD TG223

Computer simulations of draft designs have been used to compare the performance of standard 60-bird one-stage designs (similar to that outlined in the current EPA guideline OPPTS 850.2100) to the original 3-stage and the updated 3-stage and 4-stage draft TG223 designs, as explained below.

Appendix I contains a detailed report of the statistical simulations that evaluate the performance of three types of design:

- Single stage design with 50 birds. Two alternatives to the standard design, based on the OPPTS 850.2100 design. The standard design comprises 5 doses, each of which is allocated to 10 birds plus an untreated control of 10 birds. However, in this report, two alternatives to the standard design have been evaluated, both of which have 10 doses with 5 birds per dose. In an earlier evaluation both of these alternative designs were found to be superior to the design with 5 doses. One of the design alternatives has a high/low dose ratio of 20 and is better for estimating slope; the other has a high/low dose ratio of 50 and is better for the LD_{50}.
- 3-stage sequential design with 24 birds. This is the original draft TG223 sequential design recommended in a draft OECD guideline issued on October 2002. This design uses 3 stages: 4 non replicated doses in the first stage, equally spaced (on a log scale) around a provisional estimate of the LD_{50}; 10 non-replicated doses in the second stage, equally spaced (on a log scale) around an updated estimate of the LD_{50}; and 2 doses, each given to 5 birds, in the third stage.
- 4-stage sequential design with 34 birds. The updated version of the draft TG223 sequential design as described in section 4 was also evaluated. After further simulations, it was discovered that the original 3-stage design was unsatisfactory in certain situations,

particularly when the true slope of the dose response is steep. The original design has therefore been modified by the inclusion of an alternative Stage 3, the use of which depends on the outcome of Stage 2, and an optional Stage 4, the use of which depends upon the outcome of the new alternative stage 3.

Each of the above designs was evaluated by means of computer simulation. For each design nine thousand computer experiments were carried out, one thousand for each of nine combinations of true LD_{50} (5, 50, and 1500 mg/kg) and true slope (2, 5, and 10). Appendix I describes the methods used to carry out the simulations, to estimate the LD_{50} and slope for each computer-generated experiment, and to present the results. The report in Appendix I also covers all of the concerns that have been raised in the course of the development of the guideline and describes how these concerns were addressed through further simulation testing and which protocol modifications were needed.

The conclusions from the simulation exercise were as follows:

- For estimation of the LD_{50}, all three designs compared in this report give a similar performance. However, the Draft TG223, original and updated, designs require far fewer birds - either 24 or 34 - so should be preferred.
- For estimation of slope, the updated draftTG223 24/34-bird-3/4-stage design significantly outperformed the other designs. It was successful (at least 80%) in estimating the slope even when the true slope was steep. The original TG223 was less successful.
- The updated draft TG223 has been presented as a 3- or 4-stage design, in the interests of conserving animals. However, the principal of extending the number of stages can easily be applied to 5 or more in any rare circumstances where this might be considered necessary.

In addition to this major conclusion, further simulations were able to show the following:

- The performance updated Draft TG223 design is not noticeably affected by natural mortality. The updated draft TG223 design is therefore robust when considering natural or background mortality of 1% or less, which is typical of what is observed in practice in control birds. Appendix I of this report provides a historical overview, performed in 2004, of control mortality recorded in laboratories performing avian studies.
- The performance of the updated draft TG223 design can be affected by delayed mortality (i.e. mortality that occurs more than three days after treatment. However, the flexibility in the design through the ability to increase the number of days between successive stages, the ability to repeat stages and to increase the total number of stages allows the experimenter to mitigate the effects of delayed mortality without the need to repeat a study.
- The performance of the updated draft TG223 design is not noticeably affected by a poor initial guess of the LD_{50}, since correction at later stages can take place.
- The effect of errors in dosing on the performance of the updated draft TG223 design is entirely predictable and does not permit discrimination between competing designs.

As part of this validation excercise, an independent consultant has verified the simulation code by independently developing and executing the code to repeat the original simulations. In addition, the procedures used to tabulate and evaluate the simulation results were repeated independently. The report of this work and reposes by the Expert Group, is included as Appendix II of this report. One small finding mentioned in the validation report, led to a correction of the original simulation programmes. Upon rerunning the programmes small differences from the original runs were found. However, the response of the expert group was that this does not change the conclusions from the

simulations in any way. Not does it have any implications for the design specified in the draft TG223 guideline.

6. Instruments developed to enhance the reproducibility and user-friendliness of the guideline

6.1. Evaluation of clarity of the guideline through written quizzes

As part of the validation process for the draft TG223, the Expert Group has conducted a reading comprehension evaluation with written quizzes to evaluate clarity of the draft guideline language and to help ensure that laboratories conducting the study will interpret the Guideline in an identical manner. An eight question quiz (multiple choice and short answer) was developed based on the March 2007 version of the draft TG223 Draft Guideline. Four biologists from EFED (US EPA/Office of Pesticide Programs/Environmental Fate and Effects Division) volunteered to read the draft guideline and take the quiz in August 2007.

Based on the results of these quizzes and National Coordinator comments submitted in summer 2007, the draft guideline was edited and re-submitted to OECD in March 2008. The quiz was re-administered based on the March 2008 revisions. All questions in the second quiz were the same as in the first quiz, with the addition of one question focusing on identification of reversals and partials in hypothetical study data. Several EFED biologists re-took the quiz using the 2008 draft guideline. In addition, EFED biologists, independent testing laboratory employees who regularly conduct avian toxicity tests, and one avian toxicology researcher took the quiz using the revised 2008 draft guideline. A copy of the quiz given for the 2008 draft guideline, a copy of the solutions, and summaries of results from the first and second round of quizzes are provided in Appendix III.

For the first round based on the 2007 draft guideline, the respondents had difficulty identifying reversals in sequences and following the concepts of moving through the stages. For example, they were unable to identify if a study should move to Stage 3a or 3b based on results from Stages 1 and 2.

The second set of quiz-takers (2008 draft guideline) was a much larger group of 13 individuals with more diverse backgrounds. However, no one in this group was able to respond correctly to all questions. Those respondents who provided answers based on both the 2007 and the 2008 draft guidelines (three individuals) felt the revised draft was better organized and much clearer. There were several questions where the percentage of correct responses increased dramatically. For example, only two of the four individuals evaluating the 2007 draft were able correctly to identify the number of reversals in the four simple sequences provided in question 1. To address this concern, example sequences identifying partials and reversals were added to the 2008 draft. When tested on the new draft, all 13 respondents correctly identified the number of reversals in question 1. As another example, in 2007 only one of four individuals correctly answered question 5 (identifying doses for stage 2 based on results of a limit test); however, nine of 13 were able to correctly answer this question based on the 2008 draft guideline.

These improvements in correct response rate indicate that the draft guideline implemented in 2008 greatly increased comprehension of the study design. However, some questions still had poor correct response rates. This indicated that the guideline text would benefit from further revision focusing on clarity. This has been done and is reflected in the final draft of the guideline, that is being circulated together with this validation report. These results also substantiate the importance of providing to users of the draft TG223 guideline software that determines dose concentrations and performs LD_{50} calculations.

6.2. Dose calculator – SEDEC (SEquential DEsign Calculator)

SEDEC is an EXCEL® workbook designed to aid in dose selection and analysis of avian acute oral toxicity studies performed in accordance with draft TG223. Facilities are provided for the user to enter basic study information and to guide the user through the sequential calculations and selection of doses. After a study is completed, SEDEC can calculate the LD_{50}, dose response slope, and confidence intervals for both. SEDEC provides facilities for printing reports of the study results, and also provides a secure audit trail that records entry and changes to data, user decisions, the identity of the individual making entries or decisions, and the date of the action or entry.

SEDEC (designed by Dr. Timothy Springer, Wildlife International, Ltd.) was made available to all participating laboratories prior to starting the in-life phase of the ring testing. This calculator was not available for the written quizzes (Chapter 6.1), since participants were requested to have full understanding of and be able to perform all calculations themselves. This programme has been validated and the validation report is included as Appendix V of this report.

7.0 Ring Test

7.1. Purpose of the ring test

- To demonstrate the applicability and practicality of the test design described in the draft TG 223 at avian testing laboratories that perform avian testing.
- To provide estimates of the LD_{50}'s and slopes and their respective confidence limits using the draft TG 223, in order to compare with LD_{50}'s and slope estimates from previous OPPTS-design studies performed with the same chemicals (performed to FIFRA guidelines).
- To support the validity of conclusions drawn on variability and bias of LD_{50} and slope estimates in previously performed simulation-based assessments done using the tests performed according to draft TG 223 (see section 5 on statistical simulations).
- To qualitatively compare information on sublethal endpoints from TG 223 with information from historic OPPTS (FIFRA) studies.

7.2. Outline of validation design

Testing was performed with northern bobwhite quail. Two chemicals were tested in each of 5 laboratories in order to allow an assessment of the combined effects of inter- and intra-laboratory variation, for comparison with existing OPPTS-design studies in the EPA acute toxicity database. The size of the ring-test design was a compromise to minimise the use of animals and resources. Ring-test results were put to optimal use by comparing the uncertainty range found in the ring test results with that predicted from the simulation results.

7.3. Criteria for the selection of chemicals used in the ring test

The criteria for the selection of chemicals used in the ring test included the following:

- With each of the chemicals at least one OPPTS study with good performance parameters (expressed as LD_{50} and slope with their respective confidence intervals) had to be available;
- It is considered that the new guideline had to be shown to perform well for chemicals with toxicities and different slopes. Hence one chemical (2-methyl-4-chlorophenoxyacetic -

MCPA Acid) had a steep slope and was of low toxicity and one chemical had a shallow slope (isazofos) and was of high toxicity in the previously performed OPPTS-design studies;

- Permission needed to be obtained from the pesticide manufacturer, therefore chemicals of obvious commercial sensitivity were not included.
- The chemical had to be stable in a vehicle. This was the case for both chemicals selected.
- Chemicals that caused regurgitation in birds were avoided. No regurgitation was reported in either of the original studies. Regurgitation was observed for both chemicals at ring-test lab no. 1 only.
- The ring-test was performed using the same carrier and similar particle size as was used in the original OPPTS-design studies.

7.4. Criteria for evaluating results of the ring-test

For the selected chemicals, studies of the traditional design were available that showed good design performance. The LD_{50}, slope and confidence limits were estimated from the existing studies.

Evaluation of the results of the ring-test included:

- A comparison between the estimates of LD_{50} and slope obtained from the ring test studies and those obtained from the OPPTS-design studies. This was achieved through visual inspection of the results in Table 2 combined with insights obtained from the computer simulations (see chapter 5).
- A comparison of within-chemical variability in estimates of LD_{50} and slope observed from the ring-test data and variability observed in historic repeat studies using OPPTS-850.2100 and its precursor US EPA FIFRA Guideline 71-1.
- A comparison between the within-chemical variability in estimates of LD_{50} and slope observed in the ring test data and variability observed in the simulations described in Appendix I.

7.5. Participating laboratories

Participating laboratories were:

- BASF (Germany) : contact Sabine Zok
- Bayer Crop Sciences (US): contact: Mark Christ
- Huntingdon LifeSciences (UK): contact David Cameron
- Springborn Smithers (US): contact Larry Brewer
- Wildlife International, Ltd. (US): contact Joann Beavers / Patrick Hubbard

The laboratories have been given a number so that results cannot be related to a particular laboratory.

Note: Upon finalisation of this draft validation report results had not yet been obtained from one of the five laboratories. If at all possible results from this laboratory will be incorporated into the report before its finalisation.

7.6. Results, evaluation and discussion of the ring test. Detailed tables in Appendix VI

Table 2. Summary of validation ring-test results

Substance A: MCPA

Lab	LD_{50} (95% CI) (mg/kg bw)	Slope (95% CI)	Number of stages used	Number of birds used
Lab 1 [1)]	437 (360- 538)	9.67 (3.66 – 15.7)	4	34 + 5 controls
Lab 2 [2) 3)]	438 (338-544)	3.21 (1.26 and 5.16) [2)]	4	34 + 5 controls
Lab 3	333 (280 – 400)	13.2 (4.3 – 22.1)	4	34 + 5 controls
Lab 4	554 (396-845)	6.22 (1.73-10.7)	4	34 + 5 controls
Lab 5 [4)]				
Historic (FIFRA 71-1) study	377 (314-452)	11.6	Single stage study	60 (including controls)

Substance B: Isazofos

Lab	LD_{50} (95% CI) (mg/kg bw)	Slope (95% CI)	Number of stages used	Number of birds used
Lab 1 [1)]	27.4 (0 and infinity)	Could not be estimated [5)]	4	34 + 5 controls
Lab 2 [2) 3)]	24.4 (17.1 – 37.6)	3.3 (0.59 and 6.03) [2)]	3	24 + 5 controls
Lab 3	16.1 (11.7 – 23.3)	7.7 (1.99 – 13.4)	3	24 + 5 controls
Lab 4	13.8 (10.4-17.4)	6.45 (2.57-10.3)	4	34 + 5 controls
Lab 5 [4)]				
Historic (FIFRA 71-1) study	11.1 (8.3 - 14.7)	4.68	Single stage study	80 (including controls)

[1)] Some regurgitation was seen in the birds in this study; however, it does not appear to have impacted the results of the study, when comparing the LD50 values to those obtained by the other laboratories.

[2)] This laboratory performed the studies without using SEDEC. The doses selected were run with SEDEC to obtain estimates for the purpose of comparative evaluation of the ring test results.

[3)] A common control group was used for MCPA and isazofos

[4)] Results from this laboratory had not been received at the time of finalisation of the draft validation report.

[5)] This study went to 4 stages and had no reversals and no partial mortality. In this case it was not possible to estimate the slope.

In paragraph 7.1 of this report 4 main purposes of the ringtest have been outlined. The first one, which is to demonstrate the applicability and practicality of the test design described in Draft guideline 223 in typical contract laboratories that perform avian testing, has been met by this ring test. The validation management group asked for feedback from the laboratories on the method. While there were concerns raised by the laboratories, as presented in chapter 7.8, all laboratories were able to perform the test without much difficulty.

The second aim of the ringtest was to provide estimates of the LD_{50}s and slopes and their respective confidence limits for two chemicals. This was achieved and results are presented in Table 2. The results obtained in the ringtest in terms of LD_{50} were comparable to the original studies performed by Wildlife International, Ltd. using a single stage design like OPPTS 850.2100. For MCPA, the confidence limit ranges for both the LD_{50} and the slope overlap with the confidence limits ranges for all of the ring-test results. For Isazofos, the confidence limit range for the slope estimate for the OPPTS study overlaps with the confidence range exhibited by three of the ring-test studies. While the estimates of the LD_{50} were comparable between the labs, the confidence range for the historical study is outside the range exhibited by four ring test studies, however confidence limit ranges show an overlap with labs 3 and 4. Lab 1 had no confidence limits around its LD_{50} and hence no comparison could be made. A discussion of the variability found in the slopes in the ring-test is given in Chapter 7.7.

Chapter 7.7 also deals with the comparison between the variability of data obtained in this ringtest and the variability found in a sample of repeat tests taken from the EPA data base. Chapter 7.7 also deals with the third aim of the ringtest. This aim was to see if the ring-test data would support the validity of previously performed simulation-based assessments made of the variability and bias of LD_{50} and slope estimates from tests performed according to the updated draft to TG223.

The final aim of the ring test was to qualitatively compare information on sublethal endpoints from TG 223 with information from historic OPPTS studies. To this end all findings related to bodyweights, food consumption and clinical signs from the ring-test studies and from the original studies have been included in Appendix VI of this validation report. A direct comparison of clinical observations recorded in the different studies is difficult owing to the wide range of terminology used to describe the findings in the respective studies.

No regurgitation occurred in either of the original studies. Some regurgitation was seen in birds from Lab 1, however this was not seen in birds from any of the other laboratories. One possible explanation may have been a difference in dosing methodology, capsule dosing and intubation.

7.7. Evaluation of ring-test data in comparison with EPA historical Repeat data and result of simulations performed

This section describes the following:

- The statistical estimation of the variance for both LD_{50} and slope from a historic USEPA data base (http://www.ipmcenters.org/Ecotox/index.cfm).

- The estimation of variance for both LD_{50} and slope from the ring test data.

- A comparison of the variances (i.e. historic *versus* ring test) via formal statistical tests. Any statistically significant outcomes will be discussed in terms of expectations derived from the simulation exercise in Appendix I.

- Finally the variation (in LD_{50} and slope) observed in the ring test will be compared with estimates obtained in the simulation studies in Appendix I. Since the results of the

simulation exercise are presented in terms of Box-Whisker plots, the uncertainty intervals (see below) estimate from the ring test will be compared with the range shown in the simulation studies.

Historic USEPA Bobwhite data performed to USEPA FIFRA 71-1 and the OPPTS guideline

Data, which is presented in Appendix VII, comprises 15 chemicals, and 32 individual LD_{50} values. Two chemicals have three values each. The remaining chemicals have two values each. The data includes relatively few estimates of slope because the OPPTS design is poor at estimating slopes. It is assumed that repeat tests in a chemical are nested within chemicals. Under this assumption a random effects-model can be used to estimate both the variance between repeats and the variance between chemicals. Repeat tests on a chemical may, or may not have been performed at the same lab as the original test. Therefore the variance between repeats includes both intra- and inter-lab variability.

The between-repeat, within-chemical, estimate of variance for $\log_{10}(LD_{50})$ is 0.062, with 17 degrees of freedom. The within-chemical, between-repeat, estimate of variance for slope is 2.21, with 3 degrees of freedom. Very approximate uncertainty intervals around three values for LD_{50} (i.e. LD_{50} = 5, 50, 1500) have been computed as LD_{50} +/- 2*(standard deviation). These are shown in Table 3. Similarly, approximate uncertainty intervals around 3 values for slope (i.e. Slope = 2, 5, 10) have been computed. These are shown in Table 5. These values of LD_{50} and slope correspond to the values of LD_{50} and slope that were used for the computer simulation component of the design validation.

Ring Test Data

Data comprises two chemicals and eight data values – four repeats for each of the ring test chemicals. Again, it is assumed that repeat tests are nested within chemicals, so the form of analysis carried out was the same as for the historic EPA data.

The between-repeat, within-chemical, estimate of variance for $\log_{10}(LD_{50})$ was 0.014 with 6 degrees freedom. The within chemical between repeat estimate of variance for slope is 13.240 with 5 degrees freedom. Using 2*(standard deviation), very approximate uncertainty intervals were computed for three values of LD_{50} (i.e. LD_{50} = 5, 50, 1500) (Table 4). Similarly, approximate uncertainty intervals around 3 values for slope (i.e. Slope = 2, 5, 10) have been computed (Table 5).

How do the estimates of variance compare?

For LD_{50}: The variance ratio was computed with the larger estimate of variance as the numerator. In this case the variance estimated from the EPA data was larger. This gave a value of 4.52 with 17 and 6 degrees of freedom. The F-test single-sided p-value for this ratio is 0.035. This is statistically significant.

For Slope: The variance ratio is 5.99 on 5 and 3 degrees of freedom. In this case the variance estimated from the ring test data was larger. This gives a one-sided p-value of 0.036, which also is statistically significant.

Discussion of results of the evaluation

For LD_{50}, the variance from the EPA data is 4.52 times greater than the variance from the ring test. Upon consideration, this outcome is not surprising since the simulations shown in Appendix I show, for larger values of true slope, that variation between estimates of LD_{50} from draft TG223 is smaller than variation between estimates from the "OPPTS" design.

For slope, the variance of the ring test data is six times greater than the EPA data and the F-test does confirm a significant difference. This result is consistent with the results of the simulation exercise

where the simulations for draft TG223 are more successful in estimating the slope. (see figures 1.4, 1.5 and 1.6 in Appendix I). For shallow and medium slopes (slope = 2 and 5) this improvement is marginal but draft TG223 is clearly better at estimating slopes in those difficult situations in which the "OPPTS" design fails and this results in greater variance in the estimates. For steep slopes (slope =10) the difference is more dramatic. "OPPTS" is successful in 841 runs out of 3000 compared with 2425 runs out of 3000 for draft TG223. Furthermore the "OPPTS" estimates are biased towards those runs in which the data apparently show a shallower slope (all of the 841 estimates are less than 10 while the true slope is exactly 10) so the range of estimates is much lower.

How do the ring test results compare with the simulations?

For LD_{50}, the relevant comparison is between the approximate uncertainty limits in Table 4 with Box-Whisker plots in Figures 1.1, 1.2 and 1.3 of the simulations report (Appendix I). Although this is a rather crude analysis, the uncertainty ranges shown in Tables 4 are comparable with the ranges observed in the simulation results.

For slope, the relevant comparison is between the approximate uncertainty limits in Table 5 with Box-Whisker plots in Figures 1.4, 1.5 and 1.6 of the simulations report (Appendix I). Again, uncertainty ranges in Table 5 are consistent with variation shown in simulation results. In particular, the larger variance estimated from the ring test data is consistent with draft TG223 giving wider range of simulation results when compared with "OPPTS" design.

Conclusion of evaluation

From the evaluations described above we can conclude the following: the evaluation is a partnership between the statistical simulations and the ring-test results because it is not reasonable to test large numbers of birds for this purpose. If ring-test results are consistent with the statistical simulations it is reasonable to rely heavily on the statistical simulations for a full evaluation of the performance of the test design.

- Estimates of LD_{50} and slope obtained from the ring test are consistent with estimates obtained from the standard design. In three out of four cases, the standard design estimate lies within the range of estimates obtained in the ring test. In the fourth case (LD_{50} for Isazofos) the estimate from the standard design lies outside the ring test range, but confidence intervals indicate that the standard design estimate is not significantly different.
- The variance in estimates of LD_{50} from the standard design is 4.5 times larger than the variance from the ring test. The variance in estimates of slope from the ring test is 6 times larger than the variance from the standard design. Both of these results are entirely consistent with results from the computer simulations.
- The uncertainty intervals estimated for the LD_{50} and slope in the ring test are consistent with variation shown (*via* Box-Whisker plots) in the computer simulations.

In conclusion the ring test has shown that variation in estimates between labs is consistent with results from computer simulations and that we can rely on the computer simulations with confidence. Draft TG223 provided reliable estimates of LD_{50} and slope that were not notably different from equivalent estimates obtained from the standard design.

Table 3: Variation about LD_{50} based on EPA data
Variance = 0.06245180

Log10 scale			Original Scale		
Lower Limit	LD_{50}	Upper Limit	Lower Limit	LD_{50}	Upper Limit
0.1992	0.6990	1.1988	1.6	5.0	15.8
1.1992	1.6990	2.1988	15.8	50.0	158.0
2.6763	3.1761	3.6759	474.6	1500.0	4741.3

Table 4: Variation about LD_{50} based Ring Test Data
Variance = 0.013822758

Log10 scale			Original Scale		
Lower Limit	LD_{50}	Upper Limit	Lower Limit	LD_{50}	Upper Limit
0.4638	0.6990	0.9342	2.9	5.0	8.6
1.4638	1.6990	1.9342	29.1	50.0	85.9
2.9409	3.1761	3.4113	872.8	1500.0	2577.9

Table 5: Variation about slope based on EPA and Ring Test Data

EPA Variance = 2.2121			Ring Test Variance = 13.24011333		
Lower Limit	slope	Upper Limit	Lower Limit	slope	Upper Limit
0.00	2	4.97	0.00	2	9.28
2.03	5	7.97	0.00	5	12.28
7.03	10	12.97	2.72	10	17.28

7.8. Feedback from the laboratories that took part (including feedback on SEDEC).

All participating laboratories were asked to give their opinion about the practicalities of the draft TG223 guideline, using northern bobwhite as test species. The laboratories saw advantages and disadvantages to the method. A summary of these have been listed below.

Advantages as seen by the participating laboratories:

- Fewer birds are used to derive an LD_{50} value (29 – 39 birds instead of 60). This animal welfare aspect is considered of importance to all but one participating laboratory.
- A limit test will be chosen more frequently using the draft TG223 guideline than it will be using the OPPTS design. The reason is that in the draft TG223 guideline mortality is the primary endpoint and the NOEL is not required.

Disadvantages identified by the participating laboratories were related to management of birds and complexity of the design. Comments could be summarised as follows:

- The design requires more time and effort. The time taken to complete the study was considered longer and there is a slight increase in personnel resources to prepare dosages and calculate the dose levels for the next stage of the study.
- When only a few studies are run, efficient management of bird stock is more difficult. Spare birds may remain after the study if fewer stages are required than planned for. In order to minimise wastage of birds, one laboratory suggested to allow for birds to be used from different hatching batches.
- The complexity of the guideline could lead to more errors being made, but laboratories that used SEDEC found it to work very well and its use would minimise the risk of mistakes. The use of SEDEC was considered to be a challenge for some. The validation management group believes this could also be communication issue related language. A few cautionary notes were added by some of the laboratories.

Response of the Validation Management Group:

The Validation Management Group (VMG) plans to run demonstrations of SEDEC at conferences like SETAC. The VMG considers that more extensive experience with the draft TG223 and familiarisation in selecting doses and managing spare birds, will help overcome some of the concerns raised by the labs. The VMG recognises, however, that the most efficient management of birds to reduce the spare birds issue, will be at laboratories where acute oral studies are performed at a high frequency.

Overall the VMG considers that the three major benefits of the draft TG223: i) being able to go to a limit test more frequently, ii) testing fewer birds in a full LD_{50} study and iii) obtaining a slope estimate more regularly, make this design well worth dealing with the initial difficulties.

7.9. Experience gained to date with regulatory studies performed to draft OECD guideline 223

A number of studies have been conducted according to earlier drafts of TG223 that provide further insight into how it performs. All, with the exception of example 4, were conducted according to versions of the draft TG223 preceding the 2007 version, and before SEDEC was available. There are some differences in the design of the study for the presented examples (*e.g.,* design did not have a Stage 4); however, the main structure was the same. Examples 1 and 2 were conducted with formulations (several other formulations were tested as a Limit Test at the

2000mg/kg). Example 3 with a technical substance, subsequently repeated according to OPPTS820.2100. Example 4 used draft TG223 Stage 2 as a rangefinder for OPPTS850.2100. Examples 5-9 were LD_{50} only tests to better understand species sensitivity distributions using only Stages 1 and 2. Numbers of birds used are given (n=xx) excluding controls. Those identified as failed limit tests include the numbers tested in the limit test. Full datasets are showing numbers at each stage together with reversals and partial mortalities are given in Appendix VIII.

Example 1. 3 stage dose response test. 7 birds used, not 5 as described for stage 3b (n=26). LD_{50} = 440mg/kg (95% CL 258-715 mg/kg); Slope 3.2.

Example 2. Failed limit test (60% mortality) followed by Stage 1 and 2 (n=25). LD_{50} = 1734mg/kg (95% CL 1303-2273mg/kg); Slope 7.03

Example 3. Failed limit test (60% mortality) followed by Stage 1 and 2 (n=25). LD_{50} = 1278mg/kg (95% CL 625-1662mg/kg); Slope 5.7.
Same compound repeated as OPPTS 850.2100 (n=50). Results were:
LD_{50} =1206mg/kg (95% CL 897-1765); Slope 3.5.

Example 4. Failed Limit Test followed by Stage 2 (n=20)
LD_{50}= 276mg/kg (conducted as a rangefinder)
Same compound repeated as OPPTS 850.2100 (n=50). Results were:
LD_{50} = 232mg/kg (95% CL 173-313mg/kg); Slope 5.9

Example 5. 3 stage LD_{50} (n=18). LD_{50} = 1010 mg/kg.

Example 6. 2 stage LD_{50} (n=12). LD_{50} = 450mg/kg

Example 7. 2 Stage LD_{50} (n=12). LD_{50} = 166mg/kg.

Example 8. 2 Stage LD_{50} (n=12). LD_{50} = 2381mg/kg

Example 9. 2 Stage LD_{50} (n=12). LD_{50}= 348mg/kg.

8. GLP status of the validation work

All studies performed in this ringtest were performed to GLP. The simulations that were performed were subjected to a validation of which the report is appended to this report. The findings have been responded to. The SEDEC programme has been validated and the findings responded to and corrected.

9. Availability for peer review

All data generated for the purpose of the validation of OECD draft guideline 223, the SEDEC programme and the SEDEC manual will all be available for peer review on the OECD website.

10. Peer Review

Literature references

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(2) Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Series 71: Avian and Mammalian Testing § 71-1 Avian Single-Dose Oral LD_{50} Test.

(3) Office of Prevention, Pesticides and Toxic Substances (OPPTS): Series 850: Ecological Effects Test Guidelines: 850.2100 Avian Acute Oral Toxicity test, April 1996.

(4) OECD guideline 425: OECD Guidelines for the Testing of Chemicals: Acute Oral Toxicity Up-and-Down Procedure. Adopted 23 March 2006.

(5) SEquential DEsign Calculator: A Tool for Use with OECD draft TG223: Avian Acute Oral Toxicity Test. Users Guide. SEDEC Version 1.3. 29 September 2009.

(6) EPA Historic Data base: (http://www.ipmcenters.org/Ecotox/index.cfm).

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APPENDIX I

STATISTICAL SIMULATIONS SUPPORTING THE DEVELOPMENT OF OECD DRAFT TEST GUIDELINE 223 (TG223): AVIAN ACUTE ORAL TOXICITY TEST

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Introduction

One outcome of an OECD/SETAC workshop on avian toxicity testing in Pensacola, Florida, USA in 1994 (OECD/GD(96)166)– was a recommendation to evaluate the most efficient way to generate a dose-response curve with special consideration to the number of animals per dose level and the number of dose levels. Following further discussions and extensive statistical simulations of various designs the recommendation for a sequential design was made. The original TG223 design was developed between spring 2000 and autumn 2002 as an alternative to the standard single-stage 60-bird acute oral toxicity test. The generic design was a very flexible design comprising an arbitrary number of stages, doses within stages, and replicates within doses. The number of doses could vary between stages and the number of replicates could vary between doses. In all cases, doses were equally spaced (on a log scale) around a stage-specific estimate of the LD_{50}. In order to find the best designs from within this very wide class, many hundreds of computer simulations were carried out.

In the simulations, all key features of the design – total number of birds, number of stages, number of doses per stage, number of birds per dose – were varied in a systematic way in order to develop a complete picture of the performance of the design. A well-known theoretical result that guided the simulations was the knowledge that the optimal design under a probit model for estimating both LD_{50} and slope simultaneously requires half the birds be given a dose that would result in 15% kill and the other half be given a dose resulting in 85% kill. Whilst optimal design requires one to know the value of the LD_{50} and slope before carrying out the experiment, which is, of course, impossible, this theoretical result appeared likely to be useful in later stages after preliminary estimates of the LD_{50} and slope had been computed.

An efficient design is one that requires fewer birds in total. It quickly became clear to the investigators that using too many doses in stage 1 led to very inefficient designs. Looking at the more efficient designs, which had very few doses in stage 1, a pragmatic rationale seemed to be as follows: stage 1 with few birds can usually be relied on to produce a "good enough" estimate of the LD_{50}; more doses can then be used in stage 2 to produce an initial estimate of the slope and also to refine the estimate of the LD_{50}; and principles of optimal design can be used in stage 3 to further refine estimates of both the LD_{50} and slope.

In October 2002 a draft OECD guideline was issued that described a 24-bird design having 3 stages: stage 1 using 4 birds and stages 2 and 3 using 10 birds each. A large number of comments were received, particularly from the USEPA. Two meetings at USEPA offices took place in 2005 in order to tie down the precise nature of the concerns and to put in place actions that would address them. Changes were made in the overall design and programming subsequent to these two meetings that make presentation of specific prior simulation results no longer germane to the conclusions in this report regarding draft TG223 as is currently proposed. Hence all of the simulation results presented in this report were produced after the two meetings with USEPA personnel in 2005. Nevertheless, this report does give a comprehensive account of the performance of the proposed draft TG223 design.

The results presented in part 1 of this report were originally produced in order to address concerns over bias apparent in the initial draft TG223 design, but also pointed to the potential for improving accuracy when additional stages are included beyond the 3 stages in the 2002 draft Test Guideline. The apparent bias was of particular concern: in early reporting the original 3-stage draft TG223 design was shown to be far more successful in estimating the slope than the standard 60-bird design but the distribution of slope estimates was much wider and distinctly skewed towards steepness. For example, when the true slope was 2 it was not unusual to obtain slope estimates nearer to 25.

A detailed evaluation of the causes of this bias revealed a flaw in the execution of past simulations. A property of sequential studies is that some data become available, and are analysed, prior to study completion. (In this document the phrase "**working estimates**" refers to estimates of LD_{50} and slope that are computed during the study but before completion of the study. The phrase "**final estimates**" refers to estimates of LD_{50} and slope based on the data set that only becomes available at the end of a study.) The protocol for computing final estimates of LD_{50} and slope specifies that all simulated experiments be classified according to one of seven groups. Estimation of slope, via maximum likelihood, is only possible if an experiment is assigned to those groups designated C or D (for details see Tables 2 and 3). For all other groups, estimation of slope is not possible. In early simulations, at the end of stage 2 probit analysis was used to derive working estimates without attempting to classify according to groups A to G. This resulted in large numbers of slope estimates that would have been recognised as impossible had classification been carried out. In addition the slope estimates had a tendency to be steep so the lack of classification at the end of stage 2 was the major source of the apparent bias in final slope estimates. When the simulations were re-run with data allocated to groups at the end of stage 2, the bias towards steep final slope estimates was eliminated completely, but the success rate in obtaining slope estimates was also greatly reduced in some circumstances. Because of this outcome, additional types of simulations were carried out in order to explore the potential for introducing additional stages in these circumstances.

Addressing these concerns subsequently led to the design's being revised. Part 1 of this report therefore compares this updated draft TG223 design to that of:

- The standard *a priori* fixed level, 60-bird-one-stage design. In fact two designs, designated design 9 and design 11, were chosen for comparison. In a separate simulation study (not described here in detail) a total of twelve one-stage designs were compared. Some used 30 birds, some 50. Some had fewer doses and more birds per dose, whilst some used more doses and fewer birds per dose. Among the twelve, designs 9 and 11 performed the best, with design 9 being better for estimating slope and design 11 being better for estimating LD_{50}. Both designs used 10 doses and five birds per dose. Design 9 used a high-to-low dose ratio of 20, whilst design 11 used a high-to-low dose ratio of 50. It should be noted that both designs 9 and 11 differed from the more typical 60-bird design that uses 5 doses and control with 10 birds per dose. The reason for using designs 9 and 11 in this evaluation, rather than the more typical fixed- level design, is because they have been shown, in earlier simulations, to perform much better.

- A TG223 24-bird-3-stage design. This is the design described in the draft OECD guideline, dated October 2002, with one difference - the data were classified into groups at the end of stage 2 resulting in lower success rate in estimating slope but elimination of the apparent bias seen in earlier simulations.

Note 1: Throughout this document the descriptions "TG223 24-bird-3-stage design" and "original TG223 design" are used. Original refers to the TG223 design that was recommended in the draft guideline of October 2002, but with the qualification that the procedure used to obtain working estimates of the LD_{50} and slope at the end of stage 2 is different and that the success rate in estimating the slope is much poorer.

Note 2: The descriptions "TG223 24/34-bird-3/4-stage design" and "updated TG223 design" are used. Updated refers to a revised version of the original design that is different in three respects:

- Working estimates of the LD_{50} and slope at the end of stages 2 and 3 are only computed when data are classified as group C or D.
- There are two different forms for stage 3 with the results at the end of stage 2 being used to determine which is used.
- For one of the two forms of stage 3 there is an option to carry on to stage 4 - the results at the end of stage 3 being used to determine whether this happens.

This report also addresses several other issues that were originally raised as concerns by the USEPA.

- Part 2 evaluates the potential for improving performance of the standard a-priori fixed-level 60-bird design by using a range finder.
- Part 3 evaluates the robustness of the updated draft TG223 design to background mortality.
- Part 4 evaluates the robustness of the updated draft TG223 design to delayed mortality.
- Part 5 evaluates the robustness of the updated draft TG223 design to inaccurate initial guesses of the LD_{50}.
- Part 6 evaluates the robustness of the updated draft TG223 design to errors in dosing.

All simulation results are presented as box-and-whisker plots. The upper and lower edges of the box represent the 25th and 75th percentiles respectively. The line between the upper and lower edges of the box represents the median (*i.e.* the 50th percentile) and the mean is represented by a star symbol. Whiskers have been drawn from the edges of the box to the extreme values. In some cases the extreme values have been clipped to obtain a more readable plot. Clipped points are represented collectively by a square symbol, and a legend at the top of each chart indicates the number of boxes that were clipped.

Method for Simulating Designs

This section describes the method of simulating the standard 60-bird-one-stage design, the original TG223 24-bird-3-stage design, and the updated draft TG223 24/34-bird-3/4-stage design.

True LD_{50} and true slopes

One thousand experiments were simulated for each of nine combinations of true LD_{50} (5, 50, and 1500 mg/kg) and true slope (2, 5, and 10).

Initial guess of the LD_{50}

Each simulated experiment commenced with a computer simulated guess of the LD_{50}. The guess was randomly selected from a normal distribution with mean equal to the true $\log_{10}$ LD_{50} and variance consistent with the 95th and 5th percentile being a factor of ten greater or less than the true LD_{50}.

Dosing constraints

Maximum and minimum dose limits were set at 1 and 3333 mg/kg, and birds were not tested outside these limits. If the procedure for selecting doses in any stage required one or more doses to be above the maximum dose limit, the doses were reconfigured with highest dose set at the limit and the other doses selected to give the specified high-to-low-dose ratio. Similarly if the procedure required one or more of the doses to be below the minimum dose limit, the doses were reconfigured with lowest dose set at the limit and the other doses selected to give the specified high-to-low-dose ratio.

Procedure for deciding whether a bird dies or survives

The true LD_{50} and true slope for any simulated experiment represent the mean and standard deviation of the tolerance distribution for the sample of birds used in the experiment. For any specific hypothetical dose applied to a hypothetical bird the LD_{50} and slope can therefore be used to determine the exact probability that the bird dies. For each hypothetical bird in an experiment, a random number from a uniform distribution on a (0,1) scale was therefore generated. If the number generated was less than the exact probability of death then the bird was deemed to have died.

Procedure for selecting doses – standard 60-bird-one-stage design

Ten doses were equally spaced on a $\log_{10}$ scale around the initial guess of the LD_{50} with a high-to-low-dose ratio of 50 (design 11) or 20 (design 9). Each dose was given to five birds.

Procedure for selecting doses – original TG223 24-bird-3-stage design

Stage 1: Four doses were equally spaced on a $\log_{10}$ scale around the initial guess of the LD_{50} with a high-to-low-dose ratio of 50. Each dose was given to one bird. A working estimate of the LD_{50} was computed as the geometric mean of the transition doses (see Table 1).

Stage 2: Ten doses were equally spaced on a $\log_{10}$ scale around the working estimate of the LD_{50} obtained from stage 1. Assuming a working estimate of five for the slope, the extreme doses were placed at locations corresponding to probabilities of death of 0.01 and 0.99. Each dose was then given to one bird. Working estimates of the LD_{50} and slope were computed from the combined 14-bird data from stages 1 and 2 using the approaches described in Tables 2 and 3. If the working estimate of the slope was either greater than fifteen or less than one, then it was set to be fifteen or one respectively. If a working estimate of the slope was not possible, it was set to a value of five.

Stage 3: Two doses were equally spaced - at locations corresponding to probabilities of death of 0.15 and 0.85. - on a $\log_{10}$ scale around the working estimate of the LD_{50} obtained from stage 2 Each dose was given to five birds.

Procedure for selecting doses – updated draft TG223 24/34-bird-3/4-stage design

Stage 1: As described for the original TG223 design.

Stage 2: As described for the original TG223 design. At the end of stage 2 working estimates of the LD_{50} and slope were computed from the combined data from stages 1 and 2 and using the approaches described in Tables 2 and 3. The number of reversals was also computed. A reversal is a dose-

response inversion; *i.e.* mortality is lower at the higher dose of a consecutive pair of doses. If at the end of stage 2 there were two or more reversals and a working estimate of the slope had been obtained, then stage 3a was used. However if at the end of stage 2 there were fewer than two reversals and/or a working estimate of the slope was not possible, then stage 3b was used.

Stage 3a: Is identical to stage 3 of the original TG223 design.

Stage 3b: Five doses were equally spaced on a $\log_{10}$ scale around the working estimate of the LD_{50} obtained from stage 2. Assuming a working estimate of five for the slope, extreme doses were placed at locations corresponding to probabilities of death of 0.15 and 0.85. Each dose was given to two birds. At the end of the stage working estimates of the LD_{50} and slope were computed from the combined data from stages 1, 2 and 3, using the approaches described in Tables 2 and 3. The number of reversals and the number of partial kills were also computed. If there were two or more reversals or two or more partial kills, and a working estimate of the slope was obtained, then the experiment stopped there. However if there were fewer than two reversals and fewer than two partial kills, and/or a working estimate of the slope was still not possible, then the experiment continued to stage 4.

Stage 4: Identical to stage 3b, with the exception that the five doses were equally spaced on a log10 scale around a updated working estimate of the LD_{50} using all data from previous three stages.

Working estimates of LD_{50} in difficult situations

In some circumstances it was not possible, using the classification system described in Tables 2 and 3, to compute working estimate of the LD_{50} for use in stages 3a, 3b, and 4. Table 4 describes the methods that were used in these situations.

Final estimates of LD_{50} and slope

On completion of each simulated experiment, final estimates of the LD_{50} and slope were computed using the combined data from all stages and using the approach described in Tables 1 and 2.

Part 1: Performance of the updated draft TG223 design

Presentation of Results

All results are presented as box-and-whisker plots. In these, the upper and lower edges of the box represent the 25th and 75th percentiles respectively. The line between the upper and lower edges of the box represents the median (*i.e.* the 50th percentile) and the mean is represented by a star symbol. Whiskers have been drawn from the edges of the box to the extreme values. In some cases the extreme values have been clipped to obtain a more readable plot. Clipped points are represented by a square symbol, and a legend at the top of each chart indicates the number of boxes that were clipped.

How does the updated draft TG223 design compare with the original TG223 and the one- stage designs?

Figures 1.1 to 1.6 compare the performance of the updated draft TG223 24/34-bird-3/4-stage design with those of the standard 60-bird-one-stage and the original TG223 24-bird-3-stage designs.

The updated TG223 design is very successful in estimating the LD_{50} for all nine combinations of true LD_{50} and true slope. It was able to provide an estimate of the LD_{50} in over 99.9% of simulated experiments (cf. 88.5% and 75.0% using standard 60-bird designs 11 and 9 and 99.8% using the original TG223 24-bird-3-stage design). The distribution of LD_{50} estimates obtained using the updated TG223 design is very similar to those from the other designs under consideration.

For all designs under consideration, success in estimating the slope decreases as the true slope increases (*i.e.* becomes steeper). The updated TG223 24/34-bird-3/4-stage design was able to provide estimates of the slope in over 79.2% of simulated experiments, (cf. 24.1% and 42.5% using standard 60-bird-one-stage designs 9 and 11 and 51.4% using the original TG223 24-bird-3-stage design). As true slope becomes steeper success rate in estimating the slope declines for the standard 60-bird and original TG223 designs. Furthermore, when the true slope is 10 the standard 60-bird and original TG223 designs seem to be successful in subsets of the simulated experiments for which the apparent slope is relatively shallow - resulting in a bias towards shallower slopes. For example the 75th percentile of estimates from the original TG223 is smaller than the true value of 10. In contrast, when the true slope was 10 the updated TG223 was able to provide an estimate of the slope in approximately 80% of simulations.

Three stages vs four stages

Figures 2.1 to 2.6 provide more detailed comparisons between original and updated versions of the TG223. In particular, the performance of the updated TG223 at the end of stages 3 (3a or 3b) is compared with its performance at the end of stage 4.

For estimation of the LD_{50} the fourth stage provides few, if any benefits. The success rate is virtually 100% at the end of both stages, and the distribution of estimates is virtually identical.

For estimation of the slope the fourth stage is beneficial when the true slope is 10 (relatively steep). The success rate is about 6% higher and the distribution of estimates is narrower and free of bias.

Criterion for selection of the third stage

Stage 2 of the updated version of the TG223 is followed by either stage 3a or stage 3b according to whether or not a criterion is satisfied. Figures 3.1 to 3.9 show (for each of the nine combinations of true LD_{50} and slope) the variation in final estimates of slope for the original TG223 but grouped according to the criterion used to decide whether or not to use stage 3a at the end of stage 2 of updated TG223. They therefore allow us to assess the degree of success of the criterion used. Of the 9000 simulated experiments, only 2178 (*i.e.* 24%) used stage 3a.

The criterion used was as follows: If at the end of stage 2 there were two or more reversals and a working estimate of the slope was obtained, then stage 3a (*i.e.* original stage 3) was used. If, however, at the end of stage 2 there were fewer than two reversals, and/or the working estimate of the slope could not be obtained, then stage 3b was used.

Figures 3.1 to 3.9 show that the slope estimates produced at the end of stage 3 of the original TG223 24-bird-3-stage design are much more reliable if the criterion is satisfied. Of the 6822 experiments that failed the criterion, 2608 (*i.e.* 38%) had a true slope of 5 and 2986 (*i.e.* 44%) had a true slope of 10. This suggests that we are more likely to require a different stage 3 design (to that used in the original TG223 design) when the true slope is steep, or when more doses better assist slope estimation.

Criterion for use of stage four

Figures 4.1 to 4.9 show (for each of the nine combinations of true LD_{50} and slope), show variation in estimates of slope at the end of stage 3 (a or b) of the updated TG223 design grouped by the criterion used at the end of stage 3 to decide whether or not to continue to stage 4. They therefore allow us to assess the degree of success of the criterion used. Of the 9000 simulated experiments, only 2363 (*i.e.* 26%) continued to stage 4.

The criterion used was as follows: If at the end of stage 3 there were two or more reversals or two or more partial kills, and a working estimate of the slope was obtained, then the experiment stopped at stage 3. If, however, at the end of stage 3 there were fewer than two reversals and fewer than two partial kills, and/or a working estimate of the slope was not obtained, then the experiment continued to stage 4.

Figures 4.1 to 4.9 show that the slope estimates produced at the end of stage 3 (either 3a or 3b) of the updated TG223 24/34-bird-3/4-stage design are more reliable under the criterion to stop rather than the criterion to continue. Of the 2363 experiments that continued to stage 4, 1794 (*i.e.* 76%) had a true slope of 10. This suggests that we are more likely to continue to stage 4 when the true slope is steep.

Conclusion

For estimation of the LD_{50}, all four designs compared in this report give a similar performance. However, the TG223, original and updated, designs require far fewer birds – either 24 or 34 - so should be preferred.

For estimation of slope, the updated TG223 24/34-bird-3/4-stage design significantly outperformed the other designs. It was successful (at least 80%) in estimating the slope even when the true slope was steep. The original TG223 was less successful. Although modifications to the original TG223 design succeeded in eliminating bias in estimates of slope, success rate was much lower.

Although we have presented the updated TG223 as a 3- or 4-stage design, in the interests of conserving animals, the principal of extending the number of stages can easily be applied to 5 or more in any rare circumstances where this might be considered necessary.

Part 2: Potential for Improving Performance of the Standard 60-Bird Design using a Range-Finder

Method

The standard designs used were the same as in part 1. Design 11, which is better for estimation of the LD_{50}, has ten doses with five replicates per dose and a high-to–low-dose ratio of 50. Design 9, which is better for estimating the slope, has ten doses and five replicates per dose, but a high-to-low-dose ratio of 20.

Simulations of the standard design described already start with a guess of the LD_{50} that is taken from a distribution (for fuller explanation see section titled “Method for Simulating Designs”). However, the best possible guess is the true value. For each of the nine combinations of LD_{50} and slope, four different simulations were run:

- Design 11 and design 9 with the initial guess taken in the usual way (*i.e.* from a distribution).
- Design 11 and design 9 but with the initial guess of the LD_{50} set at the true value.

The approach taken here differs from that of a true range-finder. A simulated range-finder would not have delivered the true value of the LD_{50} on which to base the simulations for the main study, but it would have provided additional data values that would have improved the precision of the final estimates. It was felt, however, that the improvement in precision from additional birds would have been small.

Results

Results of simulations are presented in Figures 5.1 to 5.6

With the initial guess of the LD_{50} drawn from a distribution, the standard 60-bird one-stage design was very successful in estimating the LD_{50} for all nine combinations of true LD_{50} and slope. Design 11 (with a high-to-low-dose ratio of 50) and design 9 (with a high-to-low-dose ratio of 20) were able to provide estimates of the LD_{50} in over 88.5 and 75.0% of simulated experiments respectively. However with the initial guess of the LD_{50} drawn from a distribution, the standard 60-bird one-stage design appears less successful in estimating the slope, particularly when the true slope is high (*i.e.* steep). Design 11 and design 9 are able to provide estimates of the slope in over 81.3 and 75.7% of simulated experiments respectively when the true slope is low (*i.e.* 2 or 5), but are only able to provide estimates of the slope in over 24.1 and 42.5% of simulated experiments respectively when the true slope is high (*i.e.* 10).

Setting the initial guess of the LD_{50} at the true value led to small improvements in estimation of both slope and LD_{50}. The success rate was better but the distribution of estimates was much the same.

Conclusion

A range finder would (probably) slightly improve the performance of the standard design, particularly when steep slopes are estimated. However, the improvement is very small when compared with the much greater improvement obtained from TG223.

Part 3: Robustness of Updated TG223 Design to Natural Mortality

Method

For each simulated experiment the expected overall proportion that died, denoted q, was assumed to be made up of a proportion □ that died naturally and a proportion p, of the remaining proportion (1 - □) that died due to the test substance. Hence in this set of simulations, the probability of death q = □ + (1 -□) p. For each bird used in each simulated experiment, a random number between zero and one was selected from a uniform distribution. If the number was greater than q, the bird was deemed to have survived; otherwise it was deemed to have died.

Simulations were carried out with the probability of natural (or background) mortality □fixed at 0, 0.01, 0.05, and 0.10 (*i.e.* 0, 1, 5, and 10 %).

The probability of natural (or background) mortality that occurs in practice can be estimated using information from control birds. Recent data received from four laboratories which regularly perform avian studies show that there is very little observed control mortality. For quail and duck it is 1% or less. These data are presented in Table 5.

Results

Results of simulations are presented in Figures 6.1 to 6.6.

Increasing natural mortality from 0% to 1% has very little impact on the performance of the TG223 design. For both LD_{50} and slope, success rate and distribution of estimates are very similar. As per cent mortality increases to 5% and 10%, success rate changes little, but the distribution of estimates shifts downwards such that estimates appear biased towards values that are smaller than the true values.

In practice, therefore, since natural mortality is ordinarily 1% or less, it will have no observable impact on the performance of the TG223 design.

Conclusion

Natural (or background) mortality has no impact at levels we observe in practice.

Part 4: Robustness of UpdatedTG223 Design to Delayed Mortality

Method

For each simulated experiment the time of death was computed for each bird that died. Stage 2 started 3 days after the start of stage 1 and stage 3 started 3 days after the start stage 2 and stage 4 (if used) started 3 days after the start of stage 3. The doses for stage 2 were computed using data as was available from stage 1 - any bird that was still alive at the end of stage 1 but died before the end of the study was treated as a survivor in this computation. Similarly the doses for stage 3 were computed using the combined data as was available from stages 1 and 2 and the doses for stage 4 (if used) were computed using the combined data as was available from stages 1, 2 and 3. Each bird, and hence each stage was monitored for a total of 14 days after dosing, therefore each simulated experiment ended 14 days after exposure in stage 3 or 4 (if used). The final estimates of the LD_{50} and slope were computed using the combined data from all three stages as was available at the end of the study.

The time of death was computed in three different ways. Firstly the time of death was fixed at 0 days after exposure for all birds that died (this is the situation of no delayed mortality). Secondly the time of death was selected at random from a uniform distribution ranging between 0 and 14 days. Finally the time of death was selected from an empirical distribution formed using data provided by Wildlife International Ltd. on the day of death of 711 northern bobwhite birds. The cumulative probabilities of death are presented in Table 6. For each bird that died in each simulated experiment, a random number between 0 and 1 was selected from a uniform distribution. If the number was less than or equal to the cumulative probability of death on day 0, the bird was deemed to have died on day 0; if the number was greater than the cumulative probability of death on day 0, but less than or equal to the cumulative probability of death on day 1, the bird was deemed to have died on day 1; and so on.

An attempt was made to fit a mathematical frequency curve to the data in Table 6, however none of the fitted curves was satisfactory when compared with the empirical data (both visually and using formal lack of fit tests).

Results

Results of simulations are presented in Figures 7.1 to 7.6.

The updated TG223 design managed to provide a final estimate of the LD_{50} in over 93.9% of simulated experiments with uniform delayed mortality and over 99.2% of simulated experiments with empirical delayed mortality (cf. 100% with no delayed mortality). The distributions of estimates of the LD_{50} appear similar to those with no delayed mortality.

The TG223 design with both uniform and empirical delayed mortality appears less successful in estimating the slope, particularly when the true LD_{50} is low and the true slope is high. It managed to provide a final estimate of the slope in over 7.7% of simulated experiments with uniform delayed mortality and over 52.3% of simulated experiments with empirical delayed mortality (cf 77.8% with no delayed mortality). However, note that for the TG223 design with empirical delayed mortality, this still compares favourably with the standard 60-bird design, which was, at best, able to provide an

estimate of the slope in over 42.5% of simulated experiments (design 9 with initial guess of LD_{50} randomly selected from a distribution).

Therefore, although delayed mortality has an impact on performance of the updated TG223 design, as proposed, the design still performs better than the standard 60-bird design.

Conclusion

Delayed mortality does have an impact but still performs better than the standard 60- bird design. However, delayed mortality will, except in extreme cases, be observed before the completion of the experiment. The study director will therefore usually be in a position to take actions to mitigate the effect of the delayed mortality. For example the number of days between initiating stages can be increased, and stages can be repeated. This is one of the strengths of the design. The use of a few extra birds and a relatively small increase in the overall length of a study can be used to improve the performance of the design without the need to repeat the test as a whole.

Part 5: Robustness of Updated TG223 to Inaccurate Initial Guesses of the LD_{50}

Method

The initial guess of the LD_{50} was set at three different values: the true LD_{20}, the true $LD_{50,}$ and the true LD_{80}.

Results

Results of the simulations are presented in Figures 8.1 to 8.6.

The results of the computer simulations with the three different initial guesses of the LD_{50} all appear similar to those with zero natural mortality and zero delayed mortality. This strongly suggests that the performance of the updated TG223 design does not depend on the initial guess of the LD_{50}.

Conclusion

The updated TG223 24/32-bird 3/4-stage design is robust to misplaced starts.

Part 6: Robustness of the Updated TG223 to Errors of Dosing

It was unnecessary to address this issue by means of simulations.

Only two dosing-error scenarios are worth considering:

- Concentrations in dosing solutions incorrect by a constant factor,
- Concentrations in dosing solutions incorrect by a quantity that varies randomly between doses.

The result of case 1 is obvious. That is, the LD_{50} would be biased by an amount equal in size and direction to the constant error in dose.

The result of case 2 would be indistinguishable from increased variation in the response of birds. That is, the measured slope of the dose response curve would be decreased in proportion to the extent of random variation (error) in the dose. It has already been established how different slopes affect the performance of the test, so how the random errors in dosing would affect study performance is also known (at least qualitatively).

Conclusions

Errors in dosing have effects that are entirely predictable.

Table 1: Working estimate of the LD_{50} is from the results of stage 1

dose1	dose2	dose3	dose4	Approx. LD_{50} Estimate
O	O	O	O	$(\text{dose4} \times \text{dose5})^{1/2}$
O	O	O	X	$(\text{dose3} \times \text{dose4})^{1/2}$
O	O	X	O	$(\text{dose2} \times \text{dose3} \times \text{dose4} \times \text{dose5})^{1/4} = (\text{dose3} \times \text{dose4})^{1/2}$
O	X	O	O	$(\text{dose1} \times \text{dose2} \times \text{dose4} \times \text{dose5})^{1/4} = \text{dose3}$
X	O	O	O	$(\text{dose0} \times \text{dose1} \times \text{dose4} \times \text{dose5})^{1/4} = (\text{dose2} \times \text{dose3})^{1/2}$
O	O	X	X	$(\text{dose2} \times \text{dose3})^{1/2}$
O	X	X	O	$(\text{dose1} \times \text{dose2} \times \text{dose4} \times \text{dose5})^{1/4} = \text{dose3}$
X	X	O	O	$(\text{dose0} \times \text{dose1} \times \text{dose4} \times \text{dose5})^{1/4} = (\text{dose2} \times \text{dose3})^{1/2}$
O	X	O	X	$(\text{dose1} \times \text{dose2} \times \text{dose3} \times \text{dose4})^{1/4} = (\text{dose2} \times \text{dose3})^{1/2}$
X	O	X	O	$(\text{dose0} \times \text{dose1} \times \text{dose2} \times \text{dose3} \times \text{dose4} \times \text{dose5})^{1/6} = (\text{dose2} \times \text{dose3})^{1/2}$
X	O	O	X	$(\text{dose0} \times \text{dose1} \times \text{dose3} \times \text{dose4})^{1/4} = \text{dose2}$
O	X	X	X	$(\text{dose1} \times \text{dose2})^{1/2}$
X	O	X	X	$(\text{dose0} \times \text{dose1} \times \text{dose2} \times \text{dose3})^{1/4} = (\text{dose1} \times \text{dose2})^{1/2}$
X	X	O	X	$(\text{dose0} \times \text{dose1} \times \text{dose3} \times \text{dose4})^{1/4} = \text{dose2}$
X	X	X	O	$(\text{dose0} \times \text{dose1} \times \text{dose4} \times \text{dose5})^{1/4} = (\text{dose2} \times \text{dose3})^{1/2}$
X	X	X	X	$(\text{dose0} \times \text{dose1})^{1/2}$

Note. Survival is represented by O and death by X. Even though only 4 doses (dose1 through to dose 4) were used in the test, values for dose0 and dose5 are mentioned in the table. The values that should be used for these doses are one step up or down from the actual test doses. That is, dose0 = dose1 / step and dose5 = dose4 x step. Dose0 must be added to the computation of the approximate LD_{50} when mortality occurs at the lowest test dose, and dose5 is added when there is survival at the highest test dose.

Table 2: The classification of study outcomes

Class	Description
A	Total survival at all doses or total mortality at all doses.
B	Inconsistent results. Average log(dose) at which mortality occurs is less than or equal to average log(dose) at which survival occurs.
C	At least 2 partial kills, consistent.
D	At least one dose response inversion (*i.e.* there is at least one case in which the mortality is lower at a higher dose), consistent. This is particularly important when there is only 1 observation per dose.
E	One partial mortality bounded below by a dose(s) with 0 mortality and above by a dose(s) with full mortality, consistent, no reversals.
F	Partial mortality, but without both bounds described in E, consistent, no reversals.
G	No partial kills, with all doses with zero mortality below those with full mortality.

Table 3: Methods of estimating LD_{50}, slope, and corresponding confidence intervals.

Table 4: Working estimate of the LD_{50} used to place doses in stage 3a, 3b and 4 when a working

Class	Parameter	Procedures
A	LD_{50}	Not estimable.
	CI for LD_{50}	Binomial one sided 97.5 % confidence bound.
	Slope	Not estimable.
	CI for Slope	Not estimable.
B	LD_{50}	Not estimable.
	CI for LD_{50}	Not estimable.
	Slope	Not estimable.
	CI for Slope	Not estimable.
C&D	LD_{50}	Two parameter Maximum Likelihood estimate.
	CI for LD_{50}	Estimates based on Fieller's theorem.
	Slope	Two parameter Maximum Likelihood estimate.
	CI for Slope	Slope $\pm$ 1.96 (asymptotic variance estimate).
E	LD_{50}	Non-linear interpolation, weighted.
	CI for LD_{50}	Binomial two sided 95% confidence bounds. If one of the bounds cannot be estimated, the other becomes a one sided 97.5% bound.
	Slope	Not estimable.
	CI for Slope	Not estimable.
F	LD_{50}	Non-linear interpolation provide 0.5 survival is bracketed, else not-estimable.
	CI for LD_{50}	Binomial one sided 97.5% confidence bound
	Slope	Not estimable.
	CI for Slope	Not estimable.
G	LD_{50}	Maximum Likelihood estimate is midpoint in log scale between highest dose with complete survival and lowest dose with complete mortality.
	CI for LD_{50}	Binomial two sided 95% confidence bound. If one of the bounds cannot be estimated, the other becomes a one sided 97.5% bound.
	Slope	Not estimable.
	CI for Slope	Not estimable.

estimate of the LD_{50} could not be obtained using the methods described in Tables 1 and 2

Classification	Working LD_{50} estimate
A	Lowest dose - if total mortality at all doses, Highest dose - if total survival at all doses.
B	Lowest dose with total mortality.
F	Lowest dose - if the total number dead is greater than half of the total number exposed, Highest dose - if the total number dead is less than half of the total number exposed.

Table 5: Control mortality recorded in 105 recent avian studies performed at four laboratories

Species	Number Dead/Number Tested	Probability
Bobwhite quail	1/520	0.002
Mallard duck	1/465	0.002
Japanese quail	1/102	0.010
House sparrow	0/60	0.000
Chicken	1/35	0.029
Ring-necked pheasant	0/20	0.000
Pigeon	0/10	0.000
Zebra finch	0/10	0.000

Data provided by Wildlife International Ltd.

Table 6: Day of death for 711 northern bobwhite birds that died out of 1860 exposed (10 birds in each of 186 treatment groups)

Day of Death	Frequency	Probability	Cumulative Frequency	Cumulative Probability
0	170	0.23910	170	0.23910
1	282	0.39662	452	0.63572
2	108	0.15190	560	0.78762
3	41	0.05767	601	0.84529
4	13	0.01828	614	0.86357
5	9	0.01266	623	0.87623
6	13	0.01828	636	0.89451
7	24	0.03376	660	0.92827
8	14	0.01969	674	0.94796
9	11	0.01547	685	0.96343
10	6	0.00844	691	0.97187
11	5	0.00703	696	0.97890
12	8	0.01125	704	0.99015
13	4	0.00563	708	0.99578
14	3	0.00422	711	1.00000

Data provided by Wildlife International Ltd.

Figure 1.1: Performance of Updated TG223: true $LD_{50} = 5$

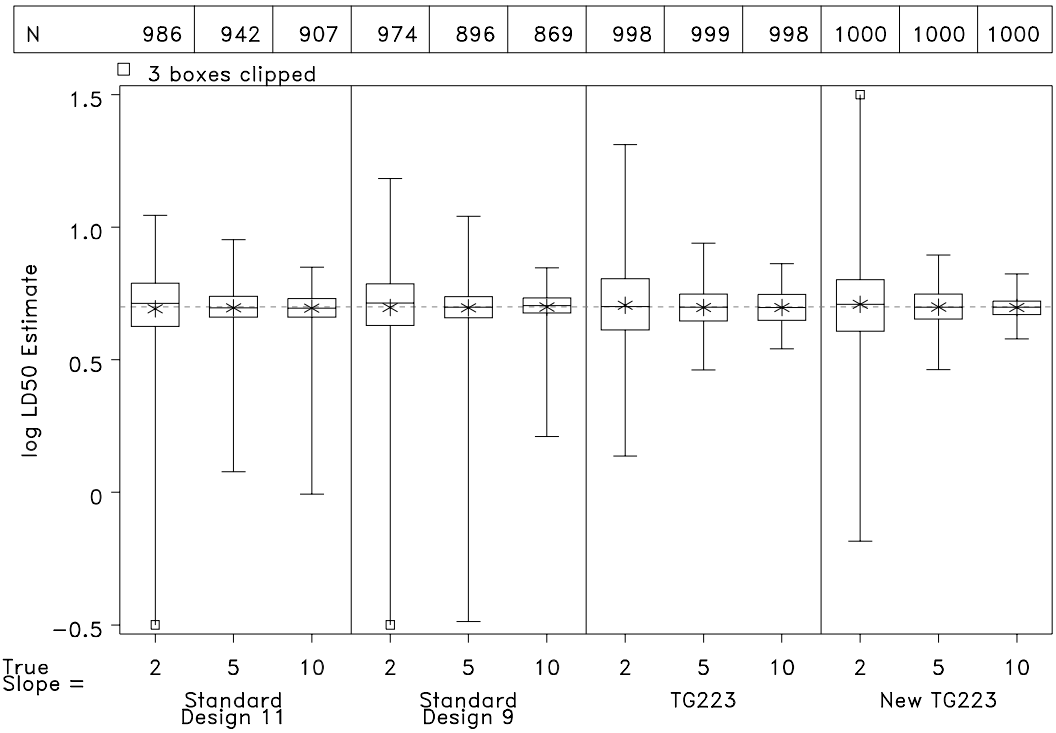


Figure 1.2: Performance of Updated TG223 - true $LD_{50} = 50$

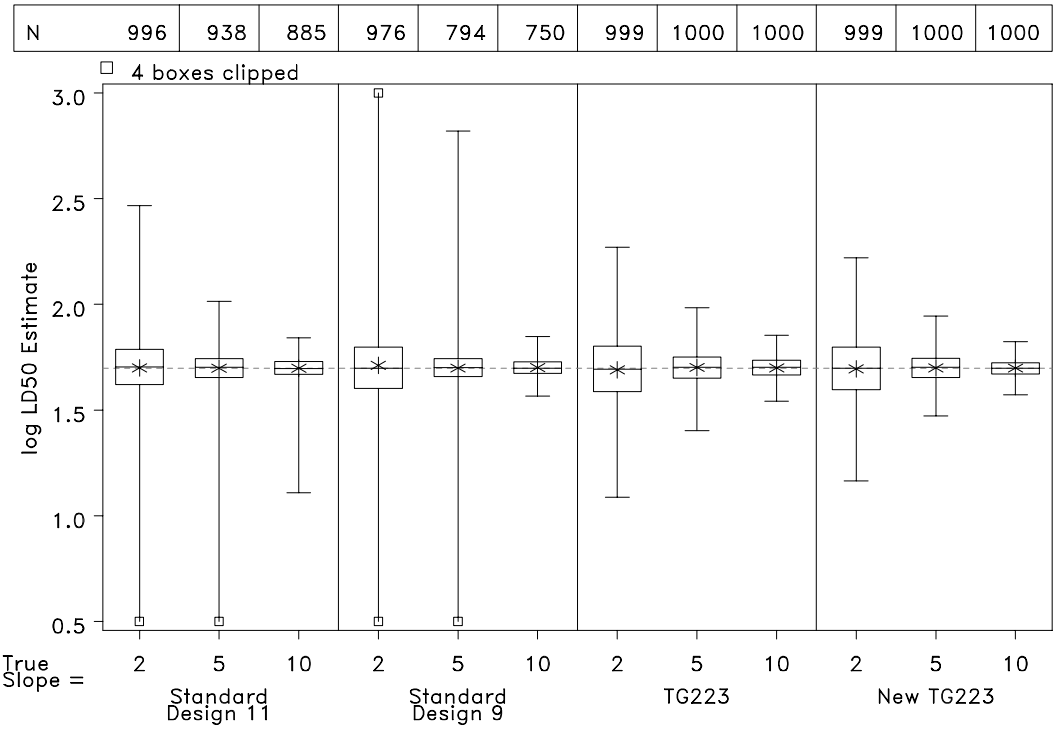


Figure 1.3: Performance of Updated TG223: true LD_{50} = 1500

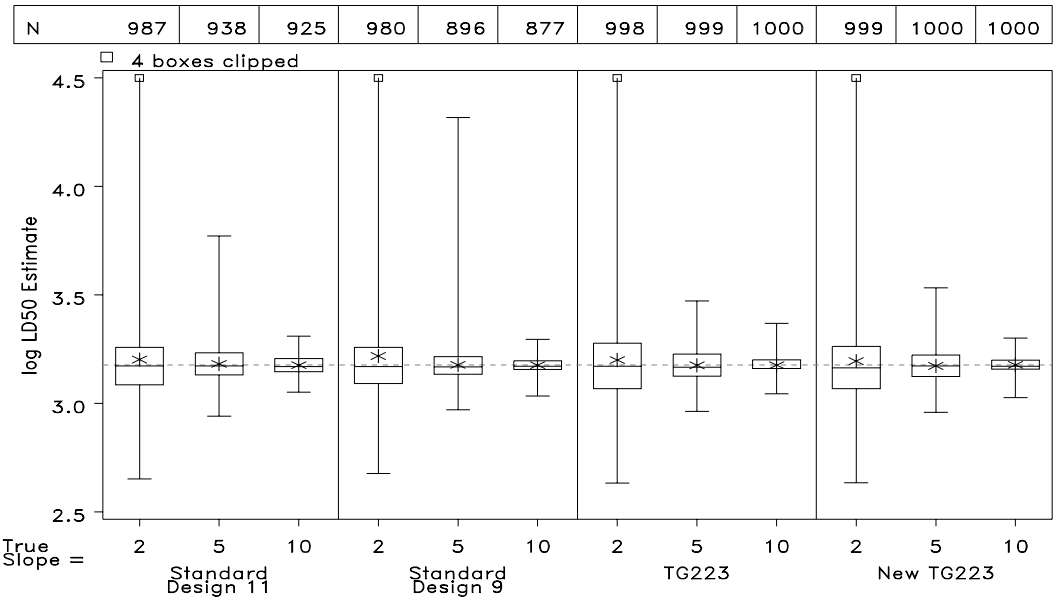


Figure 1.4: Performance of Updated TG223: true slope = 2

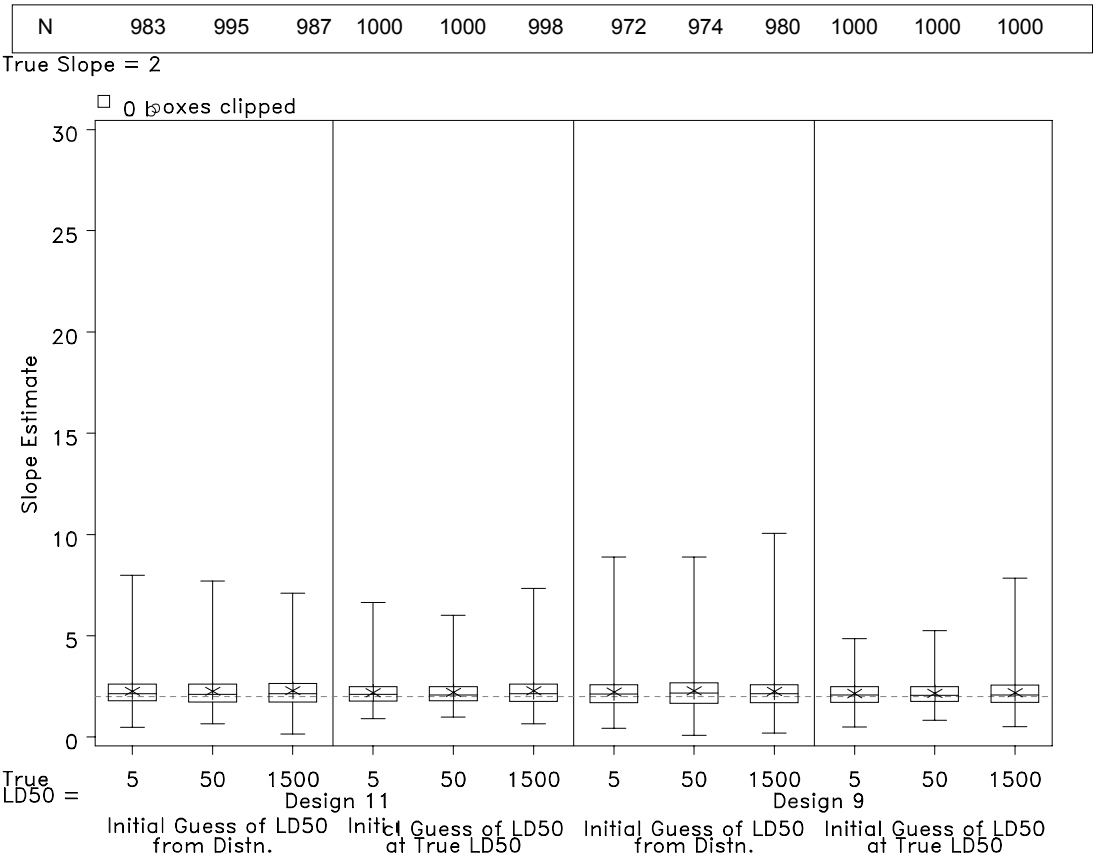


Figure 1.5: Performance of Updated TG223: true slope = 5

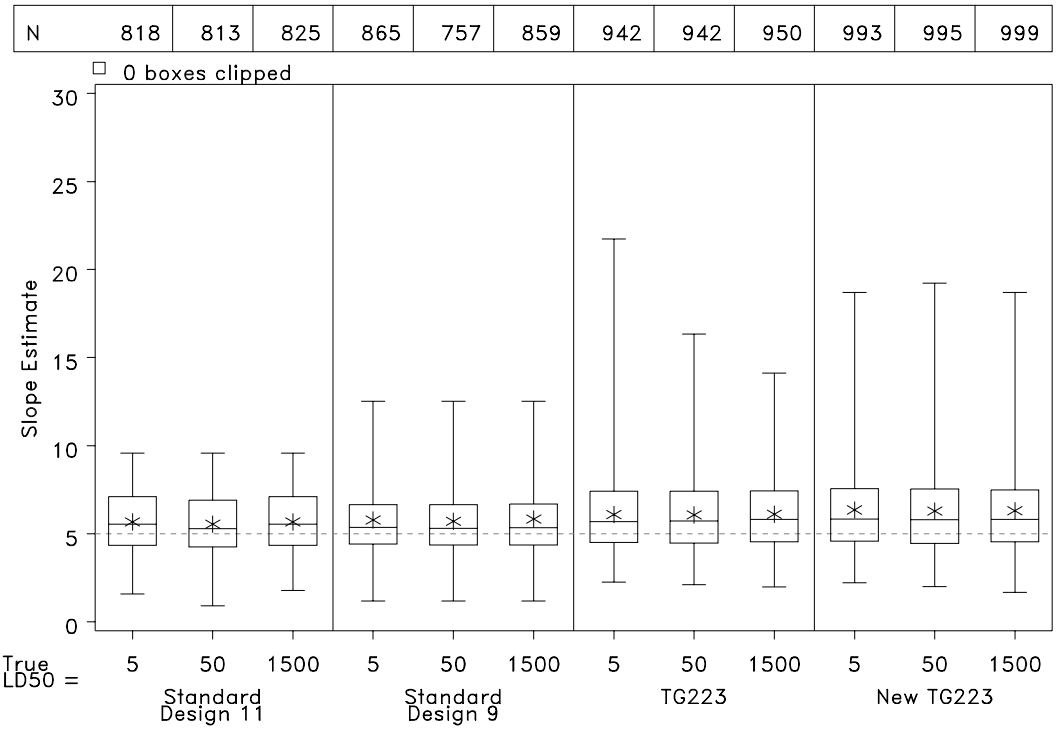


Figure 1.6: Performance of Updated TG223: true slope = 10

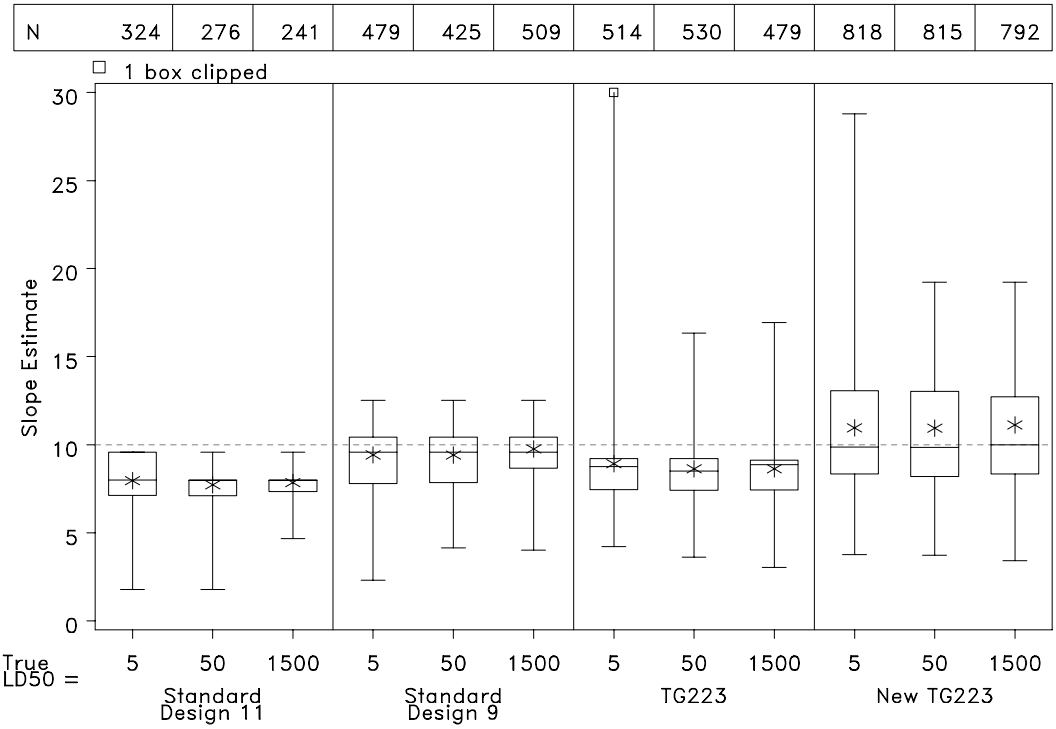


Figure 2.1: Updated TG223 - 3 stages vs. 4 stages: true $LD_{50} = 5$

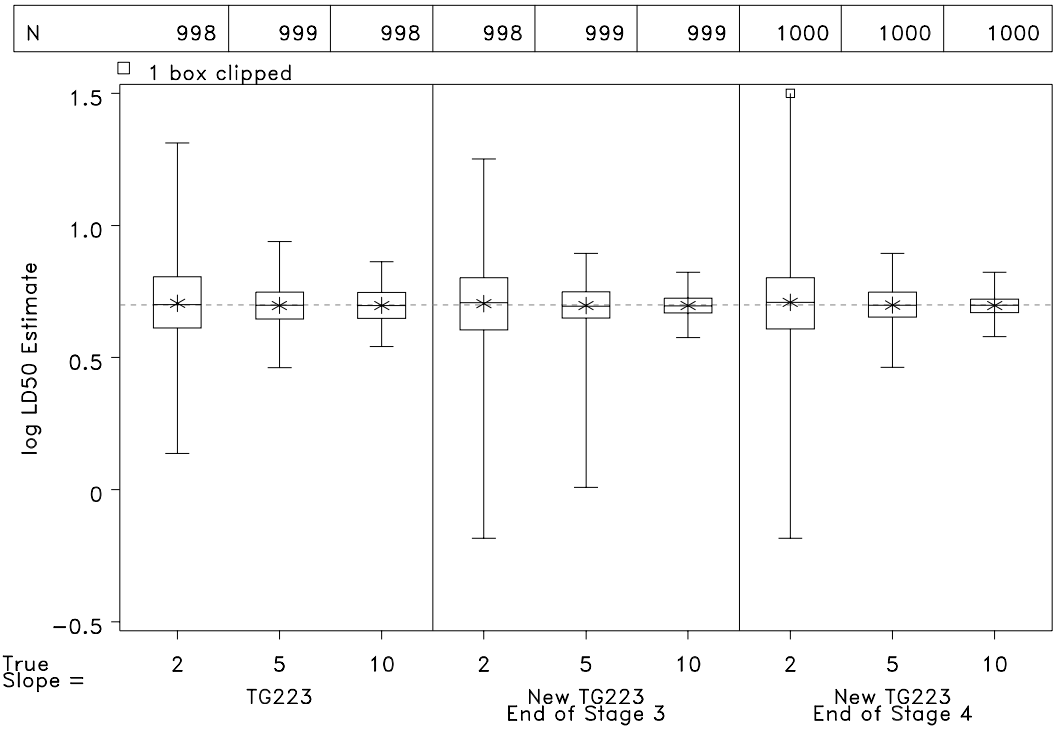


Figure 2.2: Updated TG223 - 3 stages vs. 4 stages: true $LD_{50} = 50$

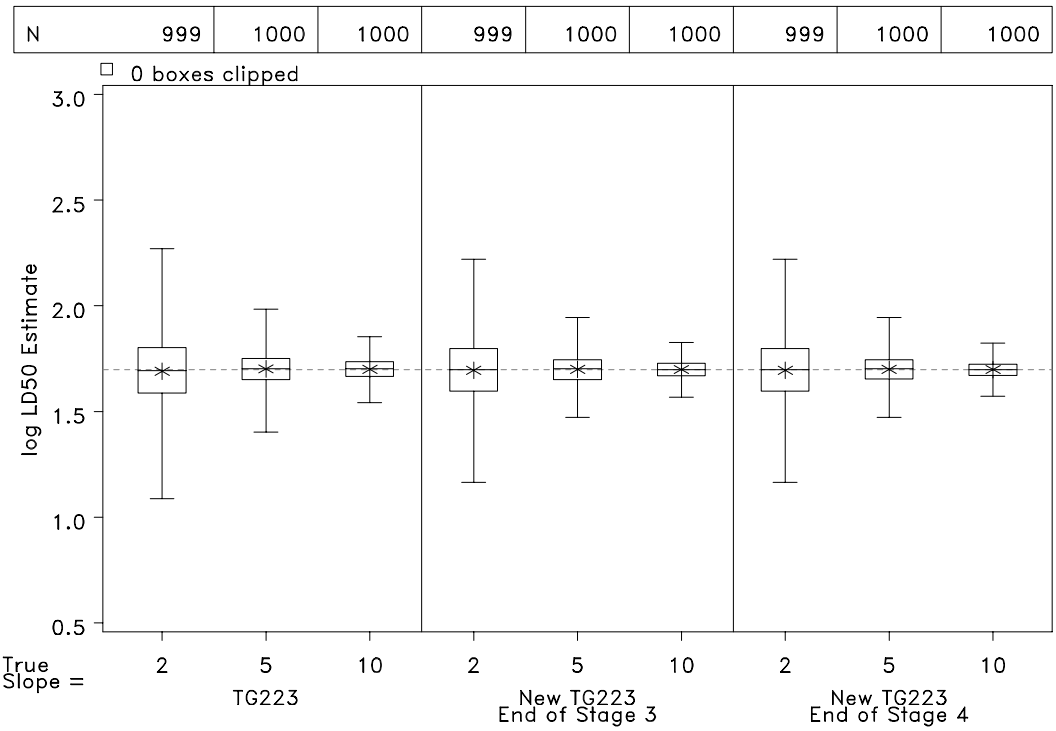


Figure 2.3: Updated TG223 - 3 stages vs. 4 stages: true LD_{50} = 1500

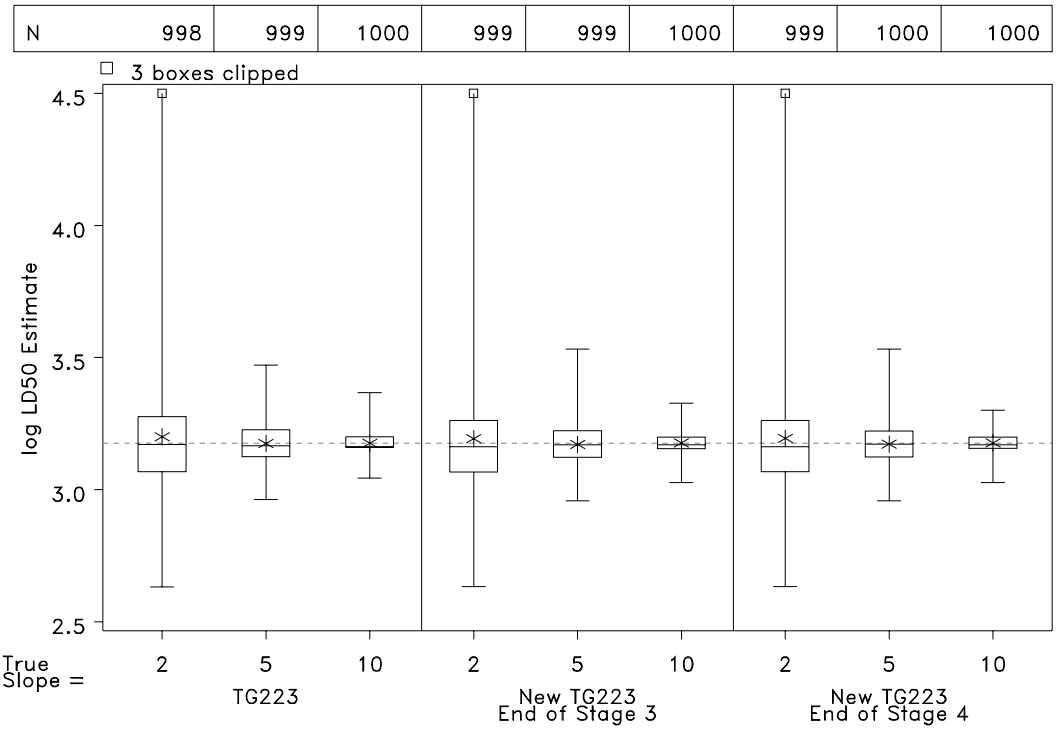


Figure 2.4: Updated TG223 - 3 stages vs. 4 stages: true slope = 2

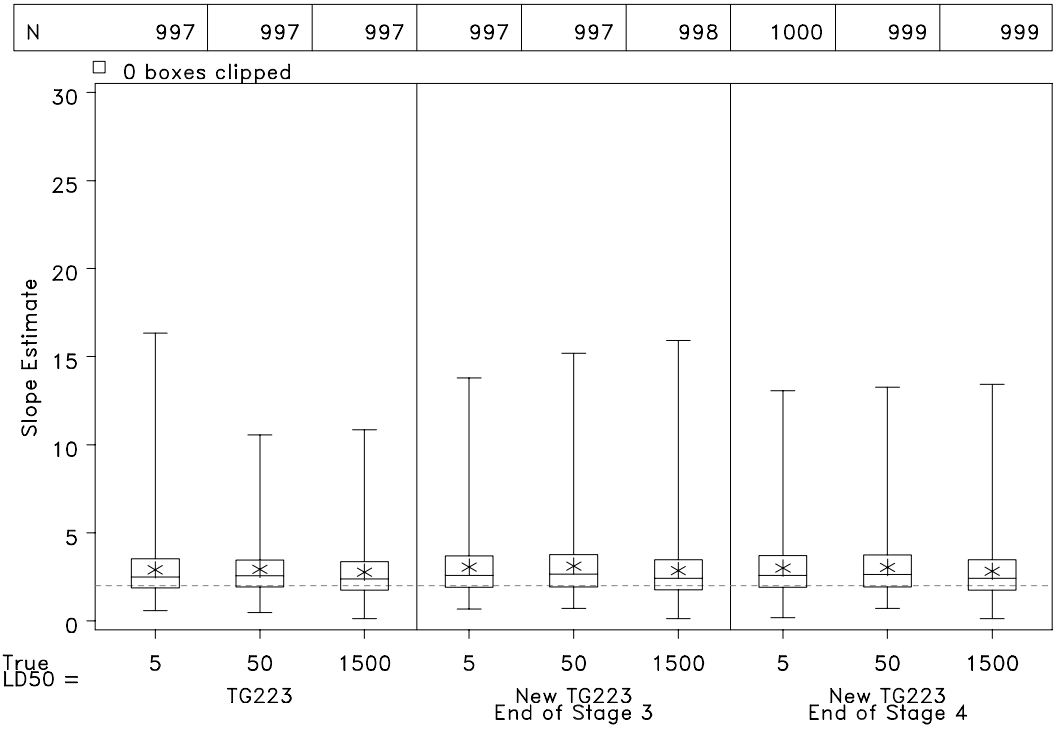


Figure 2.5: Updated TG223 - 3 stages vs. 4 stages: true slope = 5

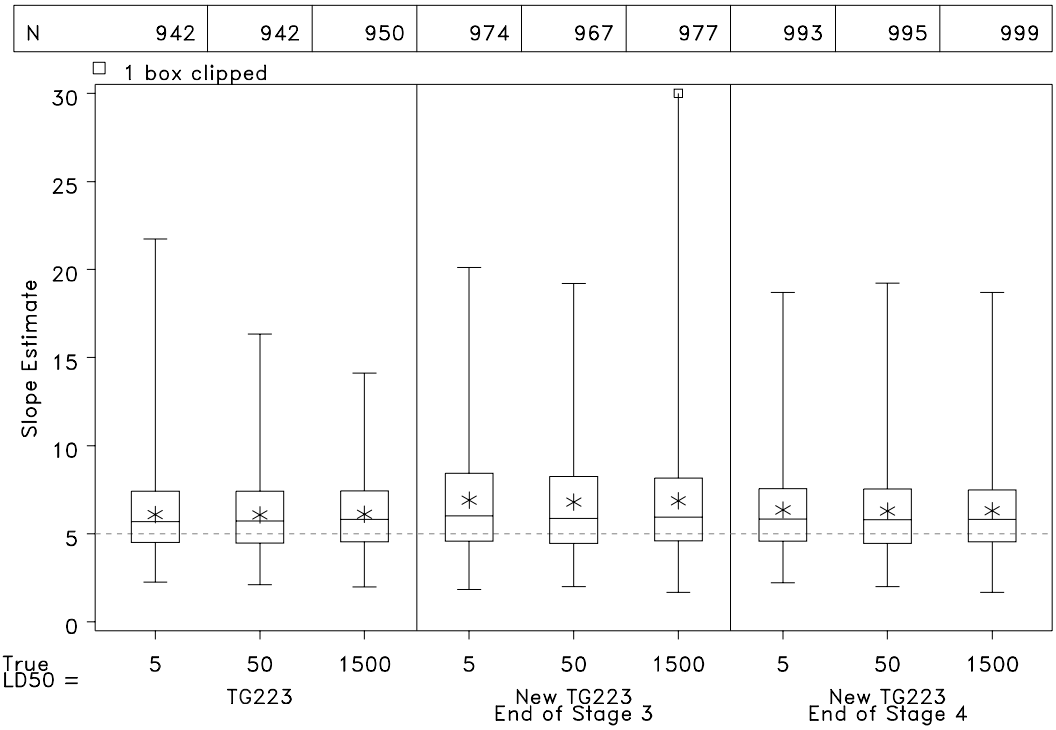


Figure 2.6: Updated TG223 - 3 stages vs. 4 stages: true slope = 10

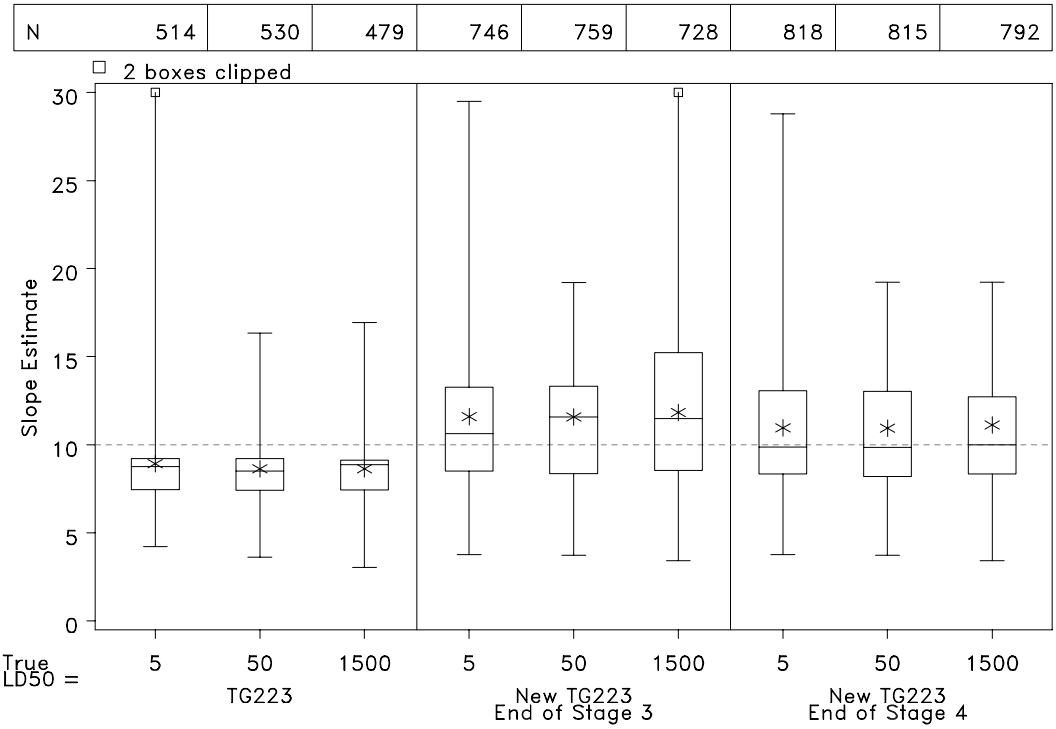


Figure 3.1: Criterion for selection of third stage; true LD_{50} = 5, true slope = 2

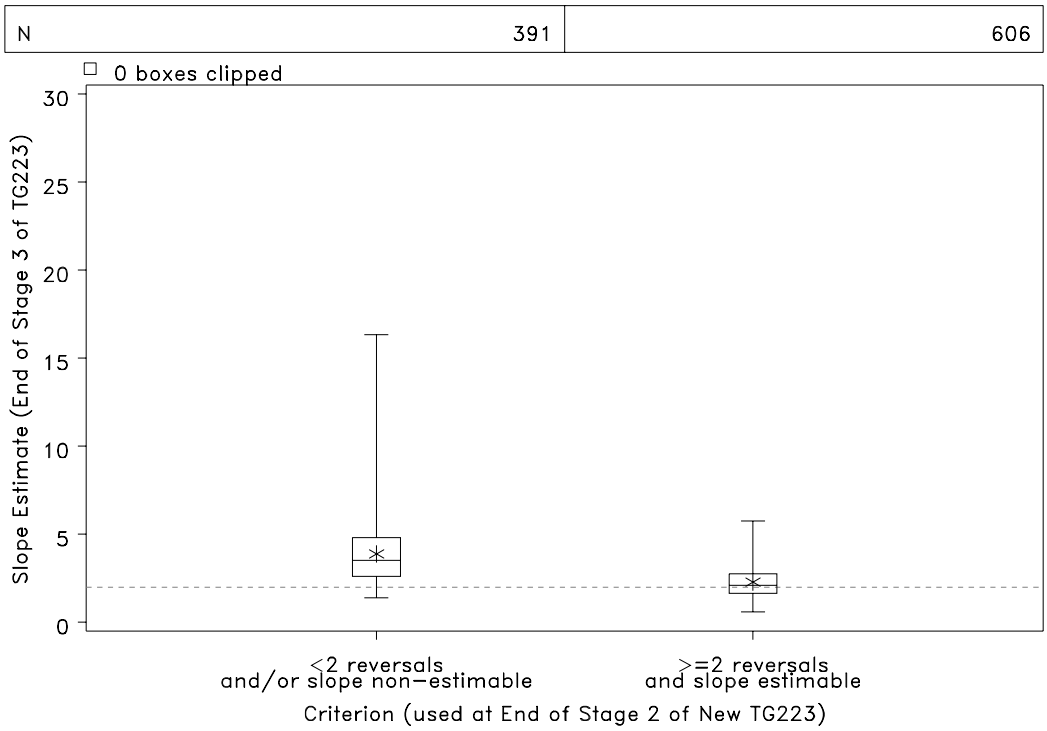


Figure 3.2: Criterion for selection of third stage: true LD_{50} = 5, the slope = 5

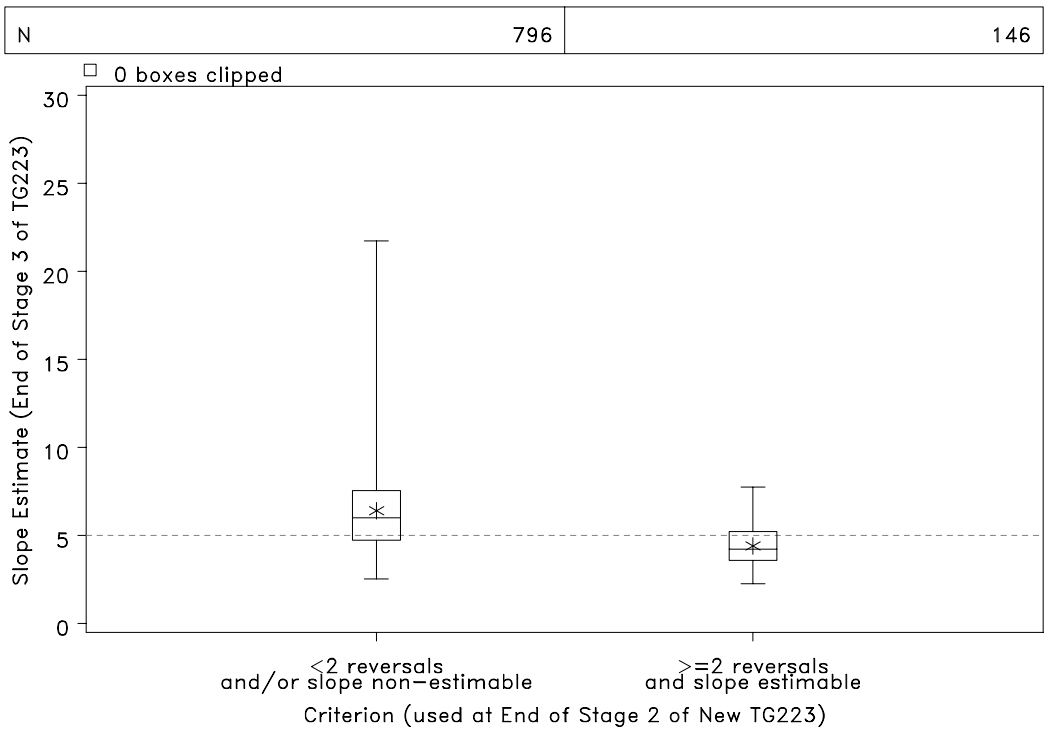


Figure 3.3: Criterion for selection of third stage: true LD_{50} = 5, true slope = 10

Figure 3.4: Criterion for selection of third stage: true LD_{50} = 50, true slope = 2

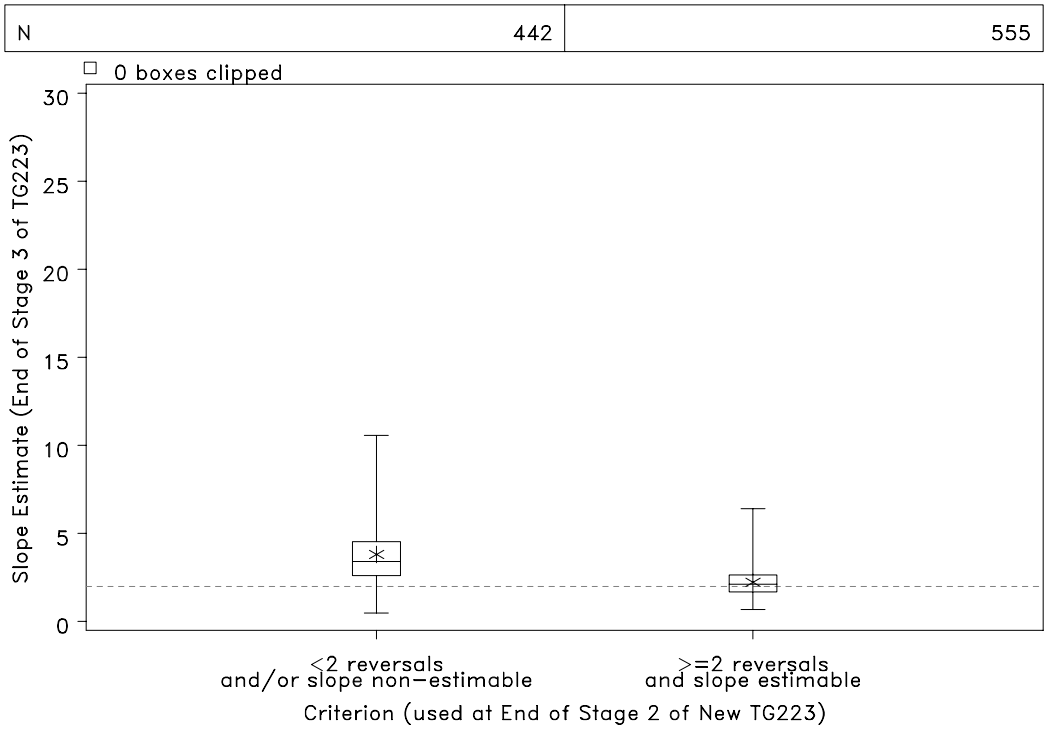


Figure 3.5: Criterion for selection of third stage: true LD_{50} = 50, true slope = 5

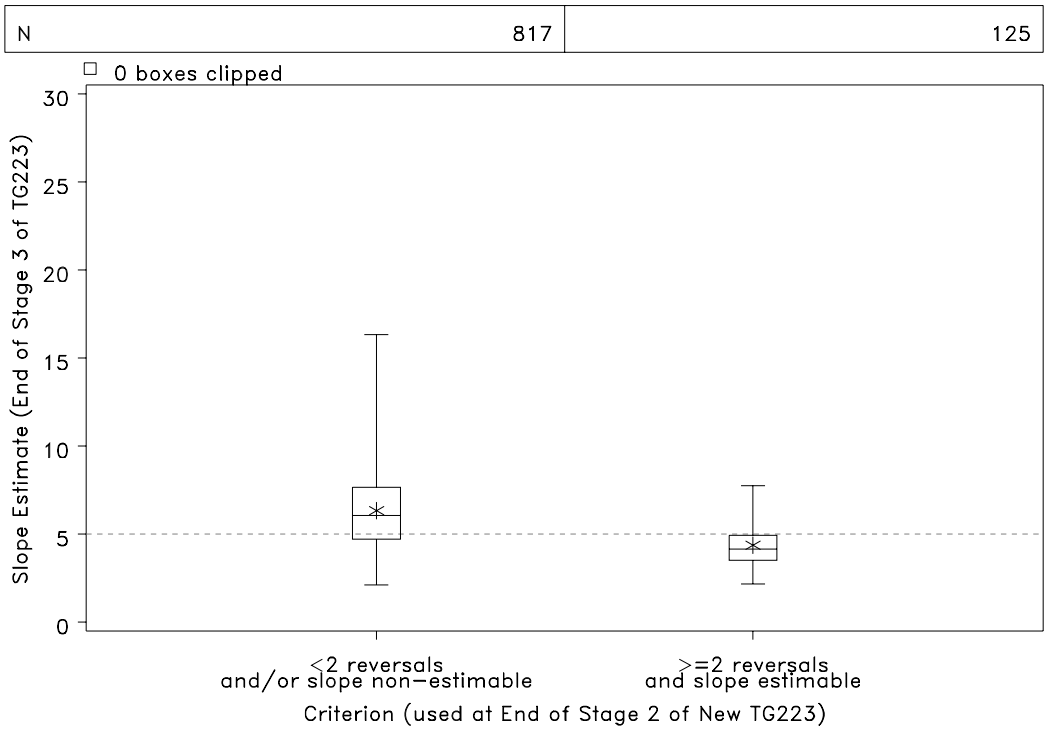


Figure 3.6: Criterion for selection of third stage: true LD_{50} = 50, true slope = 10

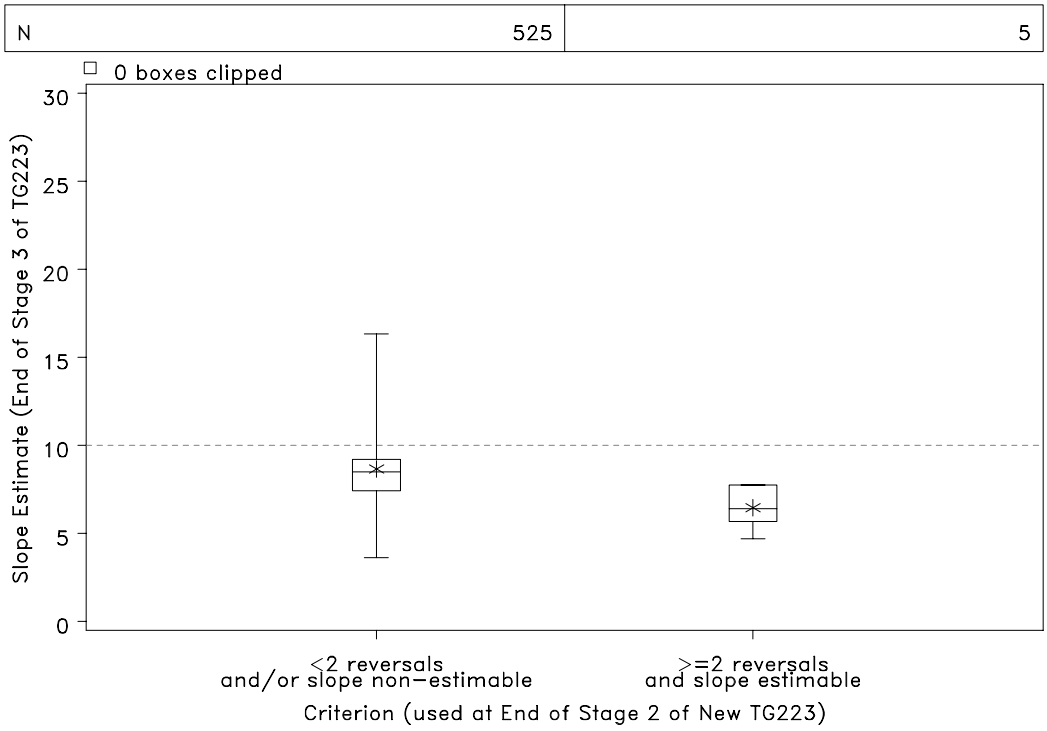


Figure 3.7: Criterion for selection of third stage: true LD_{50} = 1500, true slope = 2

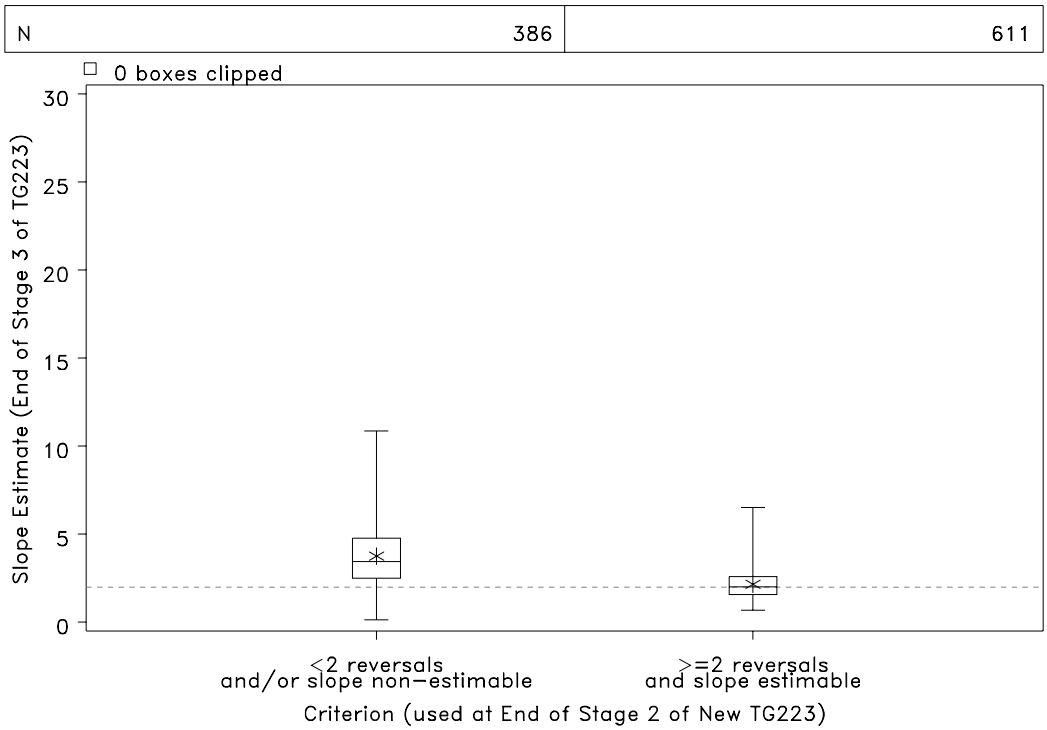


Figure 3.8: Criterion for selection of third stage: true LD_{50} = 1500, true slope = 5

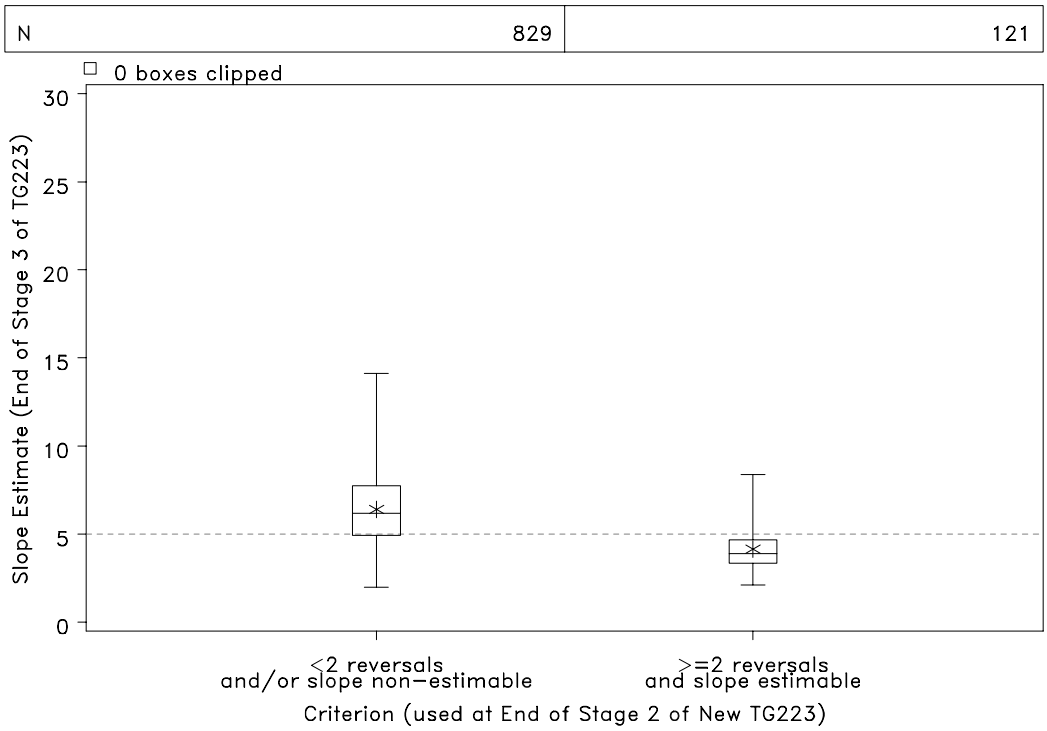


Figure 3.9: Criterion for selection of third stage: true LD_{50} = 1500, true slope = 10

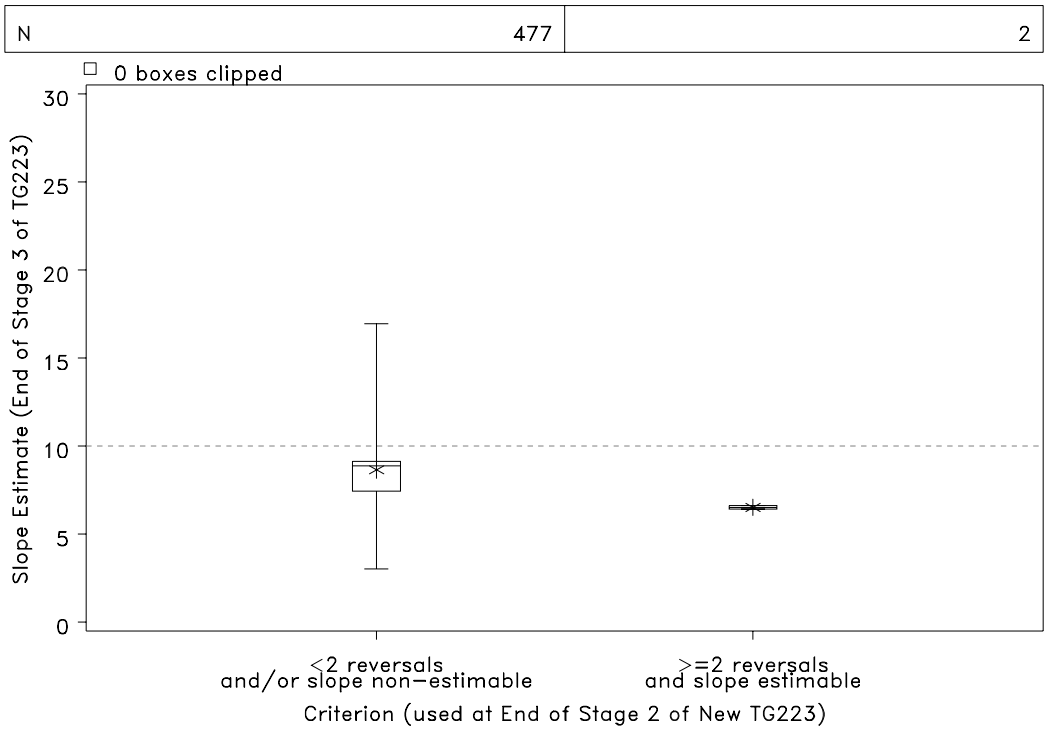


Figure 4.1: Criterion for using fourth stage: true LD_{50} = 5, true slope = 2

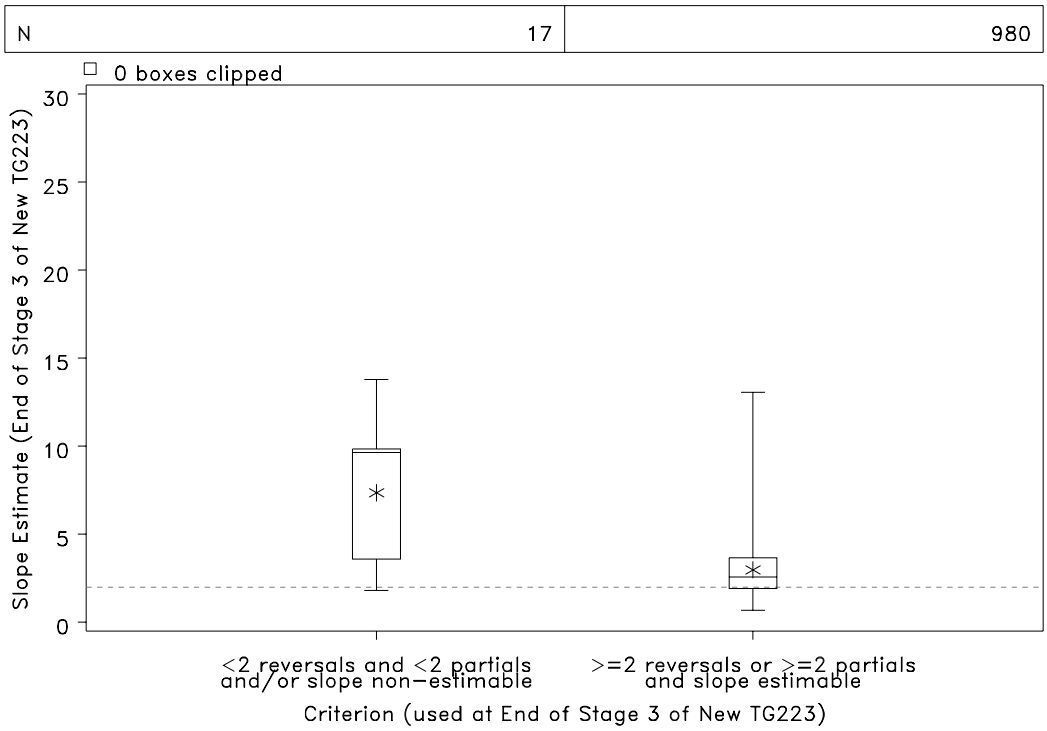


Figure 4.2: Criterion for using fourth stage: true LD_{50} = 5, true slope = 5

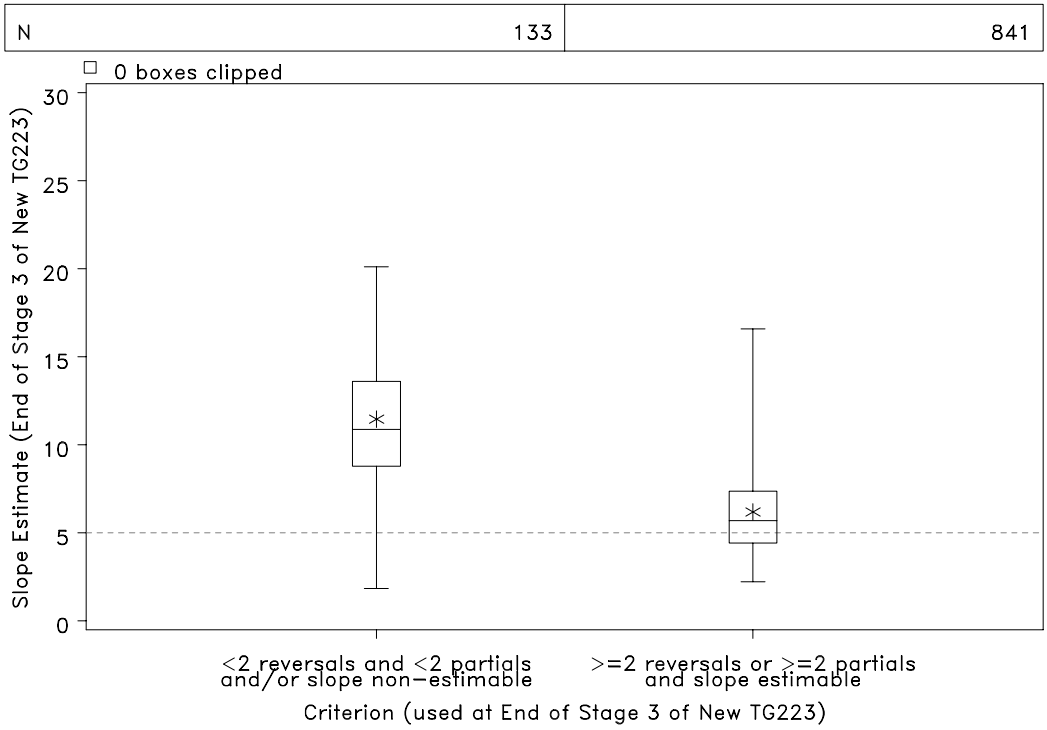


Figure 4.3: Criterion for using fourth stage: true LD_{50} = 5, true slope = 10

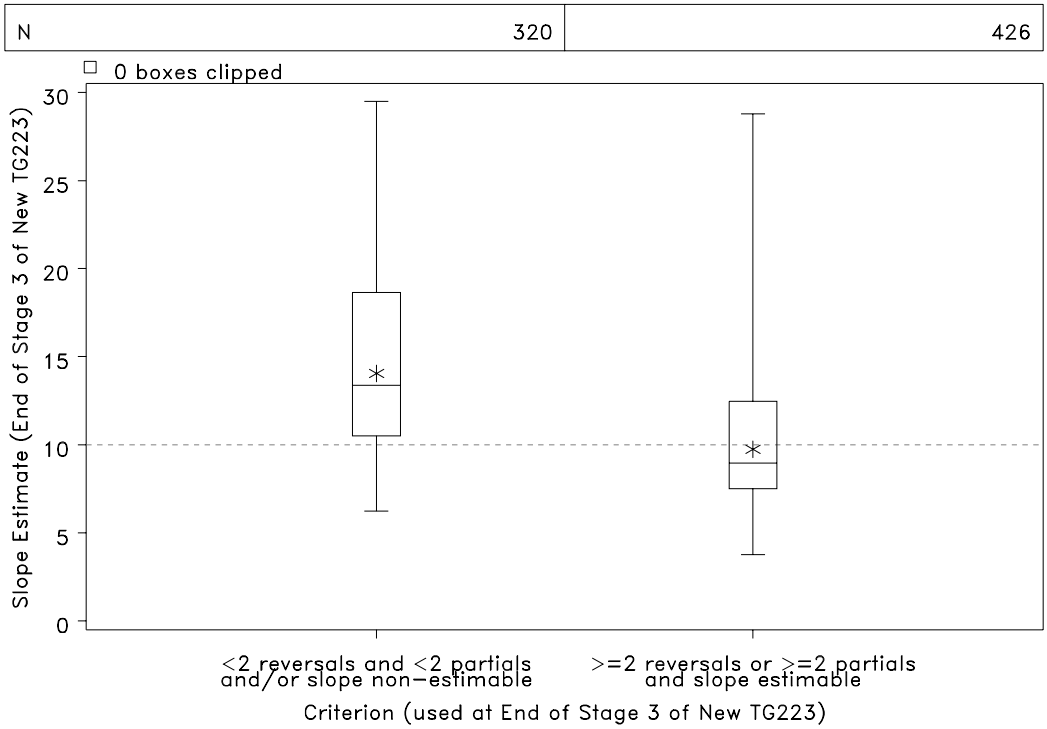


Figure 4.4: Criterion for using fourth stage: true LD_{50} = 50, true slope = 2

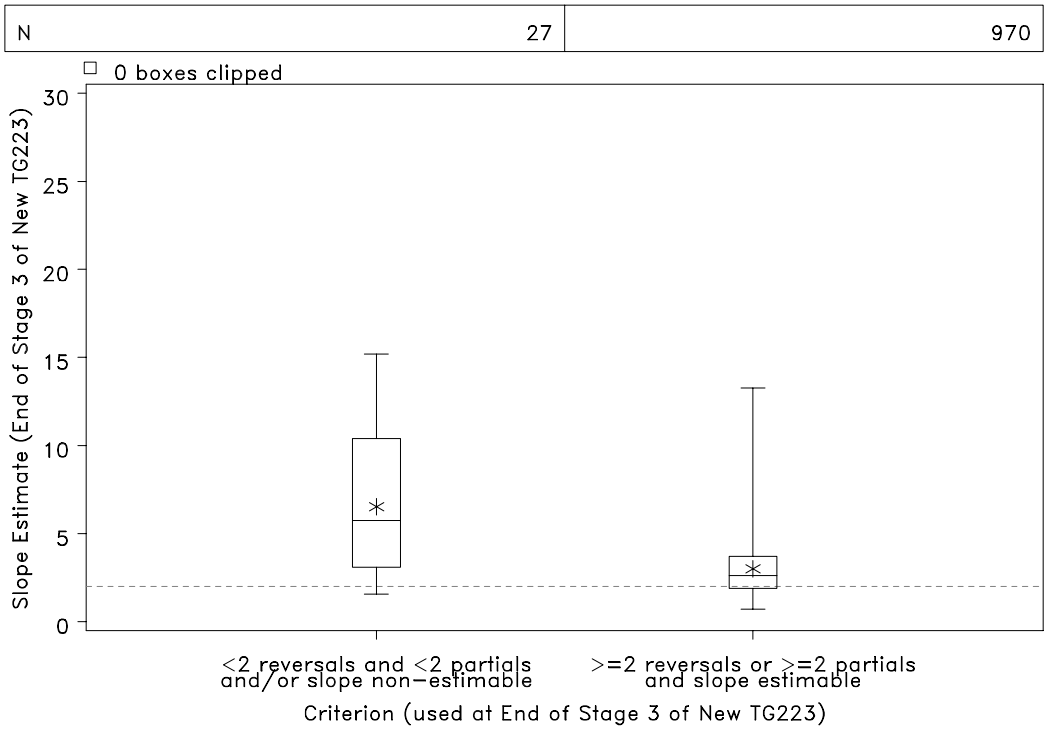


Figure 4.5: Criterion for using fourth stage: true LD_{50} = 50, true slope = 5

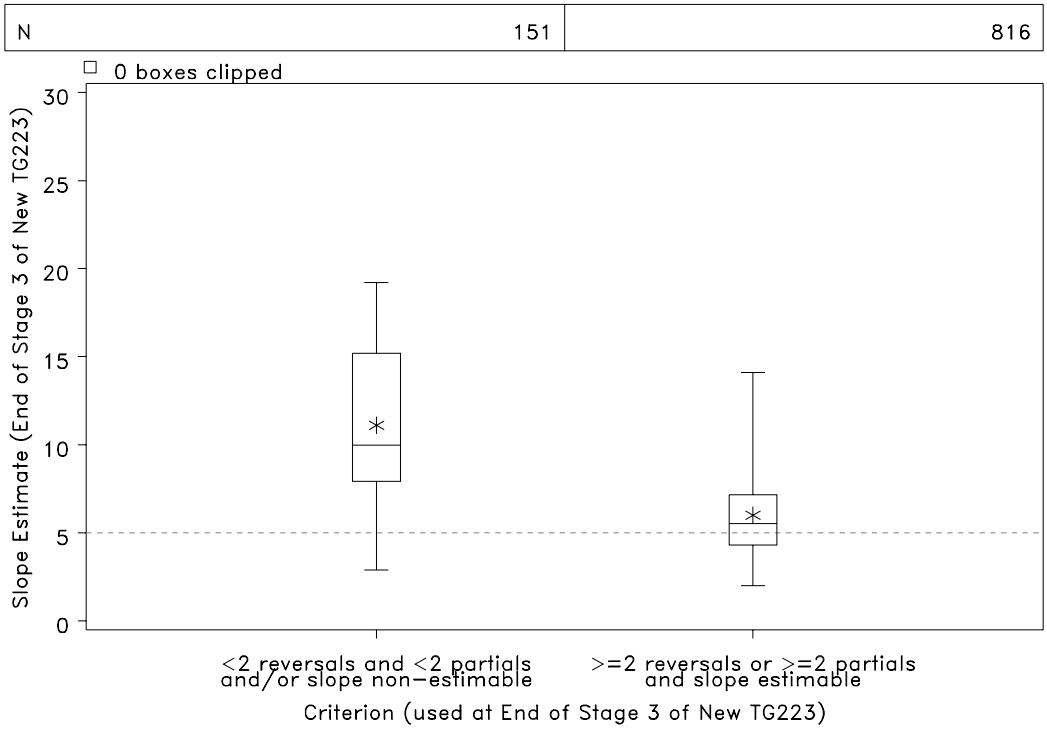


Figure 4.6: Criterion for using fourth stage: true LD_{50} = 50, true slope = 10

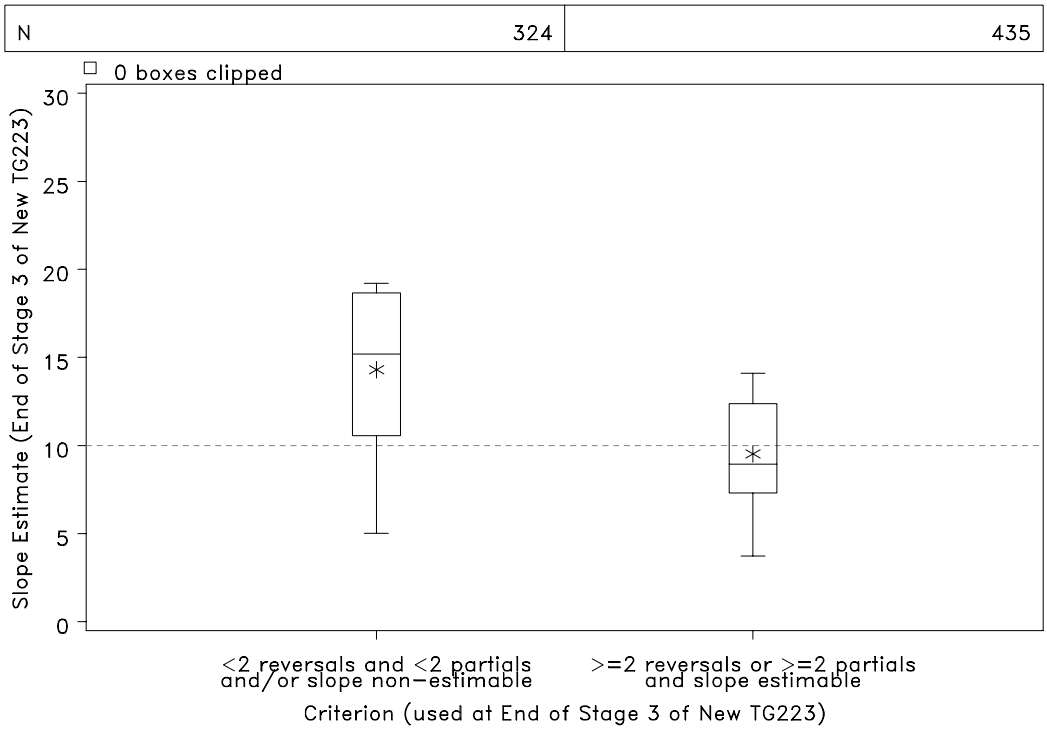


Figure 4.7: Criterion for using fourth stage: true LD_{50} = 1500, true slope = 2

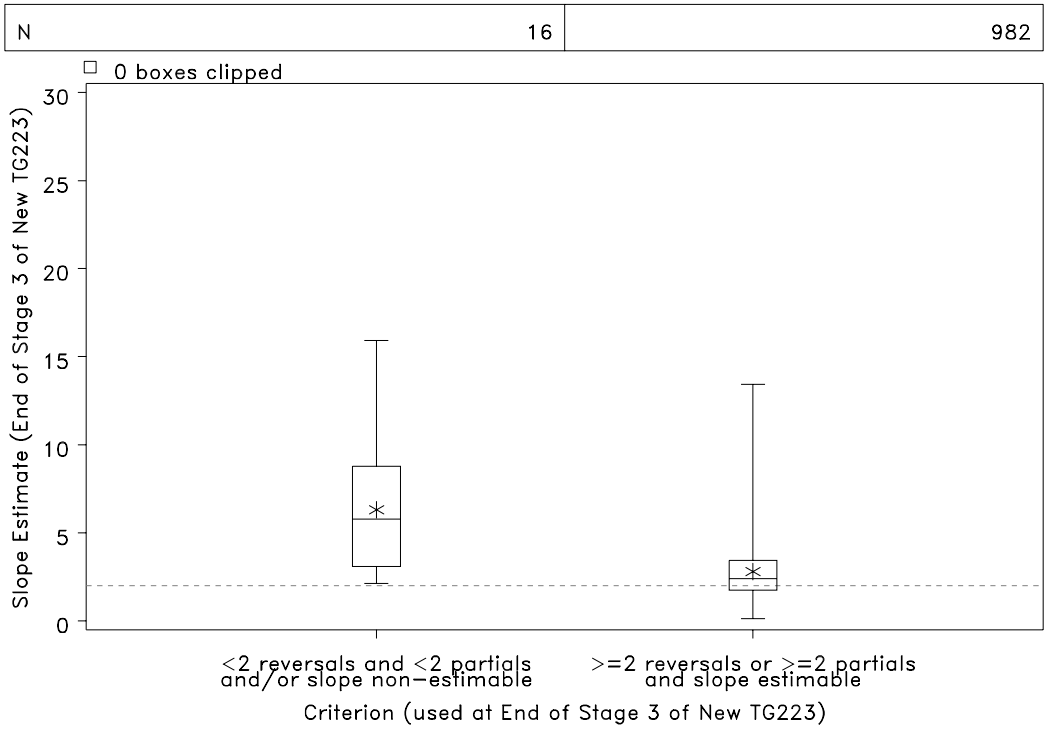


Figure 4.8: Criterion for using fourth stage: true LD_{50} = 1500, true slope = 5

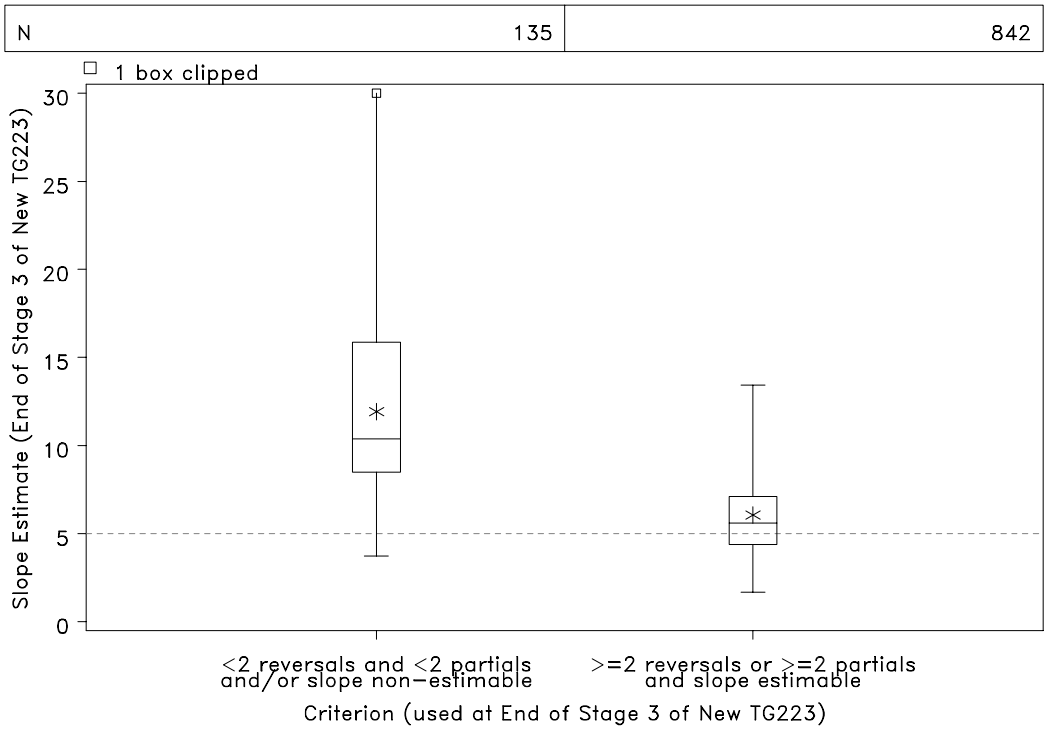


Figure 4.9: Criterion for using fourth stage: true LD_{50} = 1500, true slope = 10

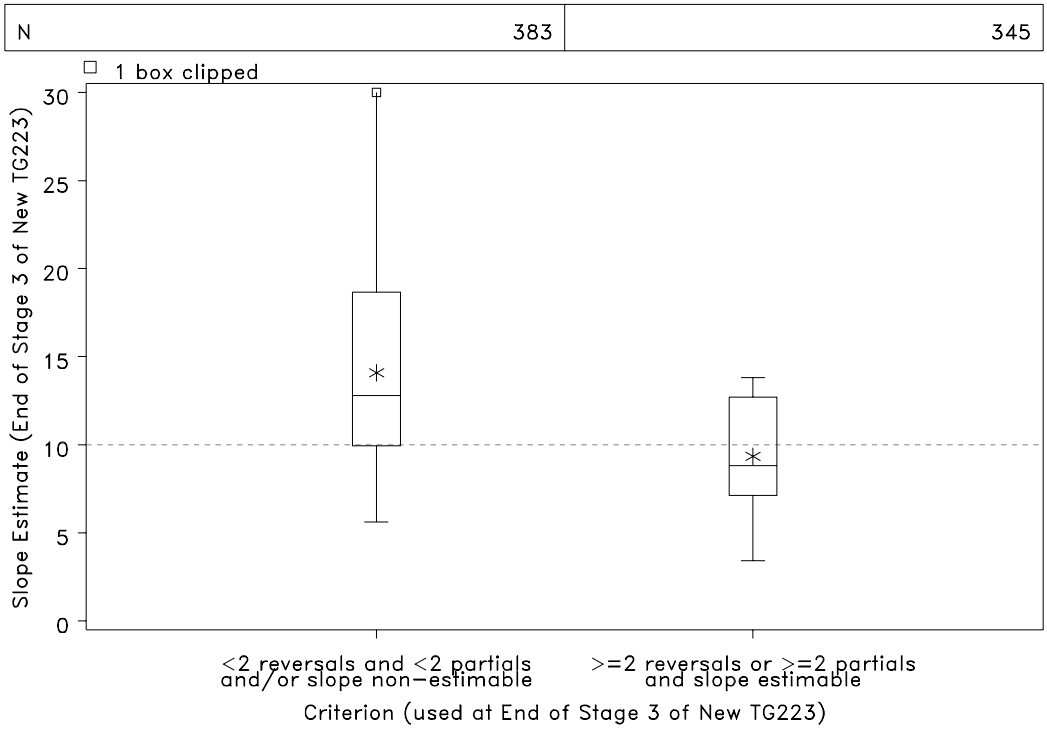


Figure 5.1: Effect of initial guess of LD_{50} on standard design: true LD_{50} = 5

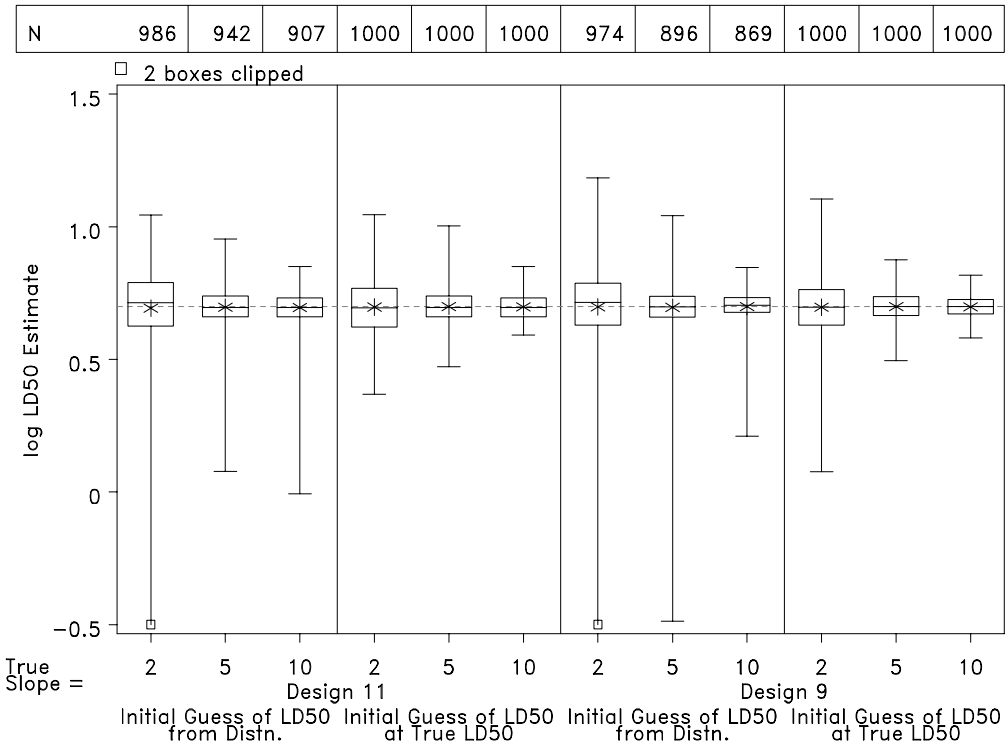


Figure 5.2: Effect of initial guess of LD_{50} on standard design: true LD_{50} = 50

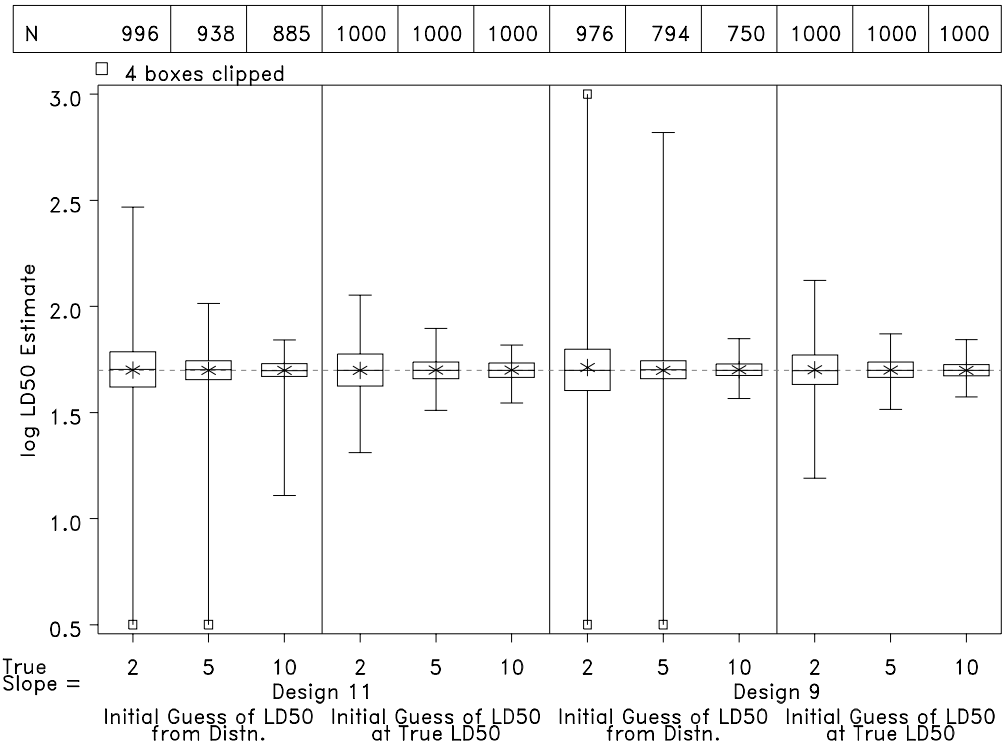


Figure 5.3: Effect of initial guess of LD_{50} on standard design: true LD_{50} = 1500

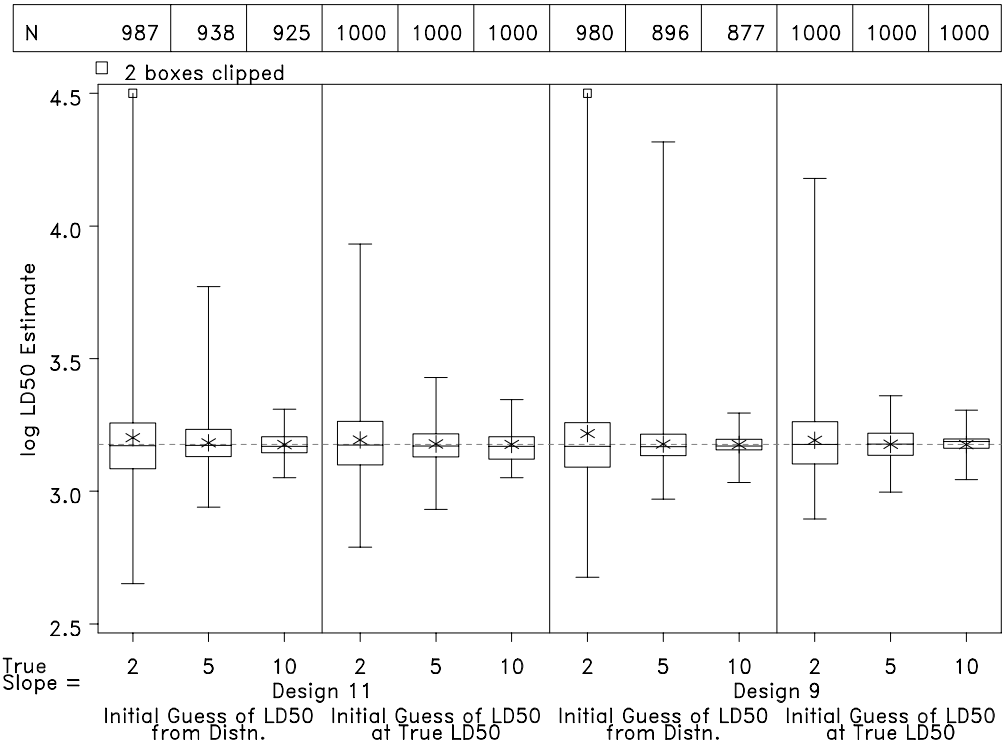


Figure 5.4: Effect of initial guess value of LD_{50} on standard design: true slope = 2

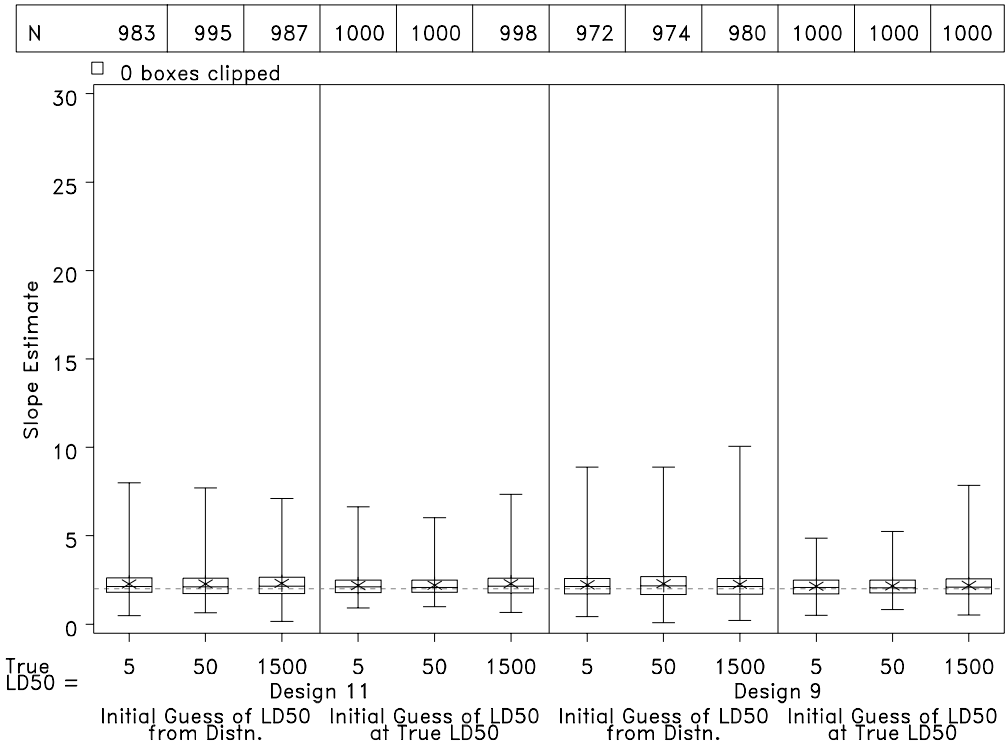


Figure 5.5: Effect of initial guess of LD_{50} on standard design: true slope = 5

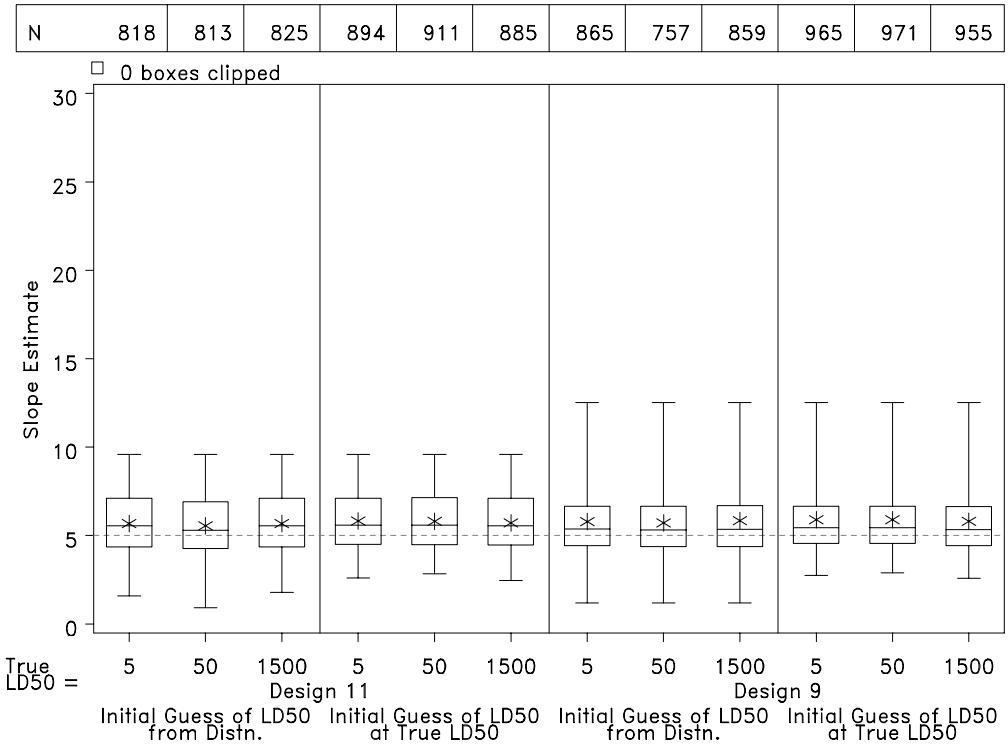


Figure 5.6: Effect of initial guess of LD_{50} on standard design: true slope = 10

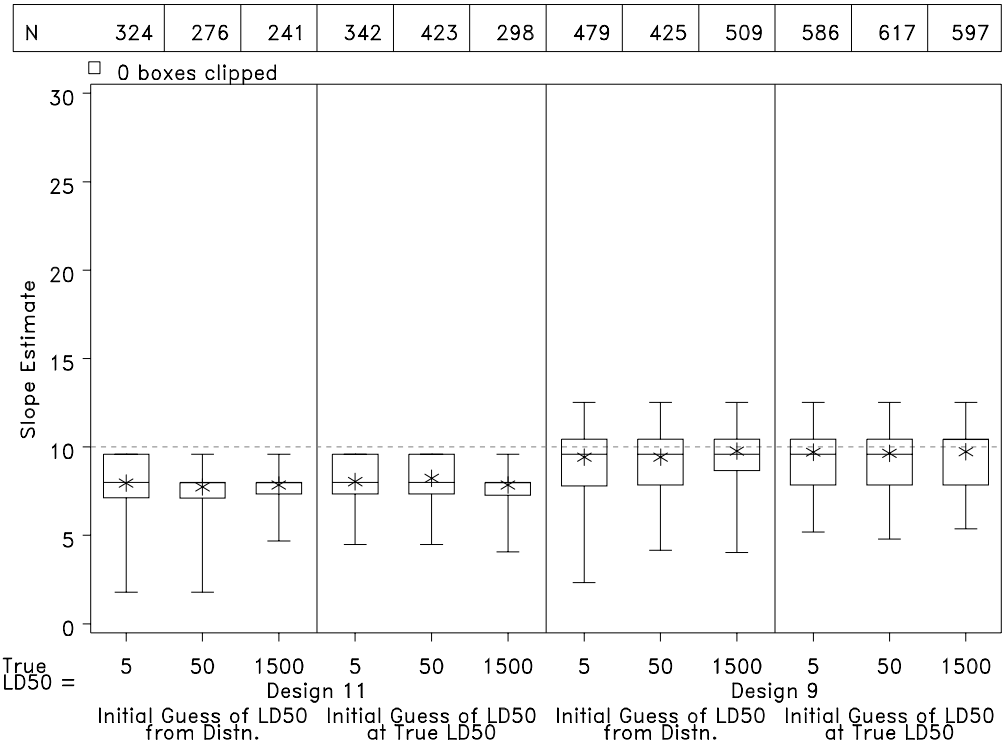


Figure 6.1: Effect of natural mortality on TG223 design: true $LD_{50} = 5$

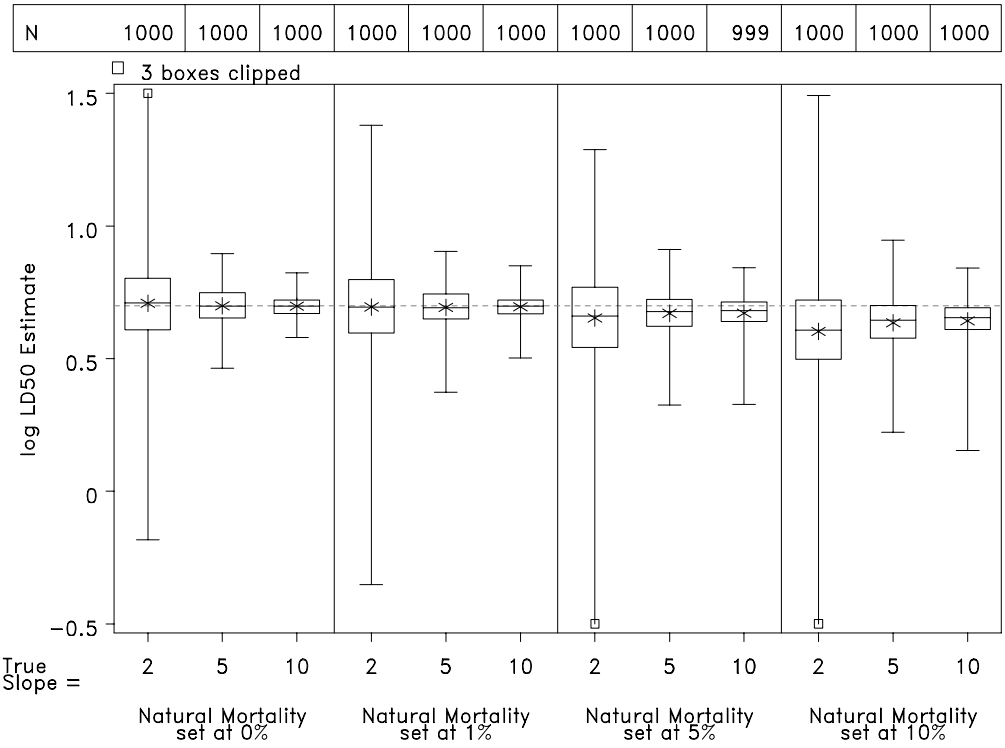


Figure 6.2: Effect of natural mortality on TG223 design: true $LD_{50} = 50$

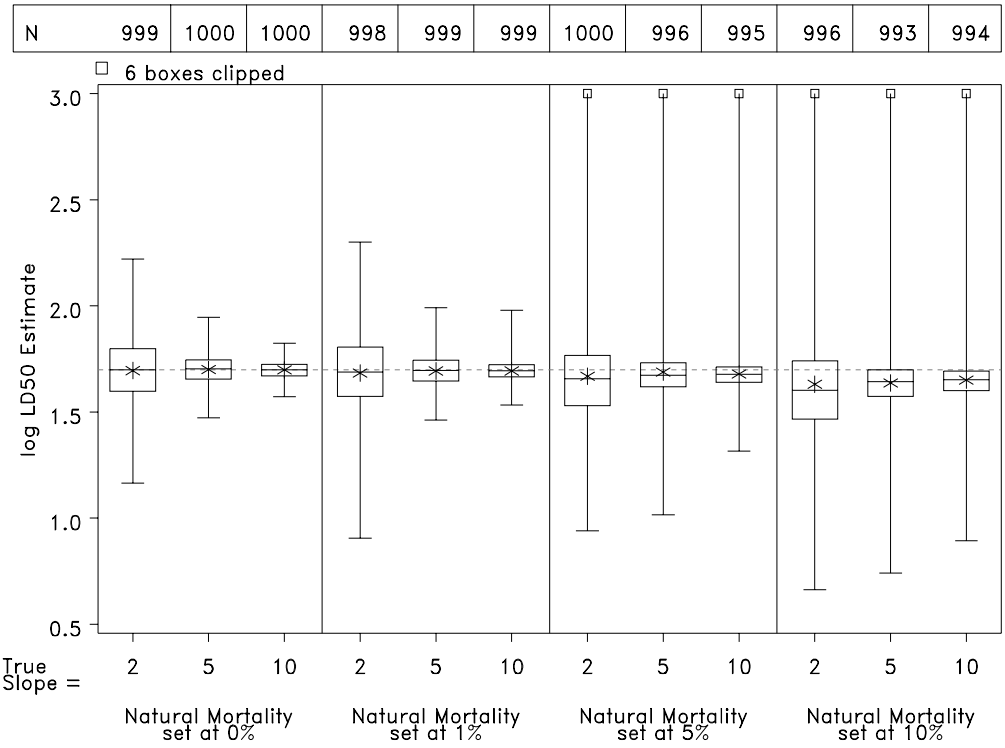


Figure 6.3: Effect of natural mortality on TG223 design: true LD_{50} = 1500

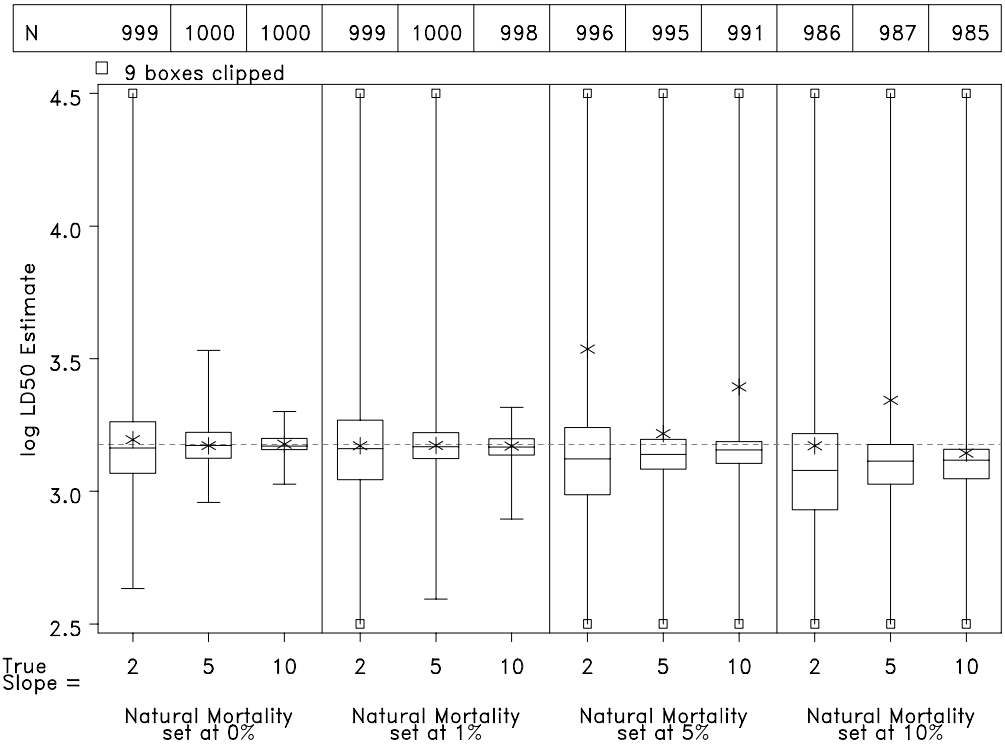


Figure 6.4: Effect of natural mortality on TG223 design: true slope = 2

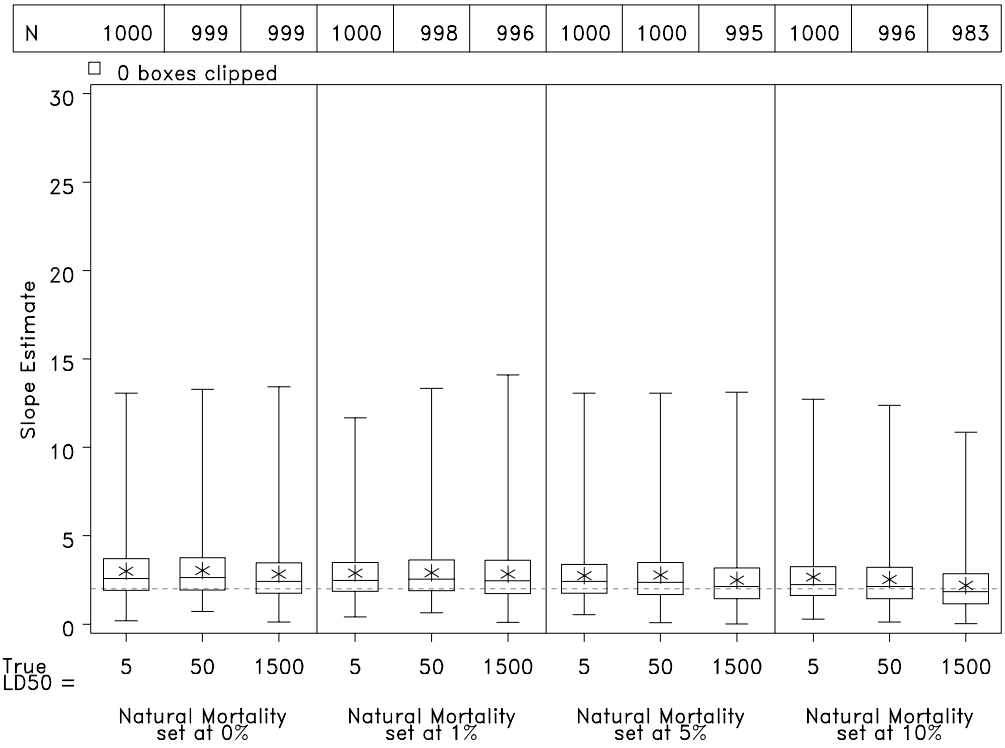


Figure 6.5: Effect of natural mortality on TG2233 design: true slope = 5

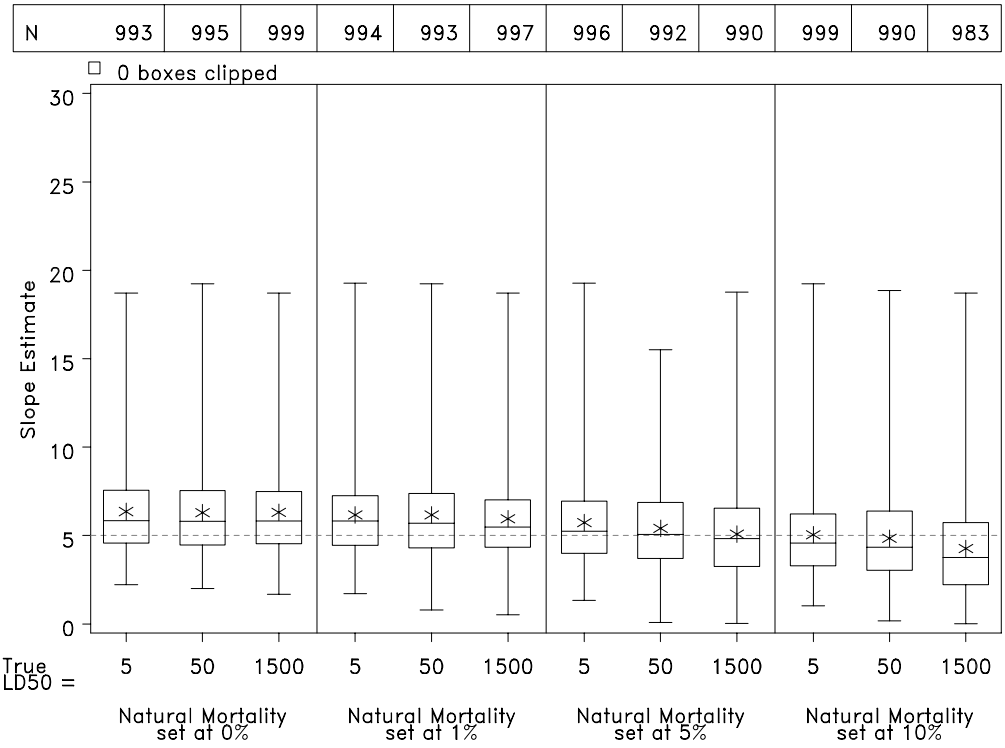


Figure 6.6: Effect of natural mortality on TG223 design: true slope = 10

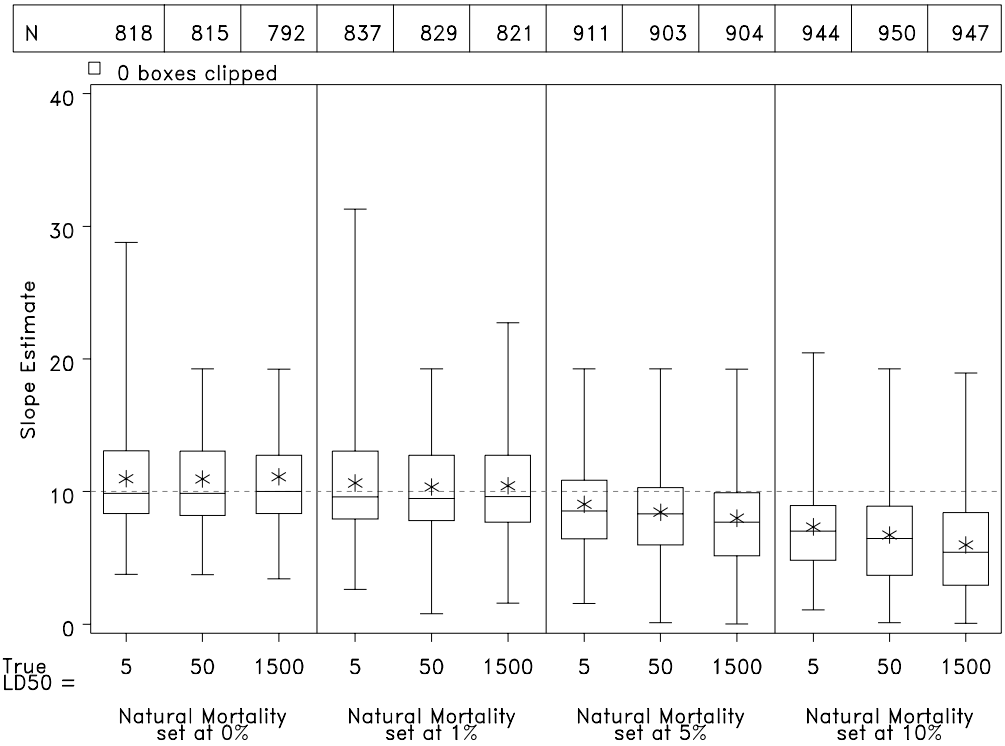


Figure 7.1: Effect of delayed mortality on TG223 design; true $LD_{50} = 5$

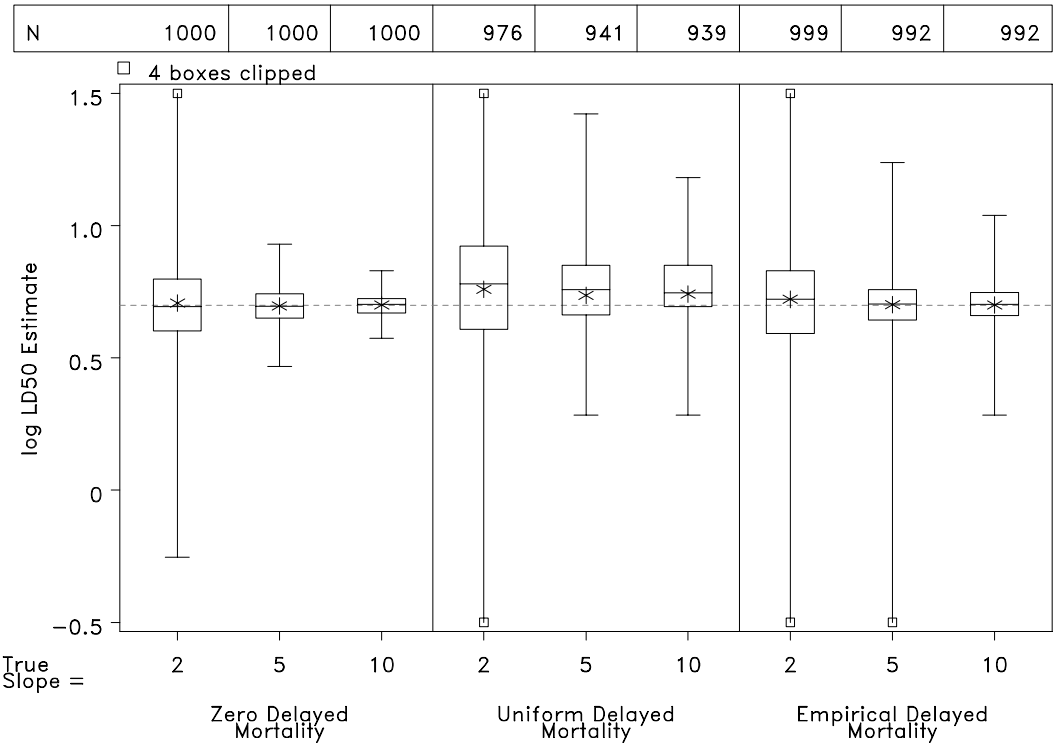


Figure 7.2: Effect of delayed mortality on TG223 design; true LD_{50} = 50

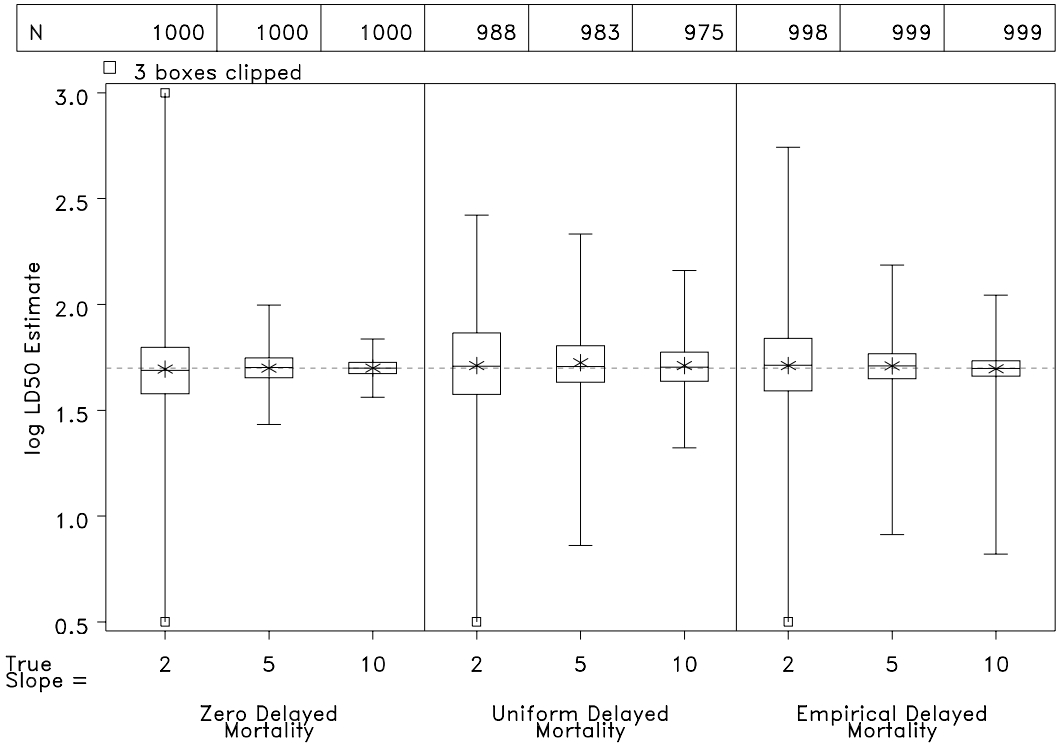


Figure 7.3: Effect of delayed mortality on TG223 design; true LD_{50} = 1500

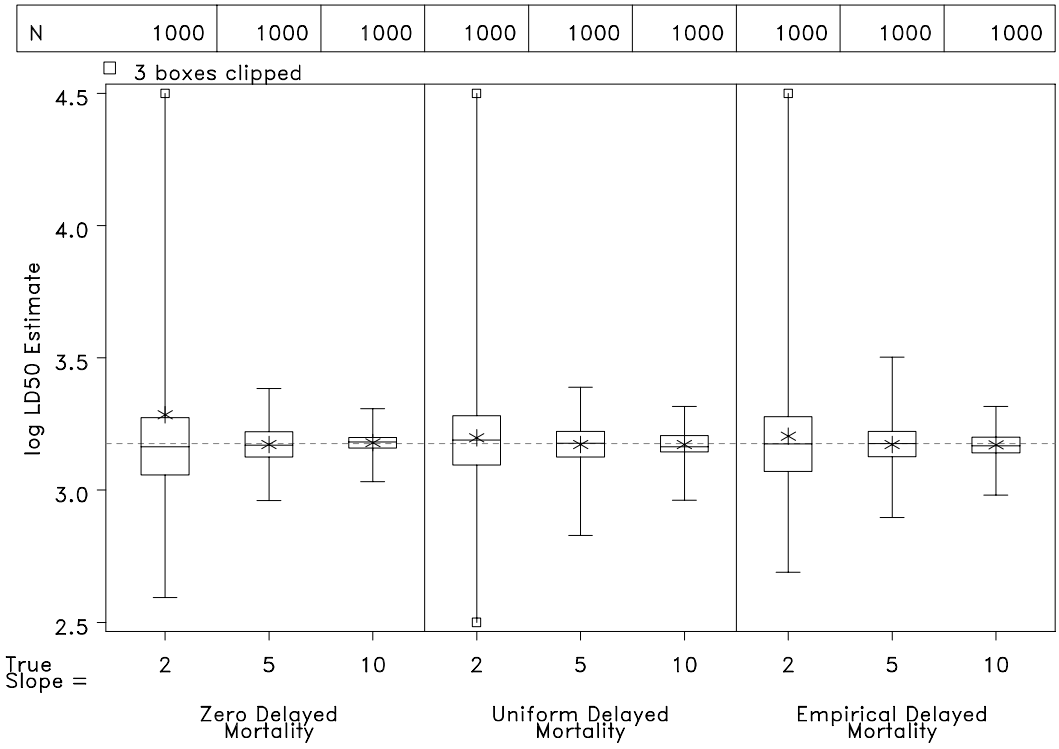


Figure 7.4: Effect of delayed mortality on TG223 design; true slope = 2

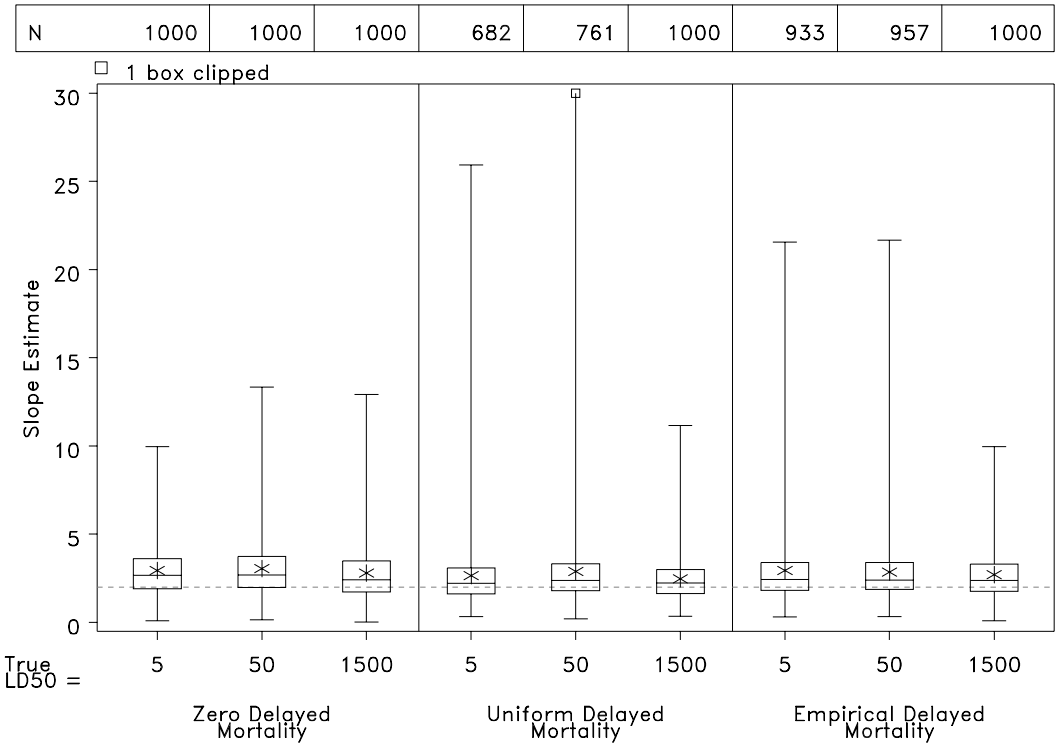


Figure 7.5: Effect of delayed mortality on TG223 design; true slope = 5

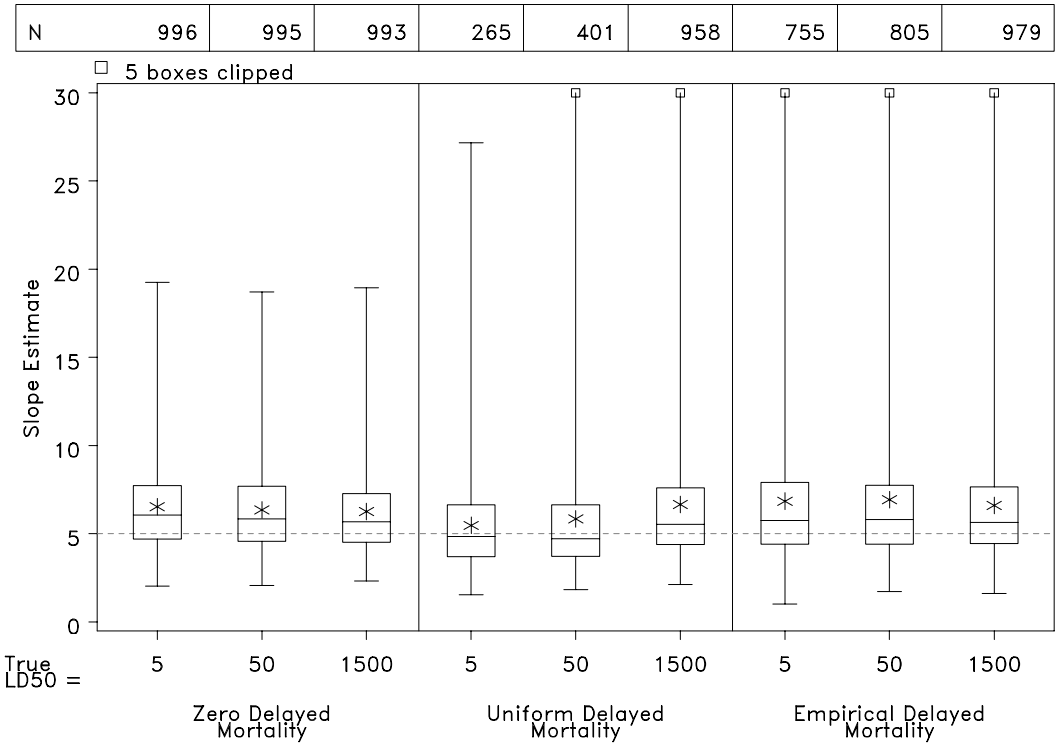


Figure 7.6: Effect of delayed mortality on TG223 design; true slope = 10

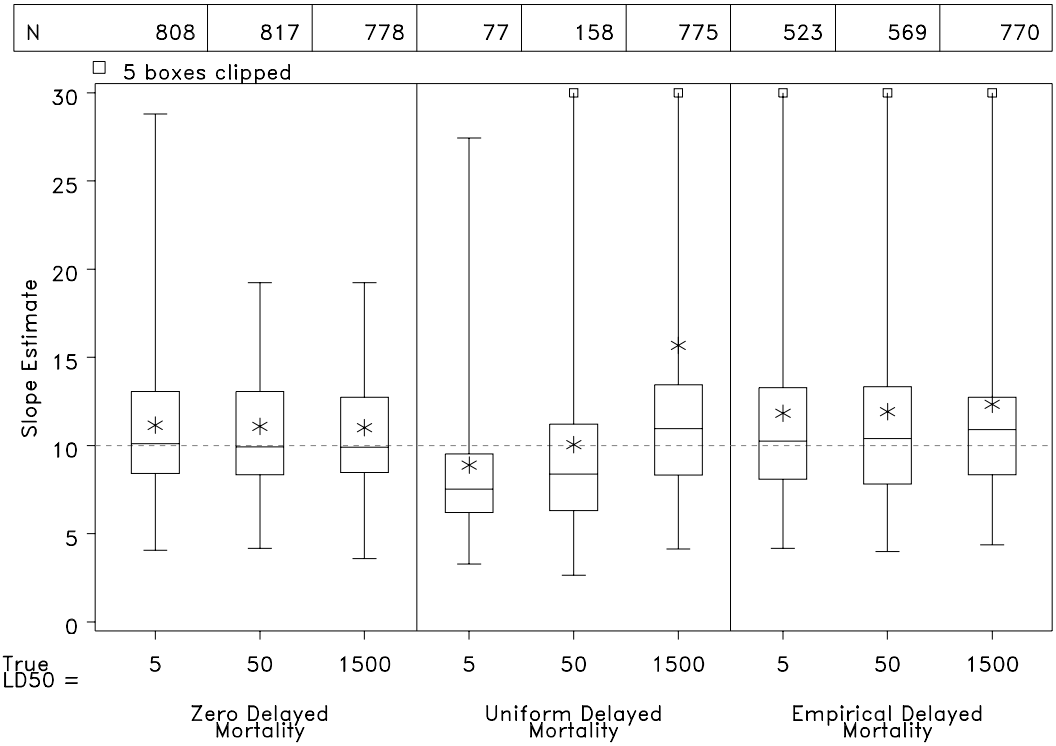


Figure 8.1: Effect of initial guess of LD_{50} on TG223 design: true LD_{50} = 5

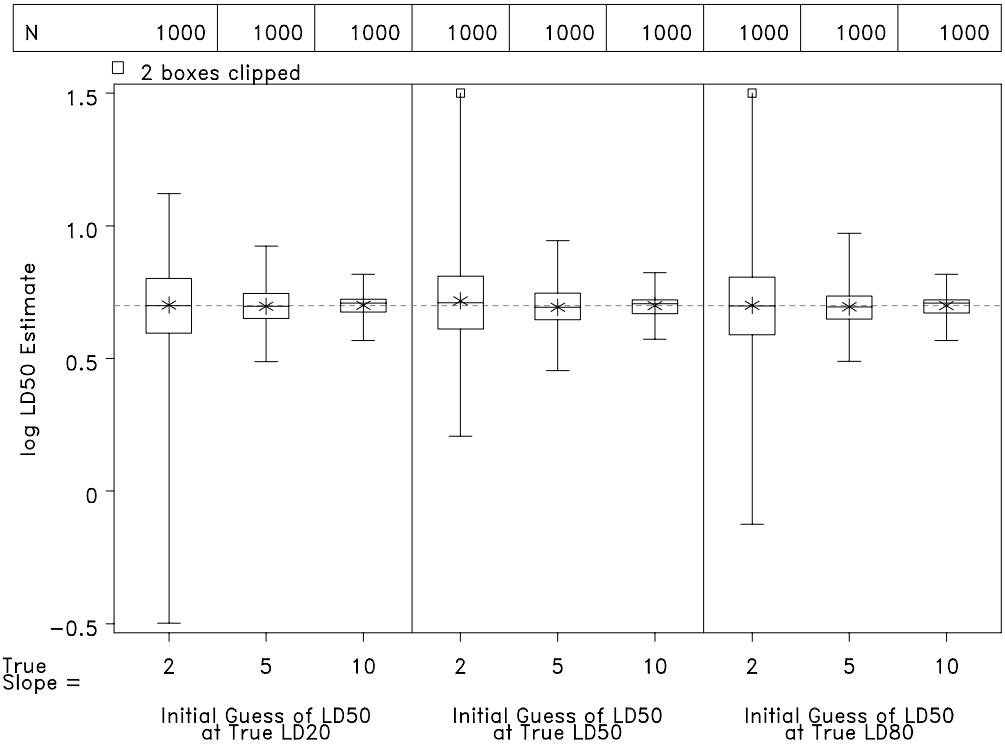


Figure 8.2: Effect of initial guess of LD_{50} on TG223 design: true LD_{50} = 50

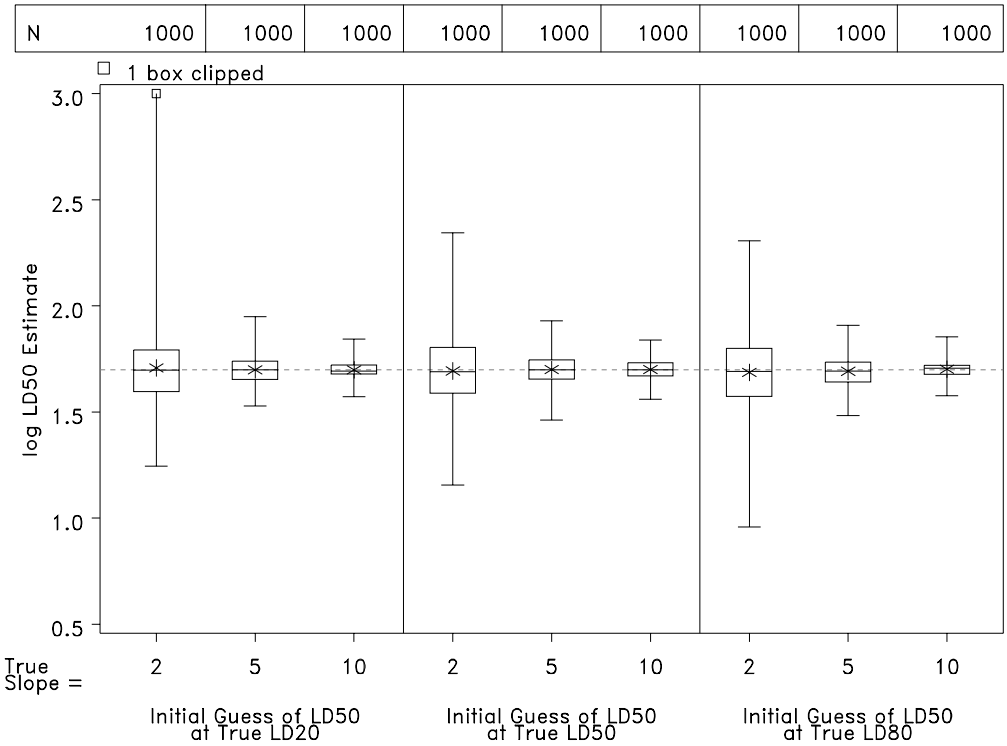


Figure 8.3: Effect of initial guess of LD_{50} on TG223 design: true LD_{50} = 1500

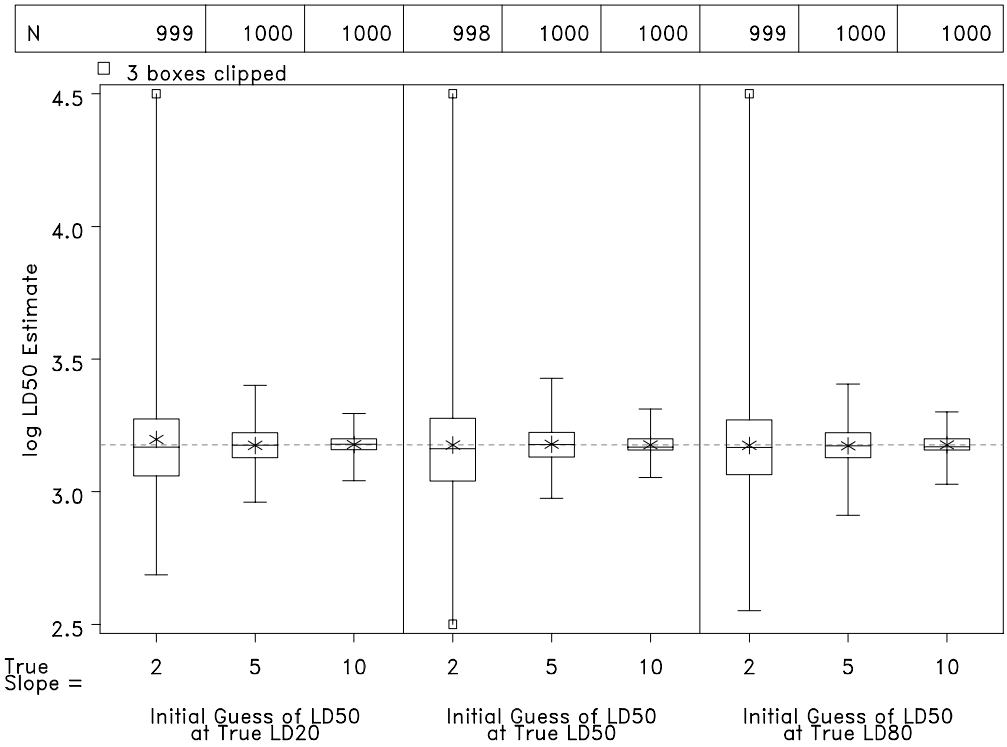


Figure 8.4: Effect of initial guess of LD_{50} on TG223 design: true slope = 2

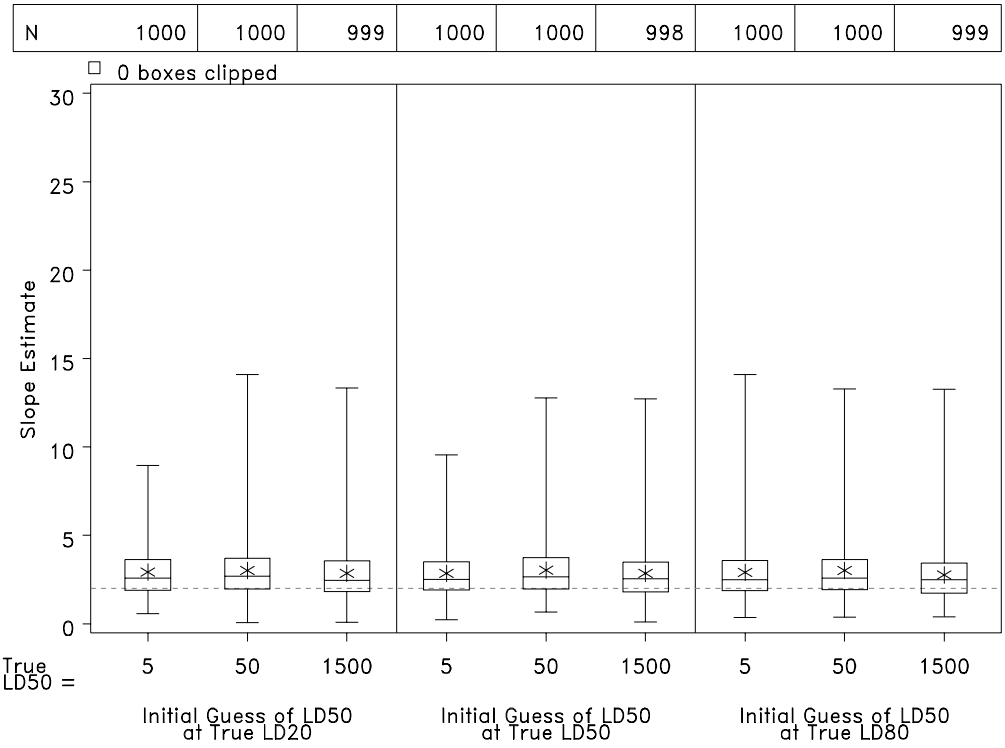


Figure 8.5: Effect of initial guess of LD_{50} on TG223 design: true slope = 5

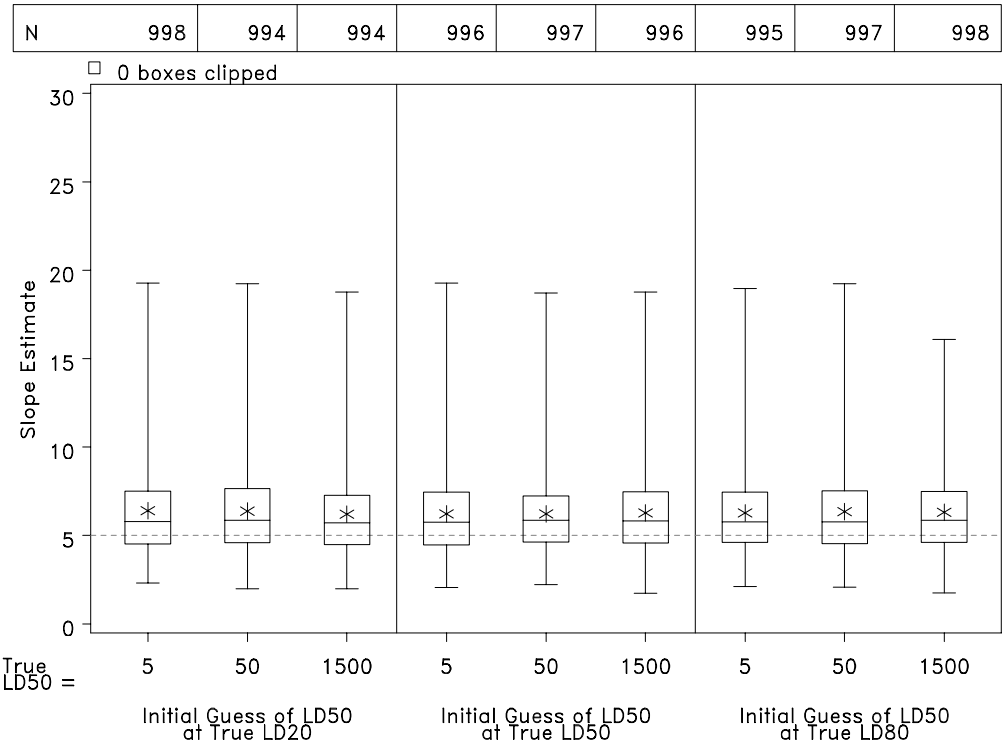
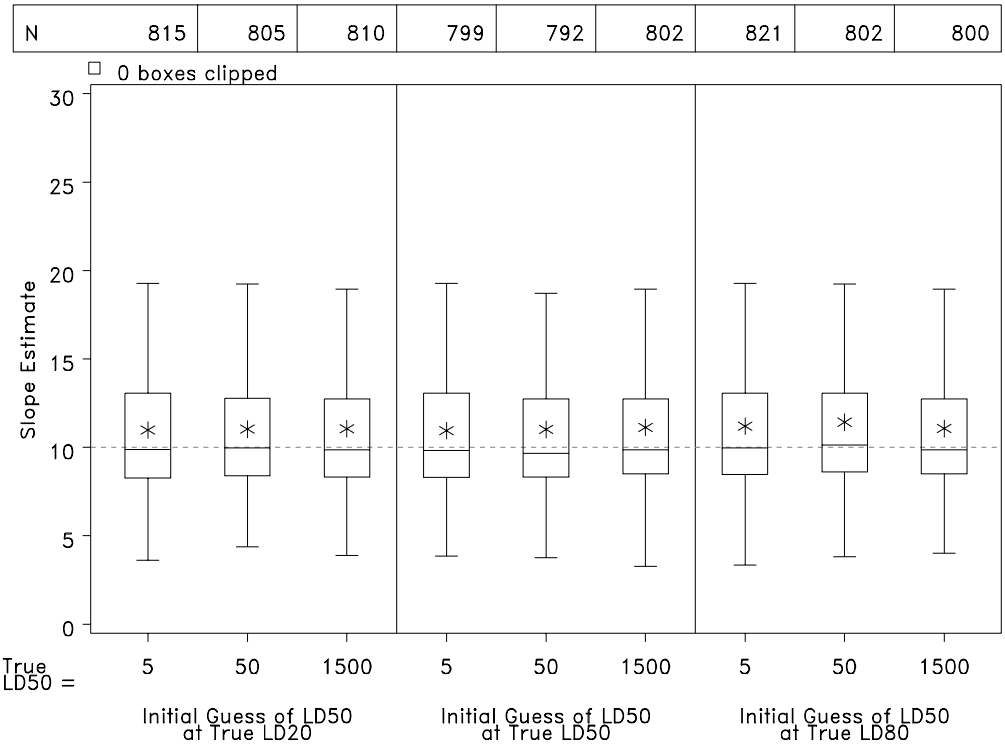


Figure 8.6: Effect of initial guess of LD_{50} on TG223 design: true slope = 10


N
815
805
810
799
792
802
821
802
800
0 boxes clipped
Slope Estimate
30
25
20
15
10
5
0
True
LD50 =
5
50
1500
5
50
1500
5
50
1500
Initial Guess of LD50
at True LD20
Initial Guess of LD50
at True LD50
Initial Guess of LD50
at True LD80

Appendix II – Validation of statistical simulations

Validation of Simulations for the

Draft OECD Test Guideline Avian Acute Oral Toxicity (TG223)

Author: Carol Yarrow

Date: 28th March 2008

Summary

Computer simulations were used to assess the performance of the proposed test design for OECD Test Guideline Avian Acute Toxicity (TG223). A validation study to independently corroborate the results of these simulations has now been successfully completed. Despite identifying a minor issue regarding the specification of random seeds, this validation study has confirmed that the original simulations carried out by Roberta Dark performed as intended and that they gave a good indication of the performance of the test under the hypothesized circumstances.

Introduction

The purpose of this report is to document the validation process for the computer simulations that were used to assess the performance of the proposed test design for TG223. These computer simulations used SAS programs to simulate and analyse test data. They have been fully described in the document entitled "Description of Simulations for the Draft OECD Test Guideline Avian Acute Oral Toxicity (TG223)" written by Roberta Dark and dated 30th January 2008 (hereafter this document is referred to as the 'Description document').

Description of Validation Process

The validation testing of the SAS programs used to simulate the proposed test design was logically conceived as three separate steps. The three steps are described in Sections 3.1, 3.2 and 3.3 below.

3.1 Step 1

The first step of the validation process was to confirm that the SAS program *analysis.sas*, which classifies and analyses dose response data, works as intended. In order to do this, the validation programmer (CSY) wrote her own SAS code to perform the classification and analysis of dose response data as described in Sections 3.1 and 3.2 of the Description document. The validation programmer did not view the original *analysis.sas* prior to writing and testing this code.

The validation code for *analysis.sas* was tested by classifying and analysing the following datasets and comparing the results with those produced by the original *analysis.sas*. In each case the results were expected to be in complete agreement.

- A set of forty datasets specifically developed to test this code. These were chosen to encompass all of the design classifications (A to G). They were provided in the SAS dataset *testdata.sas7bdat* and presented in Table 2.1 in Appendix 2 of the Description document. The results produced by *analysis.sas* for these datasets were provided in the SAS dataset

ests_testdata.sas7bdat and presented in Tables 2.2 and 2.3 in Appendix 2 of the Description document.

- A set of twelve of the nine thousand simulated datasets that had been selected to test this code. These were provided in the SAS dataset *sims_stg4_12.sas7bdat* and presented in Tables 3.1.1 to 3.1.12 in Appendix 3 of the Description document. This set of datasets comprised at least one simulated dataset from each of the nine combinations of true LD_{50} and true slope. The results produced by *analysis.sas* for these datasets were provided in the SAS dataset *ests_stg4_12.sas7bdat* and presented in Tables 3.2.1 to 3.2.12 and 3.3.1 to 3.3.12 in Appendix 3 of the Description document.

- The entire original set of nine thousand simulated experiments contained in the SAS datasets *sims_stg12.sas7bdat*, *sims_stg3.sas7bdat* and *sims_stg4.sas7bdat* (note that this was not required in the Description document, but it was done for completeness). The results produced by analysis.sas for these datasets were provided in the SAS datasets *ests_stg12.sas7bdat*, *ests_stg3.sas7bdat* and *ests_stg4.sas7bdat*.

3.1 Step 2

The second step of the validation process was to confirm that the SAS program *results.sas*, which produces boxplots and summary tables to present the results of the simulated datasets, works as intended. In order to do this, the validation programmer wrote her own SAS code to present the results of the original set of nine thousand simulated datasets. These results were provided in the SAS dataset *ests_stg4.sas7bdat*. The validation programmer did not view the original *results.sas* prior to writing and testing this code.

The results of the validation code for *results.sas* were then compared to the results of the original *results.sas*. The results were expected to be in complete agreement.

3.1 Step 3

The third and final step of the validation process was to confirm that the SAS programs *stg12.sas*, *stg3.sas* and *stg4.sas* that were used to simulate the proposed test design work as intended. In order to do this, the validation programmer wrote her own SAS code to produce a second set of nine thousand simulated datasets based on the descriptions in Sections 2 and 3 of the Description document. The validation programmer did not view the original programs prior to writing and testing this code.

This new set of simulated data was then analysed using the validation code generated in Step 1 and presented using the validation code generated in Step 2. The results were compared with the results of the original set of simulated data that are presented in Appendix 1 of the Description document. The results were not expected to be in complete agreement, but they were expected to be very similar.

Results of Validation Process

3.1Step 1

A SAS program, *csy_analysis.sas*, was developed to classify and analyse the dose response data according to Sections 3.1 and 3.2 of the Description document.

- *Csy_analysis.sas* was used to analyse and classify the test set of forty datasets in *testdata.sas7bdat*. For each of the forty datasets the consistency, number of partials, number of reversals, classification group, LD_{50} estimate, LD_{50} confidence interval, slope estimate and slope confidence interval were checked. Results were checked both manually against

the results presented in Tables 2.2 and 2.3 in the Description document and by electronic comparison with *ests_testdata.sas7bdat* using PROC COMPARE in SAS (with an equality criterion of 0.0000001). Complete agreement between the results of *csy_analysis.sas* and the original *analysis.sas* was achieved.

Issue: Note that Table 4 in the Description document does not explicitly state that the confidence interval for the LD_{50} generated using Fieller's theorem in the probit analysis should be a two-sided 95% confidence interval. However, this is the default in PROC PROBIT and this is what was generated and compared by the validation programmer.

- *Csy_analysis.sas* was used to analyse and classify the set of twelve simulated experiments that were contained in *sims_stg4_12.sas7bdat*. For each of the twelve experiments the consistency, number of partials, number of reversals, classification group, LD_{50} estimate, LD_{50} confidence interval, slope estimate and slope confidence interval were checked after Stage 2, Stage 3 and Stage 4 (if applicable). Results were checked both manually against the results presented in Tables 3.2.1 to 3.2.12 and Tables 3.3.1 to 3.3.12 in the Description document and by electronic comparison with *ests_stg4_12.sas7bdat* using PROC COMPARE in SAS (with an equality criterion of 0.0000001).

  For one of these experiments, (ID=7, Sim=115), there was initial disagreement between the LD_{50} estimate from *analysis.sas* (LD_{50}_est = 3330) and *csy_analysis.sas* (LD_{50}_est = non-estimable) after Stage 2. This experiment was classified as Group F by both programs at this stage which means that non-linear interpolation was used to estimate the LD_{50}. The non-linear interpolation procedure was described in Section 3.2.3 of the Description document. Investigation showed that the discrepancy in the LD_{50} estimates for this experiment was due to an incomplete definition for the case where the median response (50% mortality) was actually observed. This is the case where m(j)/n(j) is EQUAL to 0.5, for any particular dose level x(j).

  If the median response was observed, the original *analysis.sas* gave the LD_{50} estimate as the dose at which the median response occurred. For example, for the experiment described above (ID=7, Sim=115), 50% mortality (1 dead from 2 exposed) was observed at the highest dose of 3330. This was the value that was given to LD_{50}_est. The validation programmer felt that this was appropriate and after discussion between the original programmer and the validation programmer this omission in the description of the procedure was addressed by adding the following text to Section 3.2.3 of the Description document (note that this was included in the version dated 30th January 2008):

  "Note: If 50% mortality is observed then the estimate of the LD_{50} obtained using the non-linear interpolation procedure above is simply the dose at which 50% mortality is observed."

  Csy_analysis.sas was modified according to this definition and complete agreement between the results of *csy_analysis.sas* and the original *analysis.sas* was then achieved.

i. *Csy_analysis.sas* was used to analyse and classify the entire set of nine thousand simulated experiments that were contained in *sims_stg12.sas7bdat* (after Stage 2), *sims_stg3.sas7bdat* (after Stage 3) and *sims_stg4.sas7bdat* (after Stage 4). For each of the nine thousand experiments and after each stage the consistency, number of partials, number of reversals, classification group, LD_{50} estimate and confidence interval and slope estimate and confidence interval were checked. Results were checked by electronic comparison with *ests_stg12.sas7bdat*, *ests_stg3.sas7bdat* and *ests_stg4.sas7bdat* using PROC COMPARE in

SAS (with an equality criterion of 0.0000001). Complete agreement between the results of *csy_analysis.sas* and the original *analysis.sas* was achieved.

The SAS programs for the validation of *analysis.sas* are included in Appendix 2.

3.2Step 2

A SAS program, *csy_results.sas*, was developed to present the results of the analyses of the simulated data as described in Section 4 of the Description document.

Csy.results.sas was used to present the results of the original set of nine thousand simulated experiments provided in the SAS dataset *ests_stg4.sas7bdat*. The newly presented results were manually compared against those presented in Appendix 1 in the Description document that had been produced by the original *results.sas*. Complete agreement between the results of *csy_results.sas* and the original *results.sas* was achieved.

The SAS programs for the validation of *results.sas* are included in Appendix 3.

3.3Step 3

SAS programs *csy_stg12.sas*, *csy_stg3.sas* and *csy_stg4.sas* were developed to produce a second set of nine thousand simulated experiments based on the descriptions in Sections 2 and 3 of the Description document. This data was then analysed using *csy_analyse.sas* and the results were presented using *csy_results.sas*. These results were compared with those presented in Appendix 1 of the Description document.

Comparison of Estimates of LD_{50} and Slope

Percentiles of the final LD_{50} estimates (on a log10 scale) and final slope estimates from the original simulations are presented in Tables 1.1 and 1.2 respectively. Note that these are the same as Tables 1.1 and 1.2 in Appendix 1 of the Description document. Percentiles of the final LD_{50} estimates (on a log10 scale) and final slope estimates from the validation simulations are presented in Tables 2.1 and 2.2 respectively.

Box-and-whisker plots of the final LD_{50} estimates (on a log10 scale) and final slope estimates for the original and validation simulations are presented in Figures 1.1 to 1.6 and Figures 2.1 to 2.6 in Appendix 1 respectively. Note that Figures 1.1 to 1.6 are the same as Figures 1.1 to 1.6 in Appendix 1 of the Description document. However, they have been independently generated by the validation programmer.

Table 1.1: Percentiles of the final LD_{50} estimates (on a log10 scale) for the original simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25th	50th	75th
1	5	2	1000	0.6536	0.7537	0.8711
2	5	5	1000	0.6665	0.7107	0.7520
3	5	10	1000	0.6820	0.7041	0.7252
4	50	2	1000	1.6079	1.7199	1.8409
5	50	5	1000	1.6607	1.7115	1.7529
6	50	10	1000	1.6764	1.7010	1.7246
7	1500	2	1000	3.0614	3.1618	3.2780
8	1500	5	1000	3.1261	3.1750	3.2244
9	1500	10	1000	3.1541	3.1780	3.2004

Table 1.2: Percentiles of the final slope estimates for the original simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25th	50th	75th
1	5	2	1000	2.0524	2.7374	3.7161
2	5	5	1000	4.5727	5.8177	7.7335
3	5	10	**924**	8.6191	11.4315	14.0268
4	50	2	1000	2.0265	2.7471	3.7147
5	50	5	999	4.5232	5.7969	7.5050
6	50	10	**927**	8.6286	11.1177	14.0453
7	1500	2	1000	1.7317	2.4751	3.4170
8	1500	5	998	4.5037	5.8062	7.4932
9	1500	10	**937**	8.6645	11.3550	13.6303

Table 2.1: Percentiles of the final LD_{50} estimates (on a log10 scale) for the validation simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25th	50th	75th
1	5	2	999	0.6155	0.7092	0.8026
2	5	5	1000	0.6537	0.6993	0.7413
3	5	10	1000	0.6743	0.6985	0.7226
4	50	2	1000	1.5824	1.6993	1.8101
5	50	5	1000	1.6510	1.7004	1.7496
6	50	10	1000	1.6730	1.7000	1.7228
7	1500	2	999	3.0576	3.1541	3.2629
8	1500	5	1000	3.1364	3.1814	3.2231
9	1500	10	1000	3.1536	3.1780	3.2007

Table 2.2: Percentiles of the final slope estimates for the validation simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25th	50th	75th
1	5	2	999	1.9579	2.6306	3.8085
2	5	5	996	4.7340	5.9642	7.6272
3	**5**	**10**	**907**	8.5036	11.0611	13.2470
4	50	2	1000	1.9081	2.6275	3.6311
5	50	5	997	4.7731	6.1890	8.2340
6	**50**	**10**	**897**	8.3475	10.8522	13.3077
7	1500	2	999	1.8130	2.5223	3.5015
8	1500	5	992	4.4769	5.8230	7.5653
9	**1500**	**10**	**884**	8.6754	10.9928	13.3435

Comparison of Tables 1.1 and 2.1 shows that the distribution of the estimated LD_{50} from the original and validation sets of simulated data appear to be very similar.

Comparison of Tables 1.2 and 2.2 shows that the distribution of the estimated slopes for the original and validation sets of simulated data appear to be broadly similar. However, note that for all three values of the true LD_{50}, slightly fewer experiments with a true slope of 10 resulted with an estimate of the slope for the validation simulations (907, 897, 884) than for the original simulations (924, 927, 937).

In order to investigate whether the observed difference in the number of slope estimates was real or whether it could be explained by random variation, two further sets of simulated data, Validation2 and Validation3, were generated by using *csy_stg12.sas*, *csy_stg3.sas* and *csy_stg4.sas* with different random seeds. Table 3 shows the number of experiments that resulted in an estimate of the slope for each set of simulations for all combinations of true LD_{50} and true slope. Note that (for each set) there were 1,000 simulated experiments for each combination of true LD_{50} and slope and that the maximum number of slope estimates in each case is therefore 1,000.

Table 3: Number of final slope estimates for each set of simulated data

	True Parameters		Number of Final Slope Estimates (out of 1000)			
ID	**LD_{50}**	**Slope**	**Original**	**Validation**	**Validation2**	**Validation3**
1	5	2	1000	999	999	1000
2	5	5	1000	996	996	997
3	**5**	**10**	**924**	**907**	**904**	**884**
4	50	2	1000	1000	999	1000
5	50	5	999	997	997	997
6	**50**	**10**	**927**	**897**	**887**	**884**
7	1500	2	1000	999	1000	998
8	1500	5	998	992	996	997
9	**1500**	**10**	**937**	**884**	**871**	**881**

Inspection of Table 3 shows that the number of experiments with a true slope of 10 that gave a final estimate of the slope was consistently lower for the validation simulations than for the original simulations.

Comparison of Classification of Dose Response Data at the end of Stage 4

At the end of stages 2, 3 and 4, each experiment was classified into one of seven groups depending on the specific characteristics of the data. These seven groups were described in Section 3.1 and Table 3 of the Description document. Table 4 shows the percentage of experiments that were classified into each of the seven groups at the end of Stage 4 for the original set of simulations and for each of the three sets of validation simulations.

Inspection of Table 4 shows that at the end of Stage 4:

- 59.9% of experiments in the original set of simulations were classified as Group C compared to 57.8%, 57.8% and 57.4% in the three sets of validation simulations.
- Slightly fewer experiments were classified as each of Groups D, E and G in the original set of simulations than in each set of validation simulations.
    - Group D: 37.7% (original) vs. 38.6%, 38.3%, 38.6% (validation)
    - Group E: 1.9% (original) vs. 2.7%, 2.6%, 2.7% (validation)
    - Group G: 0.5% (original) vs. 1.0%, 1.3%, 1.3% (validation)

Table 4: Percentage of experiments classified as each group at the end of Stage 4

	Percentage of experiments (out of 9000)			
Data classification	Original	Validation	Validation2	Validation3
A	0.0%	0.0%	0.0%	0.0%
B	0.0%	0.0%	0.0%	0.0%
C	59.9%	57.8%	57.8%	57.4%
D	37.7%	38.6%	38.3%	38.6%
E	1.9%	2.7%	2.6%	2.7%
F	0.0%	0.0%	0.0%	0.0%
G	0.5%	1.0%	1.3%	1.3%

Tables 3 and 4 provide evidence that there is a small, but real, difference between the characteristics of the results for the original and validation sets of simulated data.

Investigation of Differences in Results of Simulated Data

A thorough investigation to identify the cause of the discrepancy in the results of the original set of simulated data and the validation sets of simulated data was carried out by the validation programmer. Interim results from each stage and doses tested at subsequent stages were compared. In addition, at this point the validation programmer viewed the original *stg12.sas*, *stg3.sas* and *stg4.sas* and compared them to the validation programs.

This identified that the original simulation programs had specified initial seeds for random number generation for each stage of each experiment in a systematic manner. Five different initial seeds with a constant difference of 9,000 between them were used for each experiment (for further explanation, see Sections 3.3.3.1 and 3.3.3.2 below). Comprehensive experimentation showed that using seeds that are sequential or that have a constant difference between them can result in non-independence of the random samples generated. This has been identified as the likely cause of the discrepancy in the results. The findings of the experimentation on seed behaviour are summarised in Appendix 5.

Random Number Generation

In order to simulate the data from each experiment, in both the original and the validation simulation programs, SAS random number CALL routines were used to generate random numbers as described below:

To simulate the initial guess of the LD_{50}.

> The initial guess of the LD_{50} for each experiment was determined by selecting a random number from a normal distribution. In both the original and validation simulation programs, the SAS CALL routine CALL RANNOR was used to do this.

To simulate the number of dead birds at each dose at each stage:

- Stage 1: from a total of 4 exposed birds
- Stage 2: from a total of 10 exposed birds
- Stage 3: from a total of 10 exposed birds

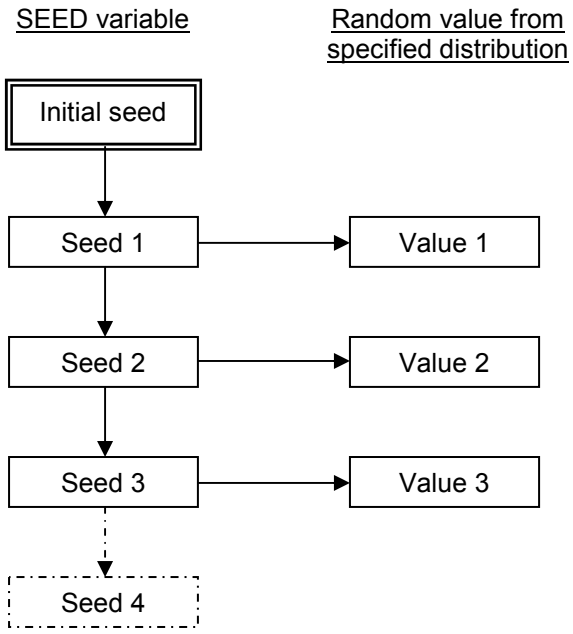
- Stage 4 (if applicable): from a total of 10 exposed birds

The number of dead birds at each dose at each stage was determined by selecting a random number from a Binomial distribution with n=number of birds exposed and p=probability of death. In both the original and validation simulation programs, the SAS CALL routine CALL RANBIN was used to do this.

A note on SAS CALL routines:

SAS CALL routines can be used to generate a random sample of numbers from a specified distribution. CALL routines require a seed variable which must be given an initial value prior to the first execution of the CALL routine; this can be user-specified or determined by the computer clock. This initial seed does not result directly in the selection of a random value from the specified distribution, but instead generates a stream of random numbers, each of which is used as a seed to select a value from the distribution. The diagram in Figure 1 below illustrates how this works.

Figure 1: Illustration of use of seeds in CALL routines



Seed specification in Original Simulations

Simulation of LD_{50} values

In order to generate an initial guess of the LD_{50}, the initial value of the seed variable, seed1, for each experiment had been specified in a systematic manner using the following SAS code:

For ID=1 to 9 and sim=1 to 1000

- seed1=1000*(ID-1)+sim; (values of 1 to 9,000)

This means that the seed variable, seed1, was given an initial value of 1 for the first experiment (ID=1 and sim=1). This initial seed of 1 generated a seed of 397204094 which was then used to select a single value from the normal distribution. The next seed in the stream is 2083249653 and this could have been used to generate the next value. However, prior to the second experiment (ID=1, sim=2) the seed variable, seed1, was re-initialised to a value of 2. This generated a seed of 794408188 which was used to select a value from the normal distribution for the second experiment. Prior to each

subsequent experiment, the seed variable, seed1, was re-initialised to a value between 3 and 9,000 in the same way.

Simulation of number of dead birds for each stage

Similarly, in order to generate the number of dead birds for each stage, the initial value of the seed variable, seed2, for each stage of each experiment had been specified in a systematic manner using the following SAS code:

For ID=1 to 9 and sim=1 to 1000:

- Stage 1: seed2=9000+1000*(ID-1)+sim; (values of 9,001 to 18,000)
- Stage 2: seed2=18000+1000*(ID-1)+sim;(values of 18,001 to 27,000)
- Stage 3: seed2=27000+1000*(ID-1)+sim;(values of 27,001 to 36,000)
- Stage 4: seed2=36000+1000*(ID-1)+sim;(values of 36,001 to 45,000)

This means that the seed variable, seed2, used to generate the number of dead birds was given a new initial value for each stage of each experiment. The initial seeds used for the first experiment (ID=1, sim=1) were 9,001 (Stage 1), 18,001 (Stage 2), 27,001 (Stage 3) and 36,001 (Stage 4). Similarly the initial seeds used for the second experiment were 9,002, 18,002, 27,002 and 36,002 and so on.

Investigations have indicated that the constant difference of 9,000 between the four initial seeds used to generate the number of dead birds for each experiment will have resulted in some degree of non-independence of the data generated at each stage. This is undesirable. The extent of correlation, however, was shown by the analyses in 4.3.4 to have a small, but ignorable, effect on the conclusions from the original simulations.

Seed specification in Validation Simulations

For each of the three validation sets of data, a single initial seed for the whole validation exercise was specified using a RETAIN statement as follows.

- Validation: retain seed 5674564;
- Validation2: retain seed 96543854;
- Validation3: retain seed 29313288l;

For each set of validation simulations, this initial seed generated a single stream of unique random seeds as shown in Figure 1. This stream of seeds was then used to determine the initial guess of the LD_{50} for each experiment and the number of dead birds at each stage in each experiment. Note that this method of specifying a single initial seed is consistent with examples of random number generation given in SAS Help[1].

Regeneration of simulated data using original programs

In order to test the hypothesis that it is the difference in the methods of seed generation that has led to the difference in the number of slope estimates generated in the original and validation sets of simulated data, the original programmer modified her simulation programs to specify the seeds in a similar way to the validation programmer. The original programmer then generated a new set of 9,000 simulated datasets using her modified programs.

The percentiles of the final LD_{50} estimates (on a log10 scale) and final slope estimates from these regenerated simulations are presented in Tables 5.1 and 5.2 respectively. Box-and-whisker plots of the final LD_{50} estimates (on a log10 scale) and final slope estimates for the regenerated simulations are presented in Figures 3.1 to 3.6 in Appendix 1.

Table 5.1: Percentiles of the final LD_{50} estimates (on a log10 scale) for the regenerated simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25^{th}	50^{th}	75^{th}
1	5	2	1000	0.6088	0.7111	0.8143
2	5	5	1000	0.6564	0.6965	0.7415
3	5	10	1000	0.6763	0.7022	0.7234
4	50	2	999	1.5917	1.7003	1.8138
5	50	5	1000	1.6536	1.6991	1.7442
6	50	10	1000	1.6742	1.6980	1.7269
7	1500	2	999	3.0541	3.1675	3.2685
8	1500	5	1000	3.1273	3.1776	3.2205
9	1500	10	1000	3.1498	3.1748	3.1995

Table 5.2: Percentiles of the final slope estimates for the regenerated simulations

ID	True Parameters		No. of Final Estimates	Percentiles of estimates		
	LD_{50}	Slope		25^{th}	50^{th}	75^{th}
1	5	2	1000	1.9652	2.6291	3.7804
2	5	5	999	4.6030	5.9673	7.7850
3	**5**	**10**	**892**	8.4920	11.1927	13.3917
4	50	2	999	1.9717	2.6838	3.7696
5	50	5	997	4.5499	5.9519	7.9676
6	**50**	**10**	**879**	8.5026	11.0064	13.6483
7	1500	2	999	1.8130	2.5148	3.4797
8	1500	5	998	4.6690	5.9257	7.6599
9	**1500**	**10**	**898**	8.6095	10.8135	13.1038

Comparison of Tables 2.1 and 5.1 and Tables 2.2 and 5.2 show that distribution of the estimated LD_{50} and slope from the regenerated set of simulations and the validation set of simulations are very similar. Further comparison of Table 5.2 with Table 3 shows that the number of regenerated experiments with a true slope of 10 that resulted in an estimate of the slope is very similar to the corresponding number for all three sets of validation simulations.

Table 6 shows the percentage of experiments from each set of simulations that were classified as each of the seven classification groups at the end of Stage 4.

Table 6: Percentage of experiments classified as each group at the end of Stage 4

	Percentage of experiments (out of 9000)				
Data classification	Original	Validation	Validation2	Validation3	Regenerated
A	0.0%	0.0%	0.0%	0.0%	0.0%
B	0.0%	0.0%	0.0%	0.0%	0.0%
C	59.9%	57.8%	57.8%	57.4%	57.8%
D	37.7%	38.6%	38.3%	38.6%	38.4%
E	1.9%	2.7%	2.6%	2.7%	2.5%
F	0.0%	0.0%	0.0%	0.0%	0.0%
G	0.5%	1.0%	1.3%	1.3%	1.3%

Inspection of Table 6 shows that the percentage of regenerated experiments that were classified as each group at the end of Stage 4 is now very similar to the corresponding percentages for the validation simulations.

The SAS programs for the validation of *stg12.sas*, *stg3.sas* and *stg4.sas* are included in Appendix 4.

Conclusions

The SAS program *analysis.sas* which analyses and classifies dose response data has been independently validated; complete agreement was reached and no errors were identified.

The SAS program *results.sas* which presents the results of the simulated datasets has been independently validated; complete agreement was reached and no errors were identified.

The SAS programs *stg12.sas, stg3.sas* and *stg4.sas* which simulate data for the proposed test design have been independently validated. There were no obvious differences in the distribution of the LD_{50} or slope estimates between the original and validation sets of simulations. A small discrepancy was identified in the number of experiments with a true slope of 10 that resulted in an estimate of the slope and this has been attributed to the way in which the seeds for random number generation were specified in the original simulations. However, this is a minor issue and has no impact on the conclusions of the original simulation.

References

1. SAS Institute Inc., "Using Random-Number Functions and CALL Routines," SAS 9.1 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004

Annex I of appendix 2
Simulation results:

Figure 1.1

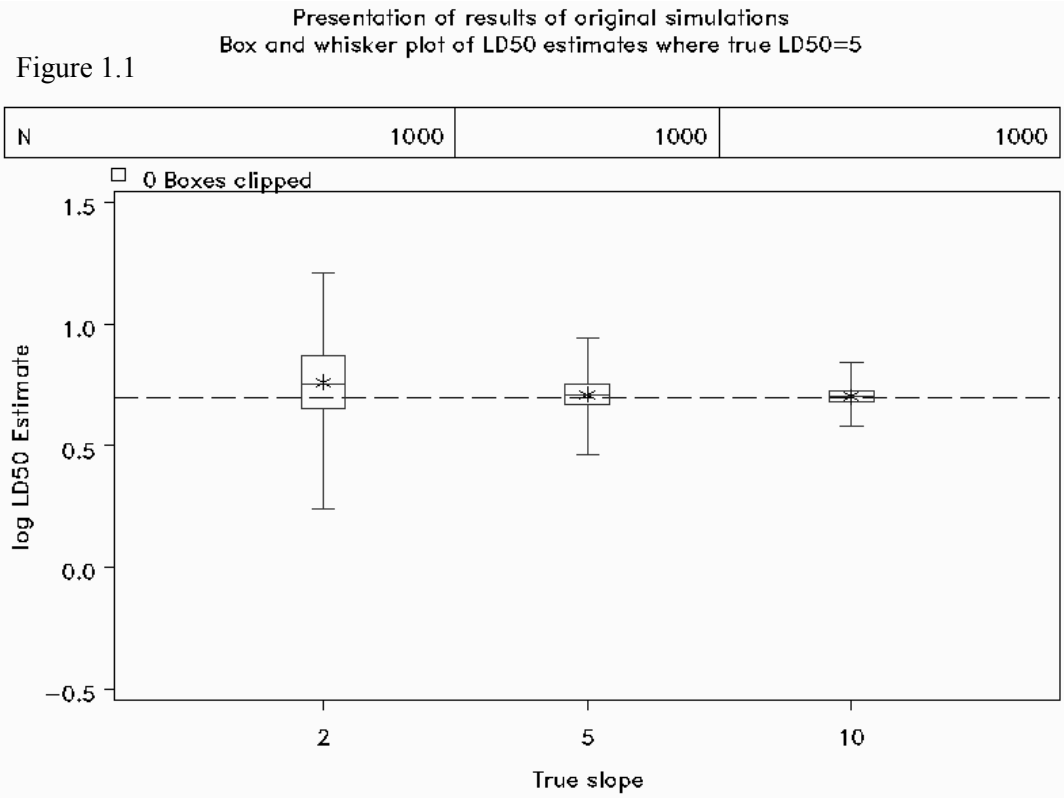


Figure 1.2

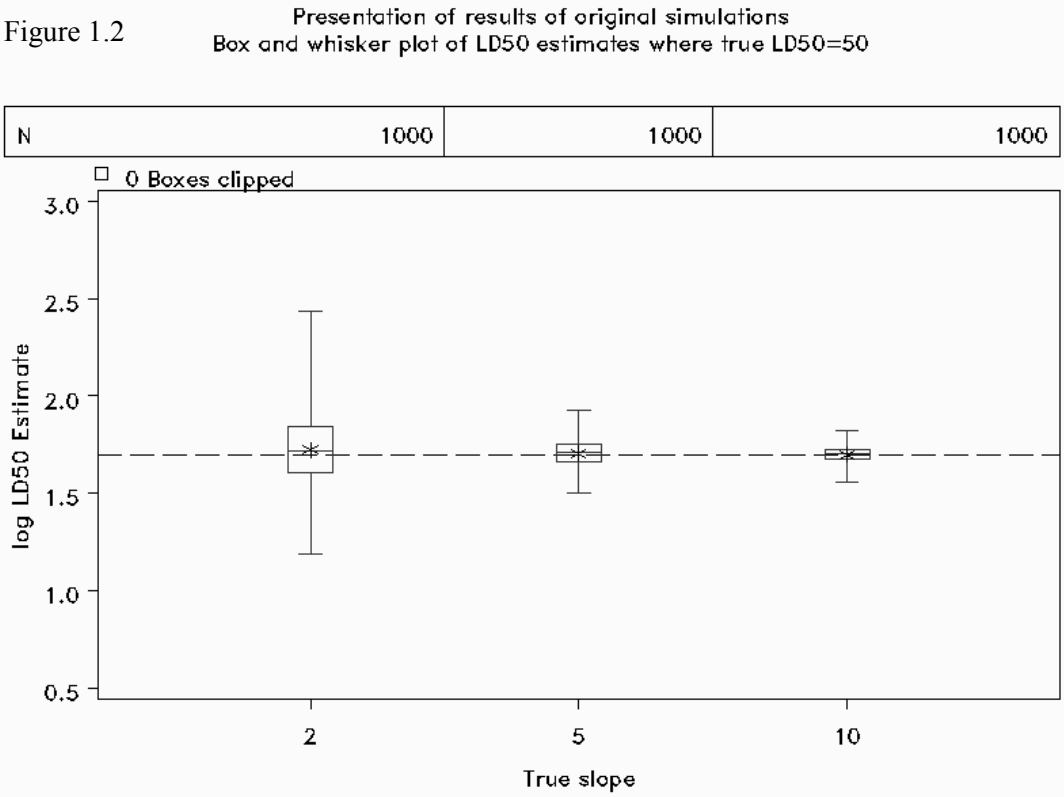


Figure 1.3

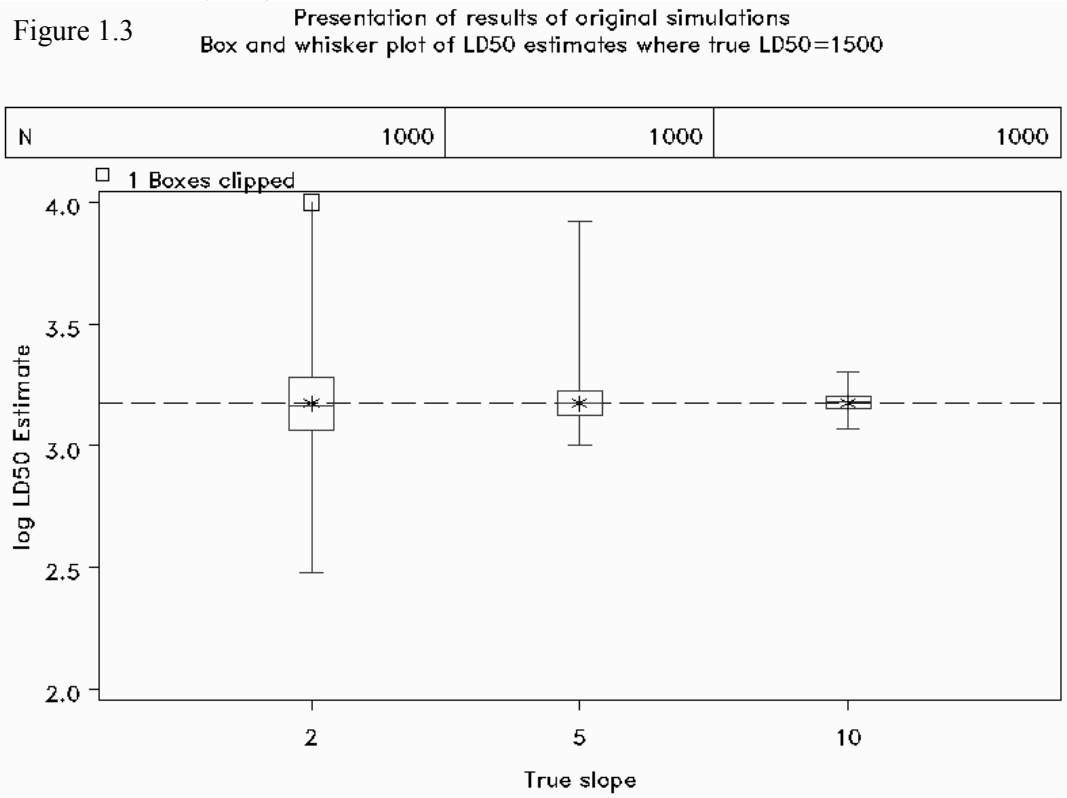


Figure 1.4

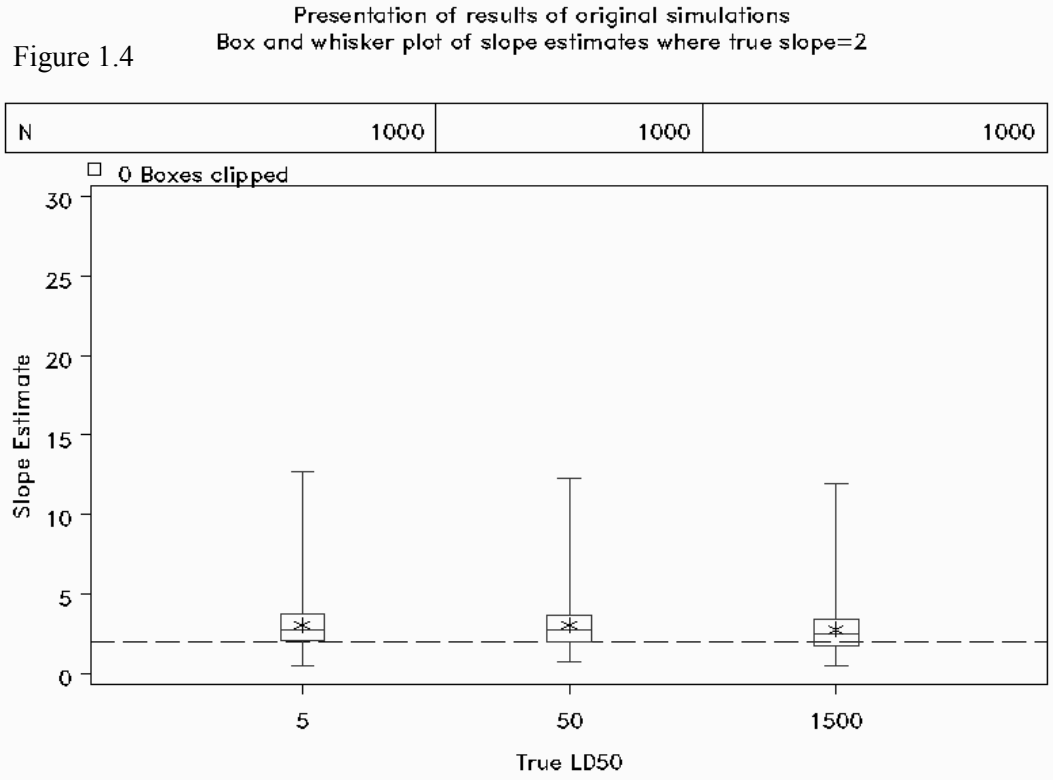


Figure 1.5

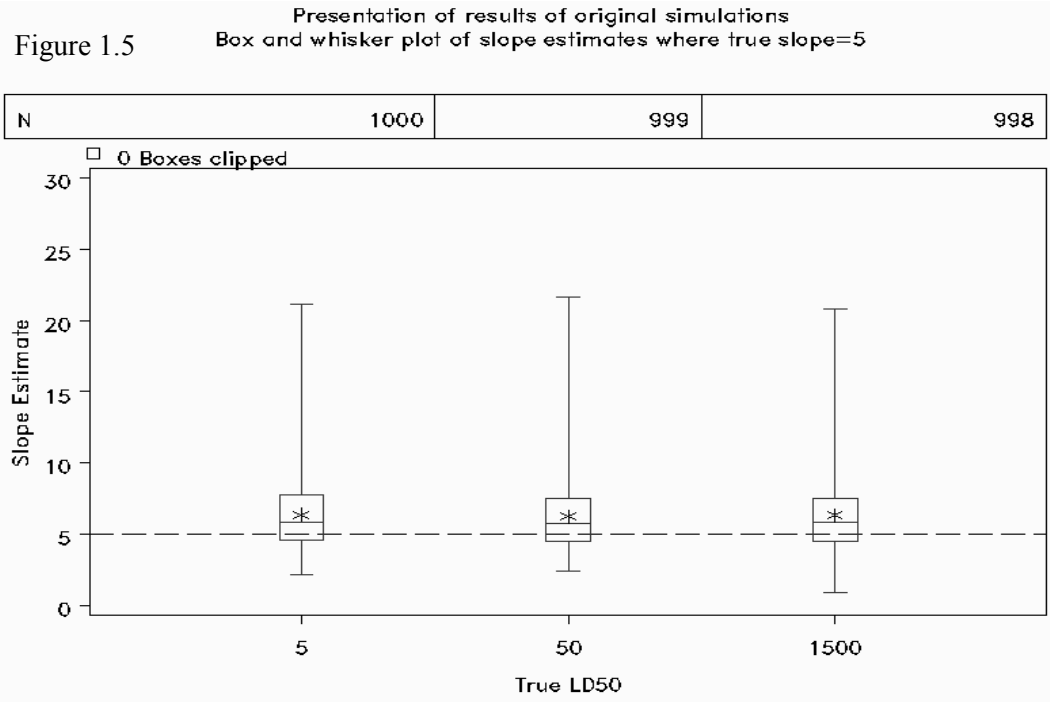


Figure 1.6

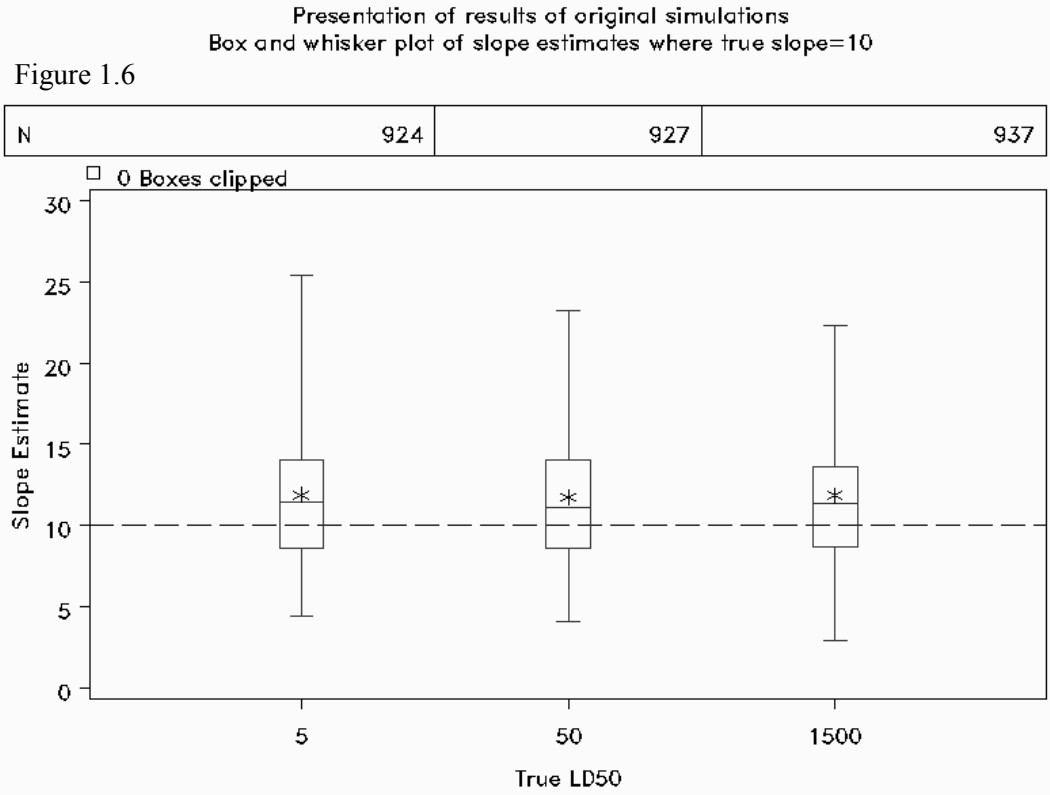


Figure 2.1 Presentation of results of validation simulations
Box and whisker plot of LD50 estimates where true LD50=5

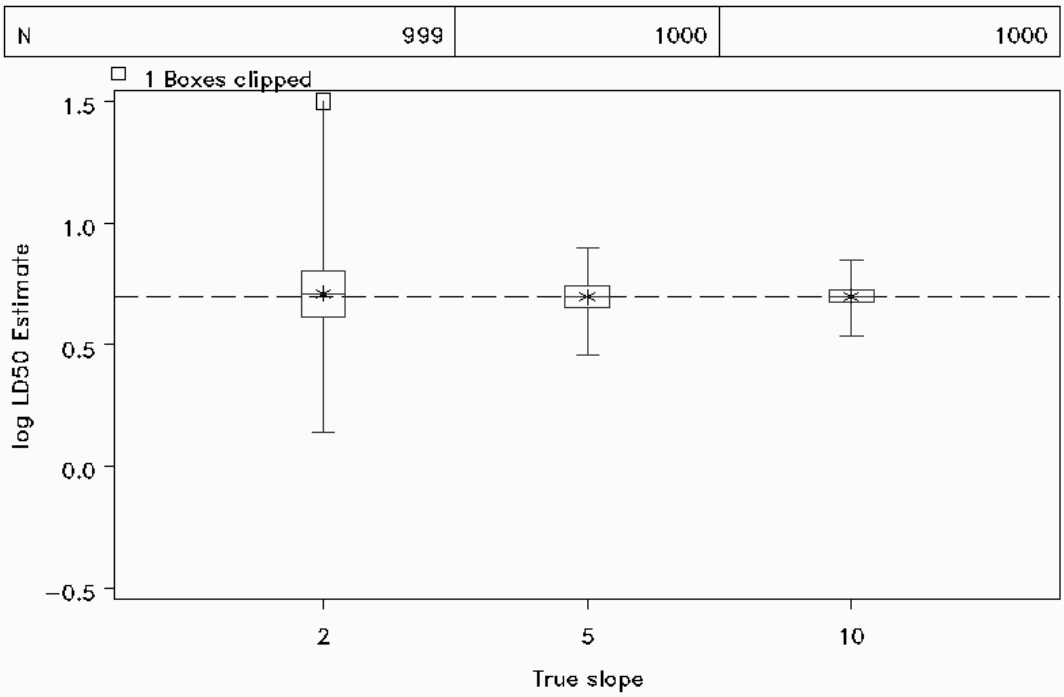


Figure 2.2 Presentation of results of validation simulations
Box and whisker plot of LD50 estimates where true LD50=50

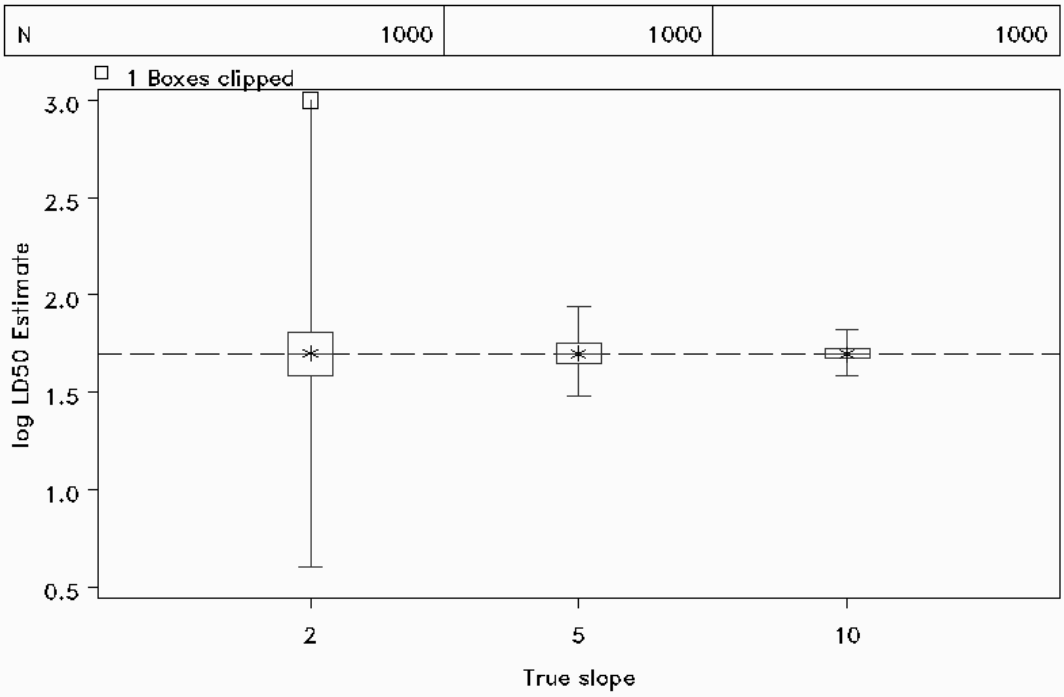


Figure 2.3 Presentation of results of validation simulations
Box and whisker plot of LD50 estimates where true LD50=1500

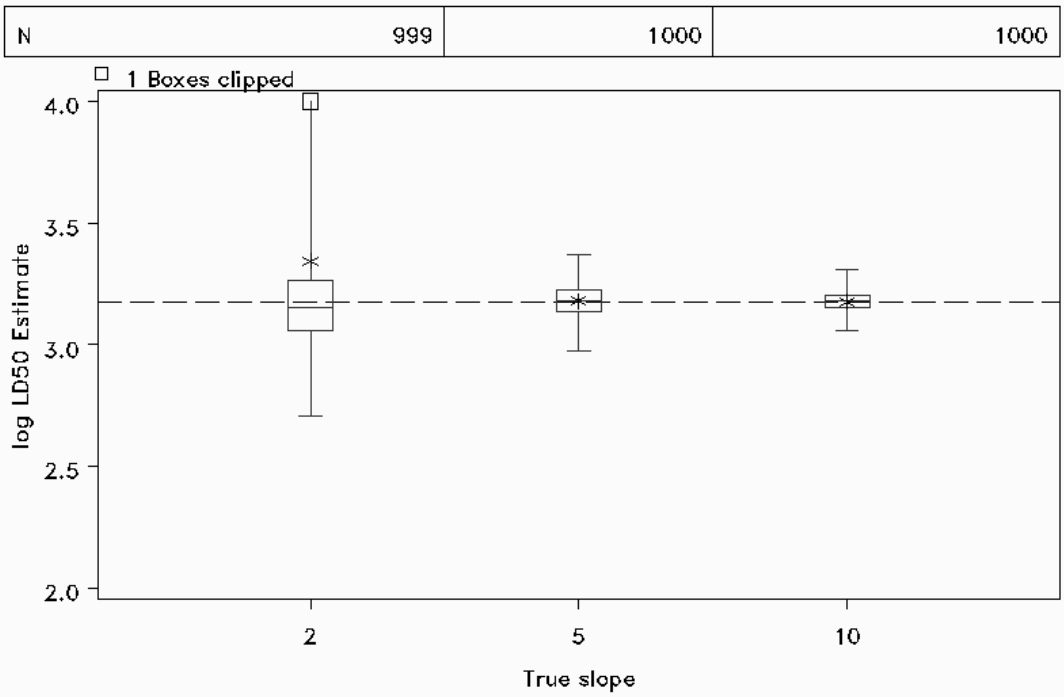


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Figure 2.4 Presentation of results of validation simulations
Box and whisker plot of slope estimates where true slope=2

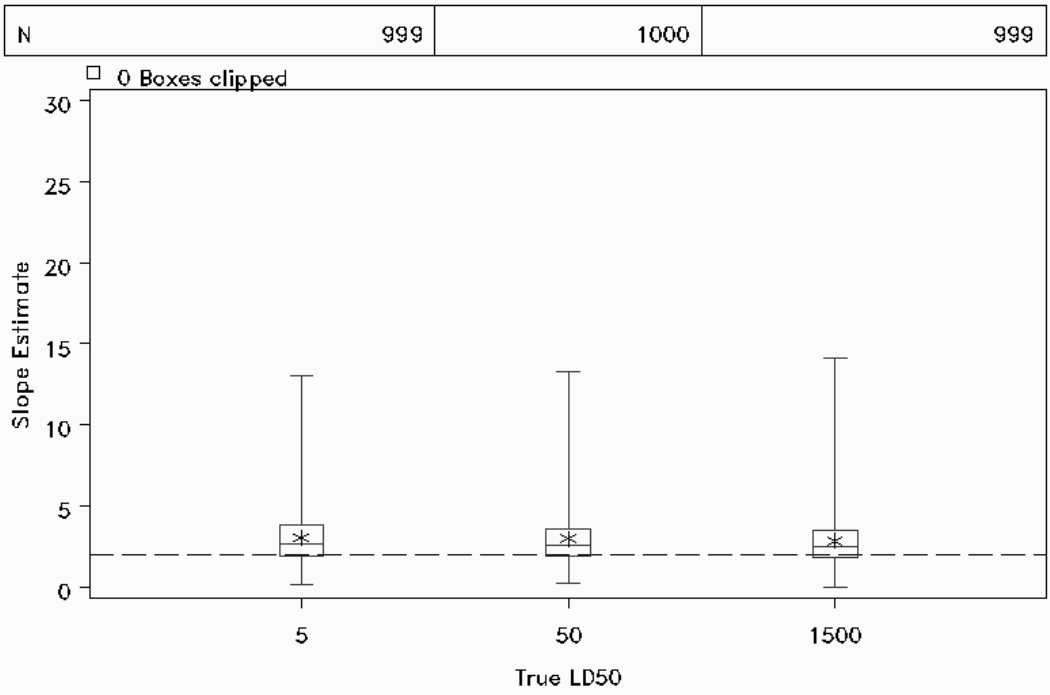


Figure 2.5

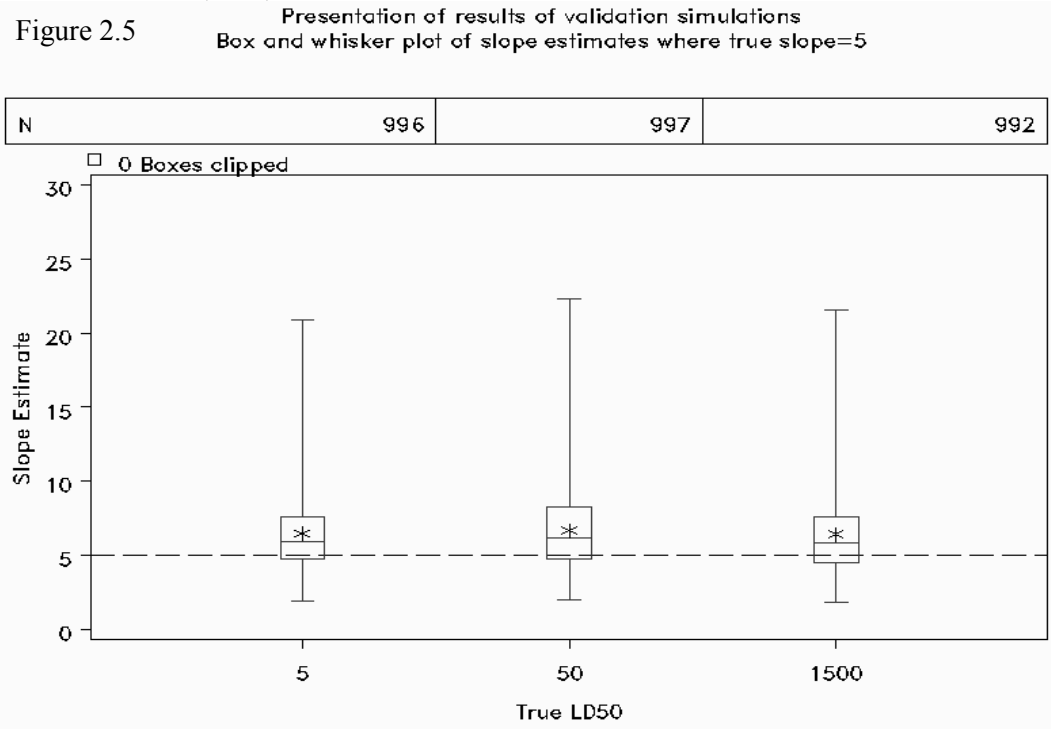


Figure 2.6

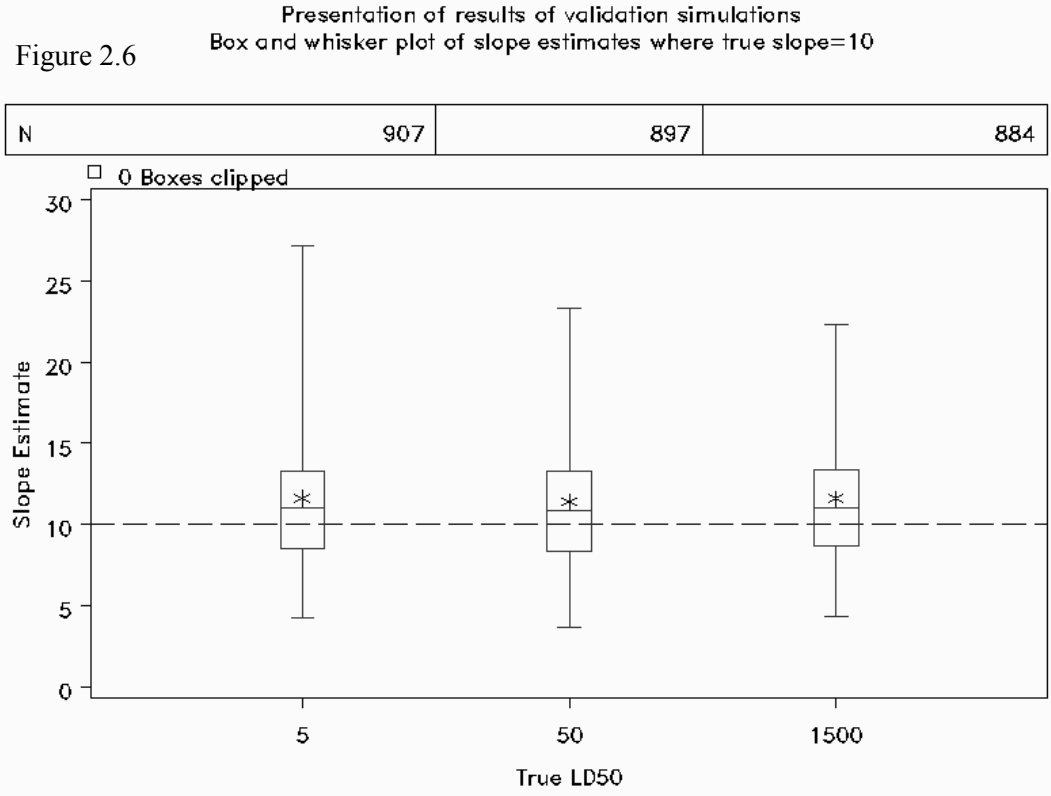


Figure 3.1 Presentation of results of regenerated simulations
Box and whisker plot of LD50 estimates where true LD50=5

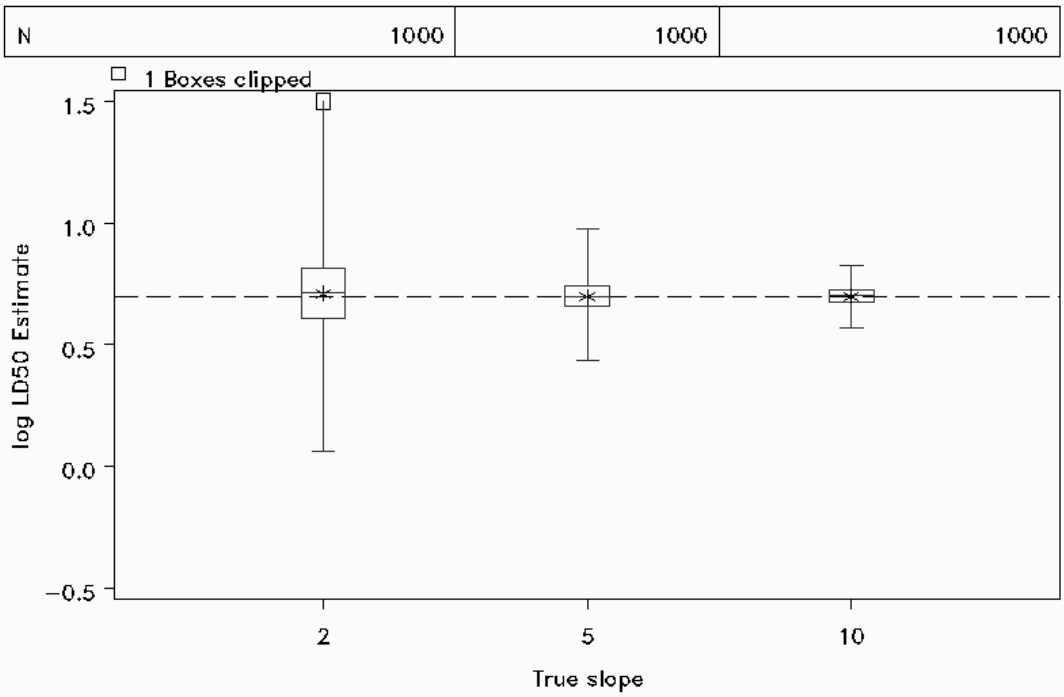


Figure 3.2 Presentation of results of regenerated simulations
Box and whisker plot of LD50 estimates where true LD50=50

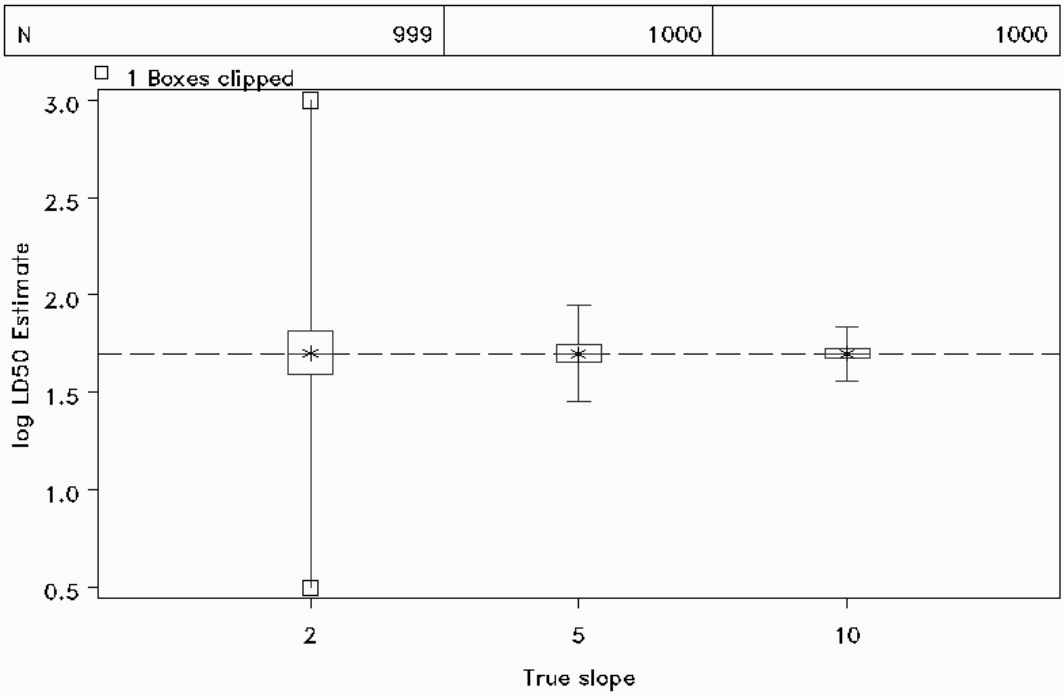


Figure 3.3 Presentation of results of regenerated simulations
Box and whisker plot of LD50 estimates where true LD50=1500

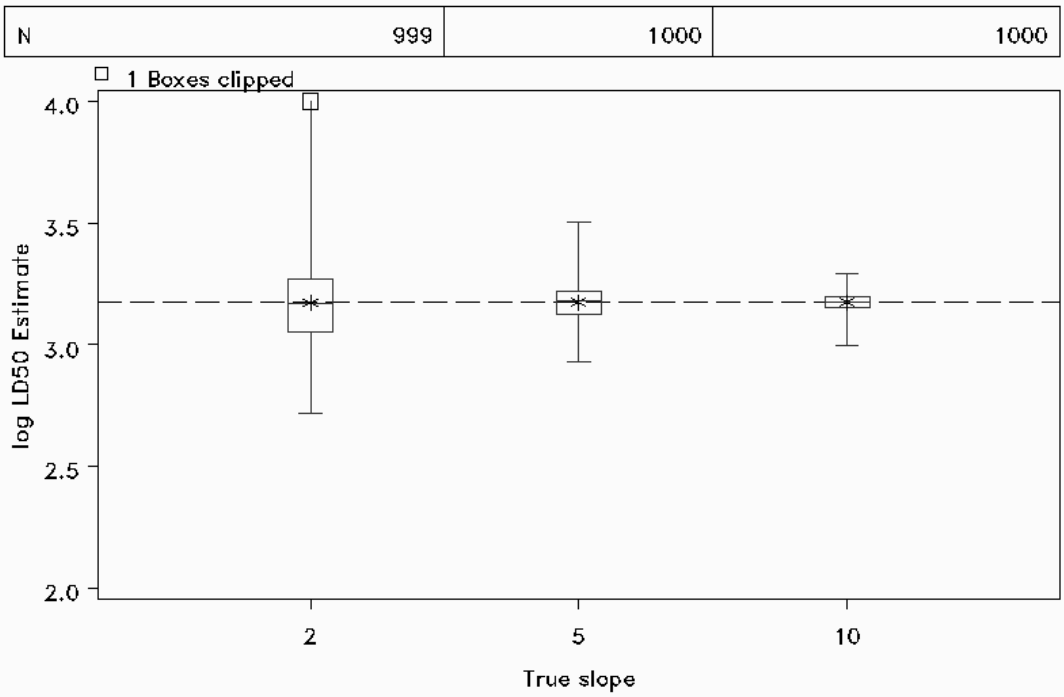


Figure 3.4 Presentation of results of regenerated simulations
Box and whisker plot of slope estimates where true slope=2

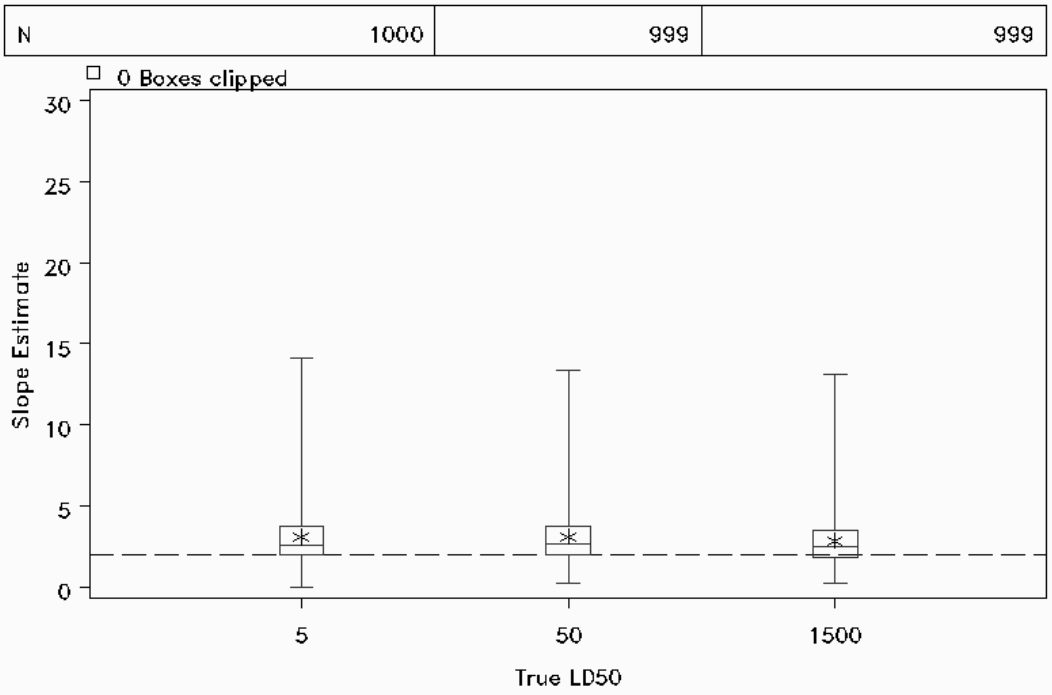


Figure 3.5

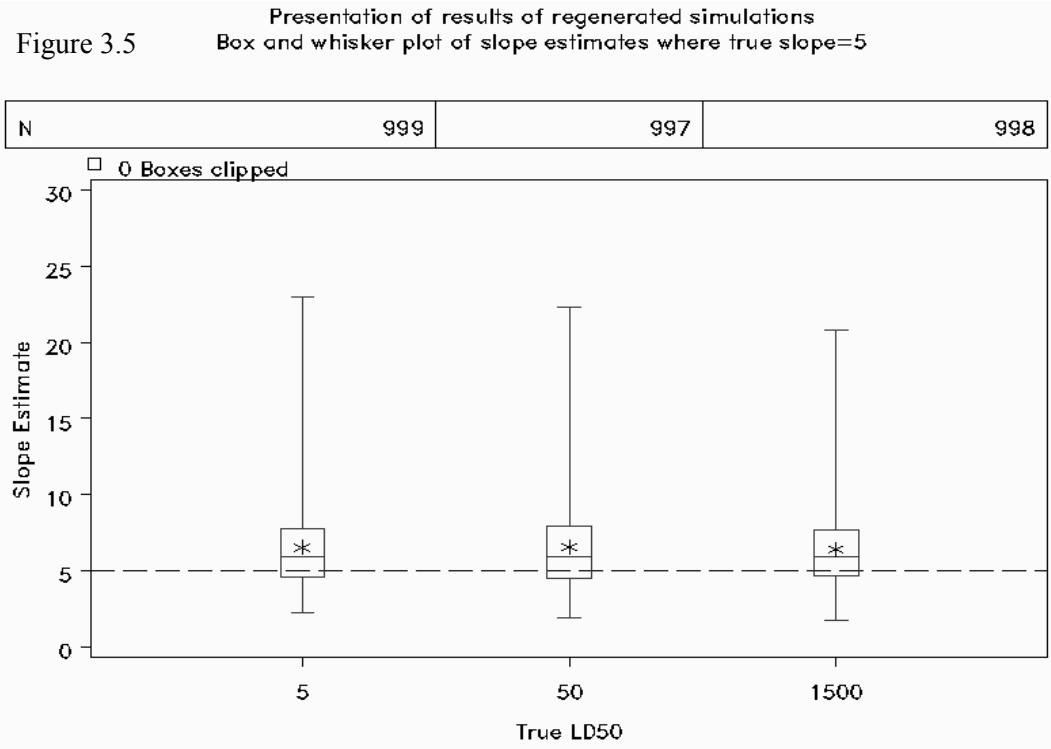
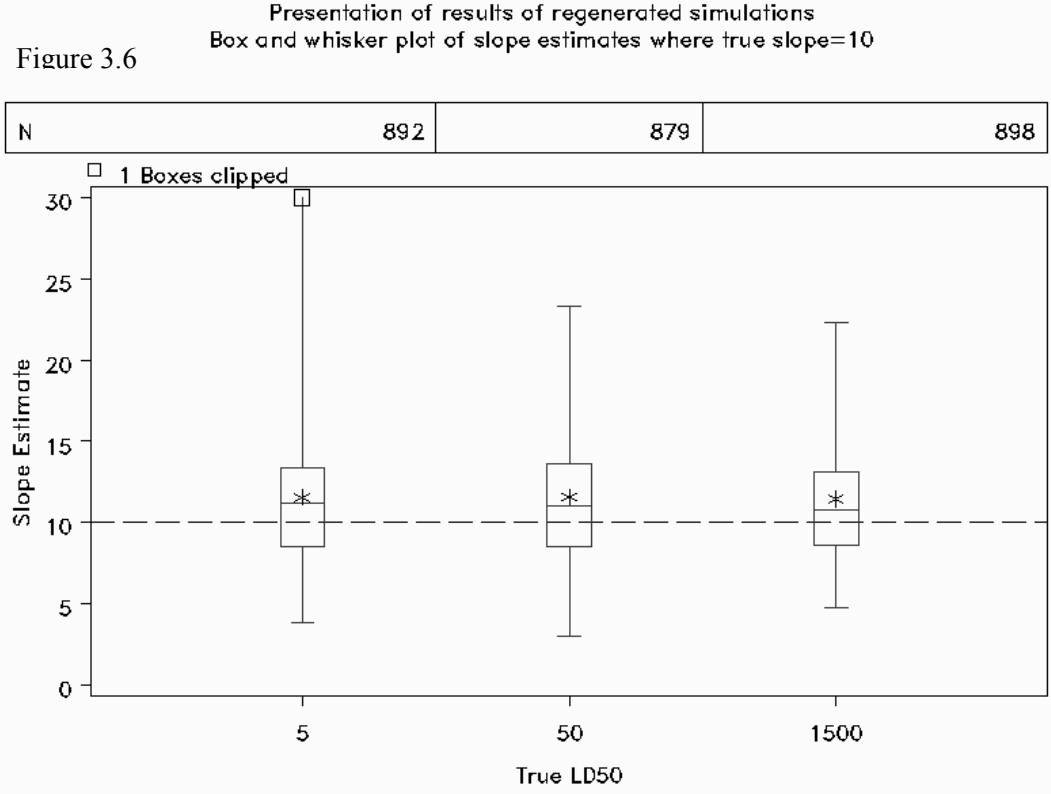


Figure 3.6



Annex 2 of Appendix 2: Programs to validate SAS program, *analysis.sas*.

```
/* VALIDATE TESTDATA.SAS                                          */
/* Validation program for TESTDATA - 40 test datasets             */
/* Author: Carol Yarrow                                           */
/* Date: 08/03/2008                                               */

libname rd "d:\2008\oecd validation\rd";
libname csy "d:\2008\oecd validation\csy";

%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";

/* Classify and analyse each of the 40 datasets in TESTDATA */

options mprint;
%analysis(lib=rd, data=testdata, nexpts=40, idvar=id, where=);

/* Compare the results for 40 test datasets */
title "Comparison of classification and analysis results for all 40 test datasets";
proc compare compare=csy.results_testdata(keep=id npartials nreversals LDclass
                              LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
          base=rd.ests_testdata(keep=id npartials nreversals LDclass
                          LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
          criterion=0.0000001 briefsummary;
  id id;
run;
/* VALIDATE 12 SIMULATIONS STAGES 1, 2, 3 AND 4.SAS               */
/* Validation program for sims_stg4_12 - 12 simulated datasets    */
/* Author: Carol Yarrow                                           */
/* Date: 08/03/2008                                               */

libname rd "d:\2008\oecd validation\rd";
libname csy "d:\2008\oecd validation\csy";

%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";

/* Create a unique experiment ID variable EXPT_ID */
data sims_stg4_12;
  set rd.sims_stg4_12;
  expt_id=(id-1)*1000+sim;
  drop j;
run;
data ests_stg4_12;
  set rd.ests_stg4_12;
  expt_id=(id-1)*1000+sim;
run;

/* Classify and analyse each of the 12 datasets in SIM_stg4_12 */
options mprint;
%analysis(lib=work, data=sims_stg4_12, first=1, nexpts=1, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=1001, nexpts=1001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=2001, nexpts=2001, idvar=expt_id, results=NO);
```

```
%analysis(lib=work, data=sims_stg4_12, first=3001, nexpts=3001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=4001, nexpts=4001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=5001, nexpts=5001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=6001, nexpts=6001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=6115, nexpts=6115, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=6169, nexpts=6169, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=6275, nexpts=6275, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=7001, nexpts=7001, idvar=expt_id, results=NO);
%analysis(lib=work, data=sims_stg4_12, first=8001, nexpts=8001, idvar=expt_id, results=NO);

/* Combine the results */
   data csy.results_sims_stg4_12;
     set results_sims_stg4_12_expt1
         results_sims_stg4_12_expt1001
         results_sims_stg4_12_expt2001
         results_sims_stg4_12_expt3001
         results_sims_stg4_12_expt4001
         results_sims_stg4_12_expt5001
         results_sims_stg4_12_expt6001
         results_sims_stg4_12_expt6115
         results_sims_stg4_12_expt6169
         results_sims_stg4_12_expt6275
         results_sims_stg4_12_expt7001
         results_sims_stg4_12_expt8001;
      format LD50_est LD50_guess LD50_ucl LD50_lcl 8.2;
   run;

/* Compare the results  */
title "Comparison of classification and analysis results for 12 simulated datasets";
title2 "After Stages 1, 2, 3 and 4";
proc compare compare=csy.results_sims_stg4_12(keep=expt_id npartials nreversals LDclass
                                    LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
          base=ests_stg4_12(keep=expt_id npartials nreversals LDclass
                              LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
          criterion=0.0000001 briefsummary;
  id expt_id;
run;


/* VALIDATE SIMULATIONS STAGES 1, 2, 3 and 4.SAS                    */
/* Validation program for sims_stg4 - 9,000 simulated datasets         */
/* Author: Carol Yarrow                                                */
/* Date: 08/03/2008                                                 */

libname rd "d:\2008\oecd validation\rd";
libname csy "d:\2008\oecd validation\csy";

%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";

/* Create a unique experiment ID variable EXPT_ID */
data sims_stg4;
  set rd.sims_stg4;
  expt_id=(id-1)*1000+sim;
```

```
  drop j;
run;
data ests_stg4;
  set rd.ests_stg4;
  expt_id=(id-1)*1000+sim;
run;

/* Classify and analyse each of the 9,000 datasets in SIM_stg4 */
proc printto log="d:\2008\oecd validation\csy\log files\log file sims_stg4.txt";
run;
%analysis(lib=work, data=sims_stg4, nexpts=9000, idvar=expt_id, where=);
proc printto;
run;

/* Compare the results  */
title "Comparison of classification and analysis results for all 9,000 simulated
      datasets";
title2 "After Stages 1, 2, 3 and 4";
proc compare compare=csy.results_sims_stg4(keep=expt_id npartials nreversals LDclass
                                LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
           base=ests_stg4(keep=expt_id npartials nreversals LDclass
                        LD50_est LD50_lcl LD50_ucl b1 b1_lcl b1_ucl)
           criterion=0.0000001 briefsummary;
  id expt_id;
run;

/* CSY_ANALYSIS.SAS */
/* Program to create a macro to classify and analyse multiple experiments
   Input data: &lib..&data
   Experiment ID of first experiment analysed = &first
   Experiment ID of last experiment analysed = &nexpts
   Experiment ID variable = &idvar
   Use &where to restrict processing to certain doses/obs if necessary */
/* Author: Carol Yarrow  Date: 08/03/2008                          */

/* Create macro to classify datasets based on Table 3 */
%include "d:\2008\oecd validation\csy\programs\macros\classify.sas";
/* Create macro to analyse datasets based on Table 4 */
%include "d:\2008\oecd validation\csy\programs\macros\analyse.sas";

/* Classify and analyse each of the &nexpts datasets in &lib..&data */
%macro analysis(lib=, data=, first=1, nexpts=, idvar=, where=, results=YES);

/* Classify and analyse result from each experiment one at a time */
  %do i=&first %to &nexpts;
    /* Reset macro vars */
    %let LD50=;
      %let LD50_lowerCL=;
      %let LD50_upperCL=;
      %let slope=;
      %let slope_lowerCL=;
      %let slope_upperCL=;
```

```
/* Extract data from single experiment. Round the dose to 2 decimal places. */
   data &data._expt&i;
     set &lib..&data;
     where &idvar=&i;
     rounded_dose=round(dose,0.01);
     /* Recalculate logdose based on rounded dose */
     logdose=log10(rounded_dose);
   run;

/* Sort by rounded_dose  */
   proc sort data=&data._expt&i;
     by rounded_dose;
     &where;
   run;

/* Combine doses (sum totals exposed, dead and alive) if rounded_dose is the same */
   proc means data=&data._expt&i noprint nway;
     var ndead nalive nexpo;
     class rounded_dose logdose;
     output out=combined_&data._expt&i sum=ndead nalive nexpo n=n;
   run;

/* Calculate no. of reversals, no. of partials and classify data for each expt */
   %classify(lib=&lib, data=&data._expt&i, idvar=&idvar, id=&i, print=YES, freq=NO);

/* Analyse the data for each experiment depending upon the class */
   %analyse(data=&data._expt&i, idvar=&idvar, id=&i);
  %end;

/* Combine results from all experiments together */
  %if &results=YES %then %do;
  data csy.results_&data;
    set
      %do j=&first %to &nexpts;
        results_&data._expt&j
      %end;
      ;
      format LD50_est LD50_guess LD50_ucl LD50_lcl 8.2;
  run;
  %end;
%mend analysis;
/* CLASSIFY.SAS                                                */
/* Macro program to classify an experiment according to the rules in Table 3 */
/* Author: Carol Yarrow                                        */
/* Date: 08/03/2008                                           */

/* Macro to classify experiment based on Table 3 */

%macro classify(lib=, data=, id=, idvar=, print=YES, freq=NO);

data classify_&data;
  &idvar=&id;
  set combined_&data end=last;
```

```
 retain Complete_Mortality Complete_Survival NPartials
       Cum_Sum_Logdose_Mortality Cum_Sum_Logdose_Survival Cum_NDead Cum_NAlive
       Previous_Mortality NReversals Dose_Complete_Survival Dose_Complete_Mortality
       Lower_Bound Upper_Bound AllDoses_Survival_LT_Mortality;

/* Calculate proportion of mortality and survival at each dose for each experiment */
 Mortality=Ndead/Nexpo;
 Survival=Nalive/Nexpo;

 /* Initialise values at the beginning of each experiment                    */
 /* Initialise Complete_Mortality and Complete_Survival to 1 if there is complete
   mortality or survival at the first dose and to 0 otherwise               */
 /* Initialise NPartials and NReversals to 0                                 */
 /* Initialise cumulative sums for logdose and ndead and nalive to 0         */
 /* Initialise Previous_Mortality to missing at first dose as no previous dose */
 /* Initialise Dose_Complete_Survival to 0                                   */

 if _n_=1 then do;
  if Mortality=1 then Complete_Mortality=1;
     else Complete_Mortality=0;
  if Survival=1 then Complete_Survival=1;
     else Complete_Survival=0;
    NPartials=0;
    Cum_Sum_Logdose_Mortality=0;
    Cum_Sum_Logdose_Survival=0;
    Cum_NDead=0;
    Cum_NAlive=0;
    Previous_Mortality=.;
    NReversals=0;
    Dose_Complete_Survival=0;
    Dose_Complete_Mortality=0;
    Lower_Bound="   ";
    Upper_Bound="   ";
    AllDoses_Survival_LT_Mortality="   ";
 end;

 /* COMPLETE MORTALITY and COMPLETE SURVIVAL */
 /* If mortality or survival at current and all subsequent doses=1 (100%) then there
   is complete mortality or survival up to this dose */
 if Mortality=1 and Complete_Mortality=1 then Complete_Mortality=1;
  else Complete_Mortality=0;
 if Survival=1 and Complete_Survival=1 then Complete_Survival=1;
  else Complete_Survival=0;

 /* PARTIAL MORTALITY */
 /* There is partial mortality at a dose if there are both survivors and deaths */
 if Mortality ne 1 and Survival ne 1 then Partial_Mortality=1;
 else Partial_Mortality=0;
 /* Sum the number of partial mortalities in an experiment */
 NPartials + Partial_Mortality;

 /* CONSISTENCY */
 /* Calculate average logdose for survival and average log dose for mortality */
```

```
/* Sum logdose for mortality and survival separately for each dose */
Sum_Logdose_Mortality=Ndead*logdose;
Sum_Logdose_Survival=Nalive*logdose;
/* Calculate cumulative sums of logdose for mortality and survival for each expt */
Cum_Sum_Logdose_Mortality + Sum_Logdose_Mortality;
Cum_Sum_Logdose_Survival + Sum_Logdose_Survival;

/* Calculate cumultive sums of the number of dead and alive birds for each expt */
Cum_Ndead + Ndead;
Cum_Nalive + Nalive;

/* Calculate the average logdose for mortality and survival on the last dose for
   each experiment */
if last then do;
  if Cum_Ndead ne 0 then
    Average_Logdose_Mortality=Cum_Sum_Logdose_Mortality/Cum_Ndead;
  if Cum_Nalive ne 0 then
    Average_Logdose_Survival=Cum_Sum_Logdose_Survival/Cum_Nalive;

/* If the average log dose for mortality is less than or equal to the average
   Logdose for survival then the results are defined as inconsistent. Don't do this
   Calculation if there is complete mortality or complete survival as one value
   will be missing and it is not relevant (as the experiment will be classified as
   Type A) */
  if Complete_Mortality ne 1 and Complete_Survival ne 1 then do;
    if Average_Logdose_Mortality<=Average_Logdose_Survival then
      Consistent="Inconsistent";
    else Consistent="Consistent";
  end;
end;

/* REVERSALS */
/* Data is sorted by dose within experiment */
/* Compare mortality in each dose to mortality in the previous dose; if it is less
   Then a reversal has occurred */
if previous_mortality ne . then do;
  if mortality lt previous_mortality then NReversals+1;
end;
previous_mortality=mortality;

/* BOUNDS */
/* If there has been complete survival at a dose then set Dose_Complete_Survival to
   1 for all subsequent doses for that experiment */
if Complete_Survival=1 then Dose_Complete_Survival=1;

/* If there is a partial mortality then if there is complete survival at a lower
   dose set Lower_Bound to "Yes" */
if Partial_Mortality=1 then do;
  if Dose_Complete_Survival=1 then Lower_Bound="Yes";
end;

/* If there is a partial mortality then if there is complete mortality at a higher
```

```
    dose
 set Upper_Bound to "Yes" */
  if NPartials>=1 then do;
    if Mortality=1 then Upper_Bound="Yes";
  end;

  /* Are all doses with complete survival below those with complete mortality? */
  /* If there has been complete mortality at a dose then set Dose_Complete_Mortality
     to 1 for all subsequent doses for that experiment. If there is a subsequent dose
     with complete survival then the answer is No. */
  if Mortality=1 then Dose_Complete_Mortality=1;
  if Dose_Complete_Mortality=1 and Survival=1 then
    AllDoses_Survival_LT_Mortality="No";

  /* Only continue and output if the last row for each experiment */
  if last;

  /* Classify each experiment */
  if Complete_Survival=1 or Complete_Mortality=1 then LDClass="A";
  if Consistent="Inconsistent" then LDClass="B";
  else if Consistent="Consistent" then do;
    if NPartials>=2 then LDClass="C";
      if NPartials<2 and NReversals>=1 then LDClass="D";
      if NPartials=1 and NReversals=0 then do;
        if Lower_Bound="Yes" and Upper_Bound="Yes" then LDClass="E";
        else if Lower_Bound="Yes" or Upper_Bound="Yes" then LDClass="F";
    end;
    if NPartials=0 and AllDoses_Survival_LT_Mortality ne "No" then LDClass="G";
  end;
  keep &idvar NPartials NReversals Consistent LDClass;
run;

%mend classify;
/* ANALYSE.SAS                                                    */
/* Macro program to analyse datasets according to the rules in Table 4  */
/* Author: Carol Yarrow                                           */
/* Date: 08/03/2008                                              */

%include "d:\2008\oecd validation\csy\programs\macros\probit.sas";
%include "d:\2008\oecd validation\csy\programs\macros\nonlinear.sas";
%include "d:\2008\oecd validation\csy\programs\macros\midpoint.sas";
%include "d:\2008\oecd validation\csy\programs\macros\justci.sas";
%include "d:\2008\oecd validation\csy\programs\macros\notest.sas";
%include "d:\2008\oecd validation\csy\programs\macros\binomial.sas";

/* Macro to analyse datasets based on classification and Table 4 */
%macro analyse(data=, idvar=, id=&i);

/* Read in class of experiment from classify result and store in macro var &CLASS */
  data _null_;
    set classify_&data;
    call symput('class',LDClass);
  run;
```

```
/* Carry out appropriate analysis for this experiment based on the classification
   result */

  /* If class=A then just estimate LD50 CI using Binomial_bounds */
  %if &class=A %then %do;
    %justci(data=&data, idvar=&idvar,  id=&id);
  %end;

  /* If class=B then nothing estimable */
  %if &class=B %then %do;
    %notest(data=&data, idvar=&idvar, id=&id);
  %end;

  /* If class=C or D then estimate LD50, slope and CIs using probit analysis */
  %if &class=C or &class=D %then %do;
    %probit(data=&data, idvar=&idvar, id=&id);
  %end;

  /* If class=E or F then estimate LD50 using non-linear interpolation provided 50%
     Mortality is bracketed, non-estimable otherwise.  Estimate LD50 CI using
     Binomial_bounds */
  %if &class=E or &class=F %then %do;
    %nonlinear(data=&data, idvar=&idvar, id=&id);
  %end;

  /* If class=G then estimate LD50 using midpoint on log10 scale between highest dose
     with complete survival and lowest dose with complete mortality Estimate LD50 CI
     using Binomial_bounds */
  %if &class=G %then %do;
    %midpoint(data=&data, idvar=&idvar, id=&id);
  %end;

  /* Include the estimate of LD50 and slope with the results of the classification */
  data results_&data;
    merge classify_&data results_&data;
      by &idvar;

    /* Save the guess of the LD50 in a macro var so that can replace if necessary
       Using rules in table 2 in order to simulate next step data */
    call symput(' LD50_guess', LD50_est);
  run;

  /* If there is no estimate of the LD50, create a working LD50 using the rules in
     Table 2 */
  %if & LD50_guess=. %then %do;
     data _null_;
       set combined_&data end=last;
       mortality=ndead/nexpo;
       retain lowdose highdose comp_mort_dose part_mort_dose LD50_guess;
       if _n_=1 then lowdose=rounded_dose;
       if last then highdose=rounded_dose;
       %if &class=A %then %do;
```

```
      /* If complete mortality at all doses then LD50_guess = lowest dose */
       if mortality=1 then LD50_guess=lowdose;
      /* If complete survival at all doses then LD50_guess = highest dose */
       if mortality=0 then LD50_guess=highdose;
    %end;
    %else %if &class=B %then %do;

     /* If there is a dose with complete mortality then LD50_guess = lowest dose
        with complete mortality */
      if mortality=1 and comp_mort_dose=. then comp_mort_dose=rounded_dose;

     /* If no doses with complete mortality then LD50=highest dose with partial
        mortality */
      if 0<mortality<1 then part_mort_dose=rounded_dose;

      if comp_mort_dose ne . then LD50_guess=comp_mort_dose;
      else LD50_guess=part_mort_dose;
    %end;
    %else %if &class=F %then %do;
      total_ndead+ndead;
      total_nexpo+nexpo;
      if last then do;
       if total_ndead/total_nexpo>0.5 then LD50_guess=lowdose;
       else if total_ndead/total_nexpo<0.5 then LD50_guess=highdose;
      end;
    %end;

    if last;
    call symput(' LD50_guess', LD50 _guess);
  run;
  %put LD50_guess=& LD50_guess;
 %end;

 /* Add the guess of the LD50for cases where it was missing */
 data results_&data;
  set results_&data;
  LD50_guess= LD50_est;
  if LD50_guess=. then LD50_guess=& LD50_guess;
 run;

%mend analyse;




/* PROBIT.SAS
   Macro program to analyse datasets using probit analysis          */
/* Author: Carol Yarrow                                             */
/* Date: 08/03/2008                                                 */

%macro probit(data=, idvar=, id=);
```

```
 /* Probit analysis - Model proportion of dead birds as a function of logdose */
 /* Use INVERSECL option to obtain CI for the LD50*/
 ods listing close;
 ods output ParameterEstimates=parameterestimates
         ProbitAnalysis=probitanalysis;
 proc probit data=combined_&data inversecl;
  model ndead/nexpo=logdose;
 run;

 /* Extract slope estimate and CIs into macro vars */
 /* Extract LD50 estimate and CIs into macro vars
   Need to find 10^estimate and 10^CL as doses are on the log10 scale */
 data results_&data;
  &idvar=&id;
  set parameterestimates(keep=parameter estimate lowerCL uppercl
                    where=(parameter="logdose")
                    rename=(estimate=b1 lowerCL=b1_lcl upperCL=b1_ucl));
  set probitanalysis(keep=probability variable lowerCL upperCL
                    where=(round(probability,0.01)=0.50)
                    rename=(variable=logLD50 lowerCL=log LD50_lowerCL
                         upperCL=logLD50_upperCL));
  call symput('Slope',put(b1,8.4));
  call symput('Slope_lowerCL',put(b1_lcl,8.4));
  call symput('Slope_upperCL',put(b1_ucl,8.4));
  LD50_est=10**log LD50;
  LD50_lcl=10**log LD50_lowerCL;
  LD50_ucl=10**log LD50_upperCL;
  call symput('',put(LD50_est,10.2));
  call symput(' LD50_lcl',put(LD50_lcl,10.2));
  call symput(' LD50_ucl',put(LD50_ucl,10.2));
  drop parameter probability;
 run;

 ods listing;
%mend probit; /* NONLINEAR.SAS
  Macro program to analyse datasets using non-linear interpolation     */
/* Author: Carol Yarrow                                                */
/* Date: 08/03/2008                                                    */

%macro nonlinear(data=, idvar=, id=);
 /* Estimate LD50 using non-linear interpolation */
 /* Inititalise macro vars to missing */
 %let J=;
 %let D=;
 %let N=;
 %let F=;
 %let LD50_est=;
 %let LD50_lcl=;
 %let LD50_ucl=;

 /* Non-linear interpolation */
 data nonlinear;
  set combined_&data;
```

```
  /* Step 1: Calculate Q for each dose level (in radians) */
  q_rad=1/2 * (arsin(sqrt(ndead/(nexpo+1))) + arsin(sqrt((ndead+1)/(nexpo+1))));
  /* Convert Q to degrees */
  q_deg=q_rad*(180/constant('PI'));
  /* Calculate the mortality at each dose */
  Mortality=ndead/nexpo;
run;

/* Transpose the mortality data to a single row with one column for each dose */
proc transpose data=nonlinear out=tran_mortality prefix=MORT_;
  var mortality;
run;

/* Step 2: Find dose level j where doses j and j+1 appear to enclose the median
   Dose. i.e. Mortality < 0.5 for dose j and mortality > 0.5 for dose j+1
   OR dose level d where median mortality is observed */
data _null_;
  set tran_mortality;
  array mortality(*) _numeric_;
  retain j;
  do i=1 to dim(mortality);

    /* If mortality=0.5 at any dose level then store the level in d */
    if mortality(i)=0.5 then d=i;
    if i<=dim(mortality)-1 then do;
      if mortality(i)<0.5 and mortality(i+1)>0.5 then j=i;
    end;
  end;

  /* Store the value of d in a macro variable &d */
  if d ne . then call symput('D',trim(left(d)));
  /* Store the value of j in a macro variable &j */
  if j ne . then call symput('J',trim(left(j)));
  /* Store N, the number of dose levels, in a macro variable &n */
  call symput('N',trim(left(dim(mortality))));
run;

/* If the median dose was observed then this is the estimated LD50*/
%if &d ne %then %do;

  /* Transpose the logdose data to give a single row with one column for each dose
     level */
  proc transpose data=nonlinear out=tran_logdose prefix=LOGDOSE_;
    var logdose;
  run;

  data _null_;
    set tran_logdose;
    array LOGDOSE(&n) LOGDOSE_1-LOGDOSE_&n;
    W=logdose(&d);
    LD50_est=10**W;
    /* Store the value of LD50_est in a macro variable & LD50_est */
    call symput(' LD50_est', LD50_est);
```

```
 run;

 /* Dose &d-1 is the highest dose below the estimated LD50*/
 /* Dose &d+1 is the lowest dose above the estimated LD50 */
 /* Find the CI for the LD50 using binomial bounds */
 %binomial(data=combined_&data, id=&id);

 data results_&data;
  &idvar=&id;
  LD50_est=&LD50_est;
  %if &LD50_lcl ne %then %do;
   LD50_lcl=&LD50_lcl;
  %end;
  %if &LD50_ucl ne %then %do;
   LD50_ucl=&LD50_ucl;
  %end;
 run;
%end;

/* If the median dose is bracketed then use non-linear interpolation to find LD50*/
%else %do;
 %if &j ne  %then %do;

  /* Transpose the Q function data to give a single row with one column for each
    dose level */
  proc transpose data=nonlinear out=tran_q prefix=Q_;
    var q_deg;
  run;

  /* Step 3: Find the fraction, f of the interval on the angular response scale
     From response q(j) to 45 degrees */
  data _null_;
    array Q(&n) Q_1-Q_&n;
    set tran_q;
    F=(45-q(&j))/(q(%eval(&j+1))-q(&j));
    /* Store the value of F in a macro variable &f */
    call symput('F',f);
   drop _name_;
  run;
  %put F= &f;

  /* Transpose the logdose data to give a single row with one column for each
     dose level */
  proc transpose data=nonlinear out=tran_logdose prefix=LOGDOSE_;
    var logdose;
  run;

  /* Step 4: Determine the log10 dose, W, that is this fraction up from
     logdose(j)and represents an estimate of the log10(LD50) */
  data _null_;
    set tran_logdose;
    array LOGDOSE(&n) LOGDOSE_1-LOGDOSE_&n;
    W=logdose(&j) + &f*(logdose(%eval(&j+1))-logdose(&j));
```

```
      LD50_est=10**W;
      /* Store the value of LD50_est in a macro variable &LD50_est */
      call symput('LD50_est',LD50_est);
    run;

    /* Find the CI for the LD50 using binomial bounds */
    %binomial(data=combined_&data, id=&id);

    data results_&data;
     &idvar=&id;
     LD50_est=&LD50_est;
     %if &LD50_lcl ne %then %do;
       LD50_lcl=&LD50


_lcl;
      %end;
      %if &LD50_ucl ne %then %do;
       LD50_ucl=&LD50_ucl;
      %end;
    run;
  %end;

  /* If the median dose is not bracketed then the LD50 is non-estimable */
  %else %if &j= %then %do;

    %let LD50_est=.;
    /* If the highest dose has less than 50% mortality then it represents the
       highest dose below the 'estimated LD50' and is dose j for purposes of
       calculating bounds */
    /* If the lowest dose has more than 50% mortality then it represents the lowest
       dose above the 'estimated LD50' and is dose j+1 for purposes of calculating
       bounds */

    data _null_;
     set tran_mortality;
     array mortality(*) _numeric_;
     if mortality(dim(mortality))<0.5 then call symput('J',dim(mortality));
    if mortality(1)>0.5 then call symput('J',0);
    run;

    /* Find the CI for the LD50 using binomial bounds */
    %binomial(data=combined_&data, id=&id);

    data results_&data;
     &idvar=&id;
     retain LD50_est;
     %if &LD50 _lcl ne %then %do;
       LD50_lcl=&LD50_lcl;
     %end;
     %if &LD50_ucl ne %then %do;
       LD50_ucl=&LD50_ucl;
     %end;
```

```
    run;
   %end;
  %end;

%mend nonlinear;

/* MIDPOINT.SAS */
/* Macro program to estimate LD50 using midpoint on log10 scale between
   highest dose with complete survival and lowest dose with complete mortality.
   Used for datasets of Type G with no partial mortalities and consistent data
   therefore these doses will be adjacent */
/* Author: Carol Yarrow                                    */
/* Date: 08/03/2008                                        */

%macro midpoint(data=, idvar=,  id=);
%let J=;
%let N=;
%let D=;
%let LD50_est=;
%let LD50_lcl=;
%let LD50_ucl=;

data midpoint;
  set combined_&data;
  retain previous_mortality j;
  /* Calculate the mortality at each dose */
  Mortality=ndead/nexpo;
  /* Set j to be the highest dose level with complete survival. j+1 will be the
     lowest dose level with complete mortality */
  if Previous_mortality=0 and mortality=1 then j=_n_-1;
  call symput('J',j);
  Previous_mortality=mortality;
run;
%put j=&j;

/* Transpose the mortality data to a single row with one column for each dose level*/
proc transpose data=midpoint out=tran_logdose prefix=LOGDOSE_;
  var logdose;
run;

/* Find the midpoint between logdose(j) and logdose(j+1) */
data _null_;
  set tran_logdose;
  array logdose(*) _numeric_;
  /* Store N, the number of dose levels, in a macro variable &n */
  call symput('N',trim(left(dim(logdose))));
  logLD50=mean(logdose(&j),logdose(%eval(&j+1)));
  LD50_est=10**logLD50;
  /* Store the value of LD50_est in a macro variable &LD50_est */
  call symput('LD50_est',LD50_est);
run;

/* Find the CI for the LD50 using binomial bounds */
```

```
%binomial(data=combined_&data, id=&id);

data results_&data;
  &idvar=&id;
  LD50_est=&LD50_est;
  %if &LD50_lcl ne %then %do;
    LD50_lcl=&LD50_lcl;
  %end;
  %if &LD50_ucl ne %then %do;
    LD50_ucl=&LD50_ucl;
  %end;
run;

%mend midpoint;
/* JUSTCI.SAS */
/* Macro program to estimate CIs for LD50 using binomial bounds, but
   missing values for LD50 and slope est and CI (dataset type A)       */
/* Author: Carol Yarrow                                                */
/* Date: 08/03/2008                                                    */

%macro justci(data=, idvar=, id=);
/* Datasets of Type A have complete mortality or complete survival at all doses */
%let D=;
%let J=;
%let N=;
%let LD50_est=;
%let LD50_lcl=;
%let LD50_ucl=;

/* Find dose level j  */
  data justci;
    set combined_&data;
    retain j;

    /* Calculate the mortality at each dose */
    Mortality=ndead/nexpo;

    /* If complete survival at all doses, then the highest dose is dose j, the
       highest dose with complete survival */
    if mortality=0 then j=_n_;

    /* If complete mortality at all doses, then the lowest dose is dose j+1, the
       lowest dose with complete mortality */
    if mortality=1 then j=0;

    call symput('J',j);
    /* Store N, the number of dose levels, in a macro variable &n */
    call symput('N',trim(left(_n_)));
  run;

/* Find the CI for the LD50 using binomial bounds */
%binomial(data=combined_&data, id=&id);
```

```
data results_&data;
  &idvar=&id;
  retain LD50_est;
  %if & LD50_lcl ne %then %do;
  LD50_lcl=&LD50_lcl;
  %end;
  %if &LD50_ucl ne %then %do;
    LD50_ucl=&LD50_ucl;
  %end;
run;

%mend justci;



/* NOTEST.SAS */
/* Macro program to give missing values for LD50 and slope ests and CIs */
/* Author: Carol Yarrow                                                  */
/* Date: 08/03/2008                                                      */

%macro notest(data=, idvar=, id=);
data results_&data;
  &idvar=&id;
run;
%mend notest;
/* BINOMIAL.SAS
   Macro program to estimate confidence limits for LD50 using Binomial confidence
   bounds    */
/* Author: Carol Yarrow                                                  */
/* Date: 08/03/2008                                                      */

%macro binomial(data=, id=);

  /* Estimate CI for LD50 using Binomial upper and/or lower 97.5% confidence bounds
     Transpose the NDEAD and NEXPO data to give a single row with one column for each
     dose level */

  proc transpose data=&data out=tran_ndead prefix=NDEAD_;
    var ndead;
  run;

  proc transpose data=&data out=tran_nexpo prefix=NEXPO_;
    var nexpo;
  run;

  proc transpose data=&data out=tran_dose prefix=DOSE_;
    var rounded_dose;
  run;

  /* Merge the NDEAD and NEXPO data together.
     Calculate the binomial cumulative distribution function for each dose level to
     find:
```

```
    - the probability of observing at MOST NDEAD responses from a binomial
      dist with sample size NEXPO and prob 0.5, CB=cdf('binom',ndead,0.5,nexpo) and
    - the probabiltiy of observing at LEAST NDEAD responses from a binomial dist
      with sample size NEXPO and prob 0.5, RB=1-cdf('binom',ndead-1,0.5,nexpo)*/

 data bounds;
  array ndead(&n) NDEAD_1-NDEAD_&n;
  array nexpo(&n) NEXPO_1-NEXPO_&n;
  array dose(&n) DOSE_1-DOSE_&n;
  array cb(&n) cb_1-cb_&n;
  array rb(&n) rb_1-rb_&n;
  merge tran_ndead tran_nexpo tran_dose;
  do i=1 to &n;
   cb(i)=cdf('binom',ndead(i),0.5,nexpo(i));
   rb(i)=1-cdf('binom',ndead(i)-1,0.5,nexpo(i));
  end;

  /* LOWER BOUND */
  /* If the median dose is observed (&d ne missing), then the highest dose below
     the LD50 is &d-1.   */
  /* Else macro variable &j represents the highest dose level below the est LD50.
    Determine whether cb(&j)<=(100-97.5).
     - If true then dose(&j) is lower 97.5% bound for the LD50*/

  %if &d ne  %then %do;
   if &d-1>0 then do;
    if cb(&d-1)<=(1-0.975) then LD50_lcl=dose(&d-1);

 /*  - If false then determine whether cb(&d-1)*cb(&d-2)<=(100-97.5).
      - If true then dose(&d-2) is lower 97.5% bound for the LD50.
        Continue stepping down through doses and multiplying until lower bound is
        found or there are no more dose levels and &d-1-k=0 */

     else do;
      l=cb(&d-1);
      do k=1 to &n;
       if &d-1-k>=1 and LD50_lcl=. then do;
         l=l*cb(&d-1-k);
         if l<=(1-0.975) then LD50_lcl=dose(&d-1-k);
       end;
      end;
    end;
   end;
  %end;

  %else %if &j ne  %then %do;
   if &j>0 then do;
    if cb(&j)<=(1-0.975) then LD50_lcl=dose(&j);
    /* - If false then determine whether cb(&j)*cb(&j-1)<=(100-97.5).
       - If true then dose(&j-1) is lower 97.5% bound for the LD50.
       Continue stepping down through doses and multiplying until lower bound is
       found or there are no more dose levels and &j-k=0 */
```

```
    else do;
     l=cb(&j);
     do k=1 to &n;
       if &j-k>=1 and LD50_lcl=. then do;
         l=l*cb(&j-k);
        if l<=(1-0.975) then LD50_lcl=dose(&j-k);
       end;
     end;
   end;
  end;
%end;

/* Store the value of LD50_lcl in a macro variable & LD50_lcl */
 call symput(' LD50_lcl', LD50 _lcl);

/* UPPER BOUND */
/* Macro variable &j+1 represents the lowest dose level above the estimated LD50.
   Determine whether rb(&j+1)<=(100-97.5).
   If true then dose(&j+1) is upper 97.5% bound for the LD50*/

 %if &d ne  %then %do;
  if &d+1<=&n then do;
    if rb(&d+1)<=(1-0.975) then LD50_ucl=dose(&d+1);

   /* If false then determine whether rb(&d+1)*rb(&d+2)<=(100-97.5).
     If true then dose(&d+2) is upper 97.5% bound for the LD50.
     Continue stepping up through doses and multiplying until upper bound is
     found or there are no more dose levels and &d+1+k>&n */

    else do;
      u=rb(&d+1);
      do k=1 to &n;
        if &d+1+k<=&n and LD50_ucl=. then do;
          u=u*rb(&d+1+k);
         if u<=(1-0.975) then LD50_ucl=dose(&d+1+k);
        end;
      end;
    end;
  end;
 %end;

%else %do;
  if &j+1<=&n then do;
    if rb(&j+1)<=(1-0.975) then LD50_ucl=dose(&j+1);

    /* If false then determine whether rb(&j+1)*rb(&j+2)<=(100-97.5).
      If true then dose(&j+2) is upper 97.5% bound for the LD50.
      Continue stepping up through doses and multiplying until upper bound is
      found or there are no more dose levels and &j+1+k>&n */

    else do;
     u=rb(&j+1);
     do k=1 to &n;
```

```
        if &j+1+k<=&n and LD50_ucl=. then do;
          u=u*rb(&j+1+k);
          if u<=(1-0.975) then LD50_ucl=dose(&j+1+k);
         end;
        end;
       end;
     end;
    %end;

 /* Store the value of LD50_ucl in a macro variable & LD50_ucl */
   call symput(' LD50_ucl', LD50_ucl);
  run;
%mend binomial;
```

Annex 3 of appendix 2: Programs to validate SAS program, results.sas.

```
/* VALIDATE RESULTS.SAS     */
/* Program to validate presentation of the results of the simulations according to
   Section 4 */
/* Input data: RD.ESTS_STG4
/* Author: Carol Yarrow                                    */
/* Date: 08/03/2008                                       */

%include "d:\2008\oecd validation\csy\programs\macros\csy_results.sas";

data ests_stg4;
  set rd.ests_stg4;
  log_ LD50 =log10(LD50_est);
  label log_ LD50="log LD50 Estimate"
       slope="True slope"
       b1="Slope Estimate"
       LD50="True LD50";
run;

%results(data=ests_stg4, title="Presentation of results of ESTS_STG4");
/* CSY_RESULTS.SAS */
/* Program to create macro to present the results of the simulations according to
   Section 4 */
/* Author: Carol Yarrow                                    */
/* Date: 08/03/2008                                       */


%macro results(data=&data, title="Presentation of results from ESTS_STG4",
            outdata="d:\temp\results.csv");

/* Produce boxplot of results */
%macro boxplot(data=, var=, group=, LD50=, slope=, where=, vaxis=, vformat=4.1,
            title=, title2=);

  goptions reset=all;
  goptions fontres=presentation ftext=duplex htext=1.3;
```

```
symbol v=star h=1.5;
%if & LD50 ne %then %let vref=%sysfunc(log10(&LD50));
%if &slope ne %then %let vref=&slope;
proc boxplot data=&data;
  title &title;
  title2 &title2;
  plot &var*&group / vref=&vref &vaxis vformat=&vformat
                clipfactor=1.5 cliplegend='# Boxes clipped' clipsubchar = '#'
                cliplegpos=top clipsymbol=square;
  insetgroup n / position=topoff;
  &where;
run;
%mend boxplot;

options mprint;
%boxplot(data=&data, var=log_LD50, group=slope, LD50=5, where=where LD50=& LD50,
        title=&title, vaxis=vaxis=(-0.5 to 1.5 by 0.5), title2="Box and whisker
        plot of LD50 estimates where true LD50=& LD50 ");

%boxplot(data=&data, var=log_ LD50, group=slope, LD50=50, where=where LD50=&LD50,
        title=&title, vaxis=vaxis=(0.5 to 3.0 by 0.5), title2="Box and whisker
        plot of LD50 estimates where true LD50=& LD50");

%boxplot(data=&data, var=log_LD50, group=slope, LD50=1500, where=where LD50=&LD50,
        title=&title, vaxis=vaxis=(2.0 to 4.0 by 0.5), title2="Box and whisker
        plot of LD50 estimates where true LD50=&LD50");

%boxplot(data=&data, var=b1, group=LD50, slope=2, where=where slope=&slope,
        title=&title, vaxis=vaxis=(0 to 30 by 5), vformat=2., title2="Box and
        whisker plot of slope estimates where true slope=&slope");

%boxplot(data=&data, var=b1, group=LD50, slope=5, where=where slope=&slope,
        title=&title, vaxis=vaxis=(0 to 30 by 5), vformat=2., title2="Box and
        whisker plot of slope estimates where true slope=&slope");

%boxplot(data=&data, var=b1, group=LD50, slope=10, where=where slope=&slope,
        title=&title, vaxis=vaxis=(0 to 30 by 5), vformat=2., title2="Box and
        whisker plot of slope estimates where true slope=&slope" );

/* Print out percentiles of final LD50 and slope estimates */
proc means data=&data nway noprint;
  var LD50_est b1;
  class LD50 slope;
  output out=percentiles n= p25= p50= p75= / autoname;
run;

/* Find the percentiles of LD50 on the log10 scale */
data percentiles;
  set percentiles;
  log_LD50_p25=log10(LD50_est_p25);
  log_LD50_p50=log10(LD50_est_p50);
  log_LD50_p75=log10(LD50_est_p75);
run;
```

```
  title &title;
  title2 "Percentiles of the final LD50 estimates on a log 10 scale";
  proc print data=percentiles label noobs;
    var LD50 slope LD50_est_n log_LD50_p25 log_LD50_p50 log_LD50_p75;
    format log_LD50_p25 log_LD50_p50 log_LD50_p75 8.4;
    label LD50_n="No. of Final Estimates"
          log_LD50_p25="25th percentile"
          log_LD50_p50="50th percentile"
          log_LD50_p75="75th percentile";
  run;

  title2 "Percentiles of the final slope estimates";
  proc print data=percentiles label;
    var LD50 slope b1_n b1_p25 b1_p50 b1_p75;
    format b1_p25 b1_p50 b1_p75 8.4;
    label b1_n="No. of Final Estimates"
          b1_p25="25th percentile"
          b1_p50="50th percentile"
          b1_p75="75th percentile";
  run;

  proc export data=percentiles outfile=&outdata dbms=csv replace;
  run;

%mend results;
```

Annex 4 of appendix 2: Programs to validate SAS programs, *stg12.sas*, *stg3.sas* and *stg4.sas*.

```
/* RUN SIMULATIONS.SAS                                          */
/* Program to simulate dose response data according to Section 2.1 and 2.2
   Output dataset CSY.CSY_STG4 and CSY.RESULTS.CSY_STG4 */
/* Author: Carol Yarrow                                         */
/* Date: 08/03/2008                                            */

libname rd "d:\2008\oecd validation\rd";
libname csy "d:\2008\oecd validation\csy";

%include "d:\2008\oecd validation\csy\programs\csy_stg12.sas";
%include "d:\2008\oecd validation\csy\programs\csy_stg3.sas";
%include "d:\2008\oecd validation\csy\programs\csy_stg4.sas";

/* Present results of simulations */
%include "d:\2008\oecd validation\csy\programs\macros\csy_results.sas";
options mprint;
%results(data=csy.results_csy_stg4, title="Presentation of results of CSY_STG4");


/* CSY_STG12.SAS                                                */
/* Program to simulate dose response data according to Section 2.1 and 2.2
   Output dataset CSY.CSY_STG12 and CSY.RESULTS_CSY_STG12 */
/* Author: Carol Yarrow                                         */
/* Date: 08/03/2008                                            */

options mprint;
%include "d:\2008\oecd validation\csy\programs\macros\dose.sas";
%include "d:\2008\oecd validation\csy\programs\macros\mortality.sas";
%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";
%global seed;

/* STAGE 1*/

/* Generate a computer simulated guess of the LD50 using random number from normal
   distribution with mean equal to true log10(LD50) and variance consistent with the
   95th and 5th percentile being a factor of 10 greater or less than the true LD50.
   This corresponds to the 5th percentile of logLD50 being 1 less than the true
   logLD50. As the 5th percentile of the  normal distribution is 1.6449 SDs
   less than the true mean, this corresponds to using a SD of 1/1.6449 */

/* Generate initial guess of LD50 for each of 9,000 experiments */
title;
data csy.LD50;
  retain seed 5674564;
  do Expt_id=1 to 9000;
    /* Select random number from the standard normal distribution */
    call rannor(seed,x);
```

```
   /* Define true LD50 and true slope values for each experiment */
   /* True LD50=5 for expts 1-3000,
           =50 for expts 3001-6001 and
           =1500 for expts 6001-9000 */
   if 1<=Expt_id<=3000 then LD50=5;
   if 3001<=Expt_ID<=6000 then LD50=50;
   if 6001<=Expt_ID<=9000 then LD50=1500;

   /* True slope=2 for expts 1-1000, 3001-4000 and 6001-7000 */
   /* True slope=5 for expts 1001-2000, 4001-5000 and 7001-8000 */
   /* True slope=10 for expts 2001-3000, 5001-6000 and 8001-9000 */
   if 1<=Expt_ID<=1000 or 3001<=Expt_ID<=4000 or 6001<=Expt_ID<=7000 then slope=2;
   if 1001<=Expt_ID<=2000 or 4001<=Expt_ID<=5000 or 7001<=Expt_ID<=8000 then
     slope=5;
   if 2001<=Expt_ID<=3000 or 5001<=Expt_ID<=6000 or 8001<=Expt_ID<=9000 then
     slope=10;

   /* Create mu as the log10 of the true LD50*/
   mu=log10(LD50);

   /* Use the random number from normal dist to generate random number from normal
      distribution with mean log10(LD50) and standard deviation of 1/1.6449 */
   log LD50_guess=mu+(1/1.6449)*x;
   LD50_guess=10**log LD50_guess;
   Slope_guess=.;

   /* Save the last value of SEED in a macro variable to use as starting seed
     for next sequence of random numbers */
   call symput('seed',seed);
   output;
  end;
  drop x mu logLD50_guess;
run;



/* Run PROC MEANS to verify that the 5th percentile and 95th percentile of the
   estimated LD50s from each distribution (for each different value of mu) are a
   factor of 10 greater or less than the true value.  Verified. */

title "Confirmation that the 5th and 95th percentiles are a factor of 10 greater or
      less than true mu";
proc means data=csy. LD50 p5 median p95;
  var LD50_guess;
  class LD50
run;

%let lowlim=1;
%let upplim=3330;

/* Generate doses for Stage 1 */
data stage1_doses;
  set csy. LD50;
```

```
  dose1= LD50_guess/sqrt(50);
  dose4= LD50_guess*sqrt(50);
  if dose1<&lowlim then do;
    dose1=&lowlim;
    dose4=dose1*50;
  end;
  if dose4>&upplim then do;
    dose4=&upplim;
    dose1=dose4/50;
  end;
  dose2=dose1*50**(1/3);
  dose3=dose1*50**(2/3);
  nexpo=1;
run;

/* Generate the mortality data for Stage 1 */
%mortality(stage=1, ndoses=4);

/* Generate the working estimate of the LD50 from Stage 1 using Table 1 */
/* Tranpose NDEAD for doses 1-4 */
proc transpose data=stage1 out=ndead_stage1;
  var ndead;
  id _name_;
  by expt_id;
run;

/* Transpose the DOSE level for doses 1-4 */
proc transpose data=stage1 out=dose_stage1;
  var dose;
  id _name_;
  by expt_id;
run;

data csy.stage1_results;
  merge ndead_stage1(rename=(dose1=ndead1 dose2=ndead2 dose3=ndead3 dose4=ndead4))
        dose_stage1;
  by expt_id;
  dose0=dose1/50**(1/3);
  dose5=dose4*50**(1/3);
  /* Apply the rules on table 1 */
  if ndead1=0 and ndead2=0 and ndead3=0 and ndead4=0 then
     LD50_guess=sqrt(dose4*dose5);
  if ndead1=0 and ndead2=0 and ndead3=0 and ndead4=1 then
     LD50_guess=sqrt(dose3*dose4);
  if ndead1=0 and ndead2=0 and ndead3=1 and ndead4=0 then
     LD50_guess=sqrt(dose3*dose4);
  if ndead1=0 and ndead2=1 and ndead3=0 and ndead4=0 then LD50_guess=dose3;
  if ndead1=1 and ndead2=0 and ndead3=0 and ndead4=0 then
     LD50_guess=sqrt(dose2*dose3);
  if ndead1=0 and ndead2=0 and ndead3=1 and ndead4=1 then
     LD50_guess=sqrt(dose2*dose3);
  if ndead1=0 and ndead2=1 and ndead3=1 and ndead4=0 then LD50_guess=dose3;
  if ndead1=1 and ndead2=1 and ndead3=0 and ndead4=0 then
```

```
    LD50_guess=sqrt(dose2*dose3);
 if ndead1=0 and ndead2=1 and ndead3=0 and ndead4=1 then
    LD50_guess=sqrt(dose2*dose3);
 if ndead1=1 and ndead2=0 and ndead3=1 and ndead4=0 then
    LD50_guess=sqrt(dose2*dose3);
 if ndead1=1 and ndead2=0 and ndead3=0 and ndead4=1 then LD50_guess=dose2;
 if ndead1=0 and ndead2=1 and ndead3=1 and ndead4=1 then
    LD50_guess=sqrt(dose1*dose2);
 if ndead1=1 and ndead2=0 and ndead3=1 and ndead4=1 then
    LD50_guess=sqrt(dose1*dose2);
 if ndead1=1 and ndead2=1 and ndead3=0 and ndead4=1 then LD50_guess=dose2;
 if ndead1=1 and ndead2=1 and ndead3=1 and ndead4=0 then
    LD50_guess=sqrt(dose2*dose3);
 if ndead1=1 and ndead2=1 and ndead3=1 and ndead4=1 then
    LD50_guess=sqrt(dose0*dose1);
 keep expt_id LD50_guess;
run;

/* STAGE 2 */
/* Combine the working LD50 estimate from Stage 1 with the true LD50 and slope */
/* Calculate the doses for Stage 2 */
data stage2_doses;
 merge csy.stage1_results csy. LD50 (drop=LD50_guess slope_guess);
 array dose(10);
 by expt_id;
 /* Calculate lowdose and highdose */
 %dose(lowprob=0.01, highprob=0.99, LD50_guess= LD50_guess, slope_guess=5);
 do i=1 to 10;
  dose(i)=lowdose*r**((i-1)/9);
 end;
 nexpo=1;
 drop i;
run;

/* Generate the mortality data for Stage 2 */
%mortality(stage=2, ndoses=10);

/* Concatenate the data from Stages 1 and 2 together */
data csy.csy_stg12;
 set stage1 stage2;
 by expt_id;
run;

/* Analyse the data from Stages 1 and 2 together */
options nomprint;
proc printto log="d:\2008\oecd validation\csy\log files\log file csy_stg12.txt";
run;
%analysis(lib=csy, data=csy_stg12, nexpts=9000, idvar=expt_id, where=);
proc printto;
run;
/* CSY_STG3.SAS                                    */
/* Program to simulate dose response data according to Section 2.1 and 2.2
   Input dataset CSY.CSY_STG12 and CSY.RESULTS_CSY_STG12
```

```
   Output dataset CSY.CSY_STG3 and CSY.RESULTS_CSY_STG3 */
/* Author: Carol Yarrow                                   */
/* Date: 08/03/2008                                       */

%include "d:\2008\oecd validation\csy\programs\macros\dose.sas";
%include "d:\2008\oecd validation\csy\programs\macros\mortality.sas";
%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";

/* STAGE 3*/
/* Combine the working LD50 and slope estimates from Stage 2 with the true LD50 and slope */

options mprint;
data stage3_doses;
  merge csy.results_csy_stg12(keep=expt_id nreversals LD50_guess b1
                        rename=(b1=slope_guess))
        csy. LD50 (drop= LD50_guess slope_guess);
  by expt_id;
  array dose(5);

  /* Calculate the doses for Stage 3 */
  /* If the working estimate of the slope is greater than 15 or less than one it is
     Set to these bounds */
  if slope_guess<1 and slope_guess ne . then slope_guess=1;
  if slope_guess>15 then slope_guess=15;

  /* If at the end of Stage 2 there are 2 or more reversals and a working estimate of
     The slope has been obtained, stage 3a is used. Otherwise Stage 3b is used */

  /* Stage 3a */
  if nreversals>=2 and slope_guess ne . then do;
    /* Calculate lowdose and highdose */
    %dose(lowprob=0.15, highprob=0.85, LD50_guess= LD50_guess,
          slope_guess=slope_guess);
    dose1=lowdose;
    dose2=highdose;
    nexpo=5;
  end;

  /* Stage 3b */
  else do;
    /* Calculate lowdose and highdose */
    %dose(lowprob=0.15, highprob=0.85, LD50_guess= LD50_guess, slope_guess=5);
    do i=1 to 5;
      dose(i)=lowdose*r**((i-1)/4);
    end;
    nexpo=2;
  end;
run;

/* Generate the mortality data for Stage 3 */
options mprint;
%mortality(stage=3, ndoses=5);
```

```
/* Concatenate the data from Stages 1, 2 and 3 together */
data csy.csy_stg3;
  set csy.csy_stg12 stage3;
  by expt_id;
run;

/* Analyse the data from Stages 1 and 2 together */
proc printto log="d:\2008\oecd validation\csy\log files\log file csy_stg3.txt";
run;
%analysis(lib=csy, data=csy_stg3, nexpts=9000, idvar=expt_id, where=);
proc printto;  run; /* CSY_STG4.SAS                                          */
/* Program to simulate dose response data according to Section 2.1 and 2.2
   Input dataset CSY.CSY_STG3 and CSY.RESULTS_CSY_STG3
   Output dataset CSY.CSY_STG4 and CSY.RESULTS_CSY_STG4 */
/* Author: Carol Yarrow                                                    */
/* Date: 08/03/2008                                                        */

%include "d:\2008\oecd validation\csy\programs\macros\dose.sas";
%include "d:\2008\oecd validation\csy\programs\macros\mortality.sas";
%include "d:\2008\oecd validation\csy\programs\macros\csy_analysis.sas";

/* STAGE 4*/
/* If there are two or more reversals and/or twwo or more partial kills at the end of
   Stage 3 then the experiment stops. Otherwise the experiment continues to stage 4*/

/* Combine the working LD50 and slope estimates from Stage 3 with the true LD50 and
   slope */

data stage4_doses;
  merge csy.results_csy_stg3(keep=expt_id nreversals npartials LD50_guess b1
                        rename=(b1=slope_guess))
        csy. LD50 (drop= LD50_guess slope_guess);
  by expt_id;
  array dose(5);

  /* If the working estimate of the slope is greater than 15 or less than one it is
     set to these bounds */
  if slope_guess<1 and slope_guess ne . then slope_guess=1;
  if slope_guess>15 then slope_guess=15;

  /* If at the end of Stage 3 there are 2 or more reversals and/or two or more
     Partial kills and a working estimate of the slope then the experiment stops.
     Else continues to Stage 4 */
  if (nreversals>=2 or npartials>=2) and slope_guess ne . then delete;
  /* Calculate the doses for Stage 4 */
  else do;
    /* Calculate lowdose and highdose */
    %dose(lowprob=0.15, highprob=0.85, LD50_guess= LD50_guess, slope_guess=5);
    do i=1 to 5;
      dose(i)=lowdose*r**((i-1)/4);
      end;
      nexpo=2;
  end;
```

```
run;

/* Generate the mortality data for Stage 4 */
options mprint;
%mortality(stage=4, ndoses=5);

/* Concatenate the data from Stages 1, 2, 3 and 4 together */
data csy.csy_stg4;
  set csy.csy_stg3 stage4;
  by expt_id;
run;

/* Analyse the data from Stages 1, 2, 3 and 4 together */
options nomprint;
proc printto log="d:\2008\oecd validation\csy\log files\log file csy_stg4.txt";
run;
%analysis(lib=csy, data=csy_stg4, nexpts=9000, idvar=expt_id, where=);
proc printto;
run;



/* DOSE.SAS                                                    */
/* Macro program to calculate lowdose and highdose based ib LD50_guess
   and Slope_guess*/
/* Author: Carol Yarrow                                        */
/* Date: 08/03/2008                                            */


%macro dose(lowlim=1, upplim=3330, lowprob=0.01, highprob=0.99, LD50_guess= LD50_guess,
slope_guess=slope_guess);
    lowdose=10**(probit(&lowprob)/&Slope_guess+log10(&LD50_guess));
    highdose=10**(probit(&highprob)/&Slope_guess+log10(&LD50_guess));
    /* Calculate the low dose to high dose ratio */
    r=highdose/lowdose;
    if lowdose<&lowlim then do;
      lowdose=&lowlim;
      highdose=lowdose*r;
    end;
    if highdose>&upplim then do;
      highdose=&upplim;
      lowdose=highdose/r;
    end;
%mend dose;
/* MORTALITY.SAS                                               */
/* Macro program to calculate mortality at each stage          */
/* Author: Carol Yarrow                                        */
/* Date: 08/03/2008                                            */


%macro mortality(stage=1, ndoses=4);

proc transpose data=stage&stage._doses out=tran_stage&stage._doses(rename=(col1=Dose));
```

```
  var dose1-dose&ndoses;
  by expt_id LD50 slope nexpo;
run;

/* Generate the mortality data for each dose */
data stage&stage;
  Stage=&stage;
  set tran_stage&stage._doses;
  if dose=. then delete;

  /* Use the current value of the macro variable SEED to initialise seed for
     random number generation */
  retain seed &seed;
  logdose=log10(dose);
  logLD50=log10(LD50);

  /* Model the probability of death at each dose using the probit model */
  if index(_NAME_,'dose')>0 then do;
    probit=(-logLD50*slope) + (slope*logdose);
    p=probnorm(probit);
    /* Generate the number dead at each dose using Binomial random number */
    if p=1 then ndead=nexpo;
      if p=0 then ndead=0;
    if p<1 and p>0 then call ranbin(seed,nexpo,p,ndead);
      nalive=nexpo-ndead;
  end;

  /* Save the last value of SEED in a macro variable to use as starting seed
     for next sequence of random numbers */
  call symput('seed',seed);
run;

%mend mortality;
```

Annex 5 of appendix 2: Summary of investigation into seed behaviour in SAS

Consequences of using systematic seeds

The advantage of specifying seeds in a systematic manner for each experiment in a simulation study is that it is easy to regenerate the data for any particular experiment as the seeds for each experiment are known. However, detailed investigation has shown that the use of these systematic seeds appears to introduce a 'non-random' element into the samples generated.

In order to illustrate this, 100 samples of 1,000 values were generated from a binomial distribution with n=10 and p=0.5 using:

a) Seeds generated in a systematic manner.
   i.e. for i=1 to 100 and j=1 to 1000:
   - seed = 1000*(i-1) + j; (1 to 1000, 1001 to 2000, 2001 to 3000 etc.)

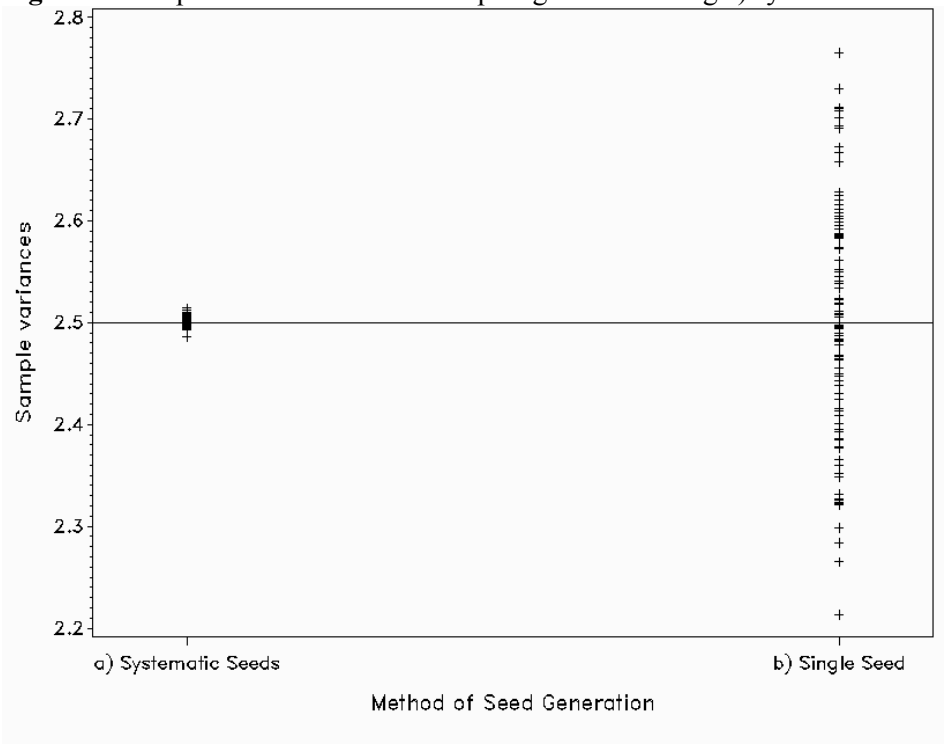
b) Seeds generated by specifying a single initial seed
   - retain seed 76807689;

As the data is binomially distributed, the expected variance within each of the 100 samples is np(1-p).

$$n*p*(1-p) = 10*0.5*0.5$$
$$= 2.5.$$

Figure 4 shows the estimated within-sample variance for each of the 100 samples generated by each method. The horizontal line shows the expected value of 2.5.

Figure 4: Sample variances for 100 samples generated using a) systematic seeds and b) a single seed



- For data generated by using both method a), systematic seeds, and method b), a single initial seed, the observed variance of each sample was centred round the expected value of 2.5.
- However, note that the spread of the within-sample variances of samples generated using systematic seeds is far less than that for samples generated using a single initial seed. In other words, the within-sample variances of those samples generated using systematic seeds is close to the expected value for each sample.

In order to determine which of these methods was producing data that behaved as we would expect, the variance of the sample means for each method was compared to its theoretical value. The expected variance of the sample means is np(1-p)/m where m is the number of values in each sample (in each case m=1000).

$$n*p*(1-p)/1000 = 2.5/1000 = 0.0025.$$

- For data generated using systematic seeds the observed variance of the sample means was **0.0000390**. This is much less (64 times less) than the expected value of 0.0025.
- For data generated using a single initial seed the observed variance of the sample means was **0.00265**. This is very close to the expected value of 0.0025.

The between-sample variation of data generated using systematic seeds in this example is therefore far less than we would expect if it were truly random. Further investigation identified that the causes of this problem are as follows:

- There is a cyclic relationship between the value of the initial seed and the value of the subsequent seed (see Figure 5 below).

- There is a direct relationship between the value of the seed used to select NDEAD from the binomial distribution and the value of NDEAD itself (see Figure 6 below).

Figure 5: Relationship between initial and subsequent values of SEED

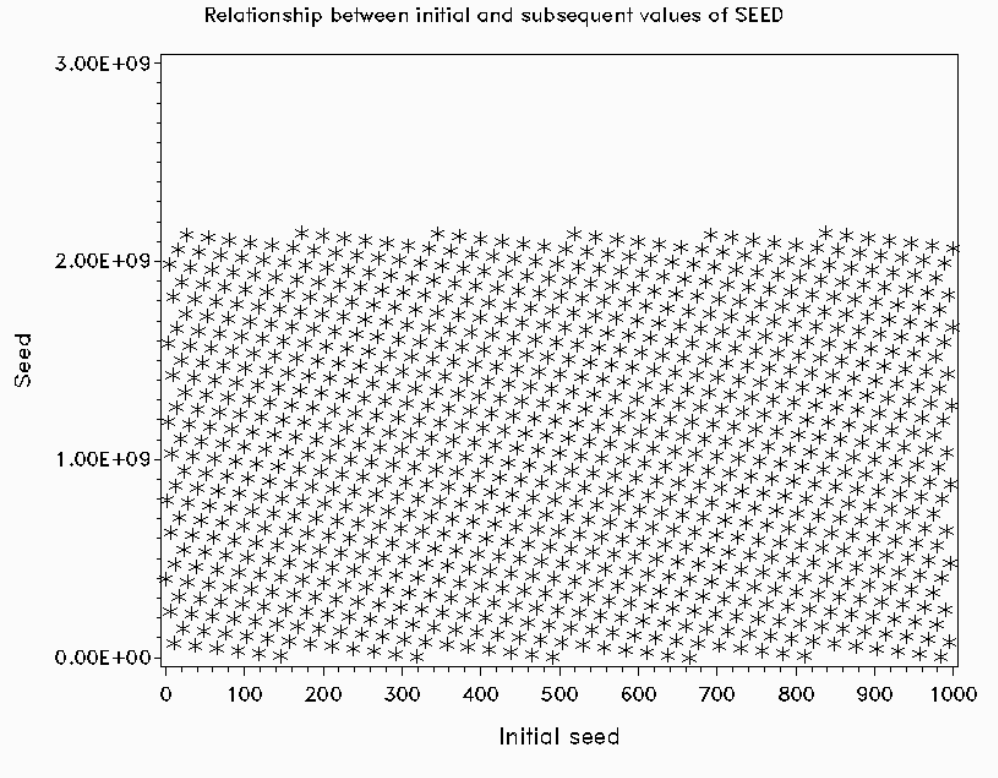
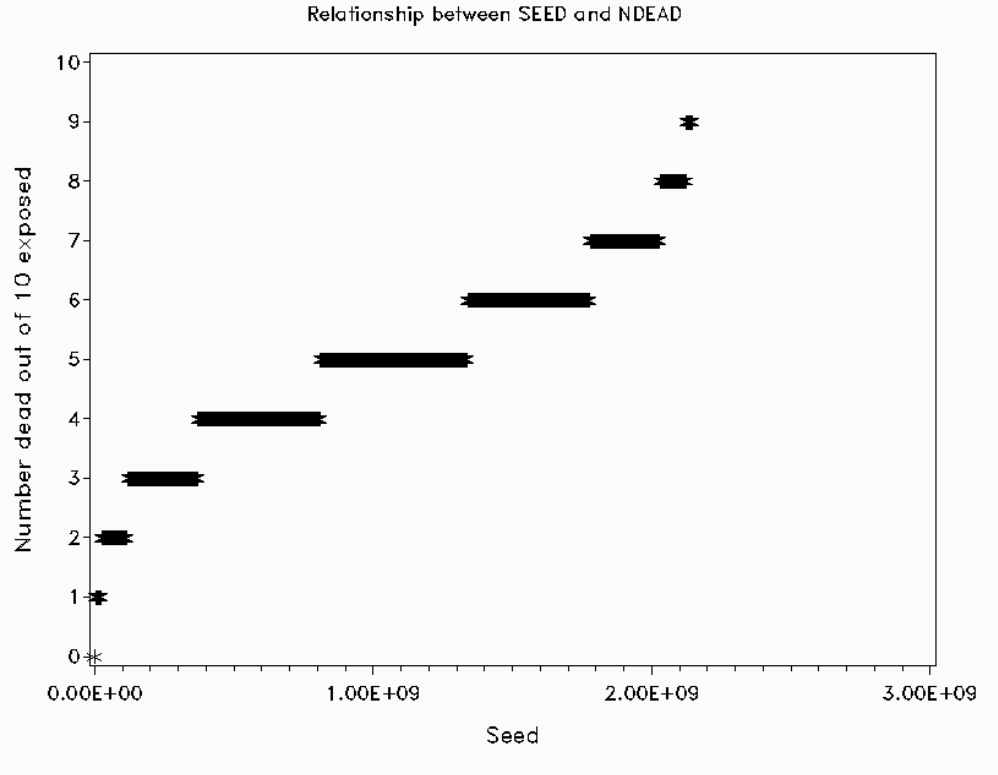


Figure 6: Relationship between value of SEED and value of NDEAD



Appendix III – Evaluation of clarity of the guideline through written quizzes.

A. TG223 Avian Acute Oral Toxicity Test - Reading Comprehension Exam without answers (March 2008)

B. TG223 Avian Acute Oral Toxicity Test - Reading Comprehension Exam with correct answers (March 2008)

C. TG223 Avian Acute Oral Toxicity Test - Results from First Quiz to Evaluate Guideline Clarity (based on March 2007 draft, summary prepared September 2007)

D. TG223 Avian Acute Oral Toxicity Test - Results from Second Quiz to Evaluate Guideline Clarity (based on March 2008 draft, summary prepared September 2009)

A. TG223 Avian Acute Oral Toxicity Test - Reading Comprehension Exam without answers (March 2008)

1. Assume a 0 represents survival and a 1 represents a kill. For each of the following sequences, identify the number of reversals. The sequences are listed to represent increasing dose (from left to right) and only one bird was tested at each dose.

   0001011111 _____
   0011101111 _____
   0101000001 _____
   0001010101 _____

2. If you conduct a limit test and one bird dosed at the limit dies, what is the next step?

3. If you conduct a limit test and a control bird dies, what is the next step?

4. Compute the working estimate of the LD_{50} based on the following Stage 1 results.

   a.

Dose	56.56	208.37	766.80	2828.4
Result *	0	0	1	0

   * 0 = survival, 1=death

   b.

Dose	26.9	99.0	364.6	1343.3
Result *	0	1	1	1

   * 0 = survival, 1=death

5. For the following scenarios, identify the correct sequence of doses for stage 2.

   a. Working estimate of LD_{50} = 425

   i.

145.6	184.7	234.3	297.3	377.3	478.7	607.3	770.6	977.7	1240.6

   ii.

145.6	215.3	318.3	470.7	696.0	1029.3	1522.1	2250.8	3328.3	4921.8

   iii.

162.7	206.4	261.9	332.3	421.6	535.0	678.8	861.3	1092.8	1386.5

   b. Limit test with 5 birds at 2000mg/kg, 3 mortalities at limit dose

   i.

391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0

   ii.

610.3	774.4	982.6	1246.7	1581.8	2007.0	2546.5	3231.1	4099.6	5201.7

   iii.

379.8	481.9	611.5	775.9	984.4	1249.0	1584.8	2010.8	2551.3	3237.2

6. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

a. Stage 1

Dose	7.1	26.0	96.0	353.5
Result	0	0	1	0

Stage 2

Dose	63.1	80.0	101.6	128.9	163.5	207.5	263.2	334.0	423.8	537.7
Result	0	0	0	0	0	1	1	1	1	1

i. 5 birds at 82.7 and 5 birds at 412.1
ii. 5 birds at 114.6 and 5 birds at 297.5
iii. 2 birds at each of the following doses:
114.5, 145.4, 184.6, 234.3, and 297.4

b. Limit test at 2000 mg/kg – observe 1 mortality out of 5 birds, signs of toxicity in 2 other birds.

Stage 2

Dose	391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0
Result	0	0	0	0	0	0	0	1	1	1

i. 2 birds at each of the following doses:
1246.0, 1581.7, 2007.8, 2548.8, 3235.5
ii. 5 birds at 1282.5 and 5 birds at 3330
iii. 2 birds at each of the following doses:
1234.8, 1567.5, 1989.8, 2525.9, 3206.5

7. What is the next step for the following scenario?
   a. Conduct limit test at 2000 mg/kg – observe 1 mortality out of 5 birds
   b. Then conduct second limit test at 2000 mg/kg – observe no additional mortalities, no signs of toxicity in any birds.

8. Under what conditions is stage 4 necessary?

9. Determine the number of partials and reversals for each of the following scenarios. The sequences are listed in increasing dose from left to right.
(this question was not in the quiz based on the 2007 draft guideline)

a. Partials _____ Reversals _____

Number of mortalities	0	0	0	1	0	1	2	1	1
Number of tested individuals	1	1	1	2	1	1	2	1	1

b. Partials _____ Reversals _____

Number of mortalities	0	0	1	3	0	1	1	4	1
Number of tested individuals	1	1	1	5	1	1	1	5	1

TG223 Avian Acute Oral Toxicity Test - Reading Comprehension Exam with correct answers (March 2008)

Assume a 0 represents survival and a 1 represents a kill. For each of the following sequences, identify the number of reversals. The sequences are listed to represent increasing dose (from left to right) and only one bird was tested at each dose.

0001011111 _____ 1
0011101111 _____ 1
0101000001 _____ 2
0001010101 _____ 3

1. If you conduct a limit test and one bird dosed at the limit dies, what is the next step?
if no signs of tox in other birds, dose 5 more birds at limit
If signs of tox in other birds, go to stage 2

2. If you conduct a limit test and a control bird dies, what is the next step?
If mortality could possibly be due to husbandry, need to redo test. If mortality could be considered incidental background (not related to conduct of test), random mortality – then add an additional 5 control birds.

3. Compute the working estimate of the LD_{50} based on the following Stage 1 results.

a.

Dose	56.56	208.37	766.80	2828.4
Result *	0	0	1	0

* 0 = survival, 1=death

1472.7

b.

Dose	26.9	99.0	364.6	1343.3
Result *	0	1	1	1

* 0 = survival, 1=death

51.6

4. For the following scenarios, identify the correct sequence of doses for stage 2.

a. Working estimate of LD_{50} = 425

i. correct

145.6	184.7	234.3	297.3	377.3	478.7	607.3	770.6	977.7	1240.6

ii. LD_{50}=425 step = 50^(1/10), Max/min value for hdose removed

145.6	215.3	318.3	470.7	696.0	1029.3	1522.1	2250.8	3328.3	4921.8

iii. correct step, LD_{50} = 475

162.7	206.4	261.9	332.3	421.6	535.0	678.8	861.3	1092.8	1386.5

b. Limit test with 5 birds at 2000mg/kg, 3 mortalities at limit dose

i. Correct

391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0

ii. Max value for hdose removed

610.3	774.4	982.6	1246.7	1581.8	2007.0	2546.5	3231.1	4099.6	5201.7

iii. Used working estimate of LD_{50}=1109

379.8	481.9	611.5	775.9	984.4	1249.0	1584.8	2010.8	2551.3	3237.2

5. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

a. Stage 1

Dose	7.1	26.0	96.0	353.5
Result	0	0	1	0

Working LD_{50}=184.2

Stage 2

Dose	63.1	80.0	101.6	128.9	163.5	207.5	263.2	334.0	423.8	537.7
Result	0	0	0	0	0	1	1	1	1	1

Working LD_{50} = 184.6

Working slope= 2.97

i. 5 birds at 82.7 and 5 birds at 412.1 correct
ii. 5 birds at 114.6 and 5 birds at 297.5 assume slope=5
iii. 2 birds at each of the following doses: correct for 3b stage
114.5, 145.4, 184.6, 234.3, and 297.4

b. Limit test at 2000 mg/kg – observe 1 mortality out of 5 birds, signs of toxicity in 2 other birds.

Working LD_{50}=2947

Stage 2

Dose	391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0
Result	0	0	0	0	0	0	0	1	1	1

Working LD_{50} = 1990

Working slope= not available (only one partial kill)

i. 2 birds at each of the following doses:
1246.0, 1581.7, 2007.8, 2548.8, 3235.5 stage 3b, calc done with 4 mortalities at 2000 mg/kg
ii. 5 birds at 1282.5 and 5 birds at 3330 stage 3a, assume slope=5
iii. 2 birds at each of the following doses: correct
1234.8, 1567.5, 1989.8, 2525.9, 3206.5

6. What is the next step for the following scenario?

a. Conduct limit test at 2000 mg/kg – observe 1 mortality out of 5 birds
b. Then conduct second limit test at 2000 mg/kg – observe no additional mortalities, no signs of toxicity in any birds.

Conclude that LD_{50} > limit dose with 0.95 probability, no more testing required.

7. Under what conditions is stage 4 necessary?

If an estimate of the slope cannot be obtained at the conclusion of stage 3b. One needs to have two or more reversals or partial kills to estimate slope.

8. Determine the number of partials and reversals for each of the following scenarios. The sequences are listed in increasing dose from left to right.

a. Partials __1___ Reversals ___1___

Number of mortalities	0	0	0	1	0	1	2	1	1
Number of tested individuals	1	1	1	2	1	1	2	1	1

b. Partials _2____ Reversals __3____

Number of mortalities	0	0	1	3	0	1	1	4	1
Number of tested individuals	1	1	1	5	1	1	1	5	1

C. TG223 Avian Acute Oral Toxicity Test - Results from First Quiz to Evaluate Guideline Clarity (based on March 2007 draft, summary prepared September 2007)

1. Assume a 0 represents survival and a 1 represents a kill. For each of the following sequences, identify the number of reversals. The sequences are listed to represent increasing dose (from left to right) and only one bird was tested at each dose.

0001011111	_____	1
0011101111	_____	1
0101000001	_____	2
0001010101	_____	3

Responses:
2 answered correctly
for sequence (3):
one person listed 1 reversal
one person listed 3 reversals

Issues to address:
Provide examples in guideline.

2. You conduct a limit test and one bird dosed at the limit dies, what is the next step?
If no signs of toxicity in other dosed birds, dose 5 more birds at limit
If signs of toxicity in other dosed birds, go to stage 2

Responses:
2 answered correctly
2 other answers were:
a) test 5 more at limit
b) if no signs of tox, dose 5 birds at limit, and if tox signs in remaining 4 birds then go to stage 2.

Issues to address:
- Ensure guidance is clear and consistent

3. If you conduct a limit test and a control bird dies, what is the next step?

If mortality could possibly be due to husbandry, need to redo test. If mortality could be considered incidental background (not related to conduct of test), random mortality – then add an additional 5 control birds.

Responses:

all four respondents answered correctly

Issues to address:

None.

4. Compute the working estimate of the LD50 based on the following Stage 1 results.

a.

Dose	56.56	208.37	766.80	2828.4
Result *	0	0	1	0

* 0 = survival, 1=death

1472.7

b.

Dose	26.9	99.0	364.6	1343.3
Result *	0	1	1	1

* 0 = survival, 1=death

51.6

Responses:

All four respondents answered correctly

Issues to address:

- None

5. For the following scenarios, identify the correct sequence of doses for stage 2.

a. Working estimate of LD50 = 425

i. correct

145.6	184.7	234.3	297.3	377.3	478.7	607.3	770.6	977.7	1240.6

ii. LD50=425 step = 50^(1/10), Max/min value for hdose removed

145.6	215.3	318.3	470.7	696.0	1029.3	1522.1	2250.8	3328.3	4921.8

iii. correct step, LD50 = 475

162.7	206.4	261.9	332.3	421.6	535.0	678.8	861.3	1092.8	1386.5

Responses:
All four respondents answered correctly

Issues to address:
- None

5. For the following scenarios, identify the correct sequence of doses for stage 2.

b. Limit test with 5 birds at 2000mg/kg, 3 mortalities at limit dose

i. Correct

391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0

ii. Max value for hdose removed

610.3	774.4	982.6	1246.7	1581.8	2007.0	2546.5	3231.1	4099.6	5201.7

iii. Used working estimate of LD50=1109

379.8	481.9	611.5	775.9	984.4	1249.0	1584.8	2010.8	2551.3	3237.2

Responses:
1 answered correctly
3 chose (ii), where maximum dose was not limited to 3330mg/kg

Issues to address:
- Ensure guidance is clear.

6. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

a. Stage 1

Dose	7.1	26.0	96.0	353.5
Result	0	0	1	0

Working LD50=184.2

Stage 2

Dose	63.1	80.0	101.6	128.9	163.5	207.5	263.2	334.0	423.8	537.7
Result	0	0	0	0	0	1	1	1	1	1

Working LD50 = 184.6
Working slope= 2.97

i. 5 birds at 82.7 and 5 birds at 412.1 correct
ii. 5 birds at 114.6 and 5 birds at 297.5 assume slope=5
iii. 2 birds at each of the following doses: correct for 3b stage
114.5, 145.4, 184.6, 234.3, and 297.4

Responses:

No one answered correctly (i)
1 chose (ii)
3 chose (iii)

Issues to address:

- Respondents were not able to determine which stage to move to (3a or 3b).
- Ensure guidance is clear.

6. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

   b. Limit test at 2000 mg/kg – observe 1 mortality out of 5 birds, signs of toxicity in 2 other birds.

   Working LD50=2947

   Stage 2

Dose	391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0
Result	0	0	0	0	0	0	0	1	1	1

   Working LD50 = 1990

   Working slope= not available (only one partial kill)

   i. 2 birds at each of the following doses:
      1246.0, 1581.7, 2007.8, 2548.8, 3235.5 stage 3b, calc done with 4 mortalities at 2000 mg/kg
   ii. 5 birds at 1282.5 and 5 birds at 3330 stage 3a, assume slope=5
   iii. 2 birds at each of the following doses: correct
      1234.8, 1567.5, 1989.8, 2525.9, 3206.5

Responses:

2 chose (i)

2 stated answer was either (i) or (iii)

Issues to address:

- Ensure guidance is clear.

7. What is the next step for the following scenario?
    a. Conduct limit test at 2000 mg/kg – observe 1 mortality out of 5 birds
    b. Then conduct second limit test at 2000 mg/kg – observe no additional mortalities, no signs of toxicity in any birds.

   Conclude that LD50 > limit dose with 0.95 probability, no more testing required.

Responses:
all four respondents answered correctly

Issues to address:
- none

8. Under what conditions is stage 4 necessary?

   If an estimate of the slope cannot be obtained at the conclusion of stage 3b. One needs to have two or more reversals or partial kills to estimate slope.

Responses:
all four respondents answered correctly

Issues to address:
- None.

D. TG223 Avian Acute Oral Toxicity Test - Results from Second Quiz to Evaluate Guideline Clarity (based on March 2008 draft, summary prepared September 2009)

1. Assume a 0 represents survival and a 1 represents a kill. For each of the following sequences, identify the number of reversals. The sequences are listed to represent increasing dose (from left to right) and only one bird was tested at each dose.

0001011111	_____	1
0011101111	_____	1
0101000001	_____	2
0001010101		3

Responses:
All 13 respondents answered correctly.

Issues to address:
None.

2. You conduct a limit test and one bird dosed at the limit dies, what is the next step?
If no signs of toxicity in other dosed birds, dose 5 more birds at limit
If signs of toxicity in other dosed birds, go to stage 2

Responses:
10 answered correctly
3 provided partially correct answers, those answers were:
a) either conduct another limit test or go to stage 2
b) dose an additional 5 birds at limit
c) If treatment related, add 5 more birds; or go to stage 1 or stage 2.

Issues to address:
- Ensure guidance is clear.
- One respondent noted concerns that one death (out of 10 birds) at the limit dose would have harmonization issues with OPPTS 850.2100 (does not allow any deaths at limit dose). Need to ensure that there are no concerns with harmonization. Respondent also noted concerns regarding inferences from 5 birds dosed at limit; even with no mortalities, cannot meet required standard for EPA endangered species assessments.

4. If you conduct a limit test and a control bird dies, what is the next step?
If mortality could possibly be due to husbandry, need to redo test. If mortality could be considered incidental background (not related to conduct of test), random mortality – then add an additional 5 control birds.

1 respondent stated could not find answer in guideline
4 provided partial/incorrect answers, those answers were: Responses:
8 answered correctly

a) restart expt with 5 new control birds
b) cause of death should be determined
c) five additional control birds should be added
d) repeat with 5 additional control birds

Issues to address:

- Ensure guidance is clear and consistent.
- Steps regarding controls needs to be in a more prominent/logical place in the guidance.

5. Compute the working estimate of the LD50 based on the following Stage 1 results.

a.

Dose	56.56	208.37	766.80	2828.4
Result *	0	0	1	0

* 0 = survival, 1=death

1472.7

b.

Dose	26.9	99.0	364.6	1343.3
Result *	0	1	1	1

* 0 = survival, 1=death

51.6

Responses:

All 13 respondents answered correctly

Issues to address:

- None

6. For the following scenarios, identify the correct sequence of doses for stage 2.

c. Working estimate of LD50 = 425

i. correct

145.6 184.7 234.3 297.3 377.3 478.7 607.3 770.6 977.7 1240.6

ii. LD50=425 step = 50^(1/10), Max/min value for hdose removed

145.6 215.3 318.3 470.7 696.0 1029.3 1522.1 2250.8 3328.3 4921.8

iii. correct step, LD50 = 475

162.7 206.4 261.9 332.3 421.6 535.0 678.8 861.3 1092.8 1386.5

Responses:

All 13 respondents answered correctly

Issues to address:

- None

6. For the following scenarios, identify the correct sequence of doses for stage 2.

   d. Limit test with 5 birds at 2000mg/kg, 3 mortalities at limit dose

      i. Correct

391.8 496.9 630.3 799.5 1014.1 1286.4 1631.7 2069.7 2625.3 3330.0

      ii. Max value for hdose removed

610.3 774.4 982.6 1246.7 1581.8 2007.0 2546.5 3231.1 4099.6 5201.7

      iii. Used working estimate of LD50=1109

379.8 481.9 611.5 775.9 984.4 1249.0 1584.8 2010.8 2551.3 3237.2

Responses:

9 answered correctly
4 chose (ii), where maximum dose was not limited to 3330mg/kg

Issues to address:

- Ensure guidance is clear.

7. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

   a. Stage 1

Dose	7.1	26.0	96.0	353.5
Result	0	0	1	0

Working LD50=184.2

Stage 2

Dose	63.1	80.0	101.6	128.9	163.5	207.5	263.2	334.0	423.8	537.7
Result	0	0	0	0	0	1	1	1	1	1

Working LD50 = 184.6
Working slope= 2.97

   i. 5 birds at 82.7 and 5 birds at 412.1 correct
   ii. 5 birds at 114.6 and 5 birds at 297.5 assume slope=5
   iii. 2 birds at each of the following doses: correct for 3b stage
   114.5, 145.4, 184.6, 234.3, and 297.4

Responses:

1 answered correctly (i)
2 chose (ii)
8 chose (iii)
1 did not answer

Issues to address:

- Most common error appears that Stage 1 and Stage 2 results were not combined to correctly determine partials/reversals (there are two reversals in the example). If reader only looks at Stage 2 data, there are no reversals and the next stage would be Stage 3b (answer iii).
- Staff in EFED noted difficulty calculating slope as the standard software used for LD_{50} calculation (Toxanal) does not perform probit calculations for data with <2 partial mortalities. Additional clarification will be added in guidance.
- Ensure guidance is clear.

7. For each of the following scenarios, identify which option shows the correct dose sequence for stage 3, where 0 = survival, 1=death.

   b. Limit test at 2000 mg/kg – observe 1 mortality out of 5 birds, signs of toxicity in 2 other birds.

Working LD50=2947

Stage 2

Dose	391.8	496.9	630.3	799.5	1014.1	1286.4	1631.7	2069.7	2625.3	3330.0
Result	0	0	0	0	0	0	0	1	1	1

Working LD50 = 1990

Working slope= not available (only one partial kill)

   j. 2 birds at each of the following doses:
      1246.0, 1581.7, 2007.8, 2548.8, 3235.5 stage 3b, calc done with 4 mortalities at 2000 mg/kg
   ii. 5 birds at 1282.5 and 5 birds at 3330 stage 3a, assume slope=5
   iv. 2 birds at each of the following doses: correct
      1234.8, 1567.5, 1989.8, 2525.9, 3206.5

Responses:

3 answered correctly (iii)
4 chose (i)
3 stated answer was either (i) or (iii)
1 chose (ii)
1 stated answer was 'none of the above'
1 did not answer

Issues to address:

- Most respondents were able to correctly determine that Stage 3b should be used. EFED staff noted difficulty calculating LD_{50} and slope as the standard software used in EFED for LD_{50} calculation (Toxanal) does not perform probit calculations for data with <2 partial mortalities.
- Some staff were looking for exact calculations for the working LD_{50} in the guidance text; this is not included for Stage 3 dose setting. Will clarify for next guideline draft revision.
- Ensure guidance is clear.

7. What is the next step for the following scenario?
   c. Conduct limit test at 2000 mg/kg – observe 1 mortality out of 5 birds
   d. Then conduct second limit test at 2000 mg/kg – observe no additional mortalities, no signs of toxicity in any birds.

Conclude that LD50 > limit dose with 0.95 probability, no more testing required.

Responses:

12 answered correctly
1 responded to go to Stage 2

Issues to address:

- Ensure guidance is clear.

8. Under what conditions is stage 4 necessary?

If an estimate of the slope cannot be obtained at the conclusion of stage 3b. One needs to have two or more reversals or partial kills to estimate slope.

Responses:

12 answered correctly
1 responded "if slope not defined or confidence intervals too wide"

Issues to address:

- Ensure guidance is clear.

Note the following question was added after the first reading comprehension results were interpreted and resulting changes made to guideline.

9. Determine the number of partials and reversals for each of the following scenarios. The sequences are listed in increasing dose from left to right.

   a. Partials __1___ Reversals ___1___

Number of mortalities	0	0	0	1	0	1	2	1	1
Number of tested individuals	1	1	1	2	1	1	2	1	1

   b. Partials _2____ Reversals __3____

Number of mortalities	0	0	1	3	0	1	1	4	1
Number of tested individuals	1	1	1	5	1	1	1	5	1

Responses:

All 13 answered the number of partials correctly for both a. and b.

Reversals for part a)

- 9 correct
- 1 responded ‘zero reversals’
- 2 responded ‘two reversals’

Reversals for part b)

- 3 answered correctly
- other answers were 1, 2, or 6 reversals

Issues to address:

Ensure guidance is clear. Provide more clear examples.

SEDEC Manual

Example of output from SEDEC programme

SEDEC V1.3 Sequential Design Calculator

Study Identification

Project Number =	Master-1
Test Substance =	Fictitious
Dose Units =	mg formulation/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD_{50} Guess =	100
Step Size =	1,2694297
Date =	28-sep-09
Initials =	TAS
Study Status Code =	43
Min % Dose Sep =	1
Stage 3 Type =	B

(Combine doses differing by < Min% for analysis)

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
14,1	1	0	34,3	1	0	62,1	2	0	62,1	2	
52,1	1	0	43,5	1	0	78,9	2	0	78,8	2	
192	1	1	55,2	1	0	100	2	1	100	2	
707	1	1	70	1	0	127	2	2	127	2	
			88,8	1	1	161	2	2	161	2	
			113	1	0						
			143	1	1						
			181	1	1						
			230	1	1						
			292	1	1						

Analysis				
Probit Analysis Results				
Itterations	Chi-square	Probability	G	N
8	6,087097931	0,992581075	0,710195894	24

Slope =	11,89865035			
95% Confidence Limits=		1,871286328	and	21,92601438

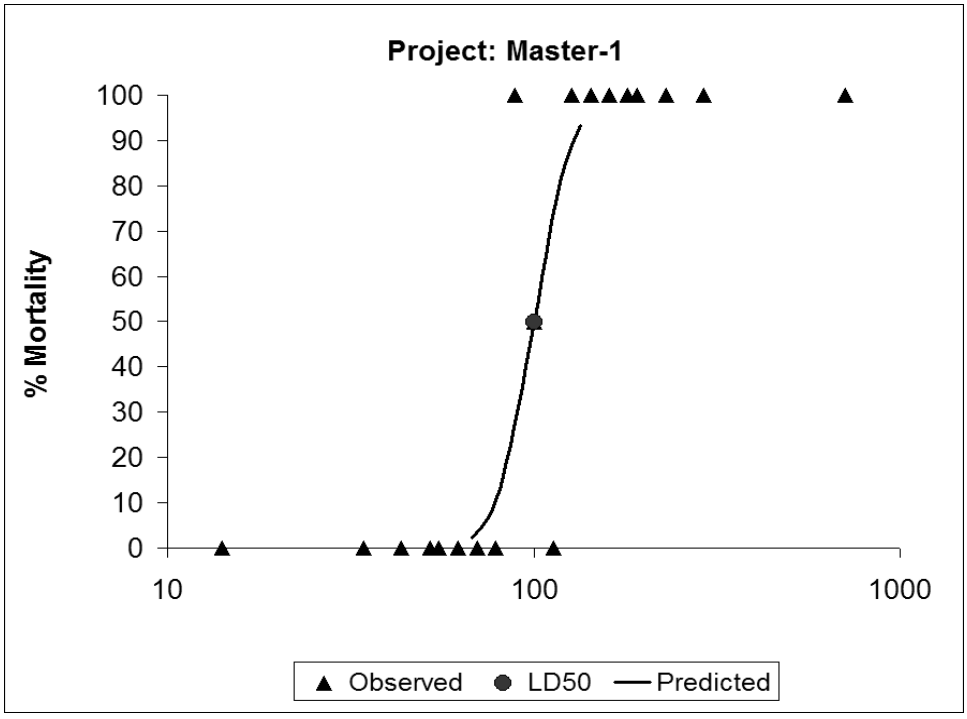
LD_{50} =	100,0872194			
95% Confidence Limits=		71,5067982	and	139,9416652

n of Reversals =	2	n of Partials =	1

Combined Data in Dose Order					
Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding	Merged Doses?
14,1	1	0	-	0	-
34,3	1	0	-	0	-
43,5	1	0	-	0	-
52,1	1	0	-	0	-
55,2	1	0	-	0	-
62,1	2	0	-	0	-
70	1	0	-	0	-
78,9	2	0	-	0	-
88,8	1	1	-	100	-
100	2	1	Rev+Partial	50	-
113	1	0	Reversal	0	-
127	2	2	-	100	-
143	1	1	-	100	-
161	2	2	-	100	-
181	1	1	-	100	-
192	1	1	-	100	-
230	1	1	-	100	-
292	1	1	-	100	-
707	1	1	-	100	-

Combined Data in Dose Order

Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding	Merged Doses?
14.1	1	0	-	0	-
34.3	1	0	-	0	-
43.5	1	0	-	0	-
52.1	1	0	-	0	-
55.2	1	0	-	0	-
62.1	2	0	-	0	-
70	1	0	-	0	-
78.9	2	0	-	0	-
88.8	1	1	-	100	-
100	2	1	Rev+Partial	50	-
113	1	0	Reversal	0	-
127	2	2	-	100	-
143	1	1	-	100	-
161	2	2	-	100	-
181	1	1	-	100	-
192	1	1	-	100	-
230	1	1	-	100	-
292	1	1	-	100	-
707	1	1	-	100	-



Appendix V –

A. Verification of the sequential design calculator SEDEC.

B.

Appendix 1. Results of analysis of 16 data sets [6)]
Appendix 2 Results of SAS results of 16 data sets 17MAR2009
Appendix 3 Query log

B. Post-Test Corrections and Additions to SEDEC

C. Verification of Post Test Corrections and Additions to SEDEC

Appendix V

A. Verification of the sequential design calculator SEDEC:

A tool for use with OECD TG223 (Avian Acute Oral Toxicity Test)

Author: Carol Yarrow, Stats Matters
Date: 17th March 2009

Summary

An exercise to verify the output of SEDEC (SEquential DEsign Calculator computer programme) using a combination of manual testing and parallel analyses in SAS has now been completed. During the course of this exercise several issues with the setup and execution of SEDEC have been identified. These have all been communicated to the SEDEC team and satisfactory solutions have been proposed. A summary of all issues identified is included within the body of this report and a full log of the queries raised and the corresponding responses is included as an Appendix.

Introduction

SEDEC was developed using Visual Basic to support an OECD guideline for carrying out a specific avian assay of chemical toxicity (TG223). This assay is sequential in nature, taking at a minimum 2-3 weeks, and each investigator must provide likelihood-based estimates, slopes and confidence intervals with data submitted to regulatory agencies. SEDEC will be made available by OECD to assist in the calculation of doses at each stage of the assay and to calculate estimates to accompany the data.

This task was a component in the verification of SEDEC. It was a logical exercise where the draft TG223 guideline (dated March 2008) directions and outcomes were matched to what SEDEC does. The testing was carried out in four steps:

1. Check SEDEC setup on the test computer using Windows Vista and Excel 2007
2. Check the decision tree and dose calculations for the limit test work appropriately
3. Check the dose calculations for Stage 1 work appropriately
4. Check the results and dose calculations for the sequential design from Stages 1 to 4

Steps 1 to 3 were accomplished by running SEDEC on test datasets and manually comparing the output produced by SEDEC to that that was expected under the TG223 guidelines. Step 4 was accomplished by developing a sequential SAS analysis to follow the guideline for the sequential test design between Stages 1 and 4. The SAS analysis analysed the data at the end of each stage, counting the number of reversals and partial kills, producing estimates of the LD_{50} and slope and making a decision and producing dose estimates for the next stage. Parallel analyses in SAS and SEDEC were then carried out for a set of sixteen test datasets that were carefully assembled to test all remaining branches of the decision tree in order to:

i) test that SEDEC's decision tree for the different test options works appropriately
ii) test the counting of reversals and partial kills
iii) assess the accuracy, precision and limitations of SEDEC's estimates and confidence intervals for the LD_{50} and slope.

Queries were sent to the SEDEC team on a weekly basis. An Excel spreadsheet was set up to log all of the queries that were identified during this process and to record the responses and proposed solutions for each issue. This log has been included as an appendix to this report. A summary of the issues identified has been given in Table 5 in the last section of this report. Where an issue was queried and found to be working as it should, it has not been included in Table 5 (although the query will appear in the query log in the Appendix together with all relevant correspondence.)

SEDEC Setup using Windows Vista and Excel 2007

The first step of the validation exercise was to confirm that SEDEC was working properly on the test computer. This computer was running Excel 2007 under Windows Vista; a configuration that had not previously been tested. SEDEC was installed and tested following the instructions in the SEDEC Users' Guide Version 1.0, dated 18 September 2008. The SEDEC qualification test described on Page 27 of the manual was performed and passed. All output was examined and compared to the screenshots in the manual. Anything that did not perform as expected or where the screenshots and the output did not match was queried.

Limit Tests

4.1 Tests of different mortality patterns

A set of fifteen limit test experiments was developed to cover all mortality patterns expected under the guideline. These experiments were run through SEDEC and the decisions (after 5 and 10 birds had been tested) and working LD_{50} (if applicable) for each experiment were compared to those expected according to the guideline. The limit dose was set at 2000 mg ai/kg for all fifteen tests. The working LD_{50}s that should be produced following the results of a limit test at 2000 mg ai/kg are shown in Table 1 on Page 6 of the draft TG223 guideline.

Table 1.1 shows the number of birds exposed, the number of birds responding and the presence or absence of toxicity in the remaining birds for the initial 5-bird limit test for each experiment. The SEDEC decision after the initial 5-bird test is shown for each experiment as either:

- 'Stage 1'= Proceed to Stage 1.
- 'Stage 2'= Proceed to Stage 2.
- 'Stage 2 or +5 birds'= Proceed to Stage 2 or test an additional 5 birds at the limit dose
- 'LD_{50}>2000'= Limit test passed

Table 1.1 also shows the number of birds exposed and the number of birds responding in the additional 5 birds tested (if applicable). Finally it shows the overall number of birds exposed, the overall number of birds responding, the overall mortality, the expected working LD_{50} according to Table 1 in the guideline and the SEDEC decision and working estimate of the LD_{50} after all (5 or 10) birds have been tested.

All SEDEC decisions were as expected according to the guidelines and the only issue identified was that the working LD_{50}s given by SEDEC were very slightly different to the expected working LD_{50}s according to Table 1 in the guidelines. These differences are negligible and have been attributed to rounding errors.

NB: Note that Experiment 2 (a 10-bird limit test with no mortalities) would not actually be expected under the guidelines. However, it was included for completeness.

Table 1.1: Verification of Limit Test Results: Decision and Estimated LD_{50}

	5-bird limit test at a dose of 2000 mg ai/kg				Additional 5 birds		Overall				
Expt ID	Number exposed	Number responding	Toxicity in others?	SEDEC Decision (5 birds)	Number exposed	Number responding	Number Exposed	Number responding	Mortality	Expected Working LD_{50}*	Final SEDEC Decision and Working LD_{50} (5 or 10 birds)
1	5	0	N	LD_{50}>2000	NA	NA	5	0	0%	NA	LD_{50}>2000
2	5	0	N	LD_{50}>2000	5	0	10	0	0%	NA	LD_{50}>2000
3	5	0	Y	LD_{50}>2000	NA	NA	5	0	0%	NA	LD_{50}>2000
4	5	1	N	Stage 2 or +5 birds	5	0	10	1	10%	NA	LD_{50}>2000
5	5	1	N	Stage 2 or +5 birds	5	1	10	2	20%	2947	Stage 2, LD_{50}= 2945
6	5	1	N	Stage 2 or +5 birds	0	0	5	1	20%	2947	Stage 2, LD_{50} = 2945
7	5	1	N	Stage 2 or +5 birds	5	2	10	3	30%	2546	Stage 2, LD_{50} = 2541
8	5	1	N	Stage 2 or +5 birds	5	3	10	4	40%	2247	Stage 2, LD_{50} = 2244
9	5	1	N	Stage 2 or +5 birds	5	4	10	5	50%	2000	Stage 2, LD_{50}= 2000
10	5	1	N	Stage 2 or +5 birds	5	5	10	6	60%	1780	Stage 2, LD_{50} = 1783
11	5	1	Y	Stage 2	NA	NA	5	1	20%	2947	Stage 2, LD_{50} = 2945
12	5	2	N/Y	Stage 2	NA	NA	5	2	40%	2247	Stage 2, LD_{50} = 2244
13	5	3	N/Y	Stage 2	NA	NA	5	3	60%	1780	Stage 2, LD_{50}= 1783
14	5	4	N/Y	Stage 2	NA	NA	5	4	80%	1357	Stage 2, LD_{50}= 1358
15	5	5	N/Y	Stage 1	NA	NA	5	5	100%	NA	Stage 1

* The expected working LD_{50} was taken from Table 1 in the guideline

Table 1.2: Verification of Limit Test results: Stage 2 Doses and Override Maximum/Minimum Dose Facility

					Dose Override			
Expt ID	Limit Dose	Number Exposed	Number responding	Mortality	Min	Max	Working LD_{50}	SEDEC Calculated Stage 2 Doses
8	2000	10	5	50%			2000	392, 497, 630, 800, 1014, 1286, 1632, 2070, 2625, 3330
16	2000	10	5	50%	1	-	2000	1, 1.27, 1.61, 2.04, 2.59, 3.28, 4.16, 5.28, 6.70, 8.50
17	2000	10	5	50%	-	2500	2000	294, 373, 473, 600, 761, 966, 1225, 1554, 1971, 2500
18	2000	10	5	50%	-	20000	2000	2353, 2985, 3786, 4802, 6091, 7726, 9800, 12431, 15767, 20000

Table 1.3: Verification of Limit Test Results: Varying Limit Doses

Expt ID	Limit Dose	Number exposed	Number responding	Mortality	Maximum Dose Override	Expected Working LD_{50}**	SEDEC Working LD_{50}	SEDEC Calculated Stage 2 Doses
19	500	10	2	20%	-	736.75	736	252, 320, 406, 515, 653, 829, 1052, 1335, 1693, 2148
20	1000	10	2	20%	-	1473.5	1472	392, 497, 630, 800, 1014, 1286, 1632, 2070, 2625, 3330
21	5000	10	2	20%	-	7367.5	7362	392, 497, 630, 800, 1014, 1286, 1632, 2070, 2625, 3330
22	5000	10	2	20%	5000	7367.5	7362	588, 746, 946, 1200, 1523, 1932, 2450, 3108, 3942, 5000

** The expected working LD_{50} was calculated by multiplying the LD_{50} from Table 1 in the guideline by 'Limit Dose'/2000.

4.2 Doses for Stage 2 and override maximum / minimum dose facility

The guideline states that when an experiment proceeds from the limit test to Stage 2 it should use the estimated LD_{50} from the limit test (working LD_{50}) to set the dose range for Stage 2. The lowest dose should be set to 0.3425 * working LD_{50} and the highest dose should be set to 2.919 * working LD_{50}. The ratio of the highest to the lowest dose is therefore 8.5. If the calculated value for the highest dose is greater than the maximum of 3330, the highest dose should be reset to 3330 and the lowest dose to 3330/8.5 = 392. The Stage 2 doses produced by SEDEC for each of experiments 1 to 15 were checked and all were found to be in accordance with the guideline.

SEDEC allows the user to accept the suggested doses (as described above) or to override the minimum or maximum dose. In order to check the doses produced after the override maximum/minimum dose facility is used, three additional experiments (Experiments 16, 17 and 18) were run through a 10 bird limit test at a dose of 2000 mg ai/kg and a mortality level of 50%. A value of 1 was used to override the minimum dose for Experiment 16 and values of 2,500 and 20,000 were used to override the maximum dose in Experiments 17 and 18 respectively. SEDEC's suggested Stage 2 doses for the 10 bird limit test with 50% mortality and no dose overrides (Experiment 8) and the Stage 2 doses that were produced for Experiments 16, 17 and 18 after the minimum or maximum doses were overridden are shown in Table 1.2.

The guideline states that the highest dose may be set lower than 3330 if limited by physical constraints and that the lowest dose should then be recalculated to maintain the 1:8.5 ratio between the highest and lowest dose. There are no specific instructions or restrictions regarding the lowest dose.

Table 1.2 shows that when the minimum dose was overridden with a value of 1, the smallest Stage 2 dose was set to the override value and the remaining nine doses were calculated at equal intervals to maintain the 1:8.5 ratio between the lowest and highest doses. Similarly, when the maximum dose was overridden, the highest Stage 2 dose was set to the override value and the remaining nine doses were calculated at equal intervals to maintain the 1:8.5 ratio between the lowest and highest doses. This behaviour is in accordance with the guidelines as given for the highest dose. However, note there is no longer any relationship between the Stage 2 doses and the expected LD_{50}s and SEDEC does not make this explicitly clear. This may be particularly relevant when overriding the maximum value as described below.

Suppose that the dose units are not given in mg ai/kg, but are in mg formulation/kg or some other unit. This may lead to a case where the upper limit of 3330 that is built into SEDEC is not appropriate and some other upper limit should be used instead. If the user uses this new upper limit to override the maximum dose (as 3330 is not relevant), the upper limit that they input will automatically become the highest dose even if it is far bigger than the working LD_{50}. Note that this is the case in Experiment 18; the maximum override value is 20000 which is far bigger than the working LD_{50} of 2000. The highest calculated dose according to the LD_{50} would have actually been 2000 * 2.919 = 5838 which is greater than 3330 but lower than the override value of 20000.

The tester therefore feels that at a minimum SEDEC should give a warning message to the user to inform them that the dose range is no longer related to the working LD_{50}. If different dose units are envisaged and SEDEC is expected to support them, the current behaviour of resetting the highest dose as the override value may not be entirely appropriate. It might be more appropriate to retain a relationship with the working LD_{50}, effectively making the override value the new upper limit. This may therefore be a matter that needs some thought.

4.3 Varying Limit Doses

Finally, in order to test limit tests at different doses (i.e. not 2000 mg ai/kg), three additional 10-bird limit tests with 20% mortality at varying doses (500, 1000, 5000) were run in SEDEC (Experiments 19, 20 and 21). The expected working LD_{50} for each test was calculated by multiplying the appropriate LD_{50} from Table 1 in the guideline by 'Limit Dose'/2000. For example, the LD_{50} for a 2000 mg ai/kg limit test with 20% mortality is given as 2947. If the test was conducted at a dose of 500, the expected LD_{50} would be 2947 * 500/2000 = 736.75. The limit dose, number of birds exposed, number of birds responding, mortality level, expected working LD_{50} and the working LD_{50} and Stage 2 doses calculated by SEDEC for these experiments are displayed in Table 1.3. In all three cases, allowing for rounding error, the working LD_{50} and Stage 2 doses calculated by SEDEC were as expected. Note that for Experiment 20 (limit dose=1000) and Experiment 21 (limit dose=5000) the dose range for Stage 2 reached the maximum dose of 3330.

A further experiment (Experiment 22) with a limit dose of 5000 and for which the maximum dose for Stage 2 was overridden to 5000 was run in SEDEC and this is also displayed in Table 1.3. As before, SEDEC set the highest dose to be equal to the maximum dose specified by the user (5000) and there was no longer any relationship between the working LD_{50} and the dose range.

Stage 1 Setup

Eight experiments that started with Stage 1 were set up in SEDEC in order to test the dose calculation for Stage 1. The guidelines explain that the doses for Stage 1 should be set up making an initial guess of the LD_{50} and by calculating the lowest dose as 0.1414 * LD_{50} guess and the highest dose as 7.071 * LD_{50} guess. If the highest dose is greater than 3330 it should be reset to 3330 and the lowest dose chosen to maintain the 1:50 ratio between the highest and lowest doses.

The first two experiments used different guesses of the LD_{50}; one was chosen to produce a highest dose that was less than 3330 and the other was chosen to produce a calculated highest dose that was greater than 3330. The other six experiments were set up to test the behaviour of the override facility for the minimum and maximum doses at Stage 1. For both minimum and maximum dose overrides, one value which should cause the doses to decrease and two values which should cause the doses to increase were tested. The first of the values which should cause the doses to increase was chosen such that the expected highest dose was under 3330 and the second was chosen such that the expected highest dose would be greater than 3330.

The LD_{50} guess, minimum and maximum dose override values, calculated high dose after overriding and the resulting Stage 1 doses produced by SEDEC are shown in Table 2.

Table 2: Stage 1 Setup

		Override Values		Expected High Dose (Dose4) after Override	Stage 1 Doses produced by SEDEC			
Expt ID	LD_{50} Guess	Min	Max		Dose1	Dose2	Dose3	Dose4
1	50			354	7.07	26	96	354
2	2000			14142	66.6	245	904	3330
3	50	1		354	1	3.68	13.6	50
4	50	60		3000	60	221	814	3000
5	50	100		5000	66.6	245	904	3330
6	50		200	200	4	14.7	54.3	200
7	50		2000	2000	40	147	543	2000
8	50		5000	5000	66.6	245	904	3330

Note that, as before, when dose overrides are used there is no longer any relationship between the LD_{50} guess and the calculated doses. For example, Experiment 3 has a LD_{50} guess of 50 which would lead to an expected dose range of 7.07 to 354 (as for Experiment 1). Setting the minimum dose to 1 causes the dose range to change to 1 to 50. Similarly, for the same value of the LD_{50} guess (50), for Experiment 7, setting the maximum dose to 2000 causes the dose range to change to 40 to 2000.

Note that for the two cases (Experiments 5 and 8) where the override of the maximum dose value caused the calculated highest dose to be greater than 3330, SEDEC has reset the highest dose to 3330. It is not clear if this is the desired behaviour as it does not contradict the guidelines. However, it does not seem to be consistent with the behaviour of the override for Stage 2, which does allow doses higher than 3330 to be produced. Note that the current behaviour for Stage 1 may also cause problems if a higher upper dose limit is appropriate because of different dose units.

Sequential Testing: Stages 1 to 4

In order to test SEDEC's decision tree and dose calculation procedures from Stages 1 to 4 and to assess the accuracy, precision and limitations of the estimation procedures, a comprehensive set of sixteen test datasets was carefully assembled. These were selected to include all branches in the decision tree between Stages 1 to 4 and to encompass a wide range of doses, mortality levels and patterns, including potential pitfalls such as very steep, very shallow and negative slope estimates and delayed mortalities. Table 3 summarises the key features of each of these datasets. The raw data for each dataset is included in Appendix 1.

Table 3: Set of sixteen test datasets for the verification of SEDEC

Dataset ID	Main Features / Mortality Pattern	Stages	Additional Features
1	Complete survival at end of Stage 2	1, 2	
2	Complete mortality at end of Stage 2	1, 2	
3	≥2 reversals at end of Stage 2	1, 2, 3a	Delayed mortality in Stage 1
4	≥2 reversals at end of Stage 2	1, 2, 3a	Shallow slope
5	≥2 reversals at end of Stage 2	1, 2, 3a	Shallow slope and low doses (<1)
6	1 reversal at end of Stage 2	1, 2, 3b	
7	1 reversal at end of Stage 2	1 ,2, 3b	Messy Stage 3 data
8	1 reversal at end of Stage 2	1, 2, 3b	High doses (3330)
9	1 reversal and 1 partial at end of Stage 3	1, 2, 3b, 4	Delayed mortality in Stage 3
10	1 partial at end of Stage 2	1, 2, 3b	High doses (3330)
11	1 partial at end of Stage 3	1, 2, 3b, 4	Override max dose (1000) in Stage 2
12	0 reversal/partials at end of Stage 2	1, 2, 3b	Steep slope
13	0 reversal/partials at end of Stage 3	1, 2, 3b, 4	Steep slope
14	0 reversal/partials at end of Stage 4	1, 2, 3b, 4	
15	Negative slope at end of Stage 2	1, 2, 3a	Delayed mortality in Stage 2
16	Negative slope at end of Stage 3	1, 2, 3b, 4	Override min dose (25) in Stage 2

Sequential Testing: Analyses of Test Datasets

The sixteen datasets listed in Table 3 were all analysed using both SEDEC and SAS. The analyses carried out in SAS were designed to match the rules and analyses specified in the draft TG223 guideline dated March 2008. The results from the two sets of analyses for each dataset were compared and any discrepancies were investigated. The following results were compared at the end of each stage.

- Decision (next stage)
- Doses for next stage
- Number of reversals
- Number of partials
- LD_{50} estimate
- LD_{50} confidence interval (last stage only)lope estimate

- Slope confidence interval (last stage only)
- Goodness of fit chi-squared value and p-value

Sequential Testing: Comparison of Results of Analyses of Test Datasets

The full data for each of the 16 test datasets and the results from the SEDEC analyses are shown in Appendix 1. The results from the SAS analyses of the 16 test datasets are shown in Appendix 2. The outcome of the comparison between the results produced by SAS and SEDEC for each of the 16 datasets are summarised in Table 4.

Table 4: Comparison between results produced by SAS and SEDEC for 16 test datasets.

Dataset ID	Discrepancies between the SAS Analysis and SEDEC
1	None. SEDEC stops after complete survival at end of Stage 2 with the message "There have been no mortalities. Consider performing a limit test or restarting the test using higher doses'.
2	The LD_{50} and slope are both reported as non-estimable by SAS at the end of Stage 2. However, SEDEC produces a negative slope estimate and a very high estimated LD_{50} (should be very low as all tested doses result in complete mortality) and then continues into Stage 3b. The high LD_{50} estimate results in the highest possible dose range for Stage 3b (the highest dose is at the upper limit of 3330). *Comment: The guideline does not cover the case of complete mortality at the end of Stage 2. However, SEDEC's behaviour in this case is not sensible and this is an error in SEDEC that needs to be resolved*.
3	None
4	Stage 3: - The confidence interval for the estimated LD_{50} is reported as non-estimable by the SAS analysis and 0 to +Infinity by SEDEC. *Comment: Confidence intervals of 0 to +Infinity should be reported as non-estimable (see query 9 in the query log)*
5	None
6	None
7	None
8	Stage 2: - The p-value for the chi-square statistic is reported as 0.48 by the SAS analysis and 0.57 by SEDEC. Note that the chi-square statistic is 10.54 in both cases. Stage 3: - The p-value of the chi-square statistic is reported as 0.54 by the SAS analysis and 0.61 by SEDEC. Note that the chi-square statistic is 14.74 in both cases. - The confidence interval for the estimated LD_{50} is reported as 244 to 2780 by the SAS analysis and 372 to 2810 by SEDEC. *Comment: This experiment has one bird tested at a dose of 3330 in Stage 1 and one bird tested at a dose of 3330 in Stage 2. SEDEC does not always combine doses repeated at different stages correctly (see query 11). Is this the cause of the discrepancies here (e.g.*

	wrong DF for the chi-square value?)
9	Stage 2: - The probit model fitted in SAS failed to converge. - The slope estimate is reported as 185 by SAS and 130 by SEDEC. *Comment: The SAS probit model failed to converge after 50 iterations and I suspect that SEDEC has also failed to converge (27 iterations). There are 0 partial kills and 0 reversals. The slope should not be reported (see queries 16 and 17)*

Table 4 (cont.): Comparison between results produced by SAS and SEDEC for 16 test datasets.

Expt ID	Discrepancies between the SAS Analysis and SEDEC
9 (cont)	Stage 4: - The number of reversals is reported as 4 by the SAS analysis and 3 by SEDEC. *Comment: Doses are repeated at Stages 3 and 4. Is this discrepancy due to failure to combine repeated doses properly?*
10	Stage 2: - The number of partials is reported as 1 by the SAS analysis and 0 by SEDEC. - The value of the chi-square statistic is reported as 0 by the SAS analysis and 2.00 by SEDEC. Note that the chi-square p-value is given as 1.00 in both cases. Stage 3: - The number of reversals is reported as 2 by the SAS analysis and 3 by SEDEC. - The value of the chi-square statistic is reported as 9.8 by SAS and 11.84 by SEDEC. - The value of the chi-square p-value is reported as 0.83 by SAS and 0.81 by SEDEC - The confidence interval for the estimated LD_{50} is reported as 3137 to 3535 by the SAS analysis and 2026.8 to +Infinity by SEDEC. *Comment: The maximum dose of 3330 is repeated at Stages 1, 2 and 3. Are these discrepancies due to failure to combine repeated doses properly?*
11	Stage 2: - The probit model fitted in SAS failed to converge. - The slope estimate is reported as 184 by SAS and 128 by SEDEC. Stage 3: - The probit model fitted in SAS failed to converge. - The slope estimate is reported as 133 by SAS and 127 by SEDEC. - The value of the chi-square statistic after Stage 3 is reported as non-estimable by SAS and <0.001 by SEDEC. *Comment: Failure to converge. 0 reversals and <2 partials. Slope should not be reported* Stage 4: - The number of partials is reported as 3 by SAS and 4 by SEDEC. - The value of the chi-square p-value is reported as 0.89 by SAS and 0.94 by SEDEC. Note that the value of the chi-square statistic is 12.79 in both cases. *Comment: Repeated doses at Stages 3 and 4*
12	Stage 2: - The probit model fitted in SAS failed to converge - The slope estimate after Stage 2 is reported as 185 by SAS and 130 by SEDEC. *Comment: Failure to converge. 0 reversals and 0 partials. Slope should not be reported*
13	Stage 2: - The probit model fitted in SAS failed to converge. - The slope estimate after Stage 2 is reported as 184 by SAS and 129 by SEDEC Stage 3: - The probit model fitted in SAS failed to converge.

	- The slope estimate after Stage 3 is reported as 365 by SAS and 250 by SEDEC. *Comment: Failure to converge. 0 partials and 0 reversals. Slope should not be reported*

Expt ID	Discrepancies between the SAS Analysis and SEDEC
14	Stage 2: - The probit model fitted in SAS failed to converge. - The slope estimate after Stage 2 is reported as 185 by SAS and 129 by SEDEC. Stage 3: - The probit model fitted in SAS failed to converge. - The slope estimate after Stage 3 is reported as 367 by SAS and 251 by SEDEC. Stage 4: - The probit model fitted in SAS failed to converge. - The slope estimate after Stage 4 is reported as 720 by SAS and 489 by SEDEC. *Comment: Failure to converge. 0 reversals and 0 partials. Slope should not be reported*
15	Stage 2: - The number of reversals is reported as 3 by SAS and 4 by SEDEC - The number of partials is reported as 1 by SAS and 0 by SEDEC - The chi-square statistic is reported as 11.8 by SAS and 14 by SEDEC - The chi-square p-value is reported as 0.38 by SAS and 0.30 by SEDEC - The LD_{50} estimate reported by SEDEC is very small (<0.01). The estimate of the LD_{50} from the SAS probit model is also very small, but as there is a negative slope, SAS uses a working LD_{50} of 1344 (average of doses tested). Stage 3: - The number of reversals is reported as 4 by SAS and 5 by SEDEC - The number of partials is reported as 1 by SAS and 0 by SEDEC - The chi-square statistic is reported as 15.1 by SAS and 19.8 by SEDEC - The chi-square p-value is reported as 0.24 by SAS and 0.14 by SEDEC - The LD_{50} CI is reported as 99 to 4621 by SAS and 135 to 4427 by SEDEC. *Comments: The max dose of 3330 has been repeated at Stages 1, 2 and 3 and this may be the cause of the difference in reversals, partials and chi-square statistic.*
16	Stage 2: - The estimated LD_{50} reported by both SAS and SEDEC is 72.4. However, there is a negative slope and the working LD50 used in the SAS analysis is 280 - The doses for Stage 3a are given as 26 and 3043 in SAS and 6.11 and 722 in SEDEC. Stage 3: - The LD_{50} estimate is reported as 336395.8 by SEDEC (very large). - The working LD_{50} reported by SAS is 802 *Comment: At the end of both Stages 2 and 3, the probit model has a negative slope. When the slope is negative, SEDEC should use a slope of 1 and the mean of the tested doses as the working* LD_{50} *in order to estimate doses for the next stage (see query 15). Note that this is not covered in the guideline. The mean of the doses tested is 280 leading to doses of 26*

	(280 0.092) and 3043 (280* 10.864) as calculated by the SAS analysis. How has SEDEC calculated the doses for Stage 3a?*

Comments about the hypothesised causes of any unresolved discrepancies between the analyses carried out in SAS and SEDEC have been included in Table 4. These will need to be verified in SEDEC before they can be established as the true cause of the discrepancies.

Note that for the comparison of doses, LD_{50} estimates and confidence intervals and slope estimates and confidence intervals, error margins were used to allow for rounding errors. Differences greater than the following error margins were reported in Table 4.

- Dose = Differences of >0.5mg ai/kg that were also >1% of SAS dose
- LD_{50} estimate = Differences of >0.5mg ai/kg that were also >1% of SAS estimate
- LD_{50} CIs = Differences of >5mg ai/kg that were also >5% of SAS value
- Slope estimate = Differences of >5 that were also >5% of SAS value
- Slope CIs = Differences of >5 that were also >5% of SAS value

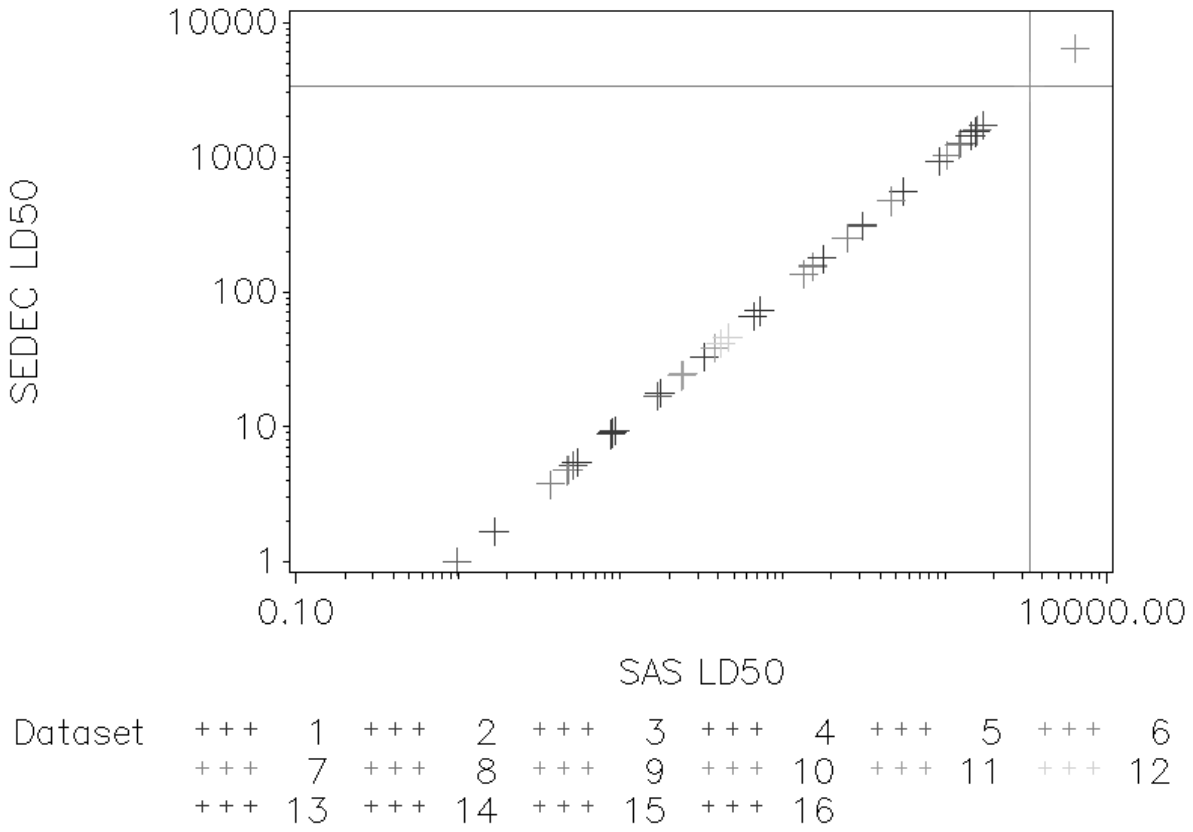
Comparison of LD_{50} Estimates

LD_{50} estimates produced by SEDEC that fell outside the range of 0.2 to 7000 mg ai/kg were investigated.

- There were three cases where SEDEC gave very large estimates for the LD_{50} (>7000). These were Dataset 2, Stages 2 and 3 and Dataset 16, Stage 3. As described above, Dataset 2 had complete mortality and SEDEC does not deal with this adequately. Dataset 16 had a negative slope for the probit model after both Stages 2 and 3 and SEDEC does not appear to be behaving as expected.
- There was one case where SEDEC gave a small estimate for the LD_{50} (<0.2). This was Dataset 15, Stage 3. This was another case where there was a negative slope for the probit model.

In all other cases, there was good agreement (difference of less than 1%) between the estimated LD_{50}s estimated by SEDEC and SAS for all stages of all 16 datasets. Figure 1 shows a plot of the SEDEC estimated LD_{50} plotted against the SAS estimated LD_{50} for each stage and for each dataset (with the exception of the extreme values described above).

Figure 1: Comparison of estimated LD50s produced by SEDEC and SAS analyses



Summary of Issues Identified

Table 5 gives a summary of all of the issues that have been identified during this exercise to verify SEDEC. The phase of testing where each issue was identified, a description of the issue and the corresponding query number from the query log are all presented. The full log of queries raised and the corresponding responses is included as Appendix 3.

References

The data analysis for this paper was generated using SAS/Base, SAS/STAT and SAS/GRAPH software, Version 9.2 of the SAS System for Windows, Copyright [2002-2003]. SAS Institute Inc., SAS, and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

Table 5: Summary of Issues Identified During SEDEC Verification

Testing Stage	Description	Query
Setup	Missing LD_{50} value in screenshot on Page 7 of the manual	1
	Missing doses in screenshot on Page 16 of the manual	2
	Run-time error with 'View Plot'. Fixed title 'Project Wildlife International No. 1' and fixed x axis values (0 to 600)	4
	Instructions for enabling macros are not applicable to Excel 2007	5
Limit Tests	Allowing a variable number of birds in 5 or 10 bird limit test leads to erroneous results; e.g. 5 mortalities from 6 exposed results in a passed limit test.	3
	When the override max dose facility is used for Stage 2 doses following a limit test, the highest dose is set to the new max dose input by the user (including when it is greater than 3330) and there is no longer any relationship between the working LD_{50} and the dose range. Similar behaviour is observed for the override min dose facility. A warning message to the user to explicitly state that there is now no relationship between the LD_{50} and the dose range would be beneficial.	8b
	If dose units other than mg ai/kg are to be supported by SEDEC then the current behaviour of setting the highest dose to be equal to (rather than less than or equal to) the override max dose may not be appropriate.	14
Stage 1 Setup	When the override max dose facility is used for Stage 1 doses, overriding the max dose with a value greater than 3330 has no effect. Similarly overriding the min dose with a a value greater than 66.6 has no effect. This is inconsistent with the behaviour observed for Stage 2 doses.	8a, 13
Stages 1 to 4	If there is complete survival after Stage 2, SEDEC stops with the message 'There have been no mortalities. Consider performing a limit test or restarting the test using higher doses'. This is not covered in the guidelines	12
	SEDEC does not deal with the situation where there is complete mortality after Stage 2 appropriately.	19
	Where confidence intervals for the LD_{50} are estimated as 0 to +Infinity they should be reported as non-estimable.	9
	SEDEC does not combine doses that have been repeated at multiple stages properly.	11
	Slope estimates and confidence intervals are reported when the probit analysis has failed to converge. In all of these cases there are <=1 partial kill and 0 reversals. The guidelines state that the slope can only be estimated when there are at least 2 partial kills or one reversal. The slope estimate and CI should therefore only be displayed when these conditions are met.	16, 17
	If the slope is negative, SEDEC does not seem to behave as expected. What does SEDEC use as the working LD_{50} to	15, 18, 20

	calculate the doses for the next stage? For dataset 16, SEDEC appears to use the LD_{50} estimate from the failed probit analysis to calculate the doses for Stage 3.	

Annex 1 of Appendix V (4 pages)

	Stage 1					Stage 2				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope
1	40,9	5,78	1	0	554,7	190	1	0		
		21,3	1	0		241	1	0		
		78,5	1	0		306	1	0		
		289	1	0		388	1	0	Nreversals	Npartials
						492	1	0		
						625	1	0		
						793	1	0	Chi-square	G
						1006	1	0		
						1276	1	0	p =	
						1619	1	0		

	Stage 1					Stage 2					Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
2	22,7	3,21	1	1	1,7	0,573	1	1	7,20E+65	-0,118	1281	2	2	1,08E+256	-0,03
		11,8	1	1		0,727	1	1	0 + Infinity	-620560 620560	1626	2	2	0 + Infinity	-297892 297891
		43,6	1	1		0,922	1	1			2065	2	2		
		161	1	1		1,17	1	1	Nreversals	Npartials	2622	2	2	Nreversals	Npartials
						1,48	1	1	0	0	3330	2	2	0	0
						1,88	1	1							
						2,39	1	1	Chi-square	G				Chi-square	G
						3,03	1	1	<0.001	2,74E+13				<0.001	9,60E+13
						3,85	1	1	p = 1					p = 1	
						4,88	1	1							

	Stage 1					Stage 2					Stage 3a				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
3	545	66,6	1	0	470,6	161	1	0	311,2	3,96	170	5	1	308,6	3,55
		245	1	0		205	1	0	3,70 927	0,12 7,81	568	5	4	164,0 510,2	1,12 5,98
		904	1	1		260	1	0							
		3330	1	1		329	1	1	Nreversals	Npartials				Nreversals	Npartials
						418	1	1	2	0				3	2
						530	1	0							
						673	1	1	Chi-square	G				Chi-square	G
						853	1	1	9,07	0,94				8,58	0,47
						1083	1	1	p = 0,70					p = 0,86	
						1374	1	1							
						245	*1*	*1*							

	Stage 1					Stage 2					Stage 3a				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
4	66	9,33	1	0	66,1	22,6	1	0	32,9	0,61	3,03	5	2	17,6	0,50
		34,4	1	1		28,7	1	1	0,00 + Infinity	-1,01 2,23	358	5	4	0,0 + Infinity	-0,19 1,19
		127	1	0		36,5	1	1							
		467	1	1		46,3	1	1	Nreversals	Npartials				Nreversals	Npartials
						58,7	1	0	3	0				4	2
						74,5	1	0							
						94,5	1	1	Chi-square	G				Chi-square	G
						120	1	1	13,95	7,06				14,48	1,94
						152	1	1	p = 0,30					p = 0,41	
						193	1	0							

	Stage 1					Stage 2						Stage 3a				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
5	62,54959	8,84	1	0	17,0	5,82	1	1	1,001	0,60	0,0921	5	0	5,2	1,21
		32,6	1	1		7,38	1	1	0,000 18,4	-1,15 2,34	10,9	5	3	0,0 17,8	0,14 2,28
		120	1	1		9,36	1	1							
		442	1	1		11,9	1	1	Nreversals	Npartials				Nreversals	Npartials
						15,1	1	1	3	0				4	1
						19,1	1	0							
						24,3	1	1	Chi-square	G				Chi-square	G
						30,8	1	0	13,33	8,65				13,47	0,78
						39,1	1	1	**p** = 0,35					**p** = 0,49	
						49,6	1	1							

	Stage 1					Stage 2						Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
6	64,28328	9,09	1	1	4,7	1,62	1	0	3,7	7,60	2,31	2	0	4,8	6,97
		33,5	1	1		2,06	1	0	0,00 + Infinity	-1,12 16,32	2,94	2	0	3,4 7,6	1,66 12,27
		123	1	1		2,61	1	0			3,73	2	0		
		455	1	1		3,31	1	1	Nreversals	Npartials	4,73	2	1	Nreversals	Npartials
						4,2	1	0	1	0	6	2	1	2	2
						5,33	1	1							
						6,77	1	1	Chi-square	G				Chi-square	G
						8,59	1	1	4,09	1,32				9,35	0,58
						10,9	1	1	**p** = 0,98					**p** = 0,93	
						13,8	1	1							

	Stage 1					Stage 2						Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
7	1007,498	66,6	1	0	470,6	161	1	0	1035,5	8,99	643	2	2	1236,5	2,13
		245	1	0		205	1	0	0,00 + Infinity	-4,46 22,45	816	2	1	618,6 7,30E+17	0,02 4,23
		904	1	1		260	1	0			1035	2	1		
		3330	1	1		329	1	0	Nreversals	Npartials	1314	2	1	Nreversals	Npartials
						418	1	0	1	0	1669	2	0	5	3
						530	1	0							
						673	1	0	Chi-square	G				Chi-square	G
						853	1	0	4,18	2,24				13,98	0,98
						1083	1	0	**p** = 0,98					**p** = 0,67	
						1374	1	1							

	Stage 1					Stage 2						Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
8	930	66,6	1	0	1735,0	392	1	0	1583,3	2,78	982	2	1	1262,2	2,70
		245	1	0		497	1	0	312,79 + Infinity	-0,06 5,61	1247	2	2	371,7 2810,3	0,34 5,05
		904	1	0		630	1	0			1583	2	1		
		3330	1	1		800	1	0	Nreversals	Npartials	2010	2	1	Nreversals	Npartials
						1014	1	1	1	0	2551	2	2	4	3
						1286	1	1							
						1632	1	1	Chi-square	G				Chi-square	G
						2070	1	0	10,54	1,04				14,74	0,76
						2625	1	0	**p** = 0,57					**p** = 0,61	
						3330	1	1							

	Stage 1				Stage 2					Stage 3b					Stage 4				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
9	250	35,4	1	0	249,8	85,6	1	0	155,4	130,32	96,4	2	0	155,1	11,87	96,2	2	1	135,7	6,90
		130	1	0		109	1	0	0,00 + Infinity	-8,1E+05 8,1E+05	122	2	1	118,3 218,8	2,09 21,64	122	2	1	101,2 169,3	2,37 11,44
		480	1	1		138	1	0			155	2	0			155	2	2		
		1768	1	1		175	1	1	**Nreversals**	**Npartials**	197	2	2	**Nreversals**	**Npartials**	197	2	1	**Nreversals**	**Npartials**
						222	1	1	0	0	250	2	2	1	1	250	2	2	3	5
						281	1	1								*155*	*2*	*1*		
						357	1	1	**Chi-square**	**G**				**Chi-square**	**G**				**Chi-square**	**G**
						453	1	1	<0.001	3,8E+07				6,48	0,68				9,31	0,43
						575	1	1	**p** = 1,00					**p** = 0,99					**p** = 0,99	
						729	1	1												

	Stage 1				Stage 2					Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
10	20000	66,6	1	0	6391,5	392	1	0	3330,0	64,64	1281	2	0	3332,0	2,97
		245	1	0		497	1	0	0,00 + Infinity	-4,9E+05 4,9E+05	1626	2	1	2026,8 + Infinity	-0,40 6,35
		904	1	0		630	1	0			2065	2	2		
		3330	1	0		800	1	0	**Nreversals**	**Npartials**	2622	2	0	**Nreversals**	**Npartials**
						1014	1	0	0	0	3330	2	1	3	2
						1286	1	0							
						1632	1	0	**Chi-square**	**G**				**Chi-square**	**G**
						2070	1	0	2,00	5,8E+07				11,84	1,29
						2625	1	0	**p** = 1,00					**p** = 0,81	
						3330	1	1							

	Stage 1				Stage 2					Stage 3b					Stage 4				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
11	100	20	1	0	38,4	13,2	1	0	23,9	128,20	14,8	2	0	23,9	127,04	14,8	2	0	24,4	8,17
		73,7	1	1		16,7	1	0	0,00 + Infinity	-5,9E+05 5,9E+05	18,8	2	0	0,0 + Infinity	-4,8E+05 4,8E+05	18,8	2	1	19,9 30,5	3,19 13,16
		271	1	1		21,2	1	0			23,9	2	1			23,9	2	1		
		1000	1	1		26,9	1	1	**Nreversals**	**Npartials**	30,3	2	2	**Nreversals**	**Npartials**	30,3	2	2	**Nreversals**	**Npartials**
						34,1	1	1	0	0	38,5	2	2	0	1	38,5	2	1	2	4
						43,2	1	1												
						54,9	1	1	**Chi-square**	**G**				**Chi-square**	**G**				**Chi-square**	**G**
						69,6	1	1	<0.001	2,1E+07				<0.001	1,4E+07				12,79	0,37
						88,3	1	1	**p** = 1,00					**p** = 1,00					**p** = 0,94	
						112	1	1												

	Stage 1				Stage 2					Stage 3b				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
12	46	6,5	1	0	46,0	15,8	1	0	46,1	130,33	28,6	2	1	41,7	8,31
		24	1	0		20	1	0	0,00 + Infinity	-8,5E+05 8,5E+05	36,3	2	0	29,9 57,1	2,16 14,47
		88,3	1	1		25,4	1	0			46,1	2	1		
		325	1	1		32,2	1	0	**Nreversals**	**Npartials**	58,5	2	2	**Nreversals**	**Npartials**
						40,9	1	0	0	0	74,2	2	2	1	2
						51,9	1	1							
						65,8	1	1	**Chi-square**	**G**				**Chi-square**	**G**
						83,5	1	1	<0.001	4,3E+07				7,20	0,55
						106	1	1	**p** = 1,00					**p** = 0,98	
						134	1	1							

	Stage 1				Stage 2					Stage 3b					Stage 4				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
13	1117	66,6	1	0	1735,0	392	1	0	1448,5	129,26	899	2	0	1538,2	250,43	954	2	0	1554,8	10,65
		245	1	0		497	1	0	0,00 + Infinity	-7,2E+05 7,2E+05	1141	2	0	0,0 + Infinity	-6,7E+05 6,7E+05	1212	2	1	1289,6 1887,6	3,96 17,34
		904	1	0		630	1	0			1448	2	0			1538	2	1		
		3330	1	1		800	1	0	Nreversals	Npartials	1839	2	2	Nreversals	Npartials	1952	2	1	Nreversals	Npartials
						1014	1	0	0	0	2334	2	2	0	0	2478	2	2	2	3
						1286	1	0												
						1632	1	1	Chi-square	G				Chi-square	G				Chi-square	G
						2070	1	1	<0.001	3,1E+07				<0.001	7,1E+06				7,70	0,39
						2625	1	1	p = 1,00					p = 1,00					p = 1,00	
						3330	1	1												

	Stage 1				Stage 2					Stage 3b					Stage 4				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
14	20	2,83	1	0	5,4	1,86	1	0	8,7	129,36	5,42	2	0	9,3	251,08	5,75	2	0	9,0	488,95
		10,4	1	1		2,36	1	0	0,00 + Infinity	-7,6E+05 7,6E+05	6,88	2	0	0,0 + Infinity	-7,2E+05 7,2E+05	7,31	2	0	0,0 + Infinity	-7,4E+05 7,4E+05
		38,4	1	1		2,99	1	0			8,73	2	0			9,27	2	2		
		141	1	1		3,8	1	0	Nreversals	Npartials	11,1	2	2	Nreversals	Npartials	11,8	2	2	Nreversals	Npartials
						4,82	1	0	0	0	14,1	2	2	0	0	14,9	2	2	0	0
						6,11	1	0												
						7,75	1	0	Chi-square	G				Chi-square	G				Chi-square	G
						9,84	1	1	<0.001	3,5E+07				<0.001	8,1E+06				<0.001	2,28E+06
						12,5	1	1	p = 1,00					p = 1,00					p = 1,00	
						15,8	1	1												

	Stage 1				Stage 2					Stage 3a					
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
15	946	66,6	1	0	1735,0	392	1	1	0,0	-0,02	28,2	5	0	926,9	1,28
		245	1	0		497	1	1	0,00 + Infinity	-1,49 1,46	3330	5	5	134,8 4426,5	0,26 2,29
		904	1	0		630	1	0			*1632*	*1*	*1*		
		3330	1	1		800	1	1	Nreversals	Npartials				Nreversals	Npartials
						1014	1	1	4	0				5	0
						1286	1	0							
						1632	1	0	Chi-square	G				Chi-square	G
						2070	1	0	14,00	7481,67				19,77	0,63
						2625	1	0	p = 0,30					p = 0,14	
						3330	1	0							

	Stage 1				Stage 2					Stage 3a					Stage 4				
ID	Working LD50	Dose	Nexp	Ndead	Working LD50	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope	Dose	Nexp	Ndead	Working LD50	Slope
16	350	25	1	1	176,7	60,5	1	0	72,4	-4,31	6,11	5	2	336395,8	-2,41E+13	1281	2	1	434,4	0,37
		92,1	1	0		76,8	1	1	0,00 + Infinity	-9,58 0,95	722	5	2	0,0 + Infinity	-2,5E+14 2,1E+14	1626	2	2	0,0 + Infinity	-0,15 0,88
		339	1	0		97,5	1	1								2065	2	2		
		1250	1	0		124	1	0	Nreversals	Npartials				Nreversals	Npartials	2622	2	2	Nreversals	Npartials
						157	1	0	3	0				4	2	3330	2	2	4	3
						199	1	0												
						253	1	0	Chi-square	G				Chi-square	G				Chi-square	G
						320	1	0	6,19	1,49				2,30E+16	91,11				21,01	1,94
						407	1	0	p = 0,91					p = <0.001					p = 0,34	

Annex 2 of Appendix V (2 pages)

Expt_ID	Stage	LD50_gues	NPartials	NReversals	LD50_est	slope_est	Chisq	p	slope_lcl	slope_ucl	LD50_lcl	LD50_ucl
1	Stage 1	555,1			555,1							
1	Stage 2		0	0								
2	Stage 1	1,67			1,67							
2	Stage 2		0	0								
3	Stage 1	470,93			470,93							
3	Stage 2	311,36	0	2	311,36	3,9664	9,0708	0,6969	-0,0204	7,9533		
3	Stage 3	308,99	2	3	308,99	3,5611	8,5764	0,8572	1,085	6,0372	161,38	510,41
4	Stage 1	66			66							
4	Stage 2	32,89	0	3	32,89	0,6091	13,9488	0,304	-1,0496	2,2678		
4	Stage 3	17,59	2	4	17,59	0,4971	14,4768	0,4148	-0,1967	1,1909		
5	Stage 1	16,98			16,98							
5	Stage 2	0,99	0	3	0,99	0,5933	13,3352	0,3452	-1,257	2,4437		
5	Stage 3	5,17	1	4	5,17	1,2075	13,4781	0,4893	0,081	2,334	0	17,6
6	Stage 1	4,74			4,74							
6	Stage 2	3,73	0	1	3,73	7,5941	4,0879	0,9818	-1,6659	16,8541		
6	Stage 3	4,78	2	2	4,78	6,9562	9,3538	0,9285	1,5533	12,3591	3,46	7,86
7	Stage 1	470,93			470,93							
7	Stage 2	1036,14	0	1	1036,14	8,9699	4,1848	0,9799	-5,3291	23,2689		
7	Stage 3	1237,47	3	5	1237,47	2,1262	13,9827	0,6683	-0,083	4,3354		
8	Stage 1	1734,93			1734,93							
8	Stage 2	1582,13	0	1	1582,13	2,7752	10,5397	0,4826	-0,175	5,7253		
8	Stage 3	1261,14	3	4	1261,14	2,6979	14,7404	0,5437	0,259	5,1368	244,2	2780,4
9	Stage 1	250			250							
9	Stage 2	155,29	0	0	155,29	184,5954	0	1	-1,05E+11	1,05E+11		
9	Stage 3	155,26	1	1	155,26	11,8937	6,451	0,9896	1,9249	21,8624	122,08	238,46
9	Stage 4	135,86	5	4	135,86	6,916	9,2907	0,9917	2,3387	11,4934	101,68	170,02
10	Stage 1	6391,55			6391,55							
10	Stage 2	3330	1	0	3330	66,8371	0	1	66,8371	66,8371	3136,58	3535,35
10	Stage 3	3330,82	2	2	3330,82	2,9772	9,8314	0,8302	-0,5453	6,4997		
11	Stage 1	38,39			38,39							
11	Stage 2	23,85	0	0	23,85	183,8369	0	1	-8,79E+10	8,79E+10		
11	Stage 3	23,85	1	0	23,85	133,6148			133,6148	133,6148	23,15	24,57

Expt_ID	Stage	LD50_gues	NPartials	NReversals	LD50_est	slope_est	Chisq	p	slope_lcl	slope_ucl	LD50_lcl	LD50_ucl
11	Stage 4	24,42	3	2	24,42	8,1795	12,787	0,8863	3,3262	13,0329	19,73	30,02
12	Stage 1	46			46							
12	Stage 2	46	0	0	46	185,0288	0	1	-1,15E+11	1,15E+11		
12	Stage 3	41,67	2	1	41,67	8,3068	7,2057	0,9807	2,1846	14,4289	31,03	59,43
13	Stage 1	1734,93			1734,93							
13	Stage 2	1447,26	0	0	1447,26	184,4729	0	1	-1,03E+11	1,03E+11		
13	Stage 3	1536,44	0	0	1536,44	365,2148	0	1	-1,12E+11	1,12E+11		
13	Stage 4	1553,38	3	2	1553,38	10,6351	7,7087	0,9963	3,8891	17,3811	1289,43	1890,78
14	Stage 1	5,43			5,43							
14	Stage 2	8,74	0	0	8,74	184,8547	0	1	-1,01E+11	1,01E+11		
14	Stage 3	9,28	0	0	9,28	366,919	0	1	-1,13E+11	1,13E+11		
14	Stage 4	9,01	0	0	9,01	719,7109	0	1	-1,32E+11	1,33E+11		
15	Stage 1	1734,93			1734,93							
15	Stage 2	1343,61	1	3	0	-0,0191	11,8107	0,378	-1,528	1,4898		
15	Stage 3	925,96	1	4	925,96	1,2761	15,0664	0,2378	0,2144	2,3379	99,05	4620,56
16	Stage 1	176,78			176,78							
16	Stage 2	279,79	0	3	72,46	-4,3159	6,187	0,9064	-9,8266	1,1948		
16	Stage 3	802,47	2	4	1,6	-0,2623	11,576	0,6403	-0,9635	0,4389		

Annex 3 of Appendix V

CY Query	TS Reply	Additional Comments
Screenshot on Page 7 from the manual 'Stage 1 doses for an estimated LD_{50} 0 of 100' . When I run this the value (100) was missing from the form	This is a bug. It was there at one time - and apparently we didn't notice when the anticipated LD50 was lost. Should be a very easy fix.	
Page 13/14 of the manual - The lowest dose (dose 1 of five) is missing from the screenshot for Stage 3. When I run the same design in SEDEC I get a lower dose of 49.7 as well as the four listest doses (63.1, 80.1, 102, 129). For stage 4, it seems that the upper dose (128) is missing in the screenshot	Images in manual needs to be replaced. Program is correct.	
Limit tests: As in the screenshot on P15 of the manual, SEDEC asks if the design is a 5 or 10 bird limit test. Having selected one of these designs (and hence the number of birds in the test) I am not sure why the form on page 16 asks for the number of birds in the limit test. Values from 5 to 10 (inclusive) can be input, although the text on P16 states that (only) 5 and 10 bird limit tests are allowed. This seems to be a duplication of information - and in addition, when I entered a value of 6 for the number of birds tested this gave erroneous results - with 5 mortalities and 6 birds it told me that the test had been passed. Is the option to enter the total number of birds here necessary? At the moment, it seems to have the potential to lead to both conflicting entry information and errors... I am not sure how SEDEC deals with the information that there are 6 birds in a 10 bird limit test etc...	The choices on the upper scroll box on this panel should be limited to 5 and 10. This should be very easy to fix. The upper scroll box does seem a little repetitious, but it allows the user to extend a 5 bird limit test by adding 5 more birds without forcing the user through study setup again. My preference is to limit the scroll to 5 and 10 but to leave the box in place.	

'View plot': This graph seems to have both a fixed title "Project: Wildlife International No. 1" and fixed x-axis values. Every time I try to produce it I get an error 'Run-time error '-2147467259 (80004001)' Method 'HasTitle' of object '_Chart' failed and the x axis values run from 0 to 600 whatever the tested doses. This means that for some tests the higher doses are off the edge of the graph (particularly when starting with a limit test at a dose of 2000). I can't see any option for entering a project title anywhere.	The graphic errors that you are seeing seem to be an Excel 2007 compatibility issue. The program was written and tested in Excel 2000. None of the things you describe occur in Excel 2000. The title is a concatenation of the words "Project number:" and the project number entered on the project set-up panel. There is no provision for entering an arbirary title.	(EM) In the Users Guide, on page 5, in item 6, you should add a sentence indicating that "The report display and any plots will be titled according to the designation you enter at 'Project number'." By the way, based on Carol's comment, it seems to be a concatenation of "Project:", not "Project number:", and whatever heading you entered at the Start New Study panel. Confirming the pattern, however, the original file appears as "WLI SIM 1023" and the one I just saved does have the title "qu", with test substance qu1, as I had entered.
I am running SEDEC on Excel 2007 under Windows Vista. The instructions on P4 in the manual about enabling macros do not seem valid for Excel 2007 as the layout of the toolbars have changed substantially. I am assuming the instructions were therefore written for an earlier version of Excel. Rather than changing the security level for macros to Medium, the advice for 2007 seems to be to create a (preferably local) folder as a 'trusted location'. I am running SEDEC on Excel 2007 under Windows Vista. This seems like it would work quite well for SEDEC as you can make a folder and all subfolders of it trusted locations and then you don't need to keep enabling macros. This is the way I am running it. Anyway, I thought that this information might be of use to you and it might be beneficial to update the user manual to include Excel 2007.	Your solution to the macro security issue seems reasonable. When you finish your review we can update the manual to include a paragraph of instructions specific to Vista/Excel2007. Create a trusted location for storing SEDEC files as suggested in CSY email of 13 Feb 2009.	

Limit test with complete mortality: For a 10-bird limit test with complete mortality, after entering the responses (all dead) SEDEC takes me straight to the report page showing 10 tested and 10 responding but no message appears. However, if I click on 'Enter responses/calculate doses' again after that I get the message "There was complete mortality in the limit test. You must restart the test using a multi dose first stage". I presume that I would want this message to appear immediately after I have entered the mortality data. For a 5-bird limit test, I cannot get this message to appear at all - just the report showing that 5 birds were tested and five died, but no further information. When I click on "Enter responses/calculate doses" I get back to the screen for entering mortality and the mortality data that I have already entered has disappeared	This message is not provided as a conclusion. If you examine the spreadsheet view you will notice that the conclusion "Limit test failed.. Total mortality. Restart test with multi-dose first stage" is provided there. The purpose of the pop-up message box is to prevent a user who has entered data indicating complete mortality occurred in a 10-bird limit test from attempting to continue the study, as this is not permitted under the guideline. The message does not appear if complete mortality occurs in a 5-bird limit test because the guideline does allow the user to continue the test under these conditions. This part of the program is working as it should.	(EM) Regarding returning to the spreadsheet view, I think you may want some kind of indication in the Users Guide that certain screens will cover the spreadsheet and "Back" is the appropriate button to press. Ordinarily I skip Back buttons.
There are some very minor differences between the LD_{50} estimates given after a Limit test in SEDEC and Table 1 on Page 4 of the guidelines (e.g. 2945 instead of 2947 for 20% mortality, 2244 instead of 2247 for 40%, 1783 instead of 1780 for 60% and 1358 instead of 1357 for 80%). This almost doesn't seem worth worrying about but it is an inconsistency.	I made the table that is in the guideline, so I am aware of these differences. If I remember correctly, because of rounding, I had to choose between apparent slight inconsistency between the guideline text and the table or slight inconsistency between guideline and SEDEC. I chose the latter	
When setting up the doses for Stage 1, overriding the maximum dose with a value of >3330 has no effect. Similarly, overriding the minimum dose with a value of >66.6 has no effect.	I believe that this is how the program should work, as no dose over 3330 is allow for animal welfare reasons.	

Otherwise (at other values and in Stage 2, overriding the maximum (or minimum value) has the effect of changing the maximum (or minimum) dose to the new value and changing all other doses to maintain the 1:50 (Stage 1) or 1:8.5 (Stage 2) ratio between the high and low dose. There is no longer any relationship between the doses and the estimated LD_{50}. I think this is what is meant to happen but is it worth a note/warning to the user to explain that this is what happens?	A warning is probably needed
There are a lot of cases where either the SAS programs do not give an estimate for the CIs and SEDEC does (although this is often 0 to infinity or very very wide). There are also some cases where SAS does give an estimate and SEDEC does not. These tend to be the cases where different methods have been used to estimate the CIs etc in my SAS programs as in Table 4 of Bertie's doc (P7). Have these methods been included in SEDEC? Would you want to give a CI of 0 to infinity or would it be better if it was displayed as non-estimable?	SEDEC implements only MLE-based methods (i.e. Finney's probit method with CI's for the LD_{50} estimated using Fieller's method. CI's off 0 to infinity should probably be reported as non-estimable. Agreed?
The following dataset is created in SEDEC with an initial LD_{50} estimate of 62.54959 and then entering the mortality data at each stage. Note that the SEDEC and SAS doses are very similar until Stage 3. At the end of Stage 2, the est LD_{50} is 1.00 (SEDEC) and 0.99 (SAS) and the estimated slope is 0.595 (SEDEC) and 0.593 (SAS). Both analyses then set the slope to 1 before calculating the doses for the next stage because the slope is less than one (P15 of guideline). However, my SAS programs set the highest dose to highdose/lowdose = 10.9/0.0921 = 118.3 and the lowest dose to 1. This was detailed in P1 of Bertie's word doc for the validation (attached) . I can't find it in the guideline - have I missed it, or should we not be restricting the lowdose to 1? I am not sure which is	Relying on very tenuous memories, I vaguely remember that setting the lowest dose to 1 was a device to prevent complications in the simulations (overflows, division by zero, or the like). I believe that we convinced ourselves that doing this would not adversely effect the interpetation of the simulations because of the rarity of the occurrrence. As far as I know, there is nothing in the guideline that specifies that this be done. SEDEC seems to be consistent with the guideline.

right here...

Stage	SEDEC dose	SAS dose	N dead	N exposed
1	8.84	8.85	0	1
1	32.6	32.59	1	1
1	120	120.06	1	1
1	442	442.29	1	1
2	5.82	5.82	1	1
2	7.38	7.38	1	1
2	9.36	9.36	1	1
2	11.9	11.88	1	1
2	15.1	15.07	1	1
2	19.1	19.12	0	1
2	24.3	24.27	1	1
2	30.8	30.79	0	1
2	39.1	39.06	1	1
2	49.6	49.56	1	1
3	0.0921	1.00	0	5
3	10.9	118.27	5	5

I am getting some errors with the calculation of number of partials and number of reversals in SEDEC; this is where a dose of 3330 has been used in both Stage 1 and Stage 2. They have not been combined in the combined data in dose order table and as a result a partial with 3330 1/1, 3330 0/1 is being treated as a reversal and so on. Can you reproduce this?	Yes, I can duplicate this. I need to allow for some "fuzz" when comparing larger concentrations so that 3330.001 is not treated as a concentration that is different from 3330.0001. This is an easy fix	
With an experiment with 0% mortality in Stage 1 and Stage 2 I get a message "There have been no mortalities. Consider performing a limit test or restarting the test using higher doses". This seems highly sensible, but I can't find this in the 'rules' anywhere. Is it part of the guidelines?	I don't think so. The logic branch had to conclude with some kind of message. Just expressing the obvious	(EM) I find two parts of the guideline that allude to this sort of situation. One is par. 36, where it says "It should be noted that in some circumstances, the LD_{50} cannot be estimated without using doses above the limit dose. Because there are constraints on the use of very high doses of test substance, it may not always be possible to estimate the LD_{50} for slightly toxic substances." The other is even less direct. That is, with 0% mort. at stage 1, the working LD_{50} is above dose 4 of stage 1 (in fact, 1.9xdose4). Then, with no reversals in in stage 2, one should go to stage 3b, from a working estimate still higher (than double initial dose 4) so the hdose will be closing on 4xidose 4. That will already be near 3330 if idose 4 was around 830, or the initial LD_{50} estimate was about 118 (and the initial ldose was about 17). So there won't be too many higher doses where one could start. Maybe, to remind folks of this, the message line should be rearranged: "There have been no mortalities. Consider restarting the test using higher doses or performing a limit test."

RE 8a and 8b:Does there remain an inconsistency here? Doses over 3330 are not allowed at Stage 1 (in that they have no effect when they are input), but can be input at Stage 2 and doses will be calculated accordingly... If no dose over 3330 is allowed for welfare reasons at Stage 1 should this not also be true for Stage 2? I would think that a warning message along the lines of 'Maximum dose allowed is 3330' would be useful for cases where a maxdose>3330 is input as well as a warning message stating that there is no longer a relationship between the suggested doses and the LD_{50} when a lower maxdose is input .	Agreed (inconsistency). Seems reasonable to me (two warning messages).
When setting up the study there is an option for units to be in different units to mg ai/kg (mg formulation/kg and a free form field). As far as I can see the only effect of entering different units in this field is that they are printed on the spreadsheet view as 'Dose Units:' However, I can see various potential problems with allowing different units at this point such as the calculation of minimum and maximum doses (if units were mg formulation/kg would 3330 still be the maximum allowable dose) and other rules in the analysis such as setting the slope to be between 1 and 15. The guidelines talk about doses in mg/kg bodyweight - (although I don't think it is specified) I would assume this to be mg ai/kg. Should there be a choice of units when setting up the study or should the units be restricted to mg ai/kg?	Perhaps it is better to limit the choice. There are quite a few complications that I can see with allowing different units. Now that I think about it, dose could be measured as an acid equivalents, titre, colony forming units (biological), or any other other oddball unit. If any of these units give a number greater than 3330, then SEDEC will shift things around. Of course units can always be rescaled (e.g. from 3330 CF to 3.33 thousand CFU). A discussion of this could be added to the manual. In retrospect, allowing the units to be changed in the program itself seems confusing.

Datasets of Type B: Inconsistent results where the average logdose at which mortality occurs is less than or equal to the average logdose at which survival occurs: SEDEC will run a probit analysis which will result in a negative slope. The slope and CIs are reported along with a message that says "The slope is negative! Examine data carefully. The LD_{50} may be meaningless". On examination, the LD50 is indeed meaningless (3E-19). In my example which had 3 reversals, the analysis then proceeded to Stage 3a, following the rule of two or more reversals *and an estimate of the slope*. In Bertie' validation doc, both LD_{50} and slope ests were specified as non-estimable if the data was inconsistent. My feeling is that all estimates and CIs should be reported as non-estimable here and SEDEC should go on to Stage 3b not 3a (I presume this would happen if the slope estimate were not reported). A warning message to examine the data carefully might still be warranted.

SEDEC has a routine to handle one situation not described in the guideline, and you are now describing this situation. If after two stages, the slope of the cumulative dose response curve is negative, SEDEC sets the slope to 1 (consistent with the guideline) and sets the working LD_{50} to be the mean of all concentrations tested (not described in the guideline). The LD_{50} estimate from the MLE routine is not used. It seems obvious in retrospect that a message to this effect should be produced (and I will add one). I chose to go on to stage 3a, and not 3b, and while that choice is clearly debatable, I think an argument can be made for going to stage 3a. For perspective, it should be remembered that the entire design is focused on preventing the occurence of such anomolous situations, and negative slopes after two stages should be extremely rare. Obvously, SEDEC should handle the negative working slope appropriately, but because of the expected rarity of the event, I am not sure there is a need to mention it in the guideline.

(EM) With respect to Tim's comment "Obvously, SEDEC should handle the negative working slope appropriately, but because of the expected rarity of the event, I am not sure there is a need to mention it in the guideline." I think we may need to mention even rare events, since someone who encounters such an event may spend a great deal of time trying to fix some aspect of his/her run, when it's the data set that's generating the problem. It may, however, be sufficient to mention it in SEDEC, and not the guideline, since it is anticipated that most folks will choose to use the software provided

E/F:/G Consistent results with no reversals and <=1 partial mortality : SEDEC attempts probit analysis and estimate and slope are reported; but I suspect that it fails to converge. If I run probit analysis on this data in SAS it fails to converge after 50 interations and the number of interations reported in SEDEC for 4 different examples was 27 in each cases - is this the maximum number of iterations? In all examples I have looked at, the CIs of slope are huge (e.g. --491032, 491162) and the CIs of the LD_{50} are zero to infinity. The estimate of the LD_{50} looks sensible (very similar to that produced by the 'other' methods described in Bertie's doc), but I suspect the estimated slope is pretty meaningless. I therefore think this might be OK if the LD_{50} was reported, but the LD_{50} CI and slope est and CI were given as non-estimable	Agreed.	
On page 11 of the Guideline it states that: "Methods for estimating LD_{50}, slope and confidence intervals for a variety of different experimental outcomes are recommended in (2). The slope can only be estimated if there are at least 2 partial kills or at least one dose response inversion." To summarise the two points above (15 and 16), I don't think SEDEC should be reporting the slope estimate for B or E/F/G. The case of E/F/G is actually covered in the above statement from the guideline as there are no reversals and <=1 partial kill in all of these cases.	Agreed. When I rework SEDEC, I'll fix this. It should be be very simple to do so	(EM) I wonder if I am looking at the wrong version of the guideline, but, in ENV/JM/TG(2008(30) (as prepared for April 2008), a) it doesn't have this sentence on page 11 and b) its reference number 2 is Chapman, Dark, and Springer. Since the latter is not yet published, it's not clear how soon it will provide illumination. The sentence in question does appear in Annex 2 on page 19, which does not have a reference list of its own. Note, however, that the references lists has at least one duplicate (3 and 7 are both Crump and Howe).
Can you confirm that for the case where there is a negative slope you estimate the working LD_{50} using the mean and not the log mean of the doses tested? I guess I am just a bit surprised because I would have expected it to be the	No reply received	

log mean, but I am sure that you have a reason!

What I wrote previously for Type A outcomes was not accurate. There do seem to be problems if there is complete mortality. It should have read:

A: Complete survival/mortality at all doses.

i) If there is complete survival at the end of Stage 2, SEDEC stops with the message "There have been no mortalities. Consider performing a limit test or restarting the test using higher doses." This seems OK.

ii) If there is complete mortality at the end of Stage 2, things can go very wrong. I get failure to converge (27 iterations), a negative slope estimate, large CIs and very high doses for Stage 3 (and therefore presumably a high working LD_{50} estimate). NB: The validation exercise used the lowest dose as the working LD_{50} estimate if there was complete mortality - I guess that either this or a warning/stop message of some sort would be appropriate? Presumably if all tested doses up to the end of Stage 2 result in mortality, the chemical is likely to be pretty toxic and not of much interest?

Oops... Good catch Carol! I neglected to cover the possibility that there might be complete mortality after stage two. In fact, I don't think that guideline properly deals with the situation. Following the guideline as written takes the investigator to stage 3b, but the guideline does not provide adequate instructions for selecting doses if this route is followed. There seem to be two possible approaches to dealing with the situation.

(1) After two stages with total morality, go to stage 3b. No working LD_{50} estimates is available, so set hdose equal to the lowest dose used in stage two,calculate ldose as hdose/2.6, and select intermediate doses as described in the guideline.

or

(2) Return to stage 1 and re-initiate the study.

(EM) If you assume all 10 animals die at stage 2, I think your "working slope" would be something less than 1.4, which is extremely shallow. Look at the box-and-whiskers plots in the simulations report for the situation when the slope was 2 compared to higher values. There was much poorer performance then. We should probably all think about this and fold those thoughts into whatever comes out of the ring test. That's probably better than trying to change SEDEC as we go.

(TS) I defiinitely agree that deciding how to to handle total mortality after stage 2 is not something that Carol and I can resolve. What SEDEC does when there are no survivors after two stages can only be resolved after Carol completes her validation and the drafting group can meet and decide how to handle the situation.

The doses for Stage 3, Dataset 16 produced by SAS and SEDEC do not match. I believe that SAS does as TS described in query 15 and am not sure what SEDEC does so I cannot find the cause of the difference

Logic suggests to me that it is not a good idea to go to stage 3b. There is really no reason to believe that the LD_{50} should be in the narrow interval (factor of 2.6) between ldose and hdose values calculated according to stage 3b directions, and it seems unlikely that a sound LD_{50} estimate would be obtained unless the doses were perfectly chosen because so few birds could contribute any information to the analysis . In my opinion, if there was total mortaility after two stages,
the investigator should go back to stage 1 and repeat it, using a much lower LD_{50} as the working estimate.

Perhaps having SEDEC
produce a message that directs the investigator to repeat stage 1 could be agreed as appropriate approach . We will need consensus on this before I make the correction to SEDEC. We might also need to add a few sentences to the guideline to cover this case.

(TS) I'm not sure I understand why having complete mortality in all tested concentrations would imply a shallow slope. I would think the total mortality situation to be most likely to occur when the LD_{50} is well below any tested concentration, and the true dose response curve has a very steep slope. Tho low-slope case should result in occassional survivors at almost any dose, whereas a very low LD_{50} and steep slope makes observation of survival at any dose well above the LD_{50} very unlikely. Perhaps the more important point to note is that complete mortality in all doses after two steps would be likely only if an incredibly bad guess at the LD_{50} (many orders of magnitude too high) was used as the basis for picking stage 1 doses.

(EM) Absolutely agree: it would be an incredibly bad guess. Or an extraordinarily potent compound. I think I was thinking small variance, not small slope (with former being reciprocal of a large slope).

Post-Test Corrections and Additions to SEDEC

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Date: 29th September 2009

Verification testing of SEDEC Version 1.1 was performed by Carol Yarrow of Stats Matters and results of testing were reported in "Verification of the sequential design calculator SEDEC: A tool for use with OECD TG223 (Avian Acute Oral Toxicity Test)", issued 26th March 2009. The verification testing included examination of the execution and output from SEDEC as well as consideration of a number of elements of the users guide. In the report, a number of deficiencies in SEDEC and the users manual were identified. Table PC1 below repeats the findings in the verification report, and links the findings to more detailed statements (queries) in Table PC2. The last column of Table PC2 describes how findings have been addressed in SEDEC Version 1.2.

Additional changes in SEDEC were requested by the expert group overseeing the development of TG223. In some cases these changes overlap with the findings in the verification report, but for completeness, changes made at the request of the group are listed below.

- Several items were added to the audit trail, including working LD_{50} values for stages 1 and 2, the type of stage 3 chosen (i.e. stage 3a or 3b), and any changes to the default minimum dose separation.

- The horizontal (dose) axis of the plot of the dose response curve was changed to a logarithmic scale, and code was added to properly set the minimum and maximum of the axis (needed because auto-scaling by EXCEL did not work properly)

- The verification report pointed out the need for SEDEC to combine response from different stages when the calculated doses were essentially identical. SEDEC version 1.2 was recoded so that by default, dose levels that are within 1% of each other are combined. The expert group requested that an option to allow the minimum separation dose to be changed, and that the implications of regrouping be discussed in the users manual. The requested changes were made and an explanation of the regrouping and a statement that caution should be applied in increasing the minimum dose separation were added to the manual. A column was added to the SEDEC report that indicates which (if any) dose groups have been combined.

- The expert group decided that when the dose response curve was negative after stage 2 of the test (a very rare occurrence), the program should direct the user to stage 3b, and that doses should be calculated using an assumed slope of 1 and a working LD_{50} equal to the geometric mean of the doses showing mortality. This change was made, and an explanation of the behavior of SEDEC added to the users manual.

- SEDEC Version 1.1 did not provide for the case of complete mortality after stage 2 of the test. As agreed by the expert group, SEDEC Version 1.2 where mortality is complete after two stages, SEDEC As decided by the expert group, SEDEC now directs the user to re-initiate the test using a multiple dose stage 1 at lower concentrations.

- SEDEC version 1.1 sometimes displayed slope estimates and confidence intervals when maximum likelihood estimates were not valid, and the expert group requested that values not

be displayed under these conditions. Maximum likelihood estimates are available when the data contain at least one reversal or two partial mortality groups. SEDEC now reports the slope and confidence intervals for the LD_{50} and slope to be "Undefined" when these conditions occur. This should serve to reduce confusion resulting from displaying inaccurate slope estimates.

Modifications to SEDEC version 1.2 address all issues identified by the verification testing of version 1.1. However, problems associated with display of the dose response plot when SEDEC is executed using Excel 2007 were not solved. The graphics module used by Excel was radically changed in Excel 2007, and none of the VBA code in SEDEC associated with graphic images is valid in Excel 2007. Because of the substantial learning curve needed to understand the module and communication with it via VBA, it has not been feasible to develop code needed to create the dose response plot when using Excel 2007. To prevent program crashes when the researcher attempts to view the plot, code was added to bypass the plot routines when Excel 2007 is used . This problem will be more fully addressed in a later revision of SEDEC.

Table PC1: Summary of Issues Identified During SEDEC Version 1.1 Verification

Testing Stage	Description	Query
Setup	Missing LD_{50} value in screenshot on Page 7 of the manual	1
	Missing doses in screenshot on Page 16 of the manual	2
	Run-time error with 'View Plot'. Fixed title 'Project Wildlife International No. 1' and fixed x axis values (0 to 600)	4
	Instructions for enabling macros are not applicable to Excel 2007	5
Limit Tests	Allowing a variable number of birds in 5 or 10 bird limit test leads to erroneous results; e.g. 5 mortalities from 6 exposed results in a passed limit test.	3
	When the override max dose facility is used for Stage 2 doses following a limit test, the highest dose is set to the new max dose input by the user (including when it is greater than 3330) and there is no longer any relationship between the working LD_{50} and the dose range. Similar behaviour is observed for the override min dose facility. A warning message to the user to explicitly state that there is now no relationship between the LD_{50} and the dose range would be beneficial.	8b
	If dose units other than mg ai/kg are to be supported by SEDEC then the current behaviour of setting the highest dose to be equal to (rather than less than or equal to) the override max dose may not be appropriate.	14
Stage 1 Setup	When the override max dose facility is used for Stage 1 doses, overriding the max dose with a value greater than 3330 has no effect. Similarly overriding the min dose with a a value greater than 66.6 has no effect. This is inconsistent with the behaviour observed for Stage 2 doses.	8a, 13
Stages 1 to 4	If there is complete survival after Stage 2, SEDEC stops with the message 'There have been no mortalities. Consider performing a limit test or restarting the test using higher doses'. This is not covered in the guidelines	12
	SEDEC does not deal with the situation where there is complete mortality after Stage 2 appropriately.	19
	Where confidence intervals for the LD_{50} are estimated as 0 to +Infinity they should be reported as non-estimable.	9
	SEDEC does not combine doses that have been repeated at multiple stages properly.	11
	Slope estimates and confidence intervals are reported when the probit analysis has failed to converge. In all of these cases there are <=1 partial kill and 0 reversals. The guidelines state that the slope can only be estimated when there are at least 2 partial kills or one reversal. The slope estimate and CI should therefore only be displayed when these conditions are met.	16, 17
	If the slope is negative, SEDEC does not seem to behave as expected. What does SEDEC use as the working LD_{50} to calculate the doses for the next stage? For dataset 16, SEDEC appears to use the LD_{50} estimate from the failed probit analysis to calculate the doses for Stage 3.	15, 18, 20

Table PC2. Changes and responses addressing findings of verification testing of SEDEC Version 1.1

No.	Date Queried	CY Query	Response/Change
1	13-Feb-09	Screenshot on Page 7 from the manual 'Stage 1 doses for an estimated LD_{50} of 100' . When I run this the value (100) was missing from the form	Corrected.
2	13-Feb-09	Page 13/14 of the manual - The lowest dose (dose 1 of five) is missing from the screenshot for Stage 3. When I run the same design in SEDEC I get a lower dose of 49.7 as well as the four listed doses (63.1, 80.1, 102, 129). For stage 4, it seems that the upper dose (128) is missing in the screenshot	Corrected.
3	13-Feb-09	Limit tests: As in the screenshot on P15 of the manual, SEDEC asks if the design is a 5 or 10 bird limit test. Having selected one of these designs (and hence the number of birds in the test) I am not sure why the form on page 16 asks for the number of birds in the limit test. Values from 5 to 10 (inclusive) can be input, although the text on P16 states that (only) 5 and 10 bird limit tests are allowed. This seems to be a duplication of information - and in addition, when I entered a value of 6 for the number of birds tested this gave erroneous results - with 5 mortalities and 6 birds it told me that the test had been passed. Is the option to enter the total number of birds here necessary? At the moment, it seems to have the potential to lead to both conflicting entry information and errors... I am not sure how SEDEC deals with the information that there are 6 birds in a 10 bird limit test etc...	SEDEC was modified to display and accept only 5 and 10 bird limit tests
4	13-Feb-09	'View plot': This graph seems to have both a fixed title "Project: Wildlife International No. 1" and fixed x-axis values. Every time I try to produce it I get an error 'Run-time error '-2147467259 (80004001)' Method 'HasTitle' of object '_Chart' failed and the x axis values run from 0 to 600 whatever the tested doses. This means that for some tests the higher doses are off the edge of the graph (particularly when starting with a limit test at a dose of 2000). I can't see any option for entering a project title anywhere.	The graphics module used by Excel was radically changed in Excel2007, and none of the VBA code in SEDEC associated with graphic images is valid in Excel 2007. Because of the substantial learning curve needed to understand and learn to communicate via VBA with the new graphics module I have been unable to develop code needed to interact with the module. To prevent program crashes, I did add code to bypass the routines that plot results if SEDEC is operating under Excel2007. I will attempt to address this issue in a later revision of SEDEC.

No.	Date Queried	CY Query	Response/Change
5	13-Feb-09	I am running SEDEC on Excel 2007 under Windows Vista. The instructions on P4 in the manual about enabling macros do not seem valid for Excel 2007 as the layout of the toolbars have changed substantially. I am assuming the instructions were therefore written for an earlier version of Excel. Rather than changing the security level for macros to Medium, the advice for 2007 seems to be to create a (preferably local) folder as a 'trusted location'. I am running SEDEC on Excel 2007 under Windows Vista. This seems like it would work quite well for SEDEC as you can make a folder and all subfolders of it trusted locations and then you don't need to keep enabling macros. This is the way I am running it. Anyway, I thought that this information might be of use to you and it might be beneficial to update the user manual to include Excel 2007.	A section was added to the manual to address macro security issues for Excel2007 users (See page 4 of the revised manual).
6	13-Feb-09	Limit test with complete mortality: For a 10-bird limit test with complete mortality, after entering the responses (all dead) SEDEC takes me straight to the report page showing 10 tested and 10 responding but no message appears. However, if I click on 'Enter responses/calculate doses' again after that I get the message "There was complete mortality in the limit test. You must restart the test using a multi dose first stage". I presume that I would want this message to appear immediately after I have entered the mortality data. For a 5-bird limit test, I cannot get this message to appear at all - just the report showing that 5 birds were tested and five died, but no further information. When I click on "Enter responses/calculate doses" I get back to the screen for entering mortality and the mortality data that I have already entered has disappeared	No changes to the code were made but, the manual was modified to indicate that SEDEC will direct the researcher to restart the test using a multidose first stage if there is complete mortality in the limit test.
7	20-Feb-09	There are some very minor differences between the LD_{50} estimates given after a Limit test in SEDEC and Table 1 on Page 4 of the guidelines (e.g. 2945 instead of 2947 for 20% mortality, 2244 instead of 2247 for 40%, 1783 instead of 1780 for 60% and 1358 instead of 1357 for 80%). This almost doesn't seem worth worrying about but it is an inconsistency.	No change was needed.

No.	Date Queried	CY Query	Response/Change
8a	20-Feb-09	When setting up the doses for Stage 1, overriding the maximum dose with a value of >3330 has no effect. Similarly, overriding the minimum dose with a value of >66.6 has no effect.	No change was needed.
8b	20-Feb-09	Otherwise (at other values and in Stage 2, overriding the maximum (or minimum value) has the effect of changing the maximum (or minimum) dose to the new value and changing all other doses to maintain the 1:50 (Stage 1) or 1:8.5 (Stage 2) ratio between the high and low dose. There is no longer any relationship between the doses and the estimated LD_{50}. I think this is what is meant to happen but is it worth a note/warning to the user to explain that this is what happens?	Text was added to the three forms in the program where it is possible to over-ride the maximum and minimum dose to warn the user of possible consequences. On each form it is stated that "Changing the maximum or minimum dose is likely to result in a less desirable distribution of doses around the LD_{50} than those initially calculated by SEDEC."
9	20-Feb-09	There are a lot of cases where either the SAS programs do not give an estimate for the CIs and SEDEC does (although this is often 0 to infinity or very very wide). There are also some cases where SAS does give an estimate and SEDEC does not. These tend to be the cases where different methods have been used to estimate the CIs etc in my SAS programs as in Table 4 of Bertie's doc (P7). Have these methods been included in SEDEC? Would you want to give a CI of 0 to infinity or would it be better if it was displayed as non-estimable?	SEDEC now reports confidence limits as "Undefined" when there is less than 1 reversal or less than 2 partial mortalities. Meaningful estimates of the confidence limits cannot be calculated using classical MLE methods when the slope cannot be estimated, and such estimates require either 2 partial moralities or 1 reversal.
10	20-Feb-09	The following dataset is created in SEDEC with an initial LD_{50} estimate of 62.54959 and then entering the mortality data at each stage. Note that the SEDEC and SAS doses are very similar until Stage 3. At the end of Stage 2, the est LD_{50} is 1.00 (SEDEC) and 0.99 (SAS) and the estimated slope is 0.595 (SEDEC) and 0.593 (SAS). Both analyses then set the slope to 1 before calculating the doses for the next stage because the slope is less than one (P15 of guideline). However, my SAS programs set the highest dose to highdose/lowdose = 10.9/0.0921 = 118.3 and the lowest dose to 1. This was detailed in P1 of Bertie's word doc for the validation (attached) . I can't find it in the guideline - have I missed it, or should we not be restricting the lowdose to 1? I am not sure which is right here...	No change was needed.
		Stage SEDEC dose SAS dose N dead N exposed	No finding to respond to.

No.	Date Queried	CY Query	Response/Change
		1 8.84 8.85 0 1 1 32.6 32.59 1 1 1 120 120.06 1 1 1 442 442.29 1 1 2 5.82 5.82 1 1 2 7.38 7.38 1 1 2 9.36 9.36 1 1 2 11.9 11.88 1 1 2 15.1 15.07 1 1 2 19.1 19.12 0 1 2 24.3 24.27 1 1 2 30.8 30.79 0 1 2 39.1 39.06 1 1 2 49.6 49.56 1 1 3 0.0921 1.00 0 5 3 10.9 118.27 5 5	
11	20-Feb-09	I am getting some errors with the calculation of number of partials and number of reversals in SEDEC; this is where a dose of 3330 has been used in both Stage 1 and Stage 2. They have not been combined in the combined data in dose order table and as a result a partial with 3330 1/1, 3330 0/1 is being treated as a reversal and so on. Can you reproduce this?	By default, SEDEC now combines dose levels that are within 1% of each other. An option was added to the 'Study Continuation" form to allow the default minimum separation dose to be changed. An explanation of the regrouping was added to page 11 of the SEDEC manual along with a statement that caution should be applied in increasing the minimum dose separation, and that as a rule of thumb, dose levels should not be combined if the precision of dose preparation is sufficient to know with reasonable certainty that one dose is greater than the other. A column was added to the SEDEC report that indicates which (if any) dose groups have been combined.
12	20-Feb-09	With an experiment with 0% mortality in Stage 1 and Stage 2 I get a message "There have been no mortalities. Consider performing a limit test or restarting the test using higher doses". This seems highly sensible, but I can't find this in the 'rules' anywhere. Is it part of the guidelines?	No change needed.
13	27-Feb-09	RE 8a and 8b:Does there remain an inconsistency here? Doses over 3330 are not allowed at Stage 1 (in that they have no effect when they are input), but can be input at Stage 2 and doses will be calculated accordingly... If no dose over 3330 is allowed for welfare reasons at Stage 1 should this not also be true for	The limitation of doses to 3330 was made consistent throughout the program code for stages 1 and 2, and SEDEC will no longer accept values greater than 3330. Text was added to the three forms in the program where it is possible to over-ride the maximum and minimum dose to

No.	Date Queried	CY Query	Response/Change
		Stage 2? I would think that a warning message along the lines of 'Maximum dose allowed is 3330' would be useful for cases where a maxdose>3330 is input as well as a warning message stating that there is no longer a relationship between the suggested doses and the LD_{50} when a lower maxdose is input .	warn the user of possible consequences. On each form it is stated "Changing the maximum or minimum dose is likely to result in a less desirable distribution of doses around the LD_{50} than those initially calculated by SEDEC." Also added was a statement of the limitation of doses to 3330 for animal welfare reasons.
14	27-Feb-09	When setting up the study there is an option for units to be in different units to mg ai/kg (mg formulation/kg and a free form field). As far as I can see the only effect of entering different units in this field is that they are printed on the spreadsheet view as 'Dose Units:' However, I can see various potential problems with allowing different units at this point such as the calculation of minimum and maximum doses (if units were mg formulation/kg would 3330 still be the maximum allowable dose) and other rules in the analysis such as setting the slope to be between 1 and 15. The guidelines talk about doses in mg/kg bodyweight - (although I don't think it is specified) I would assume this to be mg ai/kg. Should there be a choice of units when setting up the study or should the units be restricted to mg ai/kg?	A discussion of the importance of dose units, with appropriate warnings, was added to page 6 of the manual.
15	27-Feb-09	**Datasets of Type B: Inconsistent results where the average logdose at which mortality occurs is less than or equal to the average logdose at which survival occurs:** SEDEC will run a probit analysis which will result in a negative slope. The slope and CIs are reported along with a message that says "The slope is negative! Examine data carefully. The LD_{50} may be meaningless". On examination, the LD_{50} is indeed meaningless (3E-19). In my example which had 3 reversals, the analysis then proceeded to Stage 3a, following the rule of two or more reversals *and an estimate of the slope*. In Bertie' validation doc, both LD_{50} and slope ests were specified as non-estimable if the data was inconsistent. My feeling is that all estimates and CIs should be reported as non-estimable here and SEDEC should go on to Stage 3b not 3a (I presume this would	SEDEC now does the following when a negative slope is obtained after stage 2: The working slope is assumed to be 1, and the working LD_{50} is estimated as the geometric mean of doses with moralities. SEDEC proceeds to stage 3b, and calculates doses using the assumed slope and working LD_{50}. A discussion of this situation was added to page 15 of the manual.

No.	Date Queried	CY Query	Response/Change
		happen if the slope estimate were not reported). A warning message to examine the data carefully might still be warranted.	
16	27-Feb-09	**E/F:/G Consistent results with no reversals and <=1 partial mortality :** SEDEC attempts probit analysis and estimate and slope are reported; but I suspect that it fails to converge. If I run probit analysis on this data in SAS it fails to converge after 50 interations and the number of interations reported in SEDEC for 4 different examples was 27 in each cases - is this the maximum number of iterations? In all examples I have looked at, the CIs of slope are huge (e.g. --491032, 491162) and the CIs of the LD_{50} are zero to infinity. The estimate of the LD_{50} looks sensible (very similar to that produced by the 'other' methods described in Bertie's doc), but I suspect the estimated slope is pretty meaningless. I therefore think this might be OK if the LD_{50} was reported, but the LD_{50} CI and slope est and CI were given as non-estimable	SEDEC now reports confidence limits and slope as "Undefined" when there is less than 1 reversal or less than 2 partial mortalities. Meaningful estimates of the confidence limits cannot be calculated using classical MLE methods when the slope cannot be estimated, and such estimates require either two partial moralities or 1 reversal.
17	27-Feb-09	On page 11 of the Guideline it states that: "Methods for estimating LD_{50}, slope and confidence intervals for a variety of different experimental outcomes are recommended in (2). The slope can only be estimated if there are at least 2 partial kills or at least one dose response inversion." To summarise the two points above (15 and 16), I don't think SEDEC should be reporting the slope estimate for B or E/F/G. The case of E/F/G is actually covered in the above statement from the guideline as there are no reversals and <=1 partial kill in all of these cases.	See 16.
18	27-Feb-09	Can you confirm that for the case where there is a negative slope you estimate the working LD_{50} using the mean and not the log mean of the doses tested? I guess I am just a bit surprised because I would have expected it to be the log mean, but I am sure that you have a reason!	The geometric mean is used.
19	27-Feb-09	What I wrote previously for Type A outcomes was not accurate. There do seem to be problems if there is complete mortality. It should have read: **A: Complete survival/mortality at all doses.**	If there is complete mortality after stage 2, SEDEC now directs the researcher to return to stage 1 and to re-initiate the study using lower doses.

No.	Date Queried	CY Query	Response/Change
		i) If there is complete survival at the end of Stage 2, SEDEC stops with the message "There have been no mortalities. Consider performing a limit test or restarting the test using higher doses." This seems OK. ii) If there is complete mortality at the end of Stage 2, things can go very wrong. I get failure to converge (27 iterations), a negative slope estimate, large CIs and very high doses for Stage 3 (and therefore presumably a high working LD_{50} estimate). NB: The validation exercise used the lowest dose as the working LD_{50} estimate if there was complete mortality - I guess that either this or a warning/stop message of some sort would be appropriate? Presumably if all tested doses up to the end of Stage 2 result in mortality, the chemical is likely to be pretty toxic and not of much interest?	
			See19.
20	Not yet queried	The doses for Stage 3, Dataset 16 produced by SAS and SEDEC do not match. I believe that SAS does as TS described in query 15 and am not sure what SEDEC does so I cannot find the cause of the difference	The difference was indeed due to essentially identical doses being treated as separate. By default, SEDEC now combines dose levels that are within 1% of each other. A column was added to the SEDEC report that indicates which (if any) dose groups have been combined.

C. Verification of Post Test Corrections and Additions to SEDEC

Author: Carol Yarrow, Stats Matters

Date: 4th October 2009

The report entitled "Verification of the sequential design calculator SEDEC: A Tool for Use with OECD TG223 (Avian Acute Oral Toxicity Test)" that was issued on 26th March 2009 detailed several deficiencies with SEDEC and the user manual. These deficiencies were summarised in Table 5 of the above report and in a query log in its Appendix. Changes and additions to SEDEC (Version 1.2) were made as documented in Table PC2 of "Post-Test Corrections and Additions to SEDEC, by Tim Springer. This report stated that all issues identified by the verification testing of Version 1.1 had been addressed with the exception of the problems associated with the display of the dose response plot.

An exercise has now been carried out to confirm that the deficiencies identified in "Verification of SEDEC" have been resolved and implemented in SEDEC Version 1.2 and that SEDEC Version 1.2 is ready for use. A few minor issues highlighted in this exercise have been addressed in SEDEC Version 1.3 which is now ready for use.

Each of the issues summarised in Table 5 of the verification report was independently verified by examining the revised SEDEC manual dated 29 September 2009 and by analysing selected test datasets using SEDEC v1.2. Datasets 1, 2, 8, 9, 10, 11, 14, 15 and 16 described in Tables 3 and 4 of the verification report were re-analysed using SEDEC v1.2. Each of the issues listed in Table 5 (with the exception of the dose response plot) was found to be resolved in accordance with the descriptions in Table PC2. All output was rechecked against the SAS analysis that was carried out as part of the verification exercise. The results from the SEDEC v1.2 analyses of these datasets are summarised in the attached Appendix. The only output that was found to be different (apart from rounding errors) between the SAS and SEDEC results were the chi-square values and corresponding p-values for experiments where the doses within 1% of each other have been combined in SEDEC Version 1.2. This is to be expected because the SAS analysis does not combine doses in the same way. In order to test these values the original SAS analysis would have to be modified and this has not been done. However, the chi-square values and p-values from SAS and SEDEC v1.2 were in agreement in all cases where doses had not been combined.

A few very minor issues were identified and these are summarised below:

- The instructions for enabling macros on P4 of the manual should include "Excel options" before "Trust Center" in both sentences.
- On opening SEDEC in Excel 2007, there was an Excel warning message "Negative or zero values cannot be plotted correctly on log charts..."
- On the pop-up box for overriding minimum and maximum values it says 'maxim' where it should say 'maximum' (P8 and 12 of the manual).
- The example given on P11, 12, 13, 14 and 16 of the manual is not the same throughout (note different doses for Stage 2 on Pages 13 and 14). This is somewhat confusing.
- On P19 it is not clear that if the limit test is failed and there is more than one mortality SEDEC will proceed directly to Stage 2.

Appendix VI – Tables of results obtained by the ring test labs

Lab 1: Substance A: MCPA Acid: Outcome of SEDEC Programme

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	Ring Test 1
Test Substance =	A
Dose Units =	mg a.i/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD_{50} Guess =	1000
Step Size =	1,2694297
Date =	3-nov-08
Initials =	MTC
Study Status Code =	43

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
66,6	1	0	161	1	0	230	2	0	271	2	0
245	1	0	205	1	0	292	2	0	344	2	1
904	1	1	260	1	0	371	2	0	436	2	1
3330	1	1	329	1	0	471	2	1	554	2	1
			418	1	1	598	2	2	703	2	2
			530	1	1						
			673	1	1						
			853	1	1						
			1083	1	1						
			1374	1	1						

Analysis				
Probit Analysis Results				
Itterations	Chi-square	Probability	G	N
6	6,486518396	0,999445546	0,386489058	34

Slope =	9,671769928			
95% Confidence Limits=		3,659000384	and	15,68453947

LD_{50} =	436,6338357			
95% Confidence Limits=		360,1781561	and	537,8100644

n of Reversals =	3	n of Partials =	4

Combined Data in Dose Order				
Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding
66,6	1	0	-	0
161	1	0	-	0
205	1	0	-	0
230	2	0	-	0
245	1	0	-	0
260	1	0	-	0
271	2	0	-	0
292	2	0	-	0
329	1	0	-	0
344	2	1	Partial	50
371	2	0	Reversal	0
418	1	1	-	100
436	2	1	Rev+Partial	50
471	2	1	Partial	50
530	1	1	-	100
554	2	1	Rev+Partial	50
598	2	2	-	100
673	1	1	-	100
703	2	2	-	100
853	1	1	-	100
904	1	1	-	100
1083	1	1	-	100
1374	1	1	-	100
3330	1	1	-	100

Lab 1: Substance A: MCPA Acid: Outcome of SEDEC Programme – Audit Trail

SEDEC Version 1.1 Audit Trail

Project: Ring Test 1
Test Subst.: A

Last Entry: 50

Date	ID	Entry
3-11-2008	MTC	Study initialized
3-11-2008	MTC	Four dose 1st stage chosen
3-11-2008	MTC	Chose 1000
3-11-2008	MTC	Chose Dose-Response: Full
3-11-2008	Mark Chris	Stage 1 mortality entered
3-11-2008	MTC	Mortality entered for dose 161 = 0
3-11-2008	MTC	Mortality entered for dose 205 = 0
3-11-2008	MTC	Mortality entered for dose 260 = 0
3-11-2008	MTC	Mortality entered for dose 329 = 0
3-11-2008	MTC	Mortality entered for dose 418 = 1
3-11-2008	MTC	Mortality entered for dose 530 = 1
3-11-2008	MTC	Mortality entered for dose 673 = 1
3-11-2008	MTC	Mortality entered for dose 853 = 1
3-11-2008	MTC	Mortality entered for dose 1083 = 1
3-11-2008	MTC	Mortality entered for dose 1374 = 1
3-11-2008	MTC	LD50 calculated
3-11-2008	MTC	Report Printed
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved
3-11-2008	MTC	Mortality entered for dose 230 = 0
3-11-2008	MTC	Mortality entered for dose 292 = 0
3-11-2008	MTC	Mortality entered for dose 371 = 0
3-11-2008	MTC	Mortality entered for dose 471 = 1
3-11-2008	MTC	Mortality entered for dose 598 = 2
3-11-2008	MTC	LD50 calculated
3-11-2008	MTC	Report Printed
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved
3-11-2008	MTC	File saved
3-11-2008	MTC	Mortality entered for dose 271 = 0
3-11-2008	MTC	Mortality entered for dose 344 = 0
3-11-2008	MTC	Mortality entered for dose 436 = 0
3-11-2008	MTC	Mortality entered for dose 554 = 2
3-11-2008	MTC	Mortality entered for dose 703 = 2
3-11-2008	MTC	Mortality entered for dose 344 = 1
3-11-2008	MTC	Mortality entered for dose 436 = 1
3-11-2008	MTC	Mortality entered for dose 554 = 1
3-11-2008	MTC	LD50 calculated
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved

Lab 1: Subtance A: MCPA Acid – Mortality table

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control																	
0	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/5
Stage 1																	
66,6	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
245	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
904	F	0/1	1														1
3330	M	1															1
Stage 2																	
161	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
205	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
260	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
329	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
418	F	0/1	1							a	a	a	a	a	a	a	1
530	M	0/1	1							a	a	a	a	a	a	a	1
673	M	0/1	1							a	a	a	a	a	a	a	1
853	M	0/1	1							a	a	a	a	a	a	a	1
1083	M	0/1	1							a	a	a	a	a	a	a	1
1374	M	1								a	a	a	a	a	a	a	1

The LD50 value was determined to be **370.8 mg/kg (0 to Infinity)**

Change the values for the day mortality occurred, subsequent days should automatically change

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Stage 3																	
230	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
230	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2
292	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
292	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2
371	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
371	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2
471	M	0/1	1							a	a	a	a	a	a	a	
471	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	1/2
598	F	0/1	1							a	a	a	a	a	a	a	
598	F	0/1	1							a	a	a	a	a	a	a	2/2
Stage 4																	
271	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
271	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2
344	F	0/1	1							a	a	a	a	a	a	a	
344	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	1/2
436	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
436	M	0/1	0/1	1						a	a	a	a	a	a	a	1/2
554	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
554	M	0/1	1							a	a	a	a	a	a	a	1/2
703	M	0/1	1							a	a	a	a	a	a	a	
703	M	0/1	0/1	1						a	a	a	a	a	a	a	2/2

The LD50 value was determined to be **465.6 mg/kg (374.0 to 610.8)**

a=no data due to study ending on day 7

Lab 1: Substance A: MCPA Acid – Body weight

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	178	-16	162	13	175	5	180	2
	0	198	-7	191	0	191	#VALUE!	a	#VALUE!
	0	189	-6	183	-8	175	#VALUE!	a	#VALUE!
	0	183	-5	178	-2	176	#VALUE!	a	#VALUE!
	0	204	0	204	-3	201	#VALUE!	a	#VALUE!
1	66,6	181	-10	171	6	177	3	180	-1
	245	182	-22	160	15	175	5	180	-2
	904	184	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	3330	185	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
2	161	183	-15	168	-3	165	#VALUE!	a	#VALUE!
	205	202	-16	186	7	193	#VALUE!	a	#VALUE!
	260	192	-14	178	1	179	#VALUE!	a	#VALUE!
	329	192	-19	173	3	176	#VALUE!	a	#VALUE!
	418	194	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	530	196	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	673	196	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	853	187	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	1083	187	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	1374	191	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!

- No data due to mortality. a=no data due to study ending on day 7

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	230	191	-9	182	-4	178	#VALUE!	a	#VALUE!
	230	185	-7	178	1	179	#VALUE!	a	#VALUE!
	292	185	-10	175	-1	174	#VALUE!	a	#VALUE!
	292	185	-13	172	5	177	#VALUE!	a	#VALUE!
	371	185	-21	164	4	168	#VALUE!	a	#VALUE!
	371	186	-29	157	13	170	#VALUE!	a	#VALUE!
	471	179	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	471	188	-11	177	-1	176	#VALUE!	a	#VALUE!
	598	188	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	598	179	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
4	271	205	-25	180	4	184	#VALUE!	a	#VALUE!
	271	181	-16	165	1	166	#VALUE!	a	#VALUE!
	344	205	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	344	185	-23	162	6	168	#VALUE!	a	#VALUE!
	436	185	-23	162	1	163	#VALUE!	a	#VALUE!
	436	209	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	554	194	-23	171	1	172	#VALUE!	a	#VALUE!
	554	192	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	703	181	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	703	192	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!

- No data due to mortality. a=no data due to study ending on day 7

Lab 1: Substance A: MCPA Acid – Food consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	1	7	15	-5,75	-2,857143
	0	5	17	19	-6	#VALUE!
	0	7	12	14	5	#VALUE!
	0	7	12	14	5	#VALUE!
	0	7	12	16	10,75	#VALUE!
1	66,6	3	12	16	-7	3,1428571
	245	0	7	12	-9,25	3,4285714
	904	0	#VALUE!	-		
	3330	0	#VALUE!	-		
2	161	2	17	19	-4,75	#VALUE!
	205	0	8	16	-2,5	#VALUE!
	260	1	16	19	-1,75	#VALUE!
	329	0	7	16	-2	#VALUE!
	418	0	#VALUE!	-		#VALUE!
	530	0	#VALUE!	-		#VALUE!
	673	0	#VALUE!	-		#VALUE!
	853	0	#VALUE!	-		#VALUE!
	1083	0	#VALUE!	-		#VALUE!
	1374	0	#VALUE!	-		#VALUE!

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	230	0	4	14	5,75	#VALUE!
	230	0	4	14	5,75	#VALUE!
	292	0	7	18	9	#VALUE!
	292	0	7	18	9	#VALUE!
	371	0	3	9	7,75	#VALUE!
	371	0	3	9	7,75	#VALUE!
	471	1	9	17	11,5	#VALUE!
	471	1	#VALUE!	-		#VALUE!
	598	0	#VALUE!	-		#VALUE!
	598	0	#VALUE!	-		#VALUE!
4	271	1	5	16	13	#VALUE!
	271	1	5	16	13	#VALUE!
	344	0	7	11	9,25	#VALUE!
	344	0	#VALUE!	-		#VALUE!
	436	0	2	8	14,5	#VALUE!
	436	0	2	#VALUE!	-	#VALUE!
	554	0	5	14	12,75	#VALUE!
	554	0	5	#VALUE!	-	#VALUE!
	703	0	0	#VALUE!	-	#VALUE!
	703	0	#VALUE!	-		#VALUE!

Lab 1: Substance A: MCPA Acid

Clinical Observations Stage 1:

Experimental Group			Day of Test					
			0[a]				1	
(mg/kg)	Pen	Sex	Dosed	1	2	3	AM	PM
0	763	M	0925	AN	AN	AN	AN	AN
0	263	F	0919	AN	AN	AN	AN	AN
0	250	F	0944	AN	AN	AN	AN	AN
0	256	F	0944	AN	AN	AN	AN	AN
0	225	F	0943	AN	AN	AN	AN	AN
66,6	293	F	0926	K	K	AN	AN	AN
245	278	F	0928	K	K	AN	AN	AN
904	276	F	0930	K	K	K	FD	
3330	750	M	0932	L	L	FD		

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, WRITE Keys to the codes here example (AN = appeared normal, FD = found dead)

K= hyporeactivity L= immobility FD= found dead

a=no data due to study ending on day 7

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
0	763	M	AN	AN	AN	AN	AN		AN		AN	AN
0	263	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	250	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	256	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	225	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
66,6	293	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
245	278	F	AN	AN	AN	AN	AN		AN		AN	AN
904	276	F										
3330	750	M										

AN = appeared normal

Lab 1: Substance A: MCPA – Clinical observations – stage 1 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
0	763	M	AN		AN		AN		AN	
0	263	F	AN		a	a	a	a	a	a
0	250	F	AN		a	a	a	a	a	a
0	256	F	AN		a	a	a	a	a	a
0	225	F	AN		a	a	a	a	a	a
66,6	293	F	AN		AN	AN	AN	AN	AN	AN
245	278	F	AN		AN		AN		AN	
904	276	F								
3330	750	M								

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM
0	763	M	AN		AN		AN	AN	AN	
0	263	F	a	a	a	a	a	a	a	a
0	250	F	a	a	a	a	a	a	a	a
0	256	F	a	a	a	a	a	a	a	a
0	225	F	a	a	a	a	a	a	a	a
66,6	293	F	AN	AN	AN	AN	AN	AN	AN	
245	278	F	AN		AN		AN	AN	AN	
904	276	F								
3330	750	M								

AN = appeared normal

Lab 1: Substance A: MCPA Acid

Clinical Observations Stage 2:

Experimental Group			Day of Test					
			0[a]				1	
(mg/kg)	Pen	Sex	Dosed	1	2	3	AM	PM
161	292	F	0920	AN	AN	AN	AN	AN
205	300	F	0922	AN	AN	AN	AN	AN
260	746	M	0923	AN	AN	AN	AN	AN
329	290	F	0924	AN	AN	AN	AN	AN
418	291	F	0926	AN	AN	K	FD	
530	759	M	0927	K	K	K	FD	
673	762	M	0928	K	K	K	FD	
853	798	M	0930	K,X	K	K	FD	
1083	758	M	0931	K,X	K,X	K	K	FD
1374	757	M	0932	K,X	FD			

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal

K= hyporeactivity X= bird regurgitated FD= found dead

a=no data due to study ending on day 7

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
161	292	F	AN	AN	AN	AN	AN		AN		AN	AN
205	300	F	AN	AN	AN	AN	AN		AN		AN	AN
260	746	M	AN	AN	AN	AN	AN		AN		AN	AN
329	290	F	AN	AN	AN	AN	AN		AN		AN	AN
418	291	F										
530	759	M										
673	762	M										
853	798	M										
1083	758	M										
1374	757	M										

AN = appeared normal,

Lab 1: Substance A: MCPA Acid – Clinical observations – stage 2 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
161	292	F	AN		a	a	a	a	a	a
205	300	F	AN		a	a	a	a	a	a
260	746	M	AN		a	a	a	a	a	a
329	290	F	AN		a	a	a	a	a	a
418	291	F			a	a	a	a	a	a
530	759	M			a	a	a	a	a	a
673	762	M			a	a	a	a	a	a
853	798	M			a	a	a	a	a	a
1083	758	M			a	a	a	a	a	a
1374	757	M			a	a	a	a	a	a

AN = appeared normal

a=no data due to study ending on day 7

Clinical observations Stage 3:

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1	0[a] 2	0[a] 3	1 AM	1 PM
230	252	F	0947	AN	AN	AN	AN	AN
230	780	M	0947	AN	AN	AN	AN	AN
292	251	F	0950	AN	AN	AN	AN	AN
292	778	M	0950	AN	AN	AN	AN	AN
371	766	M	0952	K,X	K	K	AN	AN
371	784	M	0952	K,X	K	K	AN	AN
471	782	M	0954	K,X	K	K	FD	
471	783	M	0954	K,X	K	K	AN	AN
598	257	F	0956	K,X	K	K	FD	
598	258	F	0956	K,X	K	K	FD	

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

K= hyporeactivity X= bird regurgitated FD= found dead

a=no data due to study ending on day 7

Lab 1: Substance A: MCPA Acid – Clinical observations – stage 3, ctd.

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
230	252	F	AN	AN	AN		AN		AN	AN	AN	AN
230	780	M	AN	AN	AN		AN		AN	AN	AN	AN
292	251	F	AN	AN	AN		AN		AN	AN	AN	AN
292	778	M	AN	AN	AN		AN		AN	AN	AN	AN
371	766	M	AN	AN	AN		AN		AN	AN	AN	AN
371	784	M	AN	AN	AN		AN		AN	AN	AN	AN
471	782	M										
471	783	M	AN	AN	AN		AN		AN	AN	AN	AN
598	257	F										
598	258	F										

AN = appeared normal

Experimental Group			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
230	252	F	AN		a	a	a	a	a	a
230	780	M	AN		a	a	a	a	a	a
292	251	F	AN		a	a	a	a	a	a
292	778	M	AN		a	a	a	a	a	a
371	766	M	AN		a	a	a	a	a	a
371	784	M	AN		a	a	a	a	a	a
471	782	M			a	a	a	a	a	a
471	783	M	AN		a	a	a	a	a	a
598	257	F			a	a	a	a	a	a
598	258	F			a	a	a	a	a	a

AN = appeared normal

a=no data due to study ending on day 7

Lab 1: Substance A: MCPA Acid

Clinical observations Stage 4:

Experimental Group			Day of Test					
			0[a]				1	
(mg/kg)	Pen	Sex	Dosed	1	2	3	AM	PM
271	255	F	0946	AN	AN	AN	K	K
271	260	F	0946	AN	AN	AN	K	K
344	224	F	0949	K	K	K	FD	
344	751	M	0949	K	K	K	K,X	K
436	755	M	0951	K,X	K	K	K	K
436	761	M	0951	K,X	K	K	K	K
554	296	F	0954	K,X	K	K	K	K
554	795	M	0954	K,X	K	K	K	FD
703	747	M	0956	K,X	K,P	K,P	FD	
703	776	M	0956	K,X	K,P	K,P	K,P	K,P

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

K= hyporeactivity X= bird regurgitated P= loss of righting reflex FD= found dead

a=no data due to study ending on day 7

Experimental Group			Day of Test									
			2	2	3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
271	255	F	AN	AN	AN		AN		AN	AN	AN	AN
271	260	F	AN	AN	AN		AN		AN	AN	AN	AN
344	224	F										
344	751	M	AN	AN	AN		AN		AN	AN	AN	AN
436	755	M	AN	AN	AN		AN		AN	AN	AN	AN
436	761	M	K	FD								
554	296	F	AN	AN	AN		AN		AN	AN	AN	AN
554	795	M										
703	747	M										
703	776	M	FD									

AN = appeared normal

Lab 1: Substance A: MCPA Acid – Clinical observations stage 4, ctd.

Experimental Group (mg/kg)	Pen	Sex	7		8		9		10	
			AM	PM	AM	PM	AM	PM	AM	PM
271	255	F	AN		a	a	a	a	a	a
271	260	F	AN		a	a	a	a	a	a
344	224	F			a	a	a	a	a	a
344	751	M	AN		a	a	a	a	a	a
436	755	M	AN		a	a	a	a	a	a
436	761	M			a	a	a	a	a	a
554	296	F	AN		a	a	a	a	a	a
554	795	M			a	a	a	a	a	a
703	747	M			a	a	a	a	a	a
703	776	M			a	a	a	a	a	a

AN = appeared normal

a=no data due to study ending on day 7

Lab 1: Substance B: Isazofos : Output of SEDEC programme

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	Ring Test 2
Test Substance =	B
Dose Units =	mg a.i/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD_{50} Guess =	50
Step Size =	1,2694297
Date =	3-nov-08
Initials =	MTC
Study Status Code =	43

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
7,07	1	0	17,1	1	0	16,6	2	0	16,9	2	0
26	1	0	21,7	1	0	21,1	2	0	21,4	2	0
96	1	1	27,6	1	1	26,8	2	0	27,2	2	0
354	1	1	35	1	1	34	2	2	34,5	2	2
			44,4	1	1	43,2	2	2	43,8	2	2
			56,3	1	1						
			71,4	1	1						
			90,6	1	1						
			115	1	1						
			146	1	1						

Analysis

Probit Analysis Results				
Itterations	Chi-square	Probability	G	N
27	<0.001	1	295812,8163	34

Slope =	1874,829424			
95% Confidence Limits=		-1017820,087	and	1021569,745
LD_{50} =	27,39957726			
95% Confidence Limits=		0	and	+ Infinity

n of Reversals =	0	n of Partials =	0

Combined Data in Dose Order				
Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding
7,07	1	0	-	0
16,6	2	0	-	0
16,9	2	0	-	0
17,1	1	0	-	0
21,1	2	0	-	0
21,4	2	0	-	0
21,7	1	0	-	0
26	1	0	-	0
26,8	2	0	-	0
27,2	2	0	-	0
27,6	1	1	-	100
34	2	2	-	100
34,5	2	2	-	100
35	1	1	-	100
43,2	2	2	-	100
43,8	2	2	-	100
44,4	1	1	-	100
56,3	1	1	-	100
71,4	1	1	-	100
90,6	1	1	-	100
96	1	1	-	100
115	1	1	-	100
146	1	1	-	100
354	1	1	-	100

Lab 1: Substance B: Isazofos : Output of SEDEC programme: audit trail

SEDEC Version 1.1 Audit Trail

Project: Ring Test 2
Test Subst.: B
Last Entry: 49

Date	ID	Entry
3-11-2008	MTC	Study initialized
3-11-2008	MTC	Four dose 1st stage chosen
3-11-2008	MTC	Chose 50
3-11-2008	MTC	Chose Dose-Response: Full
3-11-2008	Mark Christ	Stage 1 mortality entered
3-11-2008	Mark Christ	File Saved
3-11-2008	Mark Christ	Stage 2 doses calculated
3-11-2008	Mark Christ	Report Printed
3-11-2008	MTC	Mortality entered for dose 17.1 = 0
3-11-2008	MTC	Mortality entered for dose 21.7 = 0
3-11-2008	MTC	Mortality entered for dose 27.6 = 1
3-11-2008	MTC	Mortality entered for dose 35 = 1
3-11-2008	MTC	Mortality entered for dose 44.4 = 1
3-11-2008	MTC	Mortality entered for dose 56.3 = 1
3-11-2008	MTC	Mortality entered for dose 71.4 = 1
3-11-2008	MTC	Mortality entered for dose 90.6 = 1
3-11-2008	MTC	Mortality entered for dose 115 = 1
3-11-2008	MTC	Mortality entered for dose 146 = 1
3-11-2008	MTC	LD_{50} calculated
3-11-2008	MTC	Report Printed
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved
3-11-2008	MTC	Mortality entered for dose 16.6 = 0
3-11-2008	MTC	Mortality entered for dose 21.1 = 0
3-11-2008	MTC	Mortality entered for dose 26.8 = 0
3-11-2008	MTC	Mortality entered for dose 34 = 2
3-11-2008	MTC	Mortality entered for dose 43.2 = 2
3-11-2008	MTC	LD_{50} calculated
3-11-2008	MTC	Report Printed
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved
3-11-2008	MTC	Mortality entered for dose 16.9 = 0
3-11-2008	MTC	Mortality entered for dose 21.4 = 0
3-11-2008	MTC	Mortality entered for dose 27.2 = 0
3-11-2008	MTC	Mortality entered for dose 34.5 = 2
3-11-2008	MTC	Mortality entered for dose 43.8 = 2
3-11-2008	MTC	LD_{50} calculated
3-11-2008	MTC	File Saved
3-11-2008	MTC	File saved

Lab 1: Substance B: Isazofos : Mortality table

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control																	
0	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/5
Stage 1																	
7,1	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
26	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
96	M	1															1/1
354	F	1															1/1
Stage 2																	
17,1	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
21,7	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/1
27,6	F	1								a	a	a	a	a	a	a	1/1
35	F	1								a	a	a	a	a	a	a	1/1
44,4	M	1								a	a	a	a	a	a	a	1/1
56,3	F	1								a	a	a	a	a	a	a	1/1
71,4	F	1								a	a	a	a	a	a	a	1/1
90,6	F	1								a	a	a	a	a	a	a	1/1
115	F	1								a	a	a	a	a	a	a	1/1
146	F	1								a	a	a	a	a	a	a	1/1

The LD50 value was determined to be **26.8 mg/kg (0 to Infinity)**

Change the values for the day mortality occurred, subsequent days should automatically change

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total	
Stage 3																		
16,6	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a		
16,6	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2	
21,1	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a		
21,1	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2	
26,8	F	1								a	a	a	a	a	a	a		<--- if stage 3a 0/5
26,8	M	1								a	a	a	a	a	a	a	2/2	
34	F	1								a	a	a	a	a	a	a		
34	F	1								a	a	a	a	a	a	a	2/2	
43,2	F	1								a	a	a	a	a	a	a		
43,2	F	1								a	a	a	a	a	a	a	2/2	<--- if stage 3a 0/5
Stage 4																		
16,9	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a		
16,9	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2	
21,4	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a		
21,4	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2	
27,2	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a		
27,2	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	a	a	a	a	a	a	a	0/2	
34,5	F	1								a	a	a	a	a	a	a		
34,5	M	1								a	a	a	a	a	a	a	2/2	
43,8	F	1								a	a	a	a	a	a	a		
43,8	F	1								a	a	a	a	a	a	a	2/2	

The LD50 value was determined to be **27.4 mg/kg (0 to Infinity)**

a = no data due to study ending on day 7

Lab 1: Substance B: Isazofos : Body

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	173	-10	163	6	169	-1	168	-5
	0	195	-5	190	-5	185	#VALUE!	a	#VALUE!
	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0
1	7,1	186	-12	174	10	184	2	186	0
	26	187	-37	150	21	171	8	179	-8
	96	187	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	354	192	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
2	17,1	180	-11	169	2	171	#VALUE!	a	#VALUE!
	21,7	188	-9	179	-1	178	#VALUE!	a	#VALUE!
	27,6	184	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	35	185	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	44,4	186	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	56,3	186	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	71,4	186	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	90,6	181	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	115	181	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	146	183	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!

- No data due to mortality. a = no data due to study ending on day 7

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	16,6	188	-6	182	0	182	#VALUE!	a	#VALUE!
	16,6	183	-9	174	-1	173	#VALUE!	a	#VALUE!
	21,1	183	-5	178	-4	174	#VALUE!	a	#VALUE!
	21,1	188	-7	181	-1	180	#VALUE!	a	#VALUE!
	26,8	189	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	26,8	184	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	34	191	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	34	184	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	43,2	184	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	43,2	191	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
4	16,9	191	-8	183	1	184	#VALUE!	a	#VALUE!
	16,9	180	-7	173	1	174	#VALUE!	a	#VALUE!
	21,4	174	-13	161	0	161	#VALUE!	a	#VALUE!
	21,4	200	-12	188	-2	186	#VALUE!	a	#VALUE!
	27,2	201	-14	187	-1	186	#VALUE!	a	#VALUE!
	27,2	176	-8	168	0	168	#VALUE!	a	#VALUE!
	34,5	204	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	34,5	176	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	43,8	177	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!
	43,8	183	#VALUE!	-	#VALUE!	-	#VALUE!	a	#VALUE!

- No data due to mortality. a = no data due to study ending on day 7

1: Substance B: Isazofos : Food consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	-11	14	16	-5,25	0,1428571
	0	9	16	17	5	#VALUE!
	0	8	13	15	3	#VALUE!
	0	8	13	15	3	#VALUE!
	0	6	12	17	11,5	#VALUE!
1	7,1	1	12	18	-9,25	1,5714286
	26	0	1	7	4,25	2,5714286
	96	#VALUE!	-	-	-	-
	354	#VALUE!	-	-	-	-
2	17,1	1	12	13	4,5	#VALUE!
	21,7	0	12	34	7	#VALUE!
	27,6	0	#VALUE!	-	-	#VALUE!
	35	0	#VALUE!	-	-	#VALUE!
	44,4	0	#VALUE!	-	-	#VALUE!
	56,3	0	#VALUE!	-	-	#VALUE!
	71,4	0	#VALUE!	-	-	#VALUE!
	90,6	0	#VALUE!	-	-	#VALUE!
	115	#VALUE!	-	-	-	#VALUE!
	146	#VALUE!	-	-	-	#VALUE!

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	16,6	1	0	31	8,75	#VALUE!
	16,6	1	0	31	8,75	#VALUE!
	21,1	1	9	15	6,25	#VALUE!
	21,1	1	9	15	6,25	#VALUE!
	26,8	0	#VALUE!	-	-	#VALUE!
	26,8	0	#VALUE!	-	-	#VALUE!
	34	0	#VALUE!	-	-	#VALUE!
	34	0	#VALUE!	-	-	#VALUE!
	43,2	0	#VALUE!	-	-	#VALUE!
	43,2	0	#VALUE!	-	-	#VALUE!
4	16,9	2	8	14	12,75	#VALUE!
	16,9	2	8	14	12,75	#VALUE!
	21,4	0	3	14	11,75	#VALUE!
	21,4	0	3	14	11,75	#VALUE!
	27,2	0	3	12	10,75	#VALUE!
	27,2	0	3	12	10,75	#VALUE!
	34,5	0	#VALUE!	-	-	#VALUE!
	34,5	0	#VALUE!	-	-	#VALUE!
	43,8	2	#VALUE!	-	-	#VALUE!
	43,8	2	#VALUE!	-	-	#VALUE!

- No data due to mortality.

a = no data due to study ending on day 7

Lab 1 – Substance B: Isazofos : Clinical observations

Clinical observations stage 1

Group (mg/kg)	Pen	Sex	0[a]				1	
			Dosed	1	2	3	AM	PM
0	277	F	0939	AN	AN	AN	AN	AN
0	771	M	0920	AN	AN	AN	AN	AN
0	754	M	0959	AN	AN	AN	AN	AN
0	791	M	0959	AN	AN	AN	AN	AN
0	283	F	0959	AN	AN	AN	AN	AN
7,1	752	M	0942	AN	AN	AN	AN	AN
26	282	F	0944	X	X	AN	AN	AN
96	748	M	0945	FD				
354	280	F	0947	FD				

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, WRITE Keys to the codes here example (AN = appeared normal, FD = found dead)

X= bird regurgitated FD= found dead

a = no data due to study ending on day 7

Group (mg/kg)	Pen	Sex	2		3		4		5		6	
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
0	277	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	771	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	754	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	791	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	283	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
7,1	752	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
26	282	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
96	748	M										
354	280	F										

AN = appeared normal

Group (mg/kg)	Pen	Sex	7		8		9		10	
			AM	PM	AM	PM	AM	PM	AM	PM
0	277	F	AN	AN	AN	AN	AN	AN	AN	AN
0	771	M	AN	AN						
0	754	M	AN	AN						
0	791	M	AN	AN						
0	283	F	AN	AN						
7,1	752	M	AN	AN	AN	AN	AN	AN	AN	AN
26	282	F	AN	AN	AN	AN	AN	AN	AN	AN
96	748	M								
354	280	F								

AN = appeared normal

Lab 1 – Substance B: Isazofos : Clinical observations

Clinical observations stage 1

Group			11		12		13		14	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
0	277	F	AN	AN	AN	AN	AN	AN	AN	AN
0	771	M								
0	754	M								
0	791	M								
0	283	F								
7,1	752	M	AN	AN	AN	AN	AN	AN	AN	AN
26	282	F	AN	AN	AN	AN	AN	AN	AN	AN
96	748	M								
354	280	F								

AN = appeared normal

Clinical observations stage 2

Experimental Group			Day of Test					
			0[a]				1	
(mg/kg)	Pen	Sex	Dosed	1	2	3	AM	PM
16,9	285	F	1001	AN	AN	AN	AN	AN
16,9	781	M	1001	AN	AN	AN	AN	AN
21,4	286	F	1003	AN	AN	AN	AN	AN
21,4	764	M	1003	AN	AN	AN	AN	AN
27,2	297	F	1005	K,X	K	K	K	K
27,2	790	M	1005	K,X	K	K	K	K
34,5	279	F	1008	K,X	FD			
34,5	799	M	1008	K,X	K	FD		
43,8	270	F	1010	K,X	FD			
43,8	298	F	1010	K,X	FD			

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

X= bird regurgitated FD= found dead K= hyporeactivity

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
17,1	772	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,7	767	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
27,6	261	F										
35	262	F										
44,4	794	M										
56,3	265	F										
71,4	284	F										
90,6	259	F										
115	266	F										
146	264	F										

AN = appeared normal,

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
17,1	772	M	AN		a	a	a	a	a	a
21,7	767	M	AN		a	a	a	a	a	a
27,6	261	F			a	a	a	a	a	a
35	262	F			a	a	a	a	a	a
44,4	794	M			a	a	a	a	a	a
56,3	265	F			a	a	a	a	a	a
71,4	284	F			a	a	a	a	a	a
90,6	259	F			a	a	a	a	a	a
115	266	F			a	a	a	a	a	a
146	264	F			a	a	a	a	a	a

AN = appeared normal

a = no data due to study ending on day 7

Clinical observations stage 3

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1	0[a] 2	0[a] 3	1 AM	1 PM
16,6	788	M	1001	AN	AN	AN	AN	AN
16,6	793	M	1001	AN	AN	AN	AN	AN
21,1	268	F	1004	AN	AN	AN	AN	AN
21,1	787	M	1004	AN	AN	AN	AN	AN
26,8	275	F	1006	AN	K	FD		
26,8	796	M	1006	AN	K	FD		
34	269	F	1007	K,X	FD			
34	294	F	1007	K,X	FD			
43,2	272	F	1010	K,X	FD			
43,2	274	F	1010	K,X	FD			

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

X= bird regurgitated FD= found dead K= hyporeactivity

Lab 1 – Substance B: Isazofos : Clinical observations

Clinical observations stage 3 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM
16,6	788	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
16,6	793	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,1	268	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,1	787	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
26,8	275	F										
26,8	796	M										
34	269	F										
34	294	F										
43,2	272	F										
43,2	274	F										

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
16,6	788	M	AN		a	a	a	a	a	a
16,6	793	M	AN		a	a	a	a	a	a
21,1	268	F	AN		a	a	a	a	a	a
21,1	787	M	AN		a	a	a	a	a	a
26,8	275	F			a	a	a	a	a	a
26,8	796	M			a	a	a	a	a	a
34	269	F			a	a	a	a	a	a
34	294	F			a	a	a	a	a	a
43,2	272	F			a	a	a	a	a	a
43,2	274	F			a	a	a	a	a	a

AN = appeared normal

Clinical signs stage 4

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	1	2	3	1 AM	1 PM
16,9	285	F	1001	AN	AN	AN	AN	AN
16,9	781	M	1001	AN	AN	AN	AN	AN
21,4	286	F	1003	AN	AN	AN	AN	AN
21,4	764	M	1003	AN	AN	AN	AN	AN
27,2	297	F	1005	K,X	K	K	K	K
27,2	790	M	1005	K,X	K	K	K	K
34,5	279	F	1008	K,X	FD			
34,5	799	M	1008	K,X	K	FD		
43,8	270	F	1010	K,X	FD			
43,8	298	F	1010	K,X	FD			

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

X= bird regurgitated FD= found dead K= hyporeactivity

Lab 1 – Substance B: Isazofos : Clinical observations

Clinical observations stage 4 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM
16,9	285	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
16,9	781	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,4	286	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,4	764	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
27,2	297	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
27,2	790	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
34,5	279	F										
34,5	799	M										
43,8	270	F										
43,8	298	F										

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
16,9	285	F	AN		a	a	a	a	a	a
16,9	781	M	AN		a	a	a	a	a	a
21,4	286	F	AN		a	a	a	a	a	a
21,4	764	M	AN		a	a	a	a	a	a
27,2	297	F	AN		a	a	a	a	a	a
27,2	790	M	AN		a	a	a	a	a	a
34,5	279	F			a	a	a	a	a	a
34,5	799	M			a	a	a	a	a	a
43,8	270	F			a	a	a	a	a	a
43,8	298	F			a	a	a	a	a	a

AN = appeared normal

Lab 2: Substance A: MCPA Acid (no SEDEC output): Mortality tables

Cage	Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
	Control																	
1	0	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
2		M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
3		F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
4		F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
5		M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/5
	Stage 1																	
6	66,6	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
7	245	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
8	904	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
9	3330	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
	Stage 2																	
10	161,2	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
11	204,5	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
12	259,5	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
13	329,3	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
14	417,8	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
15	530,1	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
16	672,5	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
17	853,3	M	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
18	1082,7	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
19	1373,7	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

The LD50 value was determined to be 597,1

Change the values for the day mortality occurred, subsequent days should automatically change

	Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
	Stage 3																	
20	370	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
21	370	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
22	469,8	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
23	469,8	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
24	596,6	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
25	596,6	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
26	757,6	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
27	757,6	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
28	962,1	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
29	962,1	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
	Stage 4																	
30	287,4	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
31	287,4	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/2
32	364,9	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
33	364,9	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
34	463,2	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
35	463,2	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
36	588	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
37	588	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/2
38	746,4	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
39	746,4	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2

The LD50 value was determined to be 437,6, Confidendence limits 338,3 - 543,9, Slope 3,212 , 95% Confidence limits: 1.2592 and 5.1638

Lab 2: Substance A: MCPA Acid : Body weights

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change	
control	0	177	9,3	186,3	-1,7	184,6	0,5	185,1	8,1	F
	0	179,3	11,4	190,7	-0,9	189,8	2,7	192,5	13,2	M
	0	183,5	10,2	193,7	-0,2	193,5	-0,8	192,7	9,2	F
	0	172,4	9,4	181,8	-1,3	180,5	2,2	182,7	10,3	F
	0	189,7	8,9	198,6	2	200,6	2,8	203,4	13,7	M
1	66,6	189,8	3,2	193	1,4	194,4	-1	193,4	3,6	F
	245	184,7	-4,1	180,6	6,3	186,9	9,4	196,3	11,6	F
	904	189,1	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	3330	204,2	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
2	161,2	200	6,4	206,4	-1,3	205,1	-2,1	203	3	M
	204,5	184,6	-1,5	183,1	4,5	187,6	11,8	199,4	14,8	F
	259,5	180,6	-12,1	168,5	5,7	174,2	4,1	178,3	-2,3	M
	329,3	171,4	-22,4	149	10	159	11,6	170,6	-0,8	M
	417,8	193,6	-25,8	167,8	12,1	179,9	8,1	188	-5,6	M
	530,1	194,7	-16,1	178,6	6,9	185,5	7,7	193,2	-1,5	M
	672,5	167,5	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	853,3	192	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	1082,7	182,1	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	1373,7	172	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change	
3	370	180,6	-14,7	165,9	1	166,9	-0,7	166,2	-14,4	F
	370	177	-22	155	7,4	162,4	13,2	175,6	-1,4	F
	469,8	176,9	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	469,8	189,3	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	596,6	171,8	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	596,6	180,1	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	757,6	193,7	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	757,6	191,1	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	962,1	194,3	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	962,1	185,6	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
4	287,4	177,2	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	287,4	179,1	-11,3	167,8	7,2	175	-8	167	-12,1	F
	364,9	193,2	-26,4	166,8	4,4	171,2	14,4	185,6	-7,6	M
	364,9	183,3	-27	156,3	3,3	159,6	18,6	178,2	-5,1	F
	463,2	165,8	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	463,2	188,9	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	588	185	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	588	172,7	-23,1	149,6	10,1	159,7	1,5	161,2	-11,5	F
	746,4	176,5	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	746,4	183,4	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F

x No data due to mortality.

Lab 2: Substance A: MCPA Acid: Food Consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day					
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14	
control	0	17,8	9,1	14,9	12,3	17,5	F
	0	17,4	9,5	15	11,9	13,8	M
	0	25,1	17,8	20	16,4	17,2	F
	0	16,6	9,6	14,6	13,4	15,2	F
	0	11,3	9,9	15,8	12,1	13,9	M
1	66,6	3,3	7,7	11,8	10,7	10,8	F
	245	2,2	8,8	13,8	12,8	15,2	F
	904	a	x	x	x	x	F
	3330	a	x	x	x	x	M
2	161,2	5,9	11,9	12,3	13,5	11,7	M
	204,5	6	15,8	13,9	14,9	12,3	F
	259,5	1,2	2,9	7,9	12,3	11,3	M
	329,3	1,4	4,4	7,3	15,3	16,9	M
	417,8	0,1	2,8	6,3	14,7	19	M
	530,1	0,6	2,9	11,1	14,9	14,4	M
	672,5	a	x	x	x	x	F
	853,3	0,3	a	x	x	x	M
	1082,7	a	x	x	x	x	M
	1373,7	a	x	x	x	x	F

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day					
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14	
3	370	0,6	6,7	15,3	12,2	11,5	F
	370	0,5	0,4	6,2	14,2	13,3	F
	469,8	a	x	x	x	x	F
	469,8	0	x	x	x	x	F
	596,6	a	x	x	x	x	F
	596,6	a	x	x	x	x	F
	757,6	a	x	x	x	x	M
	757,6	a	x	x	x	x	M
	962,1	0,1	x	x	x	x	M
	962,1	a	x	x	x	x	M
4	287,4	a	x	x	x	x	M
	287,4	1,5	4,4	13,5	12,9	10,8	F
	364,9	0	0,9	3,3	11,7	13	M
	364,9	6,6	0,5	3,9	9,1	12,2	F
	463,2	a	x	x	x	x	M
	463,2	a	x	x	x	x	F
	588	a	x	x	x	x	M
	588	0	1	9,8	12,7	13,1	F
	746,4	a	x	x	x	x	F
	746,4	a	x	x	x	x	F

x No data due to mortality.

Lab 2: Substance A: MCPA: Clinical observations

Clinical observations stage 1

Experimental Group			Day of Test					
			0[a]					1
(mg/kg)	Pen	Sex	Dosed	1040	1300	1500	1700	AM
0	1	F	1044	AN	D	(D)	AN	AN
0	2	M		AN	D	(D)	AN	AN
0	3	F	to	AN	D	D	(D)	AN
0	4	F		AN	D	D	(D)	AN
0	5	M	1103	AN	D	D	(D)	AN
			WRITE codes for observations here where AN currently is					
66,6	6	F	1044	AN	D	D	D	D
245	7	F	to	AN	D	D(A)	D(A)	D
904	8	F		AN	D	AN	AN	FD
3330	9	M	1103	AN	D	AN	AN	FD

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, WRITE Keys to the codes here example (AN = appeared normal, FD = found dead)

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Experimental Group			Day of Test				
			2	3	4	5	6
(mg/kg)	Pen	Sex	AM	AM	AM	AM	AM
0	1	F	AN	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN	AN
66,6	6	F	AN	AN	AN	AN	AN
245	7	F	AN	AN	AN	AN	AN
904	8	F	x	x	x	x	x
3330	9	M	x	x	x	x	x

AN = appeared normal

Experimental Group			Day of Test			
			7	8	9	10
(mg/kg)	Pen	Sex	AM	AM	AM	AM
0	1	F	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN
66,6	6	F	AN	AN	AN	AN
245	7	F	AN	AN	AN	AN
904	8	F	x	x	x	x
3330	9	M	x	x	x	x

AN = appeared normal

Lab 2: Substance A: MCPA: Clinical observations

Clinical observations stage 1 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
0	1	F	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN
66,6	6	F	AN	AN	AN	AN
245	7	F	AN	AN	AN	AN
904	8	F	x	x	x	x
3330	9	M	x	x	x	x

AN = appeared normal

Lab 2: Substance A: MCPA: Clinical observations

Clinical observations stage 2

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	1045	1300	1500	1700	Day of Test 1 AM
161,2	10	M	1125	AN	D	D	D	(D)
204,5	11	F		AN	D	D	D	(D)
259,5	12	M		AN	D	D	D	(D)
329,3	13	M		AN	D	D	D	D
417,8	14	M	to	AN	D	D	D	D
530,1	15	M		AN	D	D, (A)	D, (A)	D
672,5	16	F		AN	D	D, (A)	D, (A)	FD
853,3	17	M		AN	D	D, (A)	D,A	D,A
1082,7	18	M		AN	D,A	D,A	D,A	FD
1373,7	19	F	1155	AN	D,A	D,A	D,A	FD

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
161,2	10	M	AN	AN	AN	AN	AN
204,5	11	F	AN	AN	AN	AN	AN
259,5	12	M	(D)	AN	AN	AN	AN
329,3	13	M	D	D	(D)	AN	AN
417,8	14	M	D	D	(D)	AN	AN
530,1	15	M	D	D	(D)	AN	AN
672,5	16	F	x	x	x	x	x
853,3	17	M	FD	x	x	x	x
1082,7	18	M	x	x	x	x	x
1373,7	19	F	x	x	x	x	x

AN = appeared normal,

Lab 2 Substance 2: MCPA Acid: Clinical observations

Clinical Observations stage 2 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	8 AM	9 AM	10 AM
161,2	10	M	AN	AN	AN	AN
204,5	11	F	AN	AN	AN	AN
259,5	12	M	AN	AN	AN	AN
329,3	13	M	AN	AN	AN	AN
417,8	14	M	AN	AN	AN	AN
530,1	15	M	AN	AN	AN	AN
672,5	16	F	x	x	x	x
853,3	17	M	x	x	x	x
1082,7	18	M	x	x	x	x
1373,7	19	F	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
161,2	10	M	AN	AN	AN	AN
204,5	11	F	AN	AN	AN	AN
259,5	12	M	AN	AN	AN	AN
329,3	13	M	AN	AN	AN	AN
417,8	14	M	AN	AN	AN	AN
530,1	15	M	AN	AN	AN	AN
672,5	16	F	x	x	x	x
853,3	17	M	x	x	x	x
1082,7	18	M	x	x	x	x
1373,7	19	F	x	x	x	x

AN = appeared normal

Lab 2: Substance A: MCPA Acid: Clinical observations stage 3

Experimental Group			Day of Test						
			0[a]					1	
(mg/kg)	Pen	Sex	Dosed	845	1130	1330	1530	AM	AM
370	20	F	920	AN	D	D	D, (A)	D,A	
370	21	F		AN	D	D	D, (A)	D,A	
469,8	22	F		AN	D,A	D,A	D,A	FD	
469,8	23	F		AN	D,A	D,A	D,A	D,A	FD
596,6	24	F	to	AN	D	D,A	D,A	FD	
596,6	25	F		AN	D,A	D,A	D,A	FD	
757,6	26	M		AN	D,A	D,A	D,A	FD	
757,6	27	M		AN	D	D,A	D,A	FD	
962,1	28	M		AN	D	D,A	D,A	D,A	FD
962,1	29	M	940	AN	D	D,A	D,A	FD	

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Experimental Group			Day of Test				
			2	3	4	5	6
(mg/kg)	Pen	Sex	AM	AM	AM	AM	AM
370	20	F	D	(D)	AN	AN	AN
370	21	F	D	(D)	AN	AN	AN
469,8	22	F	x	x	x	x	x
469,8	23	F	x	x	x	x	x
596,6	24	F	x	x	x	x	x
596,6	25	F	x	x	x	x	x
757,6	26	M	x	x	x	x	x
757,6	27	M	x	x	x	x	x
962,1	28	M	x	x	x	x	x
962,1	29	M	x	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	7	8	9	10
			AM	AM	AM	AM
370	20	F	AN	AN	AN	AN
370	21	F	AN	AN	AN	AN
469,8	22	F	x	x	x	x
469,8	23	F	x	x	x	x
596,6	24	F	x	x	x	x
596,6	25	F	x	x	x	x
757,6	26	M	x	x	x	x
757,6	27	M	x	x	x	x
962,1	28	M	x	x	x	x
962,1	29	M	x	x	x	x

AN = appeared normal

Lab 2: Substance A: MCPA Acid: Clinical observations stage 3 ctd.

Experimental Group (mg/kg)	Pen	Sex	11 AM	12 AM	13 AM	14 AM
370	20	F	AN	AN	AN	AN
370	21	F	AN	AN	AN	AN
469,8	22	F	x	x	x	x
469,8	23	F	x	x	x	x
596,6	24	F	x	x	x	x
596,6	25	F	x	x	x	x
757,6	26	M	x	x	x	x
757,6	27	M	x	x	x	x
962,1	28	M	x	x	x	x
962,1	29	M	x	x	x	x

AN = appeared normal

Clinical observations stage 4:

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	945	1215	1415	1615	1 AM	1 AM
287,4	30	M	950	AN	D	D	D	D,A	FD
287,4	31	F		AN	D	D	D	D	
364,9	32	M		AN	D	D	D,A	D,A	
364,9	33	F		AN	D	D	D,A	D,A	
463,2	34	M	to	AN	D	D	D,A	FD	
463,2	35	F		AN	D	D	D,A	FD	
588	36	M		AN	D	D	D,A	FD	
588	37	F		AN	D	D	D,A	D	
746,4	38	F		AN	D	D	D,A	FD	
746,4	39	F	1015	AN	D	D	D,A	FD	

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Lab 2: Substance A: MCPA Acid: Clinical observations stage 4 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	2 AM	3 AM	4 AM	5 AM	6 AM
287,4	30	M	x		x	x	x	x
287,4	31	F	D		AN	AN	AN	AN
364,9	32	M	D		D	(D)	AN	AN
364,9	33	F	D		D	(D)	(D)	AN
463,2	34	M	x		x	x	x	x
463,2	35	F	x		x	x	x	x
588	36	M	x		x	x	x	x
588	37	F	D		(D)	AN	AN	AN
746,4	38	F	x		x	x	x	x
746,4	39	F	x		x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	7 AM	8 AM	9 AM	10 AM
287,4	30	M	x	x	x	x
287,4	31	F	AN	AN	AN	AN
364,9	32	M	AN	AN	AN	AN
364,9	33	F	AN	AN	AN	AN
463,2	34	M	x	x	x	x
463,2	35	F	x	x	x	x
588	36	M	x	x	x	x
588	37	F	AN	AN	AN	AN
746,4	38	F	x	x	x	x
746,4	39	F	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	11 AM	12 AM	13 AM	14 AM
287,4	30	M	x	x	x	x
287,4	31	F	AN	AN	AN	AN
364,9	32	M	AN	AN	AN	AN
364,9	33	F	AN	AN	AN	AN
463,2	34	M	x	x	x	x
463,2	35	F	x	x	x	x
588	36	M	x	x	x	x
588	37	F	AN	AN	AN	AN
746,4	38	F	x	x	x	x
746,4	39	F	x	x	x	x

AN = appeared normal

Lab 2: Substance B: Isazofos: (No SEDEC output) Mortality

Cage	Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed – Day of Test: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
	Control																	
1	0	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
2		M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
3		F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
4		F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
5		M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/5
	Stage 1																	
6	7,1	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
7	26	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
8	96	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
9	354	F	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
	Stage 2																	
10	4,65	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
11	5,9	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
12	7,49	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
13	9,5	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
14	12,06	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
15	15,3	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
16	19,41	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
17	24,64	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
18	31,26	F	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
19	39,67	F	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

The LD50 value was determined to be 20,9

Change the values for the day mortality occurred, subsequent days should automatically change

Cage	Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed – Day of Test: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total	
	Stage 3																		
20	12,95	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1		
21	12,95	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2	
22	16,45	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1		
23	16,45	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2	
24	20,88	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1		<--- if stage 3a 0/5
25	20,88	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2	
26	26,52	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1		
27	26,52	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2	
28	33,68	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1		
29	33,68	F	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	<--- if stage 3a 0/5
	Stage 4																		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	

The LD50 value was determined to be 24,4, Confidence limits 17,06 - 37,6, p = 0,8995, slope 3,3 , 95% Confidence limits = 0.5898 and 6.0262

Lab 2: Substance B: Isazofos: Body weights

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change	
control	0	177	9,3	186,3	-1,7	184,6	0,5	185,1	8,1	F
	0	179,3	11,4	190,7	-0,9	189,8	2,7	192,5	13,2	M
	0	183,5	10,2	193,7	-0,2	193,5	-0,8	192,7	9,2	F
	0	172,4	9,4	181,8	-1,3	180,5	2,2	182,7	10,3	F
	0	189,7	8,9	198,6	2	200,6	2,8	203,4	13,7	M
1	7,1	213,2	-2,3	210,9	3,8	214,7	7,2	221,9	8,7	M
	26	179,5	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	96	176,6	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	354	191,2	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
2	4,65	192,3	12,2	204,5	-4,2	200,3	0	200,3	8	F
	5,9	202,6	4,6	207,2	0,6	207,8	3,7	211,5	8,9	F
	7,49	206,4	1,5	207,9	0,2	208,1	2	210,1	3,7	M
	9,5	180,6	-0,3	180,3	0,9	181,2	5	186,2	5,6	F
	12,06	162,2	-1	161,2	5,8	167	11,1	178,1	15,9	F
	15,3	175,6	0,3	175,9	1,1	177	5,2	182,2	6,6	M
	19,41	169,8	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	24,64	188,9	-3,5	185,4	2,2	187,6	6,4	194	5,1	M
	31,26	182,1	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F
	39,67	164,7	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change	
3	12,95	181,8	-2,1	179,7	-3,3	176,4	5	181,4	-0,4	F
	12,95	213,2	5,2	218,4	-0,8	217,6	5,5	223,1	9,9	M
	16,45	193,7	1,5	195,2	0,4	195,6	9,3	204,9	11,2	M
	16,45	193,9	-1,2	192,7	1,7	194,4	3,9	198,3	4,4	M
	20,88	206,4	7,3	213,7	-4,6	209,1	5,2	214,3	7,9	F
	20,88	176	-11,4	164,6	-0,6	164	11,9	175,9	-0,1	M
	26,52	205,4	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	26,52	187,2	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	M
	33,68	186,5	-1,5	185	-3	182	10,3	192,3	5,8	F
	33,68	170,2	#VALUE!	x	#VALUE!	x	#VALUE!	x	#VALUE!	F

x No data due to mortality.

Lab 2: Substance B: Isazofos: Food consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14	
control	0	17,8	9,1	14,9	12,3	17,5	F
	0	17,4	9,5	15	11,9	13,8	M
	0	25,1	17,8	20	16,4	17,2	F
	0	16,6	9,6	14,6	13,4	15,2	F
	0	11,3	9,9	15,8	12,1	13,9	M
1	7,1	0	2,6	18,4	14,5	15,3	M
	26	x	x	x	x	x	M
	96	x	x	x	x	x	M
	354	x	x	x	x	x	F
2	4,65	4,6	8,3	14,1	15,3	12,9	F
	5,9	2,5	10,2	12,4	14	14,2	F
	7,49	5,8	10,6	9,5	12,7	12,5	M
	9,5	4,1	17,5	17,1	17,4	17,9	F
	12,06	1,8	8,3	12,9	20,5	17,7	F
	15,3	1,8	12,3	15,1	15,3	14,7	M
	19,41	a	x	x	x	x	F
	24,64	0,3	9,7	11,3	14	12,4	M
	31,26	missing	a	x	x	x	F
	39,67	x	x	x	x	x	F

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14	
3	12,95	4,3	13,4	14,2	12,8	10,1	F
	12,95	6	15,2	14	15,6	12,2	M
	16,45	1,2	8,9	18	15,1	13,3	M
	16,45	2,7	14,3	17,3	16,1	14,6	M
	20,88	4,3	14,5	16,3	14,1	12,8	F
	20,88	0,6	3,7	10,7	11,9	13,3	M
	26,52	x	x	x	x	x	M
	26,52	x	x	x	x	x	M
	33,68	1,8	6,3	10,3	11,2	11,3	F
	33,68	x	x	x	x	x	F

x No data due to mortality.

missing data in stage 2 due to failure of recording system

Lab 2: Substance B: Isazofos: Clinical observations – stage 1

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1040	0[a] 1300	0[a] 1500	0[a] 1700	1 AM
0	1	F	1044	AN	D	(D)	AN	AN
0	2	M		AN	D	(D)	AN	AN
0	3	F	to	AN	D	D	(D)	AN
0	4	F		AN	D	D	(D)	AN
0	5	M	1103	AN	D	D	(D)	AN
			WRITE codes for observations here where AN currently is					
7,1	6	M	1106	AN	D	D	D	D
26	7	M	to	AN	D,A	FD	x	x
96	8	M		AN	FD	x	x	x
354	9	F	1117	AN	FD	x	x	x

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, WRITE Keys to the codes here example (AN = appeared normal, FD = found dead)

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
0	1	F	AN	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN	AN
7,1	6	M	D	(D)	AN	AN	AN
26	7	M	x	x	x	x	x
96	8	M	x	x	x	x	x
354	9	F	x	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	8 AM	9 AM	10 AM
0	1	F	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN
7,1	6	M	AN	AN	AN	AN
26	7	M	x	x	x	x
96	8	M	x	x	x	x
354	9	F	x	x	x	x

AN = appeared normal

Lab 2: Substance B: Isazofos: Clinical observations stage 1 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
0	1	F	AN	AN	AN	AN
0	2	M	AN	AN	AN	AN
0	3	F	AN	AN	AN	AN
0	4	F	AN	AN	AN	AN
0	5	M	AN	AN	AN	AN
7,1	6	M	AN	AN	AN	AN
26	7	M	x	x	x	x
96	8	M	x	x	x	x
354	9	F	x	x	x	x

AN = appeared normal

Clinical observations – stage 2

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1045	0[a] 1300	0[a] 1500	0[a] 1700	1 AM
4,65	10	F	1045	AN	D	D	D	(D)
5,9	11	F		AN	D	D	D	(D)
7,49	12	M		AN	D	D	D	(D)
9,5	13	F		AN	D	D	D	D
12,06	14	F	to	AN	D	D	D	D
15,3	15	M		AN	D	D	D	D
19,41	16	F		AN	D	D	D	FD
24,64	17	M		AN	D	D	D	D
31,26	18	F		AN	D,A	D,A	D,A	D,A
39,67	19	F	1112	AN	D,A	D,A	FD	x

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
4,65	10	F	AN	AN	AN	AN	AN
5,9	11	F	AN	AN	AN	AN	AN
7,49	12	M	AN	AN	AN	AN	AN
9,5	13	F	(D)	AN	(D)	AN	AN
12,06	14	F	(D)	AN	(D)	AN	AN
15,3	15	M	(D)	AN	(D)	AN	AN
19,41	16	F	x	x	x	x	x
24,64	17	M	(D)	AN	AN	AN	AN
31,26	18	F	FD	x	x	x	x
39,67	19	F	x	x	x	x	x

AN = appeared normal,

Lab 2: Substance B: Isazofos: Clinical observations stage 2 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	8 AM	9 AM	10 AM
4,65	10	F	AN	AN	AN	AN
5,9	11	F	AN	AN	AN	AN
7,49	12	M	AN	AN	AN	AN
9,5	13	F	AN	AN	AN	AN
12,06	14	F	AN	AN	AN	AN
15,3	15	M	AN	AN	AN	AN
19,41	16	F	x	x	x	x
24,64	17	M	AN	AN	AN	AN
31,26	18	F	x	x	x	x
39,67	19	F	x	x	x	x

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
4,65	10	F	AN	AN	AN	AN
5,9	11	F	AN	AN	AN	AN
7,49	12	M	AN	AN	AN	AN
9,5	13	F	AN	AN	AN	AN
12,06	14	F	AN	AN	AN	AN
15,3	15	M	AN	AN	AN	AN
19,41	16	F	x	x	x	x
24,64	17	M	AN	AN	AN	AN
31,26	18	F	x	x	x	x
39,67	19	F	x	x	x	x

AN = appeared normal

Clinical observations stage 3

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	845	1130	1330	1530	1 AM	AM
12,95	20	F	850	AN	D	D	D	D	
12,95	21	M		AN	D	D	D	D	
16,45	22	M		AN	D	D	D	D	
16,45	23	M		AN	D	D	D	D	
20,88	24	F	to	AN	D	D	D	D	
20,88	25	M		AN	D	D	D	D	
26,52	26	M		AN	D,A	D,A	FD	x	
26,52	27	M		AN	D	D,A	FD	x	
33,68	28	F		AN	D	D, (A)	D, (A)	D	
33,68	29	F	910	AN	D	FD	x	x	

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal,

x = dead; Observations: D- diarrhea; (D) slight diarhea; A reduced activity; (A) slightly reduced activity.

Lab 2: Substance B: Isazofos – Clinical observations stage 3 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
12,95	20	F	(D)	AN	AN	AN	AN
12,95	21	M	(D)	AN	AN	AN	AN
16,45	22	M	(D)	AN	x	x	x
16,45	23	M	(D)	AN	x	x	x
20,88	24	F	(D)	AN	x	x	x
20,88	25	M	(D)	AN	x	x	x
26,52	26	M	x	x	x	x	x
26,52	27	M	x	x	x	x	x
33,68	28	F	(D)	(D)	AN	AN	AN
33,68	29	F	x	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	7 AM	8 AM	9 AM	10 AM
12,95	20	F	AN	AN	AN	AN
12,95	21	M	AN	AN	AN	AN
16,45	22	M	x	x	x	x
16,45	23	M	x	x	x	x
20,88	24	F	x	x	x	x
20,88	25	M	x	x	x	x
26,52	26	M	x	x	x	x
26,52	27	M	x	x	x	x
33,68	28	F	AN	AN	AN	AN
33,68	29	F	x	x	x	x

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	11 AM	12 AM	13 AM	14 AM
12,95	20	F	AN	AN	AN	AN
12,95	21	M	AN	AN	AN	AN
16,45	22	M	x	x	x	x
16,45	23	M	x	x	x	x
20,88	24	F	x	x	x	x
20,88	25	M	x	x	x	x
26,52	26	M	x	x	x	x
26,52	27	M	x	x	x	x
33,68	28	F	AM	AN	AN	AN
33,68	29	F	x	x	x	x

AN = appeared normal

Lab 3: Substance A: MCPA Acid: Outcome of SEDEC Programme

SEDEC Version1.1 Page 1 of 2

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	100-103
Test Substance =	A
Dose Units =	mg formulation/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD50 Guess =	1000
Date =	19-Nov-08
Initials =	pmh
Study Status Code =	43

Step Size = 1.2694297

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
66.6	1	0	161	1	0	230	2	0	198	2	0
245	1	0	205	1	0	292	2	2	252	2	0
904	1	1	260	1	0	371	2	1	320	2	0
3330	1	1	329	1	0	471	2	2	406	2	2
			418	1	1	598	2	2	515	2	2
			530	1	1						
			673	1	1						
			853	1	1						
			1083	1	1						
			1374	1	1						

Analysis

Probit Analysis Results

Itterations	Chi-square	Probability	G	N
7	10.39397682	0.982367168	0.450494878	34

Slope =	13.20746137		
95% Confidence Limits=	4.342756576	and	22.07216617

LD50 =	332.5140697		
95% Confidence Limits=	280.3820042	and	399.6541466

n of Reversals =	1	n of Partials =	1

Print time: 7:46 AM Printed by (Initials/Date): PH 12/18/08

Combined Data in Dose Order

Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding
66.6	1	0 -		0
161	1	0 -		0
198	2	0 -		0
205	1	0 -		0
230	2	0 -		0
245	1	0 -		0
252	2	0 -		0
260	1	0 -		0
292	2	2 -		100
320	2	0	Reversal	0
329	1	0 -		0
371	2	1	Partial	50
406	2	2 -		100
418	1	1 -		100
471	2	2 -		100
515	2	2 -		100
530	1	1 -		100
598	2	2 -		100
673	1	1 -		100
853	1	1 -		100
904	1	1 -		100
1083	1	1 -		100
1374	1	1 -		100
3330	1	1 -		100

Print time: 7:46 AM

Printed by (Initials/Date): PH 12/18/08

Lab 3: Substance A: MCPA Acid: Output SEDEC programme – Audit trail

SEDEC Version 1.1 Audit Trail

Project: 100-103
Test Subst.: A

Last Entry: 42

Date	ID	Entry
11/19/2008	pmh	Study initialized
11/19/2008	pmh	Four dose 1st stage chosen
11/19/2008	pmh	Chose 1000
11/19/2008	pmh	Chose Dose-Response: Full
11/19/2008	pmh	File Saved
11/19/2008	pmh	Stage 1 Doses Saved
11/19/2008	pmh	Stage 1 mortality entered
11/19/2008	pmh	Mortality entered for dose 161 = 0
11/19/2008	pmh	Mortality entered for dose 205 = 0
11/19/2008	pmh	Mortality entered for dose 260 = 0
11/19/2008	pmh	Mortality entered for dose 329 = 0
11/19/2008	pmh	Mortality entered for dose 418 = 1
11/19/2008	pmh	Mortality entered for dose 530 = 1
11/19/2008	pmh	Mortality entered for dose 673 = 1
11/19/2008	pmh	Mortality entered for dose 853 = 1
11/19/2008	pmh	Mortality entered for dose 1083 = 1
11/19/2008	pmh	Mortality entered for dose 1374 = 1
11/19/2008	pmh	LD50 calculated
11/19/2008	pmh	Mortality entered for dose 230 = 0
11/19/2008	pmh	Mortality entered for dose 292 = 2
11/19/2008	pmh	Mortality entered for dose 371 = 1
11/19/2008	pmh	Mortality entered for dose 471 = 2
11/19/2008	pmh	Mortality entered for dose 598 = 2
11/19/2008	pmh	LD50 calculated
11/19/2008	pmh	Mortality entered for dose 198 = 0
11/19/2008	pmh	Mortality entered for dose 252 = 0
11/19/2008	pmh	Mortality entered for dose 320 = 2
11/19/2008	pmh	Mortality entered for dose 320 = 0
11/19/2008	pmh	Mortality entered for dose 406 = 2
11/19/2008	pmh	Mortality entered for dose 515 = 2
11/19/2008	pmh	LD50 calculated
11/19/2008	pmh	Audit Trail Printed

Lab 3: Substance A: MCPA Acid: Mortality data

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control																	
0	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/5
Stage 1																	
66,6	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
245	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
904	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
3330	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Stage 2																	
161	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
205	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
260	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
329	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
418	M	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
530	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
673	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
853	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1083	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1374	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

The LD50 value was determined to be

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Stage 3																	
230	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
230	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
292	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
292	F	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
371	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
371	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/2
471	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
471	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
598	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
598	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
Stage 4																	
198	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
198	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
252	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
252	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
320	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
320	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
406	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
406	F	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2
515	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	
515	M	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	2/2

The LD50 value was determined to be 333 mg with 95% confidence intervals of 280 to 400 mg/kg.

Lab 3: Substance A: MCPA Acid: Body weights

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	221	0	221	-1	220	7	227	6
	0	210	-4	206	-2	204	4	208	-2
	0	184	2	186	-3	183	0	183	-1
	0	194	1	195	2	197	1	198	4
	0	193	3	196	-3	193	3	196	3
1	66,6	209	-2	207	2	209	1	210	1
	245	199	-19	180	9	189	3	192	-7
	904	214	-	-	-	-	-	-	-
	3330	221	-	-	-	-	-	-	-
2	161	227	-8	219	1	220	4	224	-3
	205	225	-36	189	-29	160	20	180	-45
	260	183	-16	167	10	177	11	188	5
	329	215	-13	202	-3	199	8	207	-8
	418	231	-	-	-	-	-	-	-
	530	204	-	-	-	-	-	-	-
	673	211	-	-	-	-	-	-	-
	853	216	-	-	-	-	-	-	-
	1083	209	-	-	-	-	-	-	-
	1374	226	-	-	-	-	-	-	-

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	230	204	-12	192	2	194	7	201	-3
	230	206	-20	186	4	190	9	199	-7
	292	196	-	-	-	-	-	-	-
	292	182	-	-	-	-	-	-	-
	371	222	-	-	-	-	-	-	-
	371	217	-28	189	8	197	7	204	-13
	471	229	-	-	-	-	-	-	-
	471	239	-	-	-	-	-	-	-
	598	191	-	-	-	-	-	-	-
	598	206	-	-	-	-	-	-	-
4	198	194	-21	173	5	178	8	186	-8
	198	216	-36	180	12	192	13	205	-11
	252	191	-22	169	13	182	7	189	-2
	252	223	-24	199	12	211	7	218	-5
	320	216	-22	194	9	203	6	209	-7
	320	219	-20	199	17	216	5	221	2
	406	204	-	-	-	-	-	-	-
	406	210	-	-	-	-	-	-	-
	515	246	-	-	-	-	-	-	-
	515	248	-	-	-	-	-	-	-

- No data due to mortality.

Lab 3: Substance A: MCPA Acid: Food consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	6	11	15	14	13
	0	4	11	16	16	15
	0	11	14	16	13	11
	0	52	37	20	25	29
	0	8	11	13	12	10
1	66,6	2	14	12	15	23
	245	0	3	9	15	10
	904	-	-	-	-	-
	3330	-	-	-	-	-
2	161	6	12	29	15	16
	205	8	3	4	2	9
	260	7	5	28	15	13
	329	7	5	59	20	17
	418	7	-	-	-	-
	530	-	-	-	-	-
	673	-	-	-	-	-
	853	-	-	-	-	-
	1083	-	-	-	-	-
	1374	-	-	-	-	-

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	230	4	16	15	16	15
	230	4	3	7	11	11
	292	-	-	-	-	-
	292	4	-	-	-	-
	371	4	-	-	-	-
	371	6	3	5	10	11
	471	4	-	-	-	-
	471	-	-	-	-	-
	598	-	-	-	-	-
	598	-	-	-	-	-
4	198	3	1	14	24	25
	198	3	1	5	12	13
	252	4	5	9	14	13
	252	3	5	20	16	15
	320	2	4	20	25	18
	320	2	2	8	15	14
	406	-	-	-	-	-
	406	3	-	-	-	-
	515	-	-	-	-	-
	515	-	-	-	-	-

- No data due to mortality.

Lab 3: Substance A: MCPA Acid: Clinical observations stage 1:

Experimental Group			Day of Test							
			0[a]							
(mg/kg)	Pen	Sex	Dosed	0957-1029	1040	1051	1139	1213	1322	1501	1555
0	A1	M	0917	AN	AN	AN	AN	AN	AN	AN	AN
0	A2	F		AN	AN	AN	AN	AN	AN	AN	AN
0	A3	M	to	AN	AN	AN	AN	AN	AN	AN	AN
0	A4	M		AN	AN	AN	AN	AN	AN	AN	AN
0	A5	F	0923	AN	AN	AN	AN	AN	AN	AN	AN
66,6	A6	F	0927	AN	AN	AN	11	11	11	SL11	SL11
245	A7	M	0929	AN	11SL4	11	11,14	11,SL4	11,SL4	11,14	11,14
904	A8	M	0931	10,11@1011;4,11@1020	3,4,11	2,3,4,11,13	1,5,11	1,5,11	1,5,11	FD	-
3330	A9	M	0933	4,10,11@1006;3,4,10,11,@1014	3,5,11	1,5,11	1,5,11	1,5,11	1,5,11	1,6,11	1,6,11

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 1 = depression, 2 = reduced reaction, 3 = wing droop, 4 = loss of cordination, 5 = prostrat posure, 6 = loss of righting reflex, 10 = shallow and rapid respiration, 11= ruffled appearance, 13 = lower limb weakness, 14 = lethargy, Sl = slight (used as a modifier), FD = found dead

Experimental					Day of Test									
1		Group			2		3		4		5		6	
AM	PM	(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
AN	AN	0	A1	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
AN	AN	0	A2	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
AN	AN	0	A3	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
AN	AN	0	A4	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
AN	AN	0	A5	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
SL(4,11)	SL(4,11)	66,6	A6	F	SL(11,14)	SL(4,11)	AN	AN	SL11	AN	AN	AN	AN	AN
4,11	11,SL4	245	A7	M	11	SL(4,11)	SL11	11	11	11	SL11	SL11	AN	AN
-	-	904	A8	M	-	-	-	-	-	-	-	-	-	-
FD	-	3330	A9	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 4 = loss of cordination, 11 = ruffled appearance, 14 = lethargy, SL = slight (used as a modifier)

Experimental Group			Day of Test							
			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
0	A1	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A2	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A3	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A4	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A5	F	AN	AN	AN	AN	AN	AN	AN	AN
66,6	A6	F	AN	AN	AN	AN	AN	AN	AN	AN
245	A7	M	AN	AN	AN	AN	AN	AN	AN	AN
904	A8	M	-	-	-	-	-	-	-	-
3330	A9	M	-	-	-	-	-	-	-	-

AN = appeared normal

Lab 3: Substance A: MCPA Acid: Clinical observations stage 1, ctd.:

Experimental Group (mg/kg)	Pen	Sex	Day of Test							
			11		12		13		14	
			AM	PM	AM	PM	AM	PM	AM	AM
0	A1	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A2	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A3	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A4	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A5	F	AN	AN	AN	AN	AN	AN	AN	AN
66,6	A6	F	AN	AN	AN	AN	AN	AN	AN	AN
245	A7	M	AN	AN	AN	AN	AN	AN	AN	AN
904	A8	M	-	-	-	-	-	-	-	-
3330	A9	M	-	-	-	-	-	-	-	-

AN = appeared normal

Clinical observations stage 2:

Experimental Group			Day of Test										
			0[a]									1	
(mg/kg)	Pen	Sex	Dosed	1025	1049	1113	1142	1236	1403	1506	1540	AM	PM
161	A19	M	0943	AN	2,11	2,11	2,4,11	4,11,14	4,11,14	4,11,14	4,11,14	11,SL4	SL(4,11)
205	A20	M	0944	AN	SL4,11	11,Sl4	4,11	4,11	4,11,14	4,11	4,11	11,SL4	SL(4,11)
260	A21	F	0945	AN	SL11	11,14	2,4,11,13,14	4,11,14	4,11,14	4,11,14	4,11,14	4,11,14	11,SL4
329	A22	M	0947	AN	4,11	4,11,13	4,11,13,14	4,11	3,4,11,14	4,11,14	4,11,14	4,11	11,SL4
418	A23	M	0949	10	5,10,11	4,11,13,SL10	5,11	4,11,13,14	2,5,11	5,11,14	5,11,14	5,11,14	1,5,11
530	A24	F	0951	AN	4,11	4,11,14	4,11,14	4,11,14	3,4,11,13,14	4,11,13,14	4,11,13,14	FD	-
673	A25	M	0953	10	4,11,13,SL10	4,11,14	3,5,11	5,11,14	1,5,11	1,5,11	1,5,11	FD	-
853	A26	M	0954	AN	3,5,10,11	3,5,11	1,5,11	1,5,11	FD	-	-	-	-
1083	A27	M	0957	10	4,10,11	4,10,11,13,14	3,4,11,13,14	3,4,11,13,14	1,5,11	1,5,11	1,5,11	FD	-
1374	A28	M	0959	2,10,11	5,10,11	3,5,11	1,3,5,11	1,5,11	1,5,11	1,5,11	1,5,11	FD	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 1 = depression, 2 = reduced reaction to external stimuli, 3 = wing droop, 4 = loss of cordination, 5 = prostrate posture, 10 = shallow and rapid respiration, 11 = ruffled appearance, 13 = lower limb weakness, 14 = lethargy, SL = slight (used as a modifier), FD = found dead

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
161	A19	M	11,SL4	11,SL4	11	11	11	SL11	SL11	11	AN	AN
205	A20	M	SL(4,11)	SL(4,11)	11,SL4	11,SL4	SL4	SL4	SL4	SL4	SL11	11
260	A21	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
329	A22	M	SL(4,11)	AN	AN	SL11	AN	AN	AN	AN	AN	AN
418	A23	M	FD	-	-	-	-	-	-	-	-	-
530	A24	F	-	-	-	-	-	-	-	-	-	-
673	A25	M	-	-	-	-	-	-	-	-	-	-
853	A26	M	-	-	-	-	-	-	-	-	-	-
1083	A27	M	-	-	-	-	-	-	-	-	-	-
1374	A28	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 4 = loss of cordination, 11 = ruffled appearance, SL = slight (used as a modifier), FD = found dead

Lab 3: Substance A: MCPA Acid: Clinical observations stage 2, ctd.

Experimental			Day of Test							
Group			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
161	A19	M	AN	AN	AN	AN	AN	AN	AN	AN
205	A20	M	SL11	SL11	AN	AN	WW	WW	AN	AN
260	A21	F	AN	AN	AN	AN	AN	AN	AN	AN
329	A22	M	AN	AN	AN	AN	AN	AN	AN	AN
418	A23	M	-	-	-	-	-	-	-	-
530	A24	F	-	-	-	-	-	-	-	-
673	A25	M	-	-	-	-	-	-	-	-
853	A26	M	-	-	-	-	-	-	-	-
1083	A27	M	-	-	-	-	-	-	-	-
1374	A28	M	-	-	-	-	-	-	-	-

AN = appeared normal, WW = watery white colored droopings on pan, 11 = ruffled appearance, SL = slight (used as a modifier)

Experimental			Day of Test							
Group			11		12		13		14	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
161	A19	M	AN	AN	AN	AN	AN	AN	AN	AN
205	A20	M	AN	AN	AN	AN	AN	AN	AN	AN
260	A21	F	AN	AN	AN	AN	AN	AN	AN	AN
329	A22	M	AN	AN	AN	AN	AN	AN	AN	AN
418	A23	M	-	-	-	-	-	-	-	-
530	A24	F	-	-	-	-	-	-	-	-
673	A25	M	-	-	-	-	-	-	-	-
853	A26	M	-	-	-	-	-	-	-	-
1083	A27	M	-	-	-	-	-	-	-	-
1374	A28	M	-	-	-	-	-	-	-	-

AN = appeared normal

Clinical observations stage 3:

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0905	0930	0946	1013	1118	1228	1331	1427	1536	1 AM	1 PM
230	A39	M	0829	AN	SL11	11,SL4	11,SL4	4,11	4,11	4,11,13,14	4,11,13,14	4,11	4,11	SL11
230	A40	M	0832	AN	SL(4,11)	11	2,11	3,4,11,14	4,11,13,14	4,11	4,11,14	4,11,13,14	4,11,14	4,11,14
292	A41	M	0833	AN	4,11	4,11,14	4,11,14	4,11,13,14	5,11	5,11	1,5,11	1,5,11	FD	-
292	A42	F	0836	3,11	3,4,11	3,4,11,14	3,4,11,14	4,11,13,14	4,11,13,14	3,4,11,13,14	5,11	1,5,11	1,5,11	1,5,11
371	A43	F	0837	11	4,11	3,4,11,14	3,4,11,14	3,4,11,13,14	3,4,11,13,14	4,11,13,14	4,11,13,14	3,4,11,13,14	1,5,11	FD
371	A44	F	0839	10	3,4,10,11	3,4,10,11,14	3,5,11	3,4,11,13,14	3,4,11,13,14	4,11,13,14	3,4,11,13,14	3,4,11,13,14	4,11,14	4,11,14
471	A45	M	0841	4,10,11,14	5,10,11	5,10,11	3,5,11	1,5,11	1,5,11	1,5,11	1,5,11	1,5,11	1,5,11	FD
471	A46	M	0842	SL11	SL11	SL11	4,11	4,11	4,11	5,11	1,5,11	1,5,11	FD	-
598	A47	F	0844	AN	SL(4,11)	4,11,13,14	5,11	1,5,11	1,5,11	1,5,11	1,6,11	1,6,11	FD	-
598	A48	M	0847	11,SL4	4,11	4,11	4,11,14	4,11,13,14	3,4,11,13,14	1,5,11	3,4,11,13,14	1,5,11	FD	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 1 = depression, 3 = wing droop, 4 = loss of cordination, 5 = prostrate posture, 6 = loss of righting reflex,

10 = shallow and rapid respiration, 11 = ruffled appearance, 13 = lower limb weakness, 14 = lethargy, SL = slight (used as a modifier), FD = found dead

Lab 3: Substance A: MCPA Acid: Clinical observations stage 3, ctd

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM
230	A39	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
230	A40	M	11,SL4	11	11	SL11	SL11	AN	AN	AN	AN	AN
292	A41	M	-	-	-	-	-	-	-	-	-	-
292	A42	F	FD	-	-	-	-	-	-	-	-	-
371	A43	F	-	-	-	-	-	-	-	-	-	-
371	A44	F	SL11	AN	AN	AN	AN	AN	AN	AN	AN	AN
471	A45	M	-	-	-	-	-	-	-	-	-	-
471	A46	M	-	-	-	-	-	-	-	-	-	-
598	A47	F	-	-	-	-	-	-	-	-	-	-
598	A48	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 4 = loss of cordination, 11 = ruffled appearance, SL = slight (used as a modifier), FD = found dead

Experimental Group (mg/kg)	Pen	Sex	7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
230	A39	M	AN	AN	AN	AN	AN	AN	AN	AN
230	A40	M	AN	AN	AN	AN	AN	AN	AN	AN
292	A41	M	-	-	-	-	-	-	-	-
292	A42	F	-	-	-	-	-	-	-	-
371	A43	F	-	-	-	-	-	-	-	-
371	A44	F	AN	AN	AN	AN	AN	AN	AN	AN
471	A45	M	-	-	-	-	-	-	-	-
471	A46	M	-	-	-	-	-	-	-	-
598	A47	F	-	-	-	-	-	-	-	-
598	A48	M	-	-	-	-	-	-	-	-

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	11		12		13		14	
			AM	PM	AM	PM	AM	PM	AM	PM
230	A39	M	AN	AN	AN	AN	AN	AN	AN	AN
230	A40	M	AN	AN	AN	AN	AN	AN	AN	AN
292	A41	M	-	-	-	-	-	-	-	-
292	A42	F	-	-	-	-	-	-	-	-
371	A43	F	-	-	-	-	-	-	-	-
371	A44	F	AN	AN	AN	AN	AN	AN	AN	AN
471	A45	M	-	-	-	-	-	-	-	-
471	A46	M	-	-	-	-	-	-	-	-
598	A47	F	-	-	-	-	-	-	-	-
598	A48	M	-	-	-	-	-	-	-	-

AN = appeared normal

Lab 3: Substance A: MCPA Acid: Clinical observations, stage 4, ctd

Experimental Group			Day of Test												
			0[a]										1		
(mg/kg)	Pen	Sex	Dosed	0852	0907	0921	0936	0955	1050	1244	1344	1432	1542	AM	PM
198	A59	M	0837	AN	1,10	2,4	2,4	1,2,4,11	4,11,14	4,11,14	4,11,13,14	3,4,11,3,14	3,4,11,13,14	4,11,14	4,11,13,14
198	A60	M	0839	AN	AN	4,10	3,4,11	2,4,10,11	4,11,13,14	4,11	4,11	4,11,14	4,11,14	4,11	4,11
252	A61	F	0841	AN	AN	1,4,11	1,2,4,11	1,2,4,11	4,11,14	4,11,13,14	4,11,13,14	4,11,13,14	4,11,13,14	4,11	4,11
252	A62	M	0842	AN	AN	AN	4,11	2,4,11	4,11,14	4,11	4,11	4,11	4,11,14	4,11	4,11
320	A63	M	0844	AN	4,11	AN	4,10,11	1,2,4,11	3,4,11,14	4,11,13,14	4,11,13,14	3,4,11,13,14	3,4,11,13,14	4,11	4,11,14
320	A64	F	0845	AN	1,4,11	1,4,11	1,2,4	4,11,14	2,4,11,14	4,11	2,4,11	4,11,14	4,11,14	4,11	4,11
406	A65	F	0846	AN	1,2	1,4,10	2,4,14	1,2,4,11	2,4,11,14	1,5,11	1,5,6,11	1,5,6,11,16	1,5,6,11	FD	-
406	A66	F	0847	AN	4,10	4,11	4,10,11	2,4,10,11,13	2,3,4,11,13	3,4,11,13,14	1,3,4,11,13	3,4,11,13,14	1,5,11	1,3,5,11	1,3,5,11
515	A67	M	0849	AN	2,4,10	2,4,10,11	2,3,10,13	2,5,6,10,11,13	1,5,11	1,5,11	1,5,11	1,5,6,11,16	FD	-	-
515	A68	M	0850	AN	2,4,10	2,3,4,10	2,3,10,13	2,3,4,10,11,13	1,3,11,13	3,4,11,13,14	3,4,11,13,14	1,3,4,11,13	1,5,11	FD	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 1 = depression, 2 = reduced reaction to external stimuli, 3 = wing droop, 4 = loss of cordination, 5 = prostrate posture, 6 = loss of righting reflex, 10 = shallow and rapid respiration, 11 = ruffled appearance, 13 = loer limb weakness, 14 = lethargy, 16 = minor muscle fasiculation, FD = found dead

Experimental Group			Day of Test									
			2	2	3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
198	A59	M	4,11,13,14	4,11,14	11,SL4	11	SL11	AN	AN	AN	AN	AN
198	A60	M	4,11,14	4,11,14	4,11	11,SL4	11	11	SL11	SL11	AN	AN
252	A61	F	SL(4,11)	SL(4,11)	11,SL4	11	SL11	SL11	AN	AN	AN	AN
252	A62	M	SL(4,11)	SL(4,11)	SL11	AN	AN	AN	AN	AN	AN	AN
320	A63	M	SL(4,11)	SL(4,11)	SL11	AN	AN	AN	AN	AN	AN	AN
320	A64	F	SL11,LHC	SL11,LHC	LHC	LHC	LHC	LHC	LHC	LHC	LHC	LHC
406	A65	F	-	-	-	-	-	-	-	-	-	-
406	A66	F	FD	-	-	-	-	-	-	-	-	-
515	A67	M	-	-	-	-	-	-	-	-	-	-
515	A68	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 4 = loss of cordination, 11 = ruffled appearance, 13 = lower limb weakness, 14 = lethargy, SL = slight (used as a modifier), LHC = lateral head curl, FD = found dead

Experimental Group			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
198	A59	M	AN	AN	AN	AN	AN	AN	AN	AN
198	A60	M	AN	AN	AN	AN	AN	AN	AN	AN
252	A61	F	AN	AN	AN	AN	AN	AN	AN	AN
252	A62	M	AN	AN	AN	AN	AN	AN	AN	AN
320	A63	M	AN	AN	AN	AN	AN	AN	AN	AN
320	A64	F	LHC	LHC	LHC	LHC	LHC	LHC	LHC	LHC
406	A65	F	-	-	-	-	-	-	-	-
406	A66	F	-	-	-	-	-	-	-	-
515	A67	M	-	-	-	-	-	-	-	-
515	A68	M	-	-	-	-	-	-	-	-

AN = appeared normal, LHC = lateral head curl

Lab 3: Substance A: MCPA Acid: Clinical observations, stage 4, ctd

Experimental Group (mg/kg)	Pen	Sex	11		12		13		14	
			AM	PM	AM	PM	AM	PM	AM	PM
198	A59	M	AN	AN	AN	AN	AN	AN	AN	AN
198	A60	M	AN	AN	AN	AN	AN	AN	AN	AN
252	A61	F	AN	AN	AN	AN	AN	AN	AN	AN
252	A62	M	AN	AN	AN	AN	AN	AN	AN	AN
320	A63	M	AN	AN	AN	AN	AN	AN	AN	AN
320	A64	F	LHC	LHC	LHC	LHC	LHC	LHC	LHC	LHC
406	A65	F	-	-	-	-	-	-	-	-
406	A66	F	-	-	-	-	-	-	-	-
515	A67	M	-	-	-	-	-	-	-	-
515	A68	M	-	-	-	-	-	-	-	-

Lab 3: Substance B: Isazofos: Output from SEDEC programme

SEDEC Version1.1 Page 1 of 2

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	100-104
Test Substance =	B
Dose Units =	mg formulation/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD50 Guess =	50
Date =	19-Nov-08
Initials =	pmh
Study Status Code =	43

Step Size = 1.2694297

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
7.07	1	0	4.64	1	0	8.41	2	0	9.97	2	
26	1	1	5.89	1	0	10.7	2	0	12.7	2	
96	1	1	7.48	1	0	13.5	2	1	16.1	2	
354	1	1	9.49	1	0	17.2	2	1	20.4	2	
			12	1	0	21.8	2	1	25.9	2	
			15.3	1	1						
			19.4	1	1						
			24.6	1	1						
			31.2	1	1						
			39.6	1	1						

Analysis

Probit Analysis Results				
Itterations	Chi-square	Probability	G	N
6	4.673559548	0.998552899	0.550408072	24

Slope =	7.704930517		
95% Confidence Limits=	1.988681707	and	13.42117933

LD50 =	16.06150858		
95% Confidence Limits=	11.7099155	and	23.21360558

n of Reversals =	2	n of Partials =	3

Print time: 7:48 AM Printed by (Initials/Date): PH 12/18/08

Combined Data in Dose Order

Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding
4.64	1	0	-	0
5.89	1	0	-	0
7.07	1	0	-	0
7.48	1	0	-	0
8.41	2	0	-	0
9.49	1	0	-	0
10.7	2	0	-	0
12	1	0	-	0
13.5	2	1	Partial	50
15.3	1	1	-	100
17.2	2	1	Rev+Partial	50
19.4	1	1	-	100
21.8	2	1	Rev+Partial	50
24.6	1	1	-	100
26	1	1	-	100
31.2	1	1	-	100
39.6	1	1	-	100
96	1	1	-	100
354	1	1	-	100

Lab 3: Substance B: Isazofos: Output from SEDEC programme – Audit Trail

SEDEC Version 1.1 Audit Trail

Project: 100-104
Test Subst.: B

Last Entry: 37

Date	ID	Entry
11/19/2008	pmh	Study initialized
11/19/2008	pmh	Four dose 1st stage chosen
11/19/2008	pmh	Chose 50
11/19/2008	pmh	Chose Dose-Response: Full
11/19/2008	pmh	File Saved
11/19/2008	pmh	Stage 1 Doses Saved
11/19/2008	pmh	Stage 1 mortality entered
11/19/2008	pmh	File Saved
11/19/2008	pmh	Stage 2 doses calculated
11/19/2008	pmh	Mortality entered for dose 4.64 = 0
11/19/2008	pmh	Mortality entered for dose 5.89 = 0
11/19/2008	pmh	Mortality entered for dose 7.48 = 0
11/19/2008	pmh	Mortality entered for dose 9.49 = 0
11/19/2008	pmh	Mortality entered for dose 12 = 0
11/19/2008	pmh	Mortality entered for dose 15.3 = 1
11/19/2008	pmh	Mortality entered for dose 19.4 = 1
11/19/2008	pmh	Mortality entered for dose 24.6 = 1
11/19/2008	pmh	Mortality entered for dose 31.2 = 1
11/19/2008	pmh	Mortality entered for dose 39.6 = 1
11/19/2008	pmh	LD50 calculated
11/19/2008	pmh	Mortality entered for dose 8.41 = 0
11/19/2008	pmh	Mortality entered for dose 10.7 = 0
11/19/2008	pmh	Mortality entered for dose 13.5 = 1
11/19/2008	pmh	Mortality entered for dose 17.2 = 1
11/19/2008	pmh	Mortality entered for dose 21.8 = 1
11/19/2008	pmh	LD50 calculated
11/19/2008	pmh	Audit Trail Printed

Lab 3: Substance B: Isazofos: Mortality data

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed — Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control																	
0	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/5
Stage 1																	
7,07	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
26	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
96	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
354	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Stage 2																	
4,64	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
5,89	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
7,48	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
9,49	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
12,0	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
15,3	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
19,4	F	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
24,6	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
31,2	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
39,6	F	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

The LD50 value was determined to be 16.1 with 95 % confidence intervals of 11.7 to 23.2 mg/kg.

Experimental Group (mg/kg)	Sex	Number Dead/Number Exposed — Day of Test 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Stage 3																	
8,41	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
8,41	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
10,7	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
10,7	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/2
13,5	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
13,5	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2
17,2	F	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
17,2	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2
21,8	M	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
21,8	M	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2

The LD50 value was determined to be 16.1 with 95 % confidence intervals of 11.7 to 23.2 mg/kg.

Lab 3: Substance B: Isazofos: Body weights

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	210	-4	206	2	208	4	212	2
	0	199	2	201	-2	199	4	203	4
	0	213	0	213	3	216	4	220	7
	0	189	0	189	0	189	1	190	1
	0	233	0	233	1	234	2	236	3
1	7,07	209	-28	181	9	190	10	200	-9
	26	203	-	-	-	-	-	-	-
	96	208	-	-	-	-	-	-	-
	354	204	-	-	-	-	-	-	-
2	4,64	210	-1	209	-3	206	1	207	-3
	5,89	180	7	187	-6	181	5	186	6
	7,48	228	-8	220	-2	218	5	223	-5
	9,49	190	-1	189	-6	183	9	192	2
	12	212	-4	208	1	209	1	210	-2
	15,3	208	-	-	-	-	-	-	-
	19,4	189	-	-	-	-	-	-	-
	24,6	219	-	-	-	-	-	-	-
	31,2	214	-	-	-	-	-	-	-
	39,6	181	-	-	-	-	-	-	-

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	8,41	200	-13	187	-2	185	6	191	-9
	8,41	187	-7	180	2	182	8	190	3
	10,7	188	1	189	0	189	5	194	6
	10,7	229	-13	216	8	224	10	234	5
	13,5	209	-17	192	3	195	7	202	-7
	13,5	200	-	-	-	-	-	-	-
	17,2	192	-21	171	3	174	11	185	-7
	17,2	228	-	-	-	-	-	-	-
	21,8	211	-4	207	-4	203	10	213	2
	21,8	205	-	-	-	-	-	-	-

- No data due to mortality.

Lab 3: Substance B: Isazofos: Food consumption

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	7	9	13	14	12
	0	7	17	14	14	12
	0	12	15	15	17	14
	0	20	15	14	13	18
	0	13	16	19	15	15
1	7,07	0	4	6	10	12
	26	-	-	-	-	-
	96	-	-	-	-	-
	354	-	-	-	-	-
2	4,64	9	15	23	12	12
	5,89	10	15	21	10	12
	7,48	10	18	37	12	17
	9,49	6	9	43	12	16
	12	7	12	19	12	11
	15,3	-	-	-	-	-
	19,4	-	-	-	-	-
	24,6	-	-	-	-	-
	31,2	-	-	-	-	-
	39,6	-	-	-	-	-

- No data due to mortality.

Stage	Experimental Group (mg/kg)	Estimated Mean Feed Consumption Grams/Bird/Day				
		Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	8,41	7	8	8	10	9
	8,41	7	9	12	14	11
	10,7	34	38	43	32	21
	10,7	4	6	6	14	12
	13,5	5	8	9	14	11
	13,5	-	-	-	-	-
	17,2	5	28	51	25	26
	17,2	-	-	-	-	-
	21,8	21	34	30	48	15
	21,8	-	-	-	-	-

- No data due to mortality.

Lab 3: Substance B: Isazofos: Clinical observations – stage 1

Experimental Group			Day of Test									
			0[a]								1	
(mg/kg)	Pen	Sex	Dosed	0957-1029	1040	1051	1139	1213	1322	1501	1555	AM	PM
0	A10	F	0935	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A11	F		AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A12	M	to	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A13	F		AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A14	M	0945	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
7,07	A15	F	0947	AN	11	11	11	11	11,SL4	4,11	4,11,14	4,11	11,SL4
26	A16	M	0949	AN	11	11	4,11,14	FD	-	-	-	-	-
96	A17	M	0951	2,11@1007;2,8,9,11@1026	FD	-	-	-	-	-	-	-	-
354	A18	M	0953	7,8,FD@1006	-	-	-	-	-	-	-	-	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 2 = reduced reaction to external stimuli, 4 = loss of cordination, 7 = lower limb rigidity, 8 = convulsions, 9 = salivation, 11 = ruffled appearance, 14 = lethargy, FD = found dead, Sl = slight (used as a modifier)

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
0	A10	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A11	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A12	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A13	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
0	A14	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
7,07	A15	F	SL11	SL11	11	11	SL11	SL11	SL11	SL11	SL11	AN
26	A16	M	-	-	-	-	-	-	-	-	-	-
96	A17	M	-	-	-	-	-	-	-	-	-	-
354	A18	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 11 = ruffled appearance, SL = slight (used as a modifier)

Experimental Group			Day of Test							
			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
0	A10	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A11	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A12	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A13	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A14	M	AN	AN	AN	AN	AN	AN	AN	AN
7,07	A15	F	AN	AN	AN	AN	AN	AN	AN	AN
26	A16	M	-	-	-	-	-	-	-	-
96	A17	M	-	-	-	-	-	-	-	-
354	A18	M	-	-	-	-	-	-	-	-

AN = appeared normal

Lab 3: Substance B: Isazofos: Clinical observations stage 1 ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 AM
0	A10	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A11	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A12	M	AN	AN	AN	AN	AN	AN	AN	AN
0	A13	F	AN	AN	AN	AN	AN	AN	AN	AN
0	A14	M	AN	AN	AN	AN	AN	AN	AN	AN
7,07	A15	F	AN	AN	AN	AN	AN	AN	AN	AN
26	A16	M	-	-	-	-	-	-	-	-
96	A17	M	-	-	-	-	-	-	-	-
354	A18	M	-	-	-	-	-	-	-	-

AN = appeared normal

Clinical observations stage 2:

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1035-1041	0[a] 1049	0[a] 1113	0[a] 1142	0[a] 1236	0[a] 1403	0[a] 1506	0[a] 1540	1 AM	1 PM
4,64	A29	F	1005	AN	AN	AN	11	11,SL4	11,SL4	4,11	4,11	SL(4,11)	SL11
5,89	A30	F	1007	AN	AN	AN	11,SL4	11,SL4	11,SL4	4,11	4,11	SL(4,11)	SL(4,11)
7,48	A31	F	1009	AN	SL(3,11)	4,SL11	11,SL4	4,11	4,11	4,11	4,11	SL(4,11)	SL(4,11)
9,49	A32	F	1011	AN	AN	SL(4,11)	SL(4,11)	11,SL4	11,SL4	4,11	4,11	SL(4,11)	SL(4,11)
12	A33	M	1014	AN	SL4	4,SL11	4,11	4,11	4,11	4,11,14	4,11,14	SL(4,11)	SL(4,11)
15,3	A34	F	1016	AN	SL4	4,11	4,11,14	4,11,SL13	4,8,11,13	4,11,13,14	3,4,11,13,14	FD	-
19,4	A35	F	1018	AN	SL(3,11)	4,SL11	4,11,SL14	4,11	4,11	4,11	4,11,14	FD	-
24,6	A36	M	1020	AN	SL4	4,11	4,11	FD	-	-	-	-	-
31,2	A37	M	1023	AN	4,11	4,11,13	4,11,14	4,11	6,8	FD	-	-	-
39,6	A38	F	1034	AN	2,11	4,11	5,8,11	FD	-	-	-	-	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 2 = reduced reaction, 3= wing droop, 4 = loss of cordination, 5 = prostrate posture, 6 = loss of righting reflex, 8 = convulsions, 11 = ruffled appearance, 13 = lower limb weakness, 14 = lethargy, FD = found dead, SL = slight (used as a modifier)

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	2 PM	3 AM	3 PM	4 AM	4 PM	5 AM	5 PM	6 AM	6 PM
4,64	A29	F	SL11	AN	AN	AN	AN	AN	AN	AN	AN	AN
5,89	A30	F	SL11	SL11	SL11	SL11	SL11	AN	AN	AN	AN	AN
7,48	A31	F	SL(4,11)	SL(4,11)	AN	AN	AN	AN	AN	AN	AN	AN
9,49	A32	F	SL11	AN	AN	AN	AN	11	AN	AN	AN	AN
12	A33	M	SL(4,11)	AN	AN	AN	AN	AN	AN	AN	AN	AN
15,3	A34	F	-	-	-	-	-	-	-	-	-	-
19,4	A35	F	-	-	-	-	-	-	-	-	-	-
24,6	A36	M	-	-	-	-	-	-	-	-	-	-
31,2	A37	M	-	-	-	-	-	-	-	-	-	-
39,6	A38	F	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 4 = loss of cordination, 11 = ruffled apperance, Sl = slight (used as a modifier)

Lab 3: Substance B: Isazofos: Clinical observations stage 2 – ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	7 PM	8 AM	8 PM	9 AM	9 PM	10 AM	10 PM
4,64	A29	F	AN	AN	AN	AN	AN	AN	AN	AN
5,89	A30	F	AN	AN	AN	AN	AN	AN	AN	AN
7,48	A31	F	AN	AN	AN	AN	AN	AN	AN	AN
9,49	A32	F	AN	AN	AN	AN	AN	AN	AN	AN
12	A33	M	AN	AN	AN	AN	AN	AN	AN	AN
15,3	A34	F	-	-	-	-	-	-	-	-
19,4	A35	F	-	-	-	-	-	-	-	-
24,6	A36	M	-	-	-	-	-	-	-	-
31,2	A37	M	-	-	-	-	-	-	-	-
39,6	A38	F	-	-	-	-	-	-	-	-

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	11 PM	12 AM	12 PM	13 AM	13 PM	14 AM	14 PM
4,64	A29	F	AN	AN	AN	AN	AN	AN	AN	AN
5,89	A30	F	AN	AN	AN	AN	AN	AN	AN	AN
7,48	A31	F	AN	AN	AN	AN	AN	AN	AN	AN
9,49	A32	F	AN	AN	AN	AN	AN	AN	AN	AN
12	A33	M	AN	AN	AN	AN	AN	AN	AN	AN
15,3	A34	F	-	-	-	-	-	-	-	-
19,4	A35	F	-	-	-	-	-	-	-	-
24,6	A36	M	-	-	-	-	-	-	-	-
31,2	A37	M	-	-	-	-	-	-	-	-
39,6	A38	F	-	-	-	-	-	-	-	-

AN = appeared normal

Clinical observations stage 3

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	0930	0949	1013	1045	1118	1228	1331	1427	1538	1 AM	1 PM
8,41	A49	F	0910	AN	AN	AN	AN	AN	SL(4,11)	4,11	11,SL4	4,11	AN	AN
8,41	A50	M	0912	AN	AN	AN	AN	SL11	SL(4,11)	4,11	4,11	4,11,13	SL11	SL11
10,7	A51	M	0913	AN	AN	AN	AN	AN	4,11	4,11,14	4,11	4,11,14	AN	AN
10,7	A52	M	0915	AN	SL11	2,11	2,11	11	4,11	4,11	4,11,14	4,11	11,SL4	11,SL4
13,5	A53	M	0918	AN	AN	AN	11	4,11	4,11	4,11,13,14	4,11,13,14	4,11,14,16	11,SL4	11
13,5	A54	M	0920	AN	SL4	SL4	4,11	SL(4,11)	4,9,11	FD	-	-	-	-
17,2	A55	F	0922	AN	AN	AN	11	11	4,11	4,11,14	4,11,13,14	4,11,14	1,5,11	4,11,13,14
17,2	A56	M	0924	AN	AN	SL4	4,11	4,11	8,11,13	FD	-	-	-	-
21,8	A57	M	0925	AN	AN	AN	SL11	11	4,11	4,11	4,11	4,11,14	4,11	11,SL4
21,8	A58	M	0929	AN	AN	SL4	11,SL4	4,11	FD	-	-	-	-	-

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, 2 = reduced reaction to external stimuli, 4 = loss of cordination, 5 = prostrate posture, 8 = convulsions, 9 = salivation, 11 = ruffled appearance, 13 = lower limb weakness, 14 = lethargy, 16 = minor muscle fasiculation, SL = slight (used as a modifier), FD = found dead

Lab 3: Substance B: Isazofos: Clinical observations stage 3, ctd.

Experimental Group			Day of Test									
			2		3		4		5		6	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
8,41	A49	F	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
8,41	A50	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A51	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A52	M	11	SL11	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A53	M	11	AN	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A54	M	-	-	-	-	-	-	-	-	-	-
17,2	A55	F	11	SL11	AN	AN	AN	AN	AN	AN	AN	AN
17,2	A56	M	-	-	-	-	-	-	-	-	-	-
21,8	A57	M	AN	AN	AN	AN	AN	AN	AN	AN	AN	AN
21,8	A58	M	-	-	-	-	-	-	-	-	-	-

AN = appeared normal, 11 = ruffled apperance, SL= slight (used as a modifier)

Experimental Group			7		8		9		10	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
8,41	A49	F	AN	AN	AN	AN	AN	AN	AN	AN
8,41	A50	M	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A51	M	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A52	M	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A53	M	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A54	M	-	-	-	-	-	-	-	-
17,2	A55	F	AN	AN	AN	AN	AN	AN	AN	AN
17,2	A56	M	-	-	-	-	-	-	-	-
21,8	A57	M	AN	AN	AN	AN	AN	AN	AN	AN
21,8	A58	M	-	-	-	-	-	-	-	-

AN = appeared normal

Experimental Group			11		12		13		14	
(mg/kg)	Pen	Sex	AM	PM	AM	PM	AM	PM	AM	PM
8,41	A49	F	AN	AN	AN	AN	AN	AN	AN	AN
8,41	A50	M	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A51	M	AN	AN	AN	AN	AN	AN	AN	AN
10,7	A52	M	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A53	M	AN	AN	AN	AN	AN	AN	AN	AN
13,5	A54	M	-	-	-	-	-	-	-	-
17,2	A55	F	AN	AN	AN	AN	AN	AN	AN	AN
17,2	A56	M	-	-	-	-	-	-	-	-
21,8	A57	M	AN	AN	AN	AN	AN	AN	AN	AN
21,8	A58	M	-	-	-	-	-	-	-	-

AN = appeared normal

Lab 4: Substance A: MCPA – Acid: Output SEDEC Programme

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	13836,4103
Test Substance =	MPCA
Dose Units =	mg a.i/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD_{50} Guess =	1000
Step Size =	1,2694297
Date =	20-mei-09
Initials =	jms
Study Status Code =	43

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding	Dose	N Tested	N Responding
66,6	1	0	161	1	0	292	2	0	344	2	
245	1	0	205	1	0	371	2	1	437	2	
904	1	1	260	1	0	471	2	0	554	2	
3330	1	1	329	1	0	597	2	1	703	2	
			418	1	0	758	2	1	893	2	
			530	1	1						
			673	1	1						
			853	1	1						
			1083	1	1						
			1374	1	1						

Analysis

Probit Analysis Results				
Itterations	Chi-square	Probability	G	N
5	6,776850472	0,986258549	0,522028267	24

Slope =	6,220914252		
95% Confidence Limits=	1,726208921	and	10,71561958

LD_{50} =	554,1651369		
95% Confidence Limits=	395,794625	and	844,7727822

n of Reversals =	3	n of Partials =	3

Combined Data in Dose Order

Dose	Number Exposed	Number Dead	Reversal or Partial ?	% Responding
66,6	1	0	-	0
161	1	0	-	0
205	1	0	-	0
245	1	0	-	0
260	1	0	-	0
292	2	0	-	0
329	1	0	-	0
371	2	1	Partial	50
418	1	0	Reversal	0
471	2	0	-	0
530	1	1	-	100
597	2	1	Rev+Partial	50
673	1	1	-	100
758	2	1	Rev+Partial	50
853	1	1	-	100
904	1	1	-	100
1083	1	1	-	100
1374	1	1	-	100
3330	1	1	-	100

lab 4: Substance A: MCPA – Acid: Output SEDEC Programme

SEDEC Version 1.1 Audit Trail

Project: 13836,4103
Test Subst.: MPCA

Last Entry: 40

Date	ID	Entry
20-5-2009	jms	Study initialized
20-5-2009	jms	Four dose 1st stage chosen
20-5-2009	jms	Chose 1000
20-5-2009	jms	Chose Dose-Response: Full
20-5-2009	jms	File Saved
20-5-2009	jms	Stage 1 Doses Saved
20-5-2009	jms	Stage 1 mortality entered
20-5-2009	jms	File Saved
20-5-2009	jms	Stage 2 doses calculated
20-5-2009	jms	Report Printed
20-5-2009	jms	Mortality entered for dose 161 = 0
20-5-2009	jms	Mortality entered for dose 205 = 0
20-5-2009	jms	Mortality entered for dose 260 = 0
20-5-2009	jms	Mortality entered for dose 329 = 0
20-5-2009	jms	Mortality entered for dose 418 = 0
20-5-2009	jms	Mortality entered for dose 530 = 1
20-5-2009	jms	Mortality entered for dose 673 = 1
20-5-2009	jms	Mortality entered for dose 853 = 1
20-5-2009	jms	Mortality entered for dose 1083 = 1
20-5-2009	jms	Mortality entered for dose 1374 = 1
20-5-2009	jms	LD_{50} calculated
20-5-2009	jms	Mortality entered for dose 292 = 0
20-5-2009	jms	Mortality entered for dose 371 = 1
20-5-2009	jms	Mortality entered for dose 471 = 0
20-5-2009	jms	Mortality entered for dose 597 = 1
20-5-2009	jms	Mortality entered for dose 758 = 1
20-5-2009	jms	LD_{50} calculated
20-5-2009	jms	Report Printed
20-5-2009	jms	File Saved
20-5-2009	jms	File saved

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Lab 4: Substance A: MCPA –Acid – Mortality data

Stage	Experimental Group mg a.i./kg bw	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	215,2	-7,4	207,8	-6,7	201,1	-2,1	199	-16,2
	0	228	0,1	228,1	-4,5	223,6	-1,1	222,5	-5,5
	0	189,6	1,9	191,5	-7,3	184,2	-2,3	181,9	-7,7
	0	204,3	1,5	205,8	-7,5	198,3	-5,8	192,5	-11,8
	0	198,8	7,6	206,4	-3,8	202,6	1	203,6	4,8
1	66,6	204,6	11,4	216	-7,5	208,5	2,1	210,6	6
	245	193	4,4	197,4	-5,2	192,2	8,3	200,5	7,5
	902	204,2	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	3330	185,2	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
2	161	187	-9,8	177,2	-1,2	176	11,2	187,2	0,2
	205	218,5	-5	213,5	-6,3	207,2	-2,2	205	-13,5
	260	209,2	-19,1	190,1	0,4	190,5	12,5	203	-6,2
	329	190,4	-17,8	172,6	9,6	182,2	9,1	191,3	0,9
	418	219,8	-40,1	179,7	13,6	193,3	12,9	206,2	-13,6
	530	207,7	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	673	197,4	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	853	180,9	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	1083	204,3	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	1374	190,8	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!

- No data due to mortality.

Stage	Experimental Group mg a.i./kg bw	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	292	183,2	-18,3	164,9	11,3	176,2	4,8	181	-2,2
	292	203,9	-10,2	193,7	7,1	200,8	3,9	204,7	0,8
	371	227,6	-39,1	188,5	19,4	207,9	1,1	209	-18,6
	371	180,2	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	471	199,4	-34,3	165,1	6,4	171,5	4,9	176,4	-23
	471	195,5	-22,3	173,2	3,7	176,9	0,7	177,6	-17,9
	597	182	-36	146	8,6	154,6	15,6	170,2	-11,8
	597	182,5	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	758	186,2	-31,4	154,8	17,2	172	9,4	181,4	-4,8
	758	208,5	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!

Lab 4: Substance A: MCPA –Acid – Food consumption

Stage	Experimental Group mg a.i./kg bw	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	12,3	10	12,7	11,525	11,857143
	0	5,8	16	17,1	1,8	12,928571
	0	7,4	9,5	13,8	11,2	10,7
	0	9,3	9,8	11,7	9,9	9,4142857
	0	8,4	11,5	14,4	13	13,785714
1	66,6	11,2	15,9	14,8	14,4	13,014286
	245	4,5	12,3	14,9	32,125	11,457143
	902	-0,2	0	#VALUE!	#VALUE!	#VALUE!
	3330	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
2	161	2,8	8,5	14,5	11,975	6,0428571
	205	2,6	9,7	16,4	11,175	14,228571
	260	0	3,8	10	11,15	14,071429
	329	0,1	1,7	8,4	12,45	13,814286
	418	0,1	0,1	0,8	11,25	4,0857143
	530	0	0	#VALUE!	#VALUE!	#VALUE!
	673	0,2	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	853	-0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	1083	0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	1374	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!

- No data due to mortality.

Stage	Experimental Group mg a.i./kg bw	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	292	0,2	6,9	10,5	16,125	16,085714
	292	2,4	9,3	15,1	17,45	15,485714
	371	-0,1	0,9	9,5	21,775	26,385714
	371	0	0,3	#VALUE!	#VALUE!	#VALUE!
	471	0	#VALUE!	4,3	10,9	11,728571
	471	0	5,3	10,2	12,025	13,757143
	597	0	0,2	3,3	12,6	18,857143
	597	0	0,1	#VALUE!	#VALUE!	#VALUE!
	758	0	1,8	4,8	13,3	16,285714
	758	0	#VALUE!	#VALUE!	#VALUE!	#VALUE!

Lab 4: Substance A: MCPA –Acid : Clinical observations stage 1:

Experimental Group			Day of Test					
			0[a]					1
(mg/kg)	Pen	Sex	Dosed	1040-1240	1400	1500	1600	AM
0	5	F	1000	AN	AN	AN	AN	AN
0	21	F		AN	AN	AN	AN	AN
0	22	F	to	AN	AN	AN	AN	AN
0	34	F		AN	AN	AN	AN	AN
0	37	M		AN	AN	AN	AN	AN
66,6	28	M		AN	AN	AN	AN	AN
245	2	M		AN	AN	AN	AN	AN
902	56	M		AN	hypo-R	AT, L	AT, L, WD	L
3330	1	M	1040	AN	AT, WD	AT, WD	L, WD, AT, sitting	FD

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal AT = ataxia FD = found dead

L = lethargic WD = wing droop R = responsive

Experimental Group			Day of Test				
			2	3	4	5	6
(mg/kg)	Pen	Sex	AM	AM	AM	AM	AM
0	5	F	AN	AN	AN	AN	AN
0	21	F	AN	AN	AN	AN	AN
0	22	F	AN	AN	AN	AN	AN
0	34	F	AN	AN	AN	AN	AN
0	37	M	AN	AN	AN	AN	AN
66,6	28	M	AN	AN	AN	AN	AN
245	2	M	AN	AN	AN	AN	AN
902	56	M	FD				
3330	1	M					

AN = appeared normal

Experimental Group			Day of Test			
			7	8	9	10
(mg/kg)	Pen	Sex	AM	AM	AM	AM
0	5	F	AN	AN	AN	AN
0	21	F	AN	AN	AN	AN
0	22	F	AN	AN	AN	AN
0	34	F	AN	AN	AN	AN
0	37	M	AN	AN	AN	AN
66,6	28	M	AN	AN	AN	AN
245	2	M	AN	AN	AN	AN
902	56	M				
3330	1	M				

AN = appeared normal

Lab 4: Substance A: MCPA –Acid : Clinical observations stage 1, ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
0	5	F	AN	AN	AN	AN
0	21	F	AN	AN	AN	AN
0	22	F	AN	AN	AN	AN
0	34	F	AN	AN	AN	AN
0	37	M	AN	AN	AN	AN
66,6	28	M	AN	AN	AN	AN
245	2	M	AN	AN	AN	AN
902	56	M				
3330	1	M				

AN = appeared normal

Clinical observations stage 2:

Experimental Group			Day of Test					
			0[a]					1
mg a.i./kg bw	Pen	Sex	Dosed	1000-1200	1340	1445	1540	AM
161	11	F	915	AN	AN	AN	AN	AN
205	48	F		AN	AN	AN	AN	AN
260	18	F		AN	AN	AN	AN	AN
329	42	M		AN	L, sits mostly	AT, sits mostly	L	P, AT, L
418	25	F	to	AN	AN	AN	AN	P, AT, sits mostly
530	14	F		(1), WD	sitting, WD	sitting, WD	sitting, WD	L, P, (2)
673	31	M		AN	AT	MY, AT	sitting, slow to move	FD
853	17	F		AN	AT	AT	AT, mostly sitting	FD
1083	24	M		P	laying, WD, L	laying, WD, L, LB	L, HR, WD	FD
1374	20	M	1000	WD, GF, sitting	laying, WD, LB	laying, WD, LB, HR	laying, WD, LB, HR	FD

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal
AT = ataxia
HR = hyporesponsive
(2) laying with wings outspr
(1)=standing with legs and wings slightly spread
LB = labored breathing
L = lethargic
P = piloerect
WD = wing droop
GF = gular fluttering
FD = found dead

Experimental Group			Day of Test				
			2	3	4	5	6
mg a.i./kg bw	Pen	Sex	AM	AM	AM	AM	AM
161	11	F	AN	AN	AN	AN	AN
205	48	F	AN	AN	AN	AN	AN
260	18	F	AN	AN	AN	AN	AN
329	42	M	AN	P	AN	AN	AN
418	25	F	AN	AN	AN	AN	AN
530	14	F	FD				
673	31	M					
853	17	F					
1083	24	M					
1374	20	M					

ad AN = appeared normal,

Lab 4: Substance A: MCPA –Acid : Clinical observations stage 2, ctd.

Experimental Group mg a.i./kg bw	Pen	Sex	Day of Test 7 AM	8 AM	9 AM	10 AM
161	11	F	AN	AN	AN	AN
205	48	F	AN	AN	AN	AN
260	18	F	AN	AN	AN	AN
329	42	M	AN	AN	AN	AN
418	25	F	AN	AN	AN	AN
530	14	F				
673	31	M				
853	17	F				
1083	24	M				
1374	20	M				

AN = appeared normal

Experimental Group mg a.i./kg bw	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
161	11	F	AN	AN	AN	AN
205	48	F	AN	AN	AN	AN
260	18	F	AN	AN	AN	AN
329	42	M	AN	AN	AN	AN
418	25	F	AN	AN	AN	AN
530	14	F				
673	31	M				
853	17	F				
1083	24	M				
1374	20	M				

AN = appeared normal

Clinical observations stage 3:

Experimental Group			Day of Test				
			0[a]				1
mg a.i./kg bw	Pen	Sex	1040-1240	1350	1450	1550	AM
292	12	M	AN	AT, WD, FOB	AT	laying, (1), P	AN
292	26	M	AN	AN	AN	AN	AN
371	10	M	sitting, AT	FOB, WD, SAL	FOB, WD, SAL, UNR	FOB, WD, SAL, UNR	AT
371	13	F	sitting mostly, AT	FOB, L	sitting	sitting	P, L
471	29	M	alert but hypo-R	AN	AN	AN	AN
471	52	M	AN	AN	AN	AN	AN
597	36	F	P	FOB, WD	FOB, WD	P, sitting	P, L, AT
597	49	M	PS, AT	AT, WD, FOB	FOB	FOB	P, L
758	27	M	FOB, WD	FOB, WD	AT	AT	AN
758	47	F	WD, GF, FOB	FOB, L	FOB, L, UNR	FOB, L, UNR	FD

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, SAL = excessive salivation UNR = unresponsive

AT = ataxia FOB = laying forward on breast (1) wings outspread, legs extended backwards

PS = pronounced swallowing GF = gular fluttering P = piloerect

WD = wing droop R = responsive

L = lethargic

Lab 4: Substance A: MCPA –Acid : Clinical observations stage 3, ctd.

Experimental Group			Day of Test				
			2	3	4	5	6
mg a.i./kg bw	Pen	Sex	AM	AM	AM	AM	AM
292	12	M	AN	AN	AN	AN	AN
292	26	M	AN	AN	AN	AN	AN
371	10	M	AN	D	D	AN	AN
371	13	F	FD				
471	29	M	AN	AN	AN	AN	AN
471	52	M	AN	D	D	AN	AN
597	36	F	AN	AN	AN	AN	AN
597	49	M	FD				
758	27	M	AN	AN	AN	AN	AN
758	47	F					

AN = appeared normal D = diarrhea

Experimental Group			7	8	9	10
mg a.i./kg bw	Pen	Sex	AM	AM	AM	AM
292	12	M	AN	AN	AN	AN
292	26	M	AN	AN	AN	AN
371	10	M	AN	AN	AN	AN
371	13	F				
471	29	M	AN	AN	AN	AN
471	52	M	AN	AN	AN	AN
597	36	F	AN	AN	AN	AN
597	49	M				
758	27	M	AN	AN	AN	AN
758	47	F				

AN = appeared normal

Experimental Group mg a.i./kg bw	Pen	Sex	11 AM	12 AM	13 AM	14 AM
292	12	M	AN	AN	AN	AN
292	26	M	AN	AN	AN	AN
371	10	M	AN	AN	AN	AN
371	13	F				
471	29	M	AN	AN	AN	AN
471	52	M	AN	AN	AN	AN
597	36	F	AN	AN	AN	AN
597	49	M				
758	27	M	AN	AN	AN	AN
758	47	F				

AN = appeared normal

Lab 4: Substance B: Isazofos: Output SEDEC Programme

SEDEC V1.1 Sequential Design Calculator

Study Identification

Project Number =	13836,4102
Test Substance =	Isazafos
Dose Units =	mg a.i/kg
Test Species =	Northern Bobwhite
Study Type =	Dose-Response: Full
Limit Dose =	NA
Initial LD_{50} Guess =	50
Step Size =	1,2694297
Date =	20-mei-09
Initials =	jms
Study Status Code =	43

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	Respondin	Dose	N Tested	Respondin	Dose	N Tested	Respondin	Dose	N Tested	Respondin
7,07	1	0	4,64	1	0	10,6	2	0	9,15	2	1
26	1	1	5,89	1	0	13,4	2	1	11,6	2	0
96	1	1	7,48	1	0	17	2	2	14,7	2	1
354	1	1	9,49	1	0	21,6	2	2	18,7	2	2
			12	1	0	27,5	2	2	23,8	2	2
			15,3	1	1						
			19,4	1	1						
			24,6	1	0						
			31,2	1	1						
			39,6	1	1						

Analysis

Probit Analysis Results

Itterations	Chi-square	Probability	G	N
5	25,13412037	0,290703984	0,361484023	34

Slope =	6,448256421

95% Confidence Limits=	2,571336304	and	10,32517654

LD_{50} =	13,85182087		
95% Confidence Limits=	10,45430988	and	17,40012565

n of Reversals =	2	n of Partials =	3

Combined Data in Dose Order

Dose	Exposed	Dead	Reversal or Partial ?	% Responding
4,64	1	0	-	0
5,89	1	0	-	0
7,07	1	0	-	0
7,48	1	0	-	0
9,15	2	1	Partial	50
9,49	1	0	Reversal	0
10,6	2	0	-	0
11,6	2	0	-	0
12	1	0	-	0
13,4	2	1	Partial	50
14,7	2	1	Partial	50
15,3	1	1	-	100
17	2	2	-	100
18,7	2	2	-	100
19,4	1	1	-	100
21,6	2	2	-	100
23,8	2	2	-	100
24,6	1	0	Reversal	0
26	1	1	-	100
27,5	2	2	-	100
31,2	1	1	-	100
39,6	1	1	-	100
96	1	1	-	100
354	1	1	-	100

Lab 4: Substance B: Isazofos: Output SEDEC Programme – Audit trail

SEDEC Version 1.1 Audit Trail

Project: 13836,4102
Test Subst.: Isazafos

Last Entry: 44

Date	ID	Entry
20-5-2009	jms	Study initialized
20-5-2009	jms	Four dose 1st stage chosen
20-5-2009	jms	Chose 50
20-5-2009	jms	Chose Dose-Response: Full
20-5-2009	jms	Stage 1 mortality entered
20-5-2009	jms	Mortality entered for dose 4.64 = 0
20-5-2009	jms	Mortality entered for dose 5.89 = 0
20-5-2009	jms	Mortality entered for dose 7.48 = 0
20-5-2009	jms	Mortality entered for dose 9.49 = 0
20-5-2009	jms	Mortality entered for dose 12 = 0
20-5-2009	jms	Mortality entered for dose 15.3 = 1
20-5-2009	jms	Mortality entered for dose 19.4 = 1
20-5-2009	jms	Mortality entered for dose 24.6 = 0
20-5-2009	jms	Mortality entered for dose 31.2 = 1
20-5-2009	jms	Mortality entered for dose 39.6 = 1
20-5-2009	jms	LD50 calculated
20-5-2009	jms	Mortality entered for dose 10.6 = 0
20-5-2009	jms	Mortality entered for dose 13.4 = 1
20-5-2009	jms	Mortality entered for dose 17 = 2
20-5-2009	jms	Mortality entered for dose 21.6 = 2
20-5-2009	jms	Mortality entered for dose 27.5 = 2
20-5-2009	jms	LD50 calculated
20-5-2009	jms	LD50 calculated
20-5-2009	jms	LD50 calculated
20-5-2009	jms	LD50 calculated
20-5-2009	jms	LD50 calculated
20-5-2009	jms	Mortality entered for dose 9.15 = 1
20-5-2009	jms	Mortality entered for dose 11.6 = 0
20-5-2009	jms	Mortality entered for dose 14.7 = 1
20-5-2009	jms	Mortality entered for dose 18.7 = 2
20-5-2009	jms	Mortality entered for dose 23.8 = 2
20-5-2009	jms	LD50 calculated
20-5-2009	jms	File Saved
20-5-2009	jms	File saved

Lab 4: Substance B: Isazofos: Body weights

Stage	Experimental Group (mg ai/kg bw)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
control	0	197,7	-7,7	190	-3,4	186,6	-0,4	186,2	-11,5
	0	227,7	-27,6	200,1	-6,3	193,8	0,3	194,1	-33,6
	0	208,1	8,5	216,6	-3,2	213,4	-5,7	207,7	-0,4
	0	227,6	-14,9	212,7	-15,2	197,5	-9,9	187,6	-40
	0	189,3	33,9	223,2	-4	219,2	-0,4	218,8	29,5
1	7	223,2	-5,3	217,9	-5,1	212,8	-0,2	212,6	-10,6
	26	201,5	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	96	187,4	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	354	203,7	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
2	4,64	192,5	2,3	194,8	-2,2	192,6	-2,9	189,7	-2,8
	5,89	191,8	3	194,8	0,1	194,9	-3,9	191	-0,8
	7,48	218,2	5,7	223,9	-5,9	218	-2,6	215,4	-2,8
	9,49	193,9	-10,2	183,7	5,1	188,8	0,9	189,7	-4,2
	12	183,8	-15,3	168,5	8,6	177,1	6,5	183,6	-0,2
	15,3	181,9	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	19,4	212,5	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	24,6	195,5	0,3	195,8	-2,5	193,3	-7,3	186	-9,5
	31,2	192	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	39,6	196,3	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!

- No data due to mortality.

Stage	Experimental Group (mg ai/kg bw)	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change
3	10,6	196,9	-17,7	179,2	5,9	185,1	4,6	189,7	-7,2
	10,6	198,2	-2,4	195,8	-0,3	195,5	5,1	200,6	2,4
	13,4	204,4	-7,5	196,9	2,2	199,1	1,2	200,3	-4,1
	13,4	184	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	17	183,6	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	17	202,1	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	21,6	194,3	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	21,6	242,7	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	27,5	197,2	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	27,5	192,7	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
4	9,15	191,1	-9,7	181,4	2,4	183,8	3,3	187,1	-4
	9,15	183	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	11,6	186,3	-4,9	181,4	3,8	185,2	4,9	190,1	3,8
	11,6	196	-12,9	183,1	8,1	191,2	2,6	193,8	-2,2
	14,7	204,3	-19,3	185	-1,4	183,6	4,8	188,4	-15,9
	14,7	185,3	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	18,7	192,3	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	18,7	184,2	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	23,8	205,6	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!
	23,8	184,7	#VALUE!	-	#VALUE!	-	#VALUE!	-	#VALUE!

- No data due to mortality.

Lab 4: Substance B: Isazofos: Food consumption

Stage	Experimental Group mg ai/kg bw	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
control	0	4,5	7,9	10,0	11,5	10,5
	0	12,6	14,7	16,7	17,5	16,6
	0	12,2	11,3	12,8	12,9	11,4
	0	12,6	14,0	15,5	15,2	9,8
	0	11,6	12,2	15,8	15,9	15,4
1	7	3,6	12,1	11,5	9,2	11,7
	26	0,0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	96	0,0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	354	0,0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
2	4,64	6,6	5,3	16,5	12,9	12,0
	5,89	7,4	3,8	17,3	16,1	14,2
	7,48	8,7	12,1	23,9	15,4	13,8
	9,49	0,1	1,5	11,9	13,8	13,1
	12	0,2	1,5	12,6	15,1	14,5
	15,3	0,0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	19,4	0,0	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	24,6	4,5	2,9	16,6	12,7	13,6
	31,2	0,2	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	39,6	0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!

- No data due to mortality.

Stage	Experimental Group mg ai/kg bw	Estimated Mean Feed Consumption Grams/Bird/Day Day 0-1	Day 1-2	Day 2-3	Day 3-7	Day 7-14
3	10,6	2,2	2,8	10,5	13,0	12,7
	10,6	4,3	14,9	17,9	18,4	20,0
	13,4	1,5	14,5	13,6	14,8	15,4
	13,4	0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	17	0,3	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	17	0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	21,6	1,3	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	21,6	0,1	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	27,5	0,5	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	27,5	0,3	#VALUE!	#VALUE!	#VALUE!	#VALUE!
4	9,15	0,8	10,5	14,8	16,3	18,7
	9,15	0,7	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	11,6	1,8	5,5	11,5	13,1	14,6
	11,6	0,0	1,8	9,1	13,5	13,3
	14,7	0,2	0,9	5,9	11,3	15,2
	14,7	0,8	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	18,7	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	18,7	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	23,8	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	23,8	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!

- No data due to mortality.

Lab 4: Substance B: Isazofos: Clinical observations stage 1

Experimental Group (mg/kg)	Pen	Sex	Day of Test 0[a] Dosed	1100-1300	1410	1510	1610	Day of Test 1 AM
0	1	M	1030	AN	AN	AN	AN	AN
0	7	F		AN	AN	AN	AN	AN
0	12	F	to	AN	AN	AN	AN	AN
0	14	F		AN	AN	AN	AN	AN
0	36	F		AN	AN	AN	AN	AN
7	29	F		AN	AN	AN	AN	AN
26	28	M		Laying, T				AN
96	34	M		FD				AN
354	39	F	1100	FD				AN

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal

T = tremors FD = found dead

Experimental Group (mg/kg)	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
0	1	M	AN	AN	AN	AN	AN
0	7	F	AN	AN	AN	AN	AN
0	12	F	AN	AN	AN	AN	AN
0	14	F	AN	AN	AN	AN	AN
0	36	F	AN	AN	AN	AN	AN
7	29	F	AN	AN	AN	AN	AN
26	28	M					
96	34	M					
354	39	F					

AN = appeared normal

Experimental Group (mg/kg)	Pen	Sex	Day of Test 7 AM	8 AM	9 AM	10 AM
0	1	M	AN	AN	AN	AN
0	7	F	AN	AN	AN	AN
0	12	F	AN	AN	AN	AN
0	14	F	AN	AN	AN	AN
0	36	F	AN	AN	AN	AN
7	29	F	AN	AN	AN	AN
26	28	M				
96	34	M				
354	39	F				

AN = appeared normal

Lab 4: Substance B: Isazofos: Clinical observations stage 1, ctd.

Experimental Group (mg/kg)	Pen	Sex	Day of Test 11 AM	12 AM	13 AM	14 AM
0	1	M	AN	AN	AN	AN
0	7	F	AN	AN	AN	AN
0	12	F	AN	AN	AN	AN
0	14	F	AN	AN	AN	AN
0	36	F	AN	AN	AN	AN
7	29	F	AN	AN	AN	AN
26	28	M				
96	34	M				
354	39	F				

AN = appeared normal

Clinical observations stage 2

Experimental Group mg ai/kg bw	Pen	Sex	Day of Test 0[a] Dosed	0[a] 1110-1310	0[a] 1400	0[a] 1500	0[a] 1600	1 AM
4,64	16	F	1030	AN	AN	AN	AN	AN
5,89	31	M		AN	AN	AN	AN	AN
7,48	27	F		AN	AN	AN	AN	AN
9,49	2	F		AN	AN	AN	AN	AN
12	35	M	to	AN	AN	SAL	SAL	AN
15,3	5	M		Laying, LB	FD			
19,4	21	F		AN	FD			
24,6	13	F		AN	SAL	SAL	Laying, SAL	AN
31,2	18	F		FD				
39,6	17	M	1110	FD				

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal LB = labored breathing

SAL = excessive salivation FD = found dead

Experimental Group (mg ai/kg bw	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
4,64	16	F	AN	AN	AN	AN	AN
5,89	31	M	AN	AN	AN	AN	AN
7,48	27	F	AN	AN	AN	AN	AN
9,49	2	F	AN	AN	AN	AN	AN
12	35	M	AN	AN	AN	AN	AN
15,3	5	M					
19,4	21	F					
24,6	13	F	AN	AN	AN	AN	AN
31,2	18	F					
39,6	17	M					

AN = appeared normal,

Lab 4: Substance B: Isazofos: Clinical observations stage 2, ctd.

Experimental Group			Day of Test			
			7	8	9	10
(mg ai/kg bw	Pen	Sex	AM	AM	AM	AM
4,64	16	F	AN	AN	AN	AN
5,89	31	M	AN	AN	AN	AN
7,48	27	F	AN	AN	AN	AN
9,49	2	F	AN	AN	AN	AN
12	35	M	AN	AN	AN	AN
15,3	5	M				
19,4	21	F				
24,6	13	F	AN	AN	AN	AN
31,2	18	F				
39,6	17	M				

AN = appeared normal

Experimental Group			Day of Test			
			11	12	13	14
(mg ai/kg bw	Pen	Sex	AM	AM	AM	AM
4,64	16	F	AN	AN	AN	AN
5,89	31	M	AN	AN	AN	AN
7,48	27	F	AN	AN	AN	AN
9,49	2	F	AN	AN	AN	AN
12	35	M	AN	AN	AN	AN
15,3	5	M				
19,4	21	F				
24,6	13	F	AN	AN	AN	AN
31,2	18	F				
39,6	17	M				

AN = appeared normal

Clinical observations stage 3:

Experimental Group			Day of Test					
			0[a]					1
(mg ai/kg bw	Pen	Sex	0930-1030	1030-1230	1330	1440	1550	AM
10,6	4	F	930	AN	AN	AN	AN	AN
10,6	6	M		AN	AN	AN	AN	AN
13,4	8	M		AN	AN	AN	AN	AN
13,4	33	M	TO	FD				
17	3	M		FD				
17	19	F		AN	AN	AN	AN	FD
21,6	9	F		FD				
21,6	15	M		Laying, LB, FD				
27,5	20	M		P, PS	FD			
27,5	22	F	1030	SAL, PS	FD			

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, FD = found dead SAL = excessive salivation

LB = labored breathing P = piloerect PS = pronounced swallowing

Lab 4: Substance B: Isazofos: Clinical observations stage 3 ctd.

Experimental Group (mg ai/kg bw	Pen	Sex	Day of Test 2 AM	3 AM	4 AM	5 AM	6 AM
10,6	4	F	AN	AN	AN	AN	AN
10,6	6	M	AN	AN	AN	AN	AN
13,4	8	M	AN	AN	AN	AN	AN
13,4	33	M					
17	3	M					
17	19	F					
21,6	9	F					
21,6	15	M					
27,5	20	M					
27,5	22	F					

AN = appeared normal

Experimental Group (mg ai/kg bw	Pen	Sex	7 AM	8 AM	9 AM	10 AM
10,6	4	F	AN	AN	AN	AN
10,6	6	M	AN	AN	AN	AN
13,4	8	M	AN	AN	AN	AN
13,4	33	M				
17	3	M				
17	19	F				
21,6	9	F				
21,6	15	M				
27,5	20	M				
27,5	22	F				

AN = appeared normal

Experimental Group (mg ai/kg bw	Pen	Sex	11 AM	12 AM	13 AM	14 AM
10,6	4	F	AN	AN	AN	AN
10,6	6	M	AN	AN	AN	AN
13,4	8	M	AN	AN	AN	AN
13,4	33	M				
17	3	M				
17	19	F				
21,6	9	F				
21,6	15	M				
27,5	20	M				
27,5	22	F				

AN = appeared normal

Lab 4: Substance B: Isazofos: Clinical observations stage 4.

Experimental Group			Day of Test				
			0[a]				1
mg ai/kg bw	Pen	Sex	1105-1305	1400	1455	1555	AM
9,15	26	M	AN	AN	AN	AN	AN
9,15	37	M	AN	AN	AT	Laying, WD, FD	FD
11,6	25	F	AN	AN	AN	AN	AN
11,6	42	M	P	P, PS, SAL	P	AN	AN
14,7	43	F	AN	AN	AN	AN	AN
14,7	44	F	AN	AN	AN	T	FD
18,7	24	M	SAL, AT	FD			
18,7	41	F	AN	FD			
23,8	10	F	(2)	FD			
23,8	32	M	Laying, SAL, WD, FD				

[a] - Multiple observations were done following dosing on Day 0 of the test.

AN = appeared normal, AT = ataxia WD = wing droop

SAL = excessive salivation P = piloerect

PS = pronounced swallowing T = tremors (2) = laying forward on breast with legs exten

Experimental Group			Day of Test				
			2	2	3	4	5	6
mg ai/kg bw	Pen	Sex	AM		AM	AM	AM	AM
9,15	26	M	AN		AN	AN	AN	AN
9,15	37	M						
11,6	25	F	AN		AN	AN	AN	AN
11,6	42	M	AN		AN	AN	AN	AN
14,7	43	F	AN		AN	AN	AN	AN
14,7	44	F						
18,7	24	M						
18,7	41	F						
23,8	10	F						
23,8	32	M						

AN = appeared normal

Experimental Group			7	8	9	10
mg ai/kg bw	Pen	Sex	AM	AM	AM	AM
9,15	26	M	AN	AN	AN	AN
9,15	37	M				
11,6	25	F	AN	AN	AN	AN
11,6	42	M	AN	AN	AN	AN
14,7	43	F	AN	AN	AN	AN
14,7	44	F				
18,7	24	M				
18,7	41	F				
23,8	10	F				
23,8	32	M				

AN = appeared normal

Lab 4: Substance B: Isazofos: Clinical observations stage 4, ctd

Experimental Group (mg ai/kg bw	Pen	Sex	11 AM	12 AM	13 AM	14 AM
9,15	26	M	AN	AN	AN	AN
9,15	37	M				
11,6	25	F	AN	AN	AN	AN
11,6	42	M	AN	AN	AN	AN
14,7	43	F	AN	AN	AN	AN
14,7	44	F				
18,7	24	M				
18,7	41	F				
23,8	10	F				
23,8	32	M				

AN = appeared normal

Results of original Wildlife International, Ltd Studies –

Substance A: MCPA Acid – Wildlife International, Ltd. project 222-101 – Performed in 1986: Mortality tables

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TABLE 1

CUMULATIVE MORTALITY OF BOBWHITE GAVAGED WITH MCPA ACID BY SEX

Dosage mg/kg	Number Dead/Number Exposed Day of Study 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control																
Males	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/10
Females	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
292																
Males	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/10
Females	0/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	
486																
Males	0/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	9/10
Females	1/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
810																
Males	0/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
Females	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
1350																
Males	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
Females	1/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
2250																
Males	2/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
Females	0/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	

The LD50 value was determined to be 377 mg/kg, with confidence limits (95%) of 314 mg/kg to 452 mg/kg.

Substance A: MCPA Acid – Wildlife International, Ltd. Project 222-101: Body weight and food consumption

GAVAGED WITH MCPA ACID

Dosage mg/kg	Average Body Weight in Grams								Estimated Feed Consumption Grams/Bird/Day		
	Day 0	Change	Day 3	Change	Day 7	Change	Day 14	Total Change	Days 0-3	Days 4-7	Days 8-14
Control											
Males	184±11	13	197	1	198±9	-1	197±8	13	15	16	13
Females	183±13	13	196	-2	194±12	-2	192±12	9	18	18	12
292											
Males	188±12	-12	176	10	186±18	14	200±14	12	7	19	16
Females	181±14	-14	167	15	182±16	10	192±17	11	9	27	15
486											
Males	183	-4b	138	22	160	16	176	-7	4	18	17
Females	186	*	*	*	*	*	*	*	5	*	*
810											
Males	190	*	*	*	*	*	*	*	3	*	*
Females	185	*	*	*	*	*	*	*	3	*	*
1350											
Males	182	*	*	*	*	*	*	*	4	*	*
Females	179	*	*	*	*	*	*	*	4	*	*
2250											
Males	182	*	*	*	*	*	*	*	6	*	*
Females	182	*	*	*	*	*	*	*	3	*	*

*Data not available due to total mortality.

Substance A- MCPA Acid: Wildlife International, Ltd. Project 222-101: Description of mortality and clinical observations

RESULTS

Controls

There were no mortalities in the control group. All birds were normal in appearance and behavior throughout the test period (see Table 1).

MCPA acid

There was 10% mortality (1 of 10) at the 292 mg/kg dosage; 90% mortality (9 of 10) at the 486 mg/kg dosage and 100% mortality (10 of 10) at the 810, 1350 and 2250 mg/kg dosages (see Table 1).

At the 292 mg/kg dosage signs of toxicity were apparent approximately 1 1/2 hours after dosing. One bird was found dead on the morning of Day 1. Signs of toxicity continued through Day 3 but no other mortality occurred at this dosage. All birds were normal in appearance from Day 4 until termination of the study.

At the 486 mg/kg dosage all birds displayed overt signs of toxicity approximately 1/2 hour after dosing. One bird was found dead approximately 2 3/4 hours after dosing and eight birds were found dead on the morning of Day 1. The one remaining bird showed signs of toxicity through Day 2 but by the morning of Day 3 this bird appeared normal and remained so throughout the study.

At the 810 mg/kg dosage all birds displayed overt signs of toxicity approximately 25 minutes after dosing. Nine birds were found dead on the morning of Day 1 and the one remaining bird was found dead on the morning of Day 2.

At 1350 mg/kg all birds displayed overt signs of toxicity approximately 20 minutes after dosing. One bird was found dead approximately

2 1/2 hours after dosing and the remaining birds continued to display overt signs of toxicity. At the morning observation on Day 1 seven birds were found dead and the two remaining birds continued to display signs of toxicity until they were found dead on the afternoon of Day 1.

At 2250 mg/kg all birds displayed overt signs of toxicity approximately ten minutes after dosing. Two birds were found dead approximately five hours after dosing and the remaining birds were found dead on the morning of Day 1.

Overt signs of toxicity typical of intoxication with MCPA acid included depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, prostrate posture, a ruffled appearance, lower limb weakness and lethargy.

At 292 mg/kg there appeared to be a dose responsive reduction in body weight and feed consumption for the first three days of the study when compared to the controls (see Table 2). At 486 mg/kg, there was a more pronounced loss of body weight and a reduction in food consumption in the one surviving male for the first three days of the study when compared to the controls. Birds at 810 mg/kg, 1350 mg/kg and 2250 mg/kg did not survive until the Day 3 body weight interval, therefore no comparisons were made.

In conclusion, the bobwhite acute oral LD50 value for MCPA acid for this study was determined to be 377 mg/kg with a 95% confidence interval of 314 mg/kg to 452 mg/kg. The no-observed-effect dosage was less than 252 mg/kg, the lowest dosage tested based on overt signs of toxicity, reduction in body weight and feed consumption and one death at 292 mg/kg.

Substance B: Isazofos – Wildlife International, Ltd. project 108-223 – Performed in 1983: Mortality tables

TABLE 1

CUMULATIVE MORTALITY OF BOBWHITE

GAVAGED WITH CGA-12223 TECHNICAL BY SEX

Dosage mg/kg		Number Dead/Number Exposed													
		Day of Death													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Control	M	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
	F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/10
5.62	M	0/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5	
	F	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/10
10.0	M	0/5	1/5	1/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	
	F	0/5	1/5	1/5	1/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	4/10
17.8	M	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
	F	2/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5	8/10

TABLE 1

CUMULATIVE MORTALITY OF BOBWHITE
PAGE 2

GAVAGED WITH CGA-12223 TECHNICAL BY SEX

Dosage mg/kg		Number Dead/Number Exposed — Day of Death															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
31.6	M	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
	F	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
56.2	M	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
	F	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
100	M	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
	F	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10
178	M	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	
	F	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10

The LD50 value was determined to be 11.1 mg/kg, with confidence limits (95%) of 8.3 mg/kg to 14.7 mg/kg.

Subance B: Isazofos – Wildlife International, Ltd. project 108-223 – Performed in 1983: Body weight and food consumption tables:

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TABLE 2

AVERAGE BODY WEIGHT AND ESTIMATED FEED CONSUMPTION OF BOBWHITE

GAVAGED WITH CGA-12223 TECHNICAL

Dosage	Average Body Weight in Grams**							Estimated Feed Consumption Grams/Bird/Day		
mg/kg	Day 0	Δ	Day 3	Δ	Day 7	Δ	Day 14	Total Δ	Days 0-3	Days 4-7	Days 8-14
Control	210	+14	224	+ 1	225	+ 6	231	+21	26	21	24
5.62	214	0	214	+ 6	220	+18	238	+24	17	17	28
10.0	200	-20	180	+13	193	+ 2	195	- 5	8	16	29
17.8	214	-27	187	- 1	186	+23	209	- 5	7	14	35
31.6	220		*		*		*		*	*	*
56.2	203		*		*		*		*	*	*
100	216		*		*		*		*	*	*
178	204		*		*		*		*	*	*

* Data not available due to total mortality.

** Δ indicates the change in body weight in grams over the indicated period of time. Total Δ indicates the change in body weight in grams over the entire test.

Substance B: Isazofos – Wildlife International, Ltd. project 108-223 – Performed in 1983: Description of mortalities and clinical observations.

RESULTS:

Controls - There were no mortalities in the control group (see Table 1). All birds were normal in appearance and behavior throughout the test period.

CGA-12223 - There was 10 percent mortality at the 5.62 mg/kg dosage, 40 percent mortality at the 10.0 mg/kg dosage, 80 percent mortality at the 17.8 mg/kg dosage and 100 percent mortality at the 31.6 mg/kg, 56.2 mg/kg, 100 mg/kg and 178 mg/kg dosages (see Table 1).

*General Electric Institute, General Electric Company, Nela Park, Cleveland, Ohio.

At the 5.62 mg/kg dosage a few birds displayed lethargy progressing to depression, reduced reaction to external stimuli (sound and movement), loss of coordination and lower limb weakness approximately one hour after dosing. On Day 1 one bird was found dead and one female continued to display lethargy, reduced reaction to external stimuli (sound and movement), loss of coordination and lower limb weakness. The eight other birds appeared asymptomatic on Day 1, and all nine surviving birds appeared normal on Day 2. One cock at this dosage was noted with foot lesions on Days 6 through 11. This bird displayed lower limb weakness on Days 6 and 7, and a ruffled appearance was also noted on Day 7. These symptoms were apparently related to the foot lesions. Otherwise, all birds appeared normal until the termination of the study.

At the 10.0 mg/kg dosage, symptoms of toxicity included lethargy progressing to depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness and a ruffled appearance. Symptoms of toxicity were first apparent on Day 0. On Day 1 two birds were found dead and four birds displayed symptoms of depression, reduced reaction to external stimuli (sound and movement), loss of coordination and lower limb weakness. Symptoms of toxicity continued to be apparent in four birds on Day 2. On Day 3 one bird was found dead, while two hens still displayed symptoms of toxicity. An additional bird was found dead on Day 4, but all surviving birds appeared normal from Day 4 until the termination of the study.

At the 17.8 mg/kg dosage, symptoms of toxicity became evident approximately one hour after dosing. Symptoms of toxicity observed

included lethargy, depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, prostrate posture and salivation. Of the ten birds in the 17.8 mg/kg dosage group, seven died on Day 0 (within approximately four hours of dosing). An additional mortality was found on Day 1. Both surviving hens had recovered by Day 3 and appeared normal until the termination of the study.

At dosages of 31.6 mg/kg to 178 mg/kg mortality began to occur within approximately one-half hour of dosing and all birds died within four hours of dosing. Symptoms of toxicity observed prior to death included lethargy, depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, prostrate posture and salivation. In addition, lacrimation was observed at the 56.2 mg/kg and 100 mg/kg dosages.

There was a dosage related loss in average body weight by the surviving birds at the 10.0 mg/kg and 17.8 mg/kg dosages for the first three days of the study. During those first three days there was also a reduction in feed consumption by surviving birds at the 10.0 mg/kg through 17.8 mg/kg dosages. A slight reduction in feed consumption continued to be observed at the 17.8 mg/kg dosage through Day 7, but an increase in feed consumption was observed at this dosage for the final seven days of the study (see Table 2).

In conclusion, the acute oral LD50 value of CGA-12223 Technical in the bobwhite was determined to be 11.1 mg/kg, with confidence limits (95%) of 8.3 mg/kg to 14.7 mg/kg. There was mortality at all dosages

tested. Mortality ranged from 10 percent at 5.62 mg/kg to 100 percent at dosages of 31.6 mg/kg and greater. Based upon mortality and symptoms of toxicity, the no-observed-effect level in this study is less than 5.62 mg/kg, the lowest level tested.

Appendix VII – A. Studies from EPA data base used to evaluate ring-test results.

CHEMICAL	SHAUGHNES	CAS_NO	USEPATTER	COMMONNA	AGE	GUIDELINE	AI	STUDYTIME	DOSETYPE	TGL	TOXICITY	TOXLEVEL	CL	CURVESLOP	NGL	NOEL
3-Iodo-2-propynyl butyl carb	107801	55406-53-6	Fungicide	Bobwhite qua	21 WKS	71-1	97.5	21 D	LD50		970	MGK	717-1389	3.2		292
3-Iodo-2-propynyl butyl carb	107801	55406-53-6	Fungicide	Bobwhite qua	23WKS	71-1	98.2	14 D	LD50		749	MGK	552-1004	3.7	<	292
Arsenic acid	006801	7778-39-4	Herbicide	Bobwhite qua	27 WKS	71-1	75	21 D	LD50		28.9	MGK	21.5-46.4	N.R.		N.R.
Arsenic acid	006801	7778-39-4	Herbicide	Bobwhite qua	18 WKS	71-1	76.1	14 D	LD50		46	MGK	25-100	N.R.		N.R.
Bromethalin	112802	63333-35-7	Rodenticide	Bobwhite qua	17WKS	71-1	96.3	14 D	LD50		4.6	MGK	3.6-5.8	3.64		1.0
Bromethalin	112802	63333-35-7	Rodenticide	Bobwhite qua	25WKS	71-1	96.3	14 D	LD50		11.04	MGK	9.3-13.1	7.24		N.R.
Calcium hypochlorite	014701	7778-54-3	Algicide	Bobwhite qua	14 D	71-1	65	14 D	LD50		3474	MGK	2532-4766	N.R.		N.R.
Calcium hypochlorite	014701	7778-54-3	Algicide	Bobwhite qua	27 WKS	71-1	65	14 D	LD50		1502	MGK	1097-2561	3.196		175
Chlorethoxyfos	129006	54593-83-8	Insecticide	Bobwhite qua	17WKS	71-1	5G	14 D	LD50		721	MGK	486-1350	N.R.	<	292
Chlorethoxyfos	129006	54593-83-8	Insecticide	Bobwhite qua	30WKS	71-1	5G	14 D	LD50		556	MGK	476-648	7.0		249
Copper sulfate, pentahydrate	024401	7758-99-8	Algicide	Bobwhite qua	20 WKS	71-1	99	14 D	LD50		340	MGK	270-400	N.R.		N.R.
Copper sulfate, pentahydrate	024401	7758-99-8	Algicide	Bobwhite qua	N.R.	71-1	99	14 D	LD50		357.9	MGK	0-INF	9.46		120
DBNPA	101801	10222-01-2	Microbicide	Bobwhite qua	21WKS	71-1	100	14 D	LD50		354	MGK	250-500	N.R.		250
DBNPA	101801	10222-01-2	Microbicide	Bobwhite qua	17WKS	71-1	Tech	21 D	LD50		150	MGK	118-191	N.R.		63
Disulfoton	032501	298-04-4	Insecticide	Bobwhite qua	N.R.	71-1	Tech	14 D	LD50		12.0	MGK	7-19	N.R.		N.R.
Disulfoton	032501	298-04-4	Insecticide	Bobwhite qua	20 WKS	71-1	98.7	14 D	LD50		39	MGK	28.6-139.4	4.75		11.0
Dodine	044301	2439-10-3	Fungicide	Bobwhite qua	Juv	71-1	95	14 D	LD50		690	MGK	511-932	N.R.		N.R.
Dodine	044301	2439-10-3	Fungicide	Bobwhite qua	18 wk	71-1	95.3	21 D	LD50		981	PPM	824-1156	10.19	<	400
Ethephon	099801	16672-87-0	Herbicide	Bobwhite qua	20 WKS	71-1	75	14 D	LD50		794	MGK	694-908	N.R.		N.R.
Ethephon	099801	16672-87-0	Herbicide	Bobwhite qua	22 WKS	71-1	75	14 D	LD50		1072	MGK	907-1267	N.R.		N.R.
Hydantoin, 1-bromo-3-chlorc	006315	16079-88-2	Microbicide	Bobwhite qua	18WKS	71-1	96	14 D	LD50		1839	MGK	1350-2250	N.R.		1350
Hydantoin, 1-bromo-3-chlorc	006315	16079-88-2	Microbicide	Bobwhite qua	6MONS	71-1	99	14 D	LD50		1070	MGK	781-1542	4.36		250
Methylisothiazolinone(Actici	107103	26172-55-4	Microbicide	Bobwhite qua	31WKS	71-1	13.1	14 D	LD50		62.5	MGK	53.2-73.7	10.69		N.R.
Methylisothiazolinone(Actici	107103	26172-55-4	Microbicide	Bobwhite qua	14 D	71-1	14	14 D	LD50		690	MGK	450-1059	N.R.		N.R.
Octhilinone	099901	26530-20-1	Microbicide	Bobwhite qua	Juv	71-1	88.7	21 D	LD50		346	MGK	297-403	N.R.		159
Octhilinone	099901	26530-20-1	Microbicide	Bobwhite qua	19WKS	71-1	98.5	21 D	LD50		660	MGK	553-795	7.9		N.R.
Octhilinone	099901	26530-20-1	Microbicide	Bobwhite qua	2WKS	71-1	95.9	14 D	LD50		384	MGK	324-455	7.65	<	171
Sodium chlorite	020502	7758-19-2	Microbicide	Bobwhite qua	Adult	71-1	80	14 D	LD50		467	MGK	372 - 585	NA		250
Sodium chlorite	020502	7758-19-2	Microbicide	Bobwhite qua	N.R.	71-1	83	>80hr	LD50		660	MGK	540-810	N.R.		N.R.
Sodium chlorite	020502	7758-19-2	Microbicide	Bobwhite qua	Adult	71-1	80	26 wk	LD50		395	MGK	272-573	NA		NR
Sodium hypochlorite	014703	7681-52-9	Microbicide	Bobwhite qua	N.R.	71-1	9	N.R.	LD50		6800	MGK	5400-8400	N.R.		N.R.
Sodium hypochlorite	014703	7681-52-9	Microbicide	Bobwhite qua	N.R.	71-1	12.1	21 D	LD50		4009	MGK	3100-5390	N.R.		N.R.

Appendix VII – B:

This section of Appendix VII displays
1) the SAS® code followed by
2) the SAS® log followed by
3) the resulting analyses
first, for the analysis of 15 chemicals with historic bobwhite quail data, in order to obtain comparison values, and then, for the two chemicals and four laboratories of the ring test. In each instance, the LD_{50}s were analysed and then the slopes.

```
/*
…-data-LD50 and slope … 08-25-09.sas
*/

PROC IMPORT OUT= WORK.D1
        DATAFILE= "C:\[…]-data.xls"
        DBMS=EXCEL REPLACE;
    SHEET="Sheet1$";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;


data d1; set d1;
if chemical = "" then delete; **removes extra blank observations**;
if chemical = "Acetochlor" or chemical = "Amitraz  degradate" or
   chemical = "Indoxacarb(DPX-MP062)" then delete;
logtox = log10(toxicity);
slope=curveslope;
if slope=0 then slope=.;
keep chemical toxicity slope logtox;


proc print;
  title "analysis of historic OPPTS data, multiple studies per chemical";
  run;

proc glm;
title2 " analysis for log10-LD50";
class chemical;
model logtox = chemical;
run;

proc glm;
title2 " analysis for slope";
class chemical;
model slope = chemical;
run;

/*  combinedhistoric-ringld50slopeAug26am.log */

NOTE: Copyright (c) 2002-2008 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software 9.2 (TS1M0)
     Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0070034933.
NOTE: This session is executing on the XP_PRO  platform.



NOTE: SAS initialization used:
     real time           11.60 seconds
```

```
    cpu time          2.40 seconds

1   /*
2   [...]-data-LD50 and slope CSH 08-25-09.sas
3   */
4
5   PROC IMPORT OUT= WORK.D1
6               DATAFILE= "C:\[...]-data.xls"
8               DBMS=EXCEL REPLACE;
9         SHEET="Sheet1$";
10         GETNAMES=YES;
11         MIXED=NO;
12         SCANTEXT=YES;
13         USEDATE=YES;
14         SCANTIME=YES;
15  RUN;

NOTE: WORK.D1 data set was successfully created.
NOTE: PROCEDURE IMPORT used (Total process time):
    real time          9.04 seconds
    cpu time           1.17 seconds


16
17
18   data d1; set d1;
19   if chemical = "" then delete; **removes extra blank observations**;
20   if chemical = "Acetochlor" or chemical = "Amitraz  degradate" or
21      chemical = "Indoxacarb(DPX-MP062)" then delete;
22   logtox = log10(toxicity);
23   slope=curveslope;
24   if slope=0 then slope=.;
25   keep chemical toxicity slope logtox;
26
27

NOTE: Character values have been converted to numeric values at the places given by:
    (Line):(Column).
    22:16
NOTE: There were 41 observations read from the data set WORK.D1.
NOTE: The data set WORK.D1 has 32 observations and 4 variables.
NOTE: DATA statement used (Total process time):
    real time          0.39 seconds
    cpu time           0.07 seconds


28   proc print;
29     title "analysis of historic OPPTS data, multiple studies per chemical";
30     run;

NOTE: There were 32 observations read from the data set WORK.D1.
NOTE: PROCEDURE PRINT used (Total process time):
    real time          0.54 seconds
```

cpu time 0.15 seconds

```
31
32  proc glm;
33  title2 " analysis for log10-LD50";
34  class chemical;
35  model logtox = chemical;
36  run;

37
```

NOTE: PROCEDURE GLM used (Total process time):
real time 0.56 seconds
cpu time 0.10 seconds

```
38  proc glm;
39  title2 " analysis for slope";
40  class chemical;
41  model slope = chemical;
42  run;

/*  combinedhistoric-ringld50slopeAug26am.lst */  [run about 11:30 am, not 8:52 am!]
```

analysis of historic OPPTS data, multiple studies per chemical 1
08:52 Wednesday, August 26, 2009

Obs	CHEMICAL	TOXICITY	logtox	slope
1	3-Iodo-2-propynyl butyl carbamate (IPBC)	970	2.98677	3.200
2	3-Iodo-2-propynyl butyl carbamate (IPBC)	749	2.87448	3.700
3	Arsenic acid	28.9	1.46090	.
4	Arsenic acid	46	1.66276	.
5	Bromethalin	4.6	0.66276	3.640
6	Bromethalin	11.04	1.04297	7.240
7	Calcium hypochlorite	3474	3.54083	.
8	Calcium hypochlorite	1502	3.17667	3.196
9	Chlorethoxyfos	721	2.85794	.
10	Chlorethoxyfos	556	2.74507	7.000
11	Copper sulfate, pentahydrate	340	2.53148	.
12	Copper sulfate, pentahydrate	357.9	2.55376	9.460
13	DBNPA	354	2.54900	.
14	DBNPA	150	2.17609	.
15	Disulfoton	12.0	1.07918	.
16	Disulfoton	39	1.59106	4.750
17	Dodine	690	2.83885	.
18	Dodine	981	2.99167	10.190
19	Ethephon	794	2.89982	.
20	Ethephon	1072	3.03019	.
21	Hydantoin, 1-bromo-3-chloro-5,5-dimethyl-	1839	3.26458	.
22	Hydantoin, 1-bromo-3-chloro-5,5-dimethyl-	1070	3.02938	4.360
23	Methylisothiazolinone(Acticide14 formulation)	62.5	1.79588	10.690

24	Methylisothiazolinone(Acticide14 formulation)	690	2.83885	.
25	Octhilinone	346	2.53908	.
26	Octhilinone	660	2.81954	7.900
27	Octhilinone	384	2.58433	7.650
28	Sodium chlorite	467	2.66932	.
29	Sodium chlorite	660	2.81954	.
30	Sodium chlorite	395	2.59660	.
31	Sodium hypochlorite	6800	3.83251	.
32	Sodium hypochlorite	4009	3.60304	.

analysis of historic OPPTS data, multiple studies per chemical 2
analysis for log10-LD50 08:52 Wednesday, August 26, 2009

The GLM Procedure

Class Level Information

Class	Levels	Values
CHEMICAL	15	3-Iodo-2-propynyl butyl carbamate (IPBC) Arsenic acid Bromethalin Calcium hypochlorite Chlorethoxyfos Copper sulfate, pentahydrate DBNPA Disulfoton Dodine Ethephon Hydantoin, 1-bromo-3-chloro-5,5-dimethyl- Methylisothiazolinone(Acticide14 formulation) Octhilinone Sodium chlorite Sodium hypochlorite

Number of Observations Read	32
Number of Observations Used	32

analysis of historic OPPTS data, multiple studies per chemical 3
analysis for log10-LD50 08:52 Wednesday, August 26, 2009

The GLM Procedure

Dependent Variable: logtox

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	16.71161087	1.19368649	19.11	<.0001
Error	17	1.06168055	0.06245180		
Corrected Total	31	17.77329141			

R-Square	Coeff Var	Root MSE	logtox Mean
0.940265	9.794750	0.249904	2.551403

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHEMICAL	14	16.71161087	1.19368649	19.11	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHEMICAL	14	16.71161087	1.19368649	19.11	<.0001

analysis of historic OPPTS data, multiple studies per chemical 4
analysis for slope 08:52 Wednesday, August 26, 2009

The GLM Procedure

Class Level Information

Class	Levels	Values
CHEMICAL	15	3-Iodo-2-propynyl butyl carbamate (IPBC) Arsenic acid Bromethalin Calcium hypochlorite Chlorethoxyfos Copper sulfate, pentahydrate DBNPA Disulfoton Dodine Ethephon Hydantoin, 1-bromo-3-chloro-5,5-dimethyl- Methylisothiazolinone(Acticide14 formulation) Octhilinone Sodium chlorite Sodium hypochlorite

Number of Observations Read	32
Number of Observations Used	13

analysis of historic OPPTS data, multiple studies per chemical 5
analysis for slope 08:52 Wednesday, August 26, 2009

The GLM Procedure

Dependent Variable: slope

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	82.66710631	9.18523403	4.15	0.1343
Error	3	6.63625000	2.21208333		
Corrected Total	12	89.30335631			

R-Square	Coeff Var	Root MSE	slope Mean
0.925689	23.30191	1.487307	6.382769

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CHEMICAL	9	82.66710631	9.18523403	4.15	0.1343

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CHEMICAL	9	82.66710631	9.18523403	4.15	0.1343

```
/*
ringtest-LD50-addl 09-14-09.sas
*/

data d2;

input chemical lab $ tox ;
logtox = log10(tox);
if lab = "oppts" then studytype="A "; else studytype="NA";

cards;

1 oppts      377
1 [lab1]     437
1 [lab2]     438
1 [lab3]     333
1 [lab5]     .
1 [lab4]     554

2 oppts      11.1
2 [lab1]     26.9
2 [lab2]     24.4
2 [lab3]     16.1
2 [lab5]     .
2 [lab4]     13.8

3 oppts      1206
3 [labx]     1278

;

data d8; set d2;
if lab = "oppts" or chemical = 3 then delete;

proc glm data=d8;
title 'ringtests-LD50 – oppts, chem 3 deleted (4 labs and 2 chems),model=chemical';
class chemical lab;
model logtox = chemical;
run;

NOTE: Copyright (c) 2002-2003 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) 9.1 (TS1M3)
    Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0045713013.
NOTE: This session is executing on the XP_PRO  platform.

NOTE: SAS initialization used:
    real time          15.68 seconds
    cpu time           2.37 seconds

1   /*
2   ringtest-LD50-addl 09-14-09.sas
3   */
4
```

```
5   data d2;
6
7   input chemical lab $ tox ;
8   logtox = log10(tox);
9   if lab = "oppts" then studytype="A "; else studytype="NA";
10
11  cards;

NOTE: SAS went to a new line when INPUT statement reached past the end of a line.
NOTE: Missing values were generated as a result of performing an operation on missing values.
      Each place is given by: (Number of times) at (Line):(Column).
      2 at 8:10
NOTE: The data set WORK.D2 has 14 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time          0.53 seconds
      cpu time           0.12 seconds


30  ;

49  data d8; set d2;
50  if lab = "oppts" or chemical = 3 then delete;
51

NOTE: There were 14 observations read from the data set WORK.D2.
NOTE: The data set WORK.D8 has 10 observations and 5 variables.
NOTE: DATA statement used (Total process time):
      real time          0.00 seconds
      cpu time           0.00 seconds


52  proc glm data=d8;
53  title 'ringtests-LD50 - oppts, chem 3 deleted (4 labs and 2 chems),model=chemical';
54  class chemical lab;
55  model logtox = chemical;
56  run;

57
58

NOTE: PROCEDURE GLM used (Total process time):
      real time          0.03 seconds
      cpu time           0.03 seconds

                ringtests-LD50 - all data included except chem. 3                    2
                                        10:06 Monday, September 14, 2009

              Obs    chemical    lab        tox      logtox    studytype

               1         1       oppts      377.0    2.57634     A
               2         1       [lab1]     437.0    2.64048     NA
               3         1       [lab2]     438.0    2.64147     NA
               4         1       [lab3]     333.0    2.52244     NA
```

5	1	[lab5]	.	.	NA
6	1	[lab4]	554.0	2.74351	NA
7	2	oppts	11.1	1.04532	A
8	2	[lab1]	26.9	1.42975	NA
9	2	[lab2]	24.4	1.38739	NA
10	2	[lab3]	16.1	1.20683	NA
11	2	[lab5]	.	.	NA
12	2	[lab4]	13.8	1.13988	NA

ringtests-LD50 - oppts, chem 3 deleted (4 labs and 2 chems),model=chemical 5
10:06 Monday, September 14, 2009

The GLM Procedure

Class Level Information

Class	Levels	Values
chemical	2	1 2
lab	5	[lab1 lab2 lab3 lab5 lab4] [one lab is labx]

Number of Observations Read 10
Number of Observations Used 8

ringtests-LD50 - oppts, chem 3 deleted (4 labs and 2 chems),model=chemical 6
10:06 Monday, September 14, 2009

The GLM Procedure

Dependent Variable: logtox

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.62351609	3.62351609	262.05	<.0001
Error	6	0.08296549	0.01382758		
Corrected Total	7	3.70648159			

R-Square	Coeff Var	Root MSE	logtox Mean
0.977616	5.987401	0.117591	1.963970

Source	DF	Type I SS	Mean Square	F Value	Pr > F
chemical	1	3.62351609	3.62351609	262.05	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
chemical	1	3.62351609	3.62351609	262.05	<.0001

```
/*
ringtest-slope-addl 09-14-09.sas
*/

data d2;

input chemical lab $ tox slope;
logtox = log10(tox);
if lab = "oppts" then studytype="A "; else studytype="NA";

cards;

1 oppts        377  11.6
1 [lab1]       437  9.67
1 [lab2]       438  3.21
1 [lab3]       333  13.2
1 [lab5]         .  .
1 [lab4]       554  6.22

2 oppts        11.1  4.68
2 [lab1]       26.9   .
2 [lab2]       24.4  3.3
2 [lab3]       16.1  7.7
2 [lab5]         .   .
2 [lab4]       13.8  6.45

3 oppts        1206  3.5
3 [labx]       1278  5.7

;

data d8; set d2;
if lab = "oppts" or chemical = 3 then delete;

proc glm data=d8;
title 'ringtests-slope – oppts, chem 3 deleted (4 labs and 2 chems), model=chemical';
class chemical lab;
model slope = chemical;
run;


72   data d2;
73
74   input chemical lab $ tox slope;
75   logtox = log10(tox);
76   if lab = "oppts" then studytype="A "; else studytype="NA";
77
78   cards;

NOTE: SAS went to a new line when INPUT statement reached past the end of a line.
NOTE: Missing values were generated as a result of performing an operation on missing values.
      Each place is given by: (Number of times) at (Line):(Column).
```

```
    2 at 75:10
NOTE: The data set WORK.D2 has 14 observations and 6 variables.
NOTE: DATA statement used (Total process time):
    real time          0.01 seconds
    cpu time           0.01 seconds


97   ;
98
116  data d8; set d2;
117  if lab = "oppts" or chemical = 3 then delete;
118

NOTE: There were 14 observations read from the data set WORK.D2.
NOTE: The data set WORK.D8 has 10 observations and 6 variables.
NOTE: DATA statement used (Total process time):
    real time          0.00 seconds
    cpu time           0.00 seconds


119  proc glm data=d8;
120  title 'ringtests-slope - oppts, chem 3 deleted (4 labs and 2 chems), model=chemical';
121  class chemical lab;
122  model slope = chemical;
123  run;

124

NOTE: PROCEDURE GLM used (Total process time):
    real time          0.01 seconds
    cpu time           0.00 seconds


                ringtests-slope - all data included except chem. 3                  10
                                        10:06 Monday, September 14, 2009

          Obs    chemical    lab         tox     slope     logtox     studytype

            1        1       oppts       377.0    11.60    2.57634       A
            2        1       [lab1]      437.0     9.67    2.64048       NA
            3        1       [lab2]      438.0     3.21    2.64147       NA
            4        1       [lab3]      333.0    13.20    2.52244       NA
            5        1       [lab5]        .        .        .           NA
            6        1       [lab4]      554.0     6.22    2.74351       NA
            7        2       oppts        11.1     4.68    1.04532       A
            8        2       [lab1]       26.9      .      1.42975       NA
            9        2       [lab2]       24.4     3.30    1.38739       NA
           10        2       [lab3]       16.1     7.70    1.20683       NA
           11        2       [lab5]        .        .        .           NA
           12        2       [lab4]       13.8     6.45    1.13988       NA
       ringtests-slope - oppts, chem 3 deleted (4 labs and 2 chems), model=chemical        13
                                        10:06 Monday, September 14, 2009
```

The GLM Procedure

Class Level Information

Class	Levels	Values
chemical	2	1 2
lab	5	[lab1 lab2 lab3 lab5 lab4] [one lab is labx]

Number of Observations Read 10
Number of Observations Used 7

ringtests-slope - oppts, chem 3 deleted (4 labs and 2 chems), model=chemical 14
10:06 Monday, September 14, 2009

The GLM Procedure

Dependent Variable: slope

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.74297619	8.74297619	0.66	0.4534
Error	5	66.20056667	13.24011333		
Corrected Total	6	74.94354286			

R-Square	Coeff Var	Root MSE	slope Mean
0.116661	51.19774	3.638697	7.107143

Source	DF	Type I SS	Mean Square	F Value	Pr > F
chemical	1	8.74297619	8.74297619	0.66	0.4534

Source	DF	Type III SS	Mean Square	F Value	Pr > F
chemical	1	8.74297619	8.74297619	0.66	0.4534

```
data d10; set d2;
if lab = "[lab1]" or lab = "oppts" or chemical = 3 then delete;

proc glm  data=d10;
title 'ringtests-ld50 - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical';
class studytype chemical;
model logtox = chemical;
run;

proc glm  data=d10;
title 'ringtests-slope - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical';
class studytype chemical;
model slope = chemical;
run;


134  data d10; set d2;
135  if lab = "[lab1]" or lab = "oppts" or chemical = 3 then delete;
136

NOTE: There were 14 observations read from the data set WORK.D2.
NOTE: The data set WORK.D10 has 8 observations and 6 variables.
NOTE: DATA statement used (Total process time):
      real time           0.01 seconds
      cpu time            0.01 seconds


137  proc glm  data=d10;
138  title 'ringtests-ld50 - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical'
138! ;
139  class studytype chemical;
140  model logtox = chemical;
141  run;

NOTE: The CLASS variable studytype has only one level: 'NA'.
142

NOTE: PROCEDURE GLM used (Total process time):
      real time           0.03 seconds
      cpu time            0.01 seconds


143  proc glm  data=d10;
144  title 'ringtests-slope - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems),
144! model=chemical';
145  class studytype chemical;
146  model slope = chemical;
147  run;

NOTE: The CLASS variable studytype has only one level: 'NA'.
```

ringtests-ld50 - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical 17
10:06 Monday, September 14, 2009

The GLM Procedure

Class Level Information

Class	Levels	Values
studytype	1	NA
chemical	2	1 2

Number of Observations Read	8
Number of Observations Used	6

ringtests-ld50 - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical 18
10:06 Monday, September 14, 2009

The GLM Procedure

Dependent Variable: logtox

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.90278517	2.90278517	202.76	0.0001
Error	4	0.05726538	0.01431634		
Corrected Total	5	2.96005054			

R-Square	Coeff Var	Root MSE	logtox Mean
0.980654	6.166767	0.119651	1.940254

Source	DF	Type I SS	Mean Square	F Value	Pr > F
chemical	1	2.90278517	2.90278517	202.76	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
chemical	1	2.90278517	2.90278517	202.76	0.0001

ringtests-slope - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical 19
10:06 Monday, September 14, 2009

The GLM Procedure

Class Level Information

Class	Levels	Values
studytype	1	NA
chemical	2	1 2

Number of Observations Read	8
Number of Observations Used	6

ringtests-slope - oppts, [lab1] and chem 3 deleted (3 labs and 2 chems), model=chemical 20
10:06 Monday, September 14, 2009

The GLM Procedure

Dependent Variable: slope

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.47206667	4.47206667	0.28	0.6218
Error	4	62.80853333	15.70213333		
Corrected Total	5	67.28060000			

R-Square	Coeff Var	Root MSE	slope Mean
0.066469	59.32024	3.962592	6.680000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
chemical	1	4.47206667	4.47206667	0.28	0.6218

Source	DF	Type III SS	Mean Square	F Value	Pr > F
chemical	1	4.47206667	4.47206667	0.28	0.6218

Appendix VIII – Example results of regulatory studies performed to date

A number of studies have been conducted according to earlier drafts of TG223 that provide further insight into how it performs. All were conducted according to versions of the draft TG223 preceding the 2007 version, and before SEDEC was available. There are some differences in the design of the study for the presented examples (*e.g.,* design did not have a Stage 4); however, the main structure was the same. Examples 1 and 2 were conducted with formulations (several other formulations were tested as a Limit Test at the 2000mg/kg). Example 3 with a technical substance, subsequently repeated according to OPPTS820.2100. Example 4 used TG223 Stage 2 as a rangefinder for OPPTS850.2100. Examples 5-9 were LD_{50}-only tests to better understand species sensitivity distributions using only Stages 1 and 2. Numbers of birds used are given (n=xx) excluding controls. Those identified as failed limit tests include the numbers tested in the limit test. Full datasets showing numbers at each stage together with reversals and partial mortalities are given below.

Example 1. 3 stage dose response test. 7 birds used, not 5 as described for stage 3b (n=26). LD_{50} = 440mg/kg (95% CL 258-715 mg/kg); Slope 3.2.
2 reversal and 2 partial mortalities

Stage	Dose	No. tested	No. dead	% mortality
1	47	1	0	0
2	113	1	0	0
2	154	1	0	0
1	173	1	0	0
2	209	1	0	0
3	279	7	3	43
2	283	1	0	0
2	385	1	0	0
2	523	1	1	100
1	637	1	1	100
2	711	1	1	100
3	724	7	4	57
2	966	1	1	100
1	2350	1	1	100

Example 2. 60% mortality in the Limit Test followed by Stage 1 and 2 (n=25).
LD_{50} = 1734mg/kg (95% CL 1303-2273mg/kg); Slope 7.03
1 reversal, 1 partial mortality

Stage	Dose	No. tested	No. dead	% mortality
1	610	1	0	0
1	736	1	0	0
1	888	1	0	0
2	1048	5	0	0
1	1071	1	1	100
1	1292	1	0	0
1	1558	1	0	0
1	1880	1	0	0
Limit Test	2000	5	3	60
1	2268	1	1	100
1	2736	1	1	100
2	2855	5	5	100
1	3300	1	1	100

Example 3. 60% mortality in the Limit Test followed by Stage 1 and 2 (n=25).
LD_{50} = 1278mg/kg (95% CL 625-1662mg/kg); Slope 5.7.
2 partial mortalities and 2 reversals.
This same compound was repeated to OPPTS 850-2100.
The LD_{50} = 1206mg/kg (95% CL 897-1765); Slope 3.5.

Stage	Dose	No. tested	No. dead	% mortality
1	392	1	0	0
1	497	1	0	0
1	630	1	0	0
1	800	1	0	0
1	1014	1	0	0
1	1061	2	1	50
1	1286	1	0	0
2	1347	2	2	100
1	1632	1	1	100
2	1710	2	2	100
Limit Test	2000	5	3	60
1	2070	1	1	100
2	2171	2	2	100
1	2625	1	1	100
2	2755	2	2	100
1	3330	1	1	100

Example 4. Failed Limit Test followed by Stage 2 (n=20)
LD_{50} = 276mg/kg (conducted as a rangefinder)
No reversal or partial mortalities
This same compound was repeated to OPPTS 850-2100.

The LD_{50} =232mg/kg (95% CL 173-313mg/kg; Slope 5.9.

Stage	Dose	No. tested	No. dead	% mortality
2	120	1	0	0
2	152	1	0	0
2	193	1	0	0
2	245	1	0	0
2	310	1	1	100
2	394	1	1	100
2	500	1	1	100
2	634	1	1	100
2	805	1	1	100
2	1021	1	1	100

Example 5. 3 stage LD_{50} (n=18). LD_{50} = 1010 mg/kg.
R = Partial regurgitation of the dose by some individuals
1 partial mortality and 1 reversal, both compromised by regurgitation

Stage	Dose	No. tested	No. dead	% mortality
1	23	1	0	0
1	84	1	0	0
1	311	1	0	0
3	443	1	0	0
3	507	1	0	0
3	582	1	0 (R)	0
3	667	1	1	100
3	765	1	1	100
3	876	1	1	100
3	1000	1	1	100
1	1150	2	0 (2/2 R)	0
2	1150	5	5 (4/5 R)	100
3	1150	1	1 (R)	100

Example 6. 2 stage LD_{50} (n=12). LD_{50} = 450mg/kg
R = Partial regurgitation of the dose by some individuals
1 reversal mortality

Stage	Dose	No. tested	No. dead	% mortality
1	17	1	0	0
1	67	1	0	0
2	154	1	0	0
2	209	1	0 (R)	0
1	234	1	0 (R)	0
2	285	1	0	0
2	387	1	1	100
2	526	1	0	0
2	715	1	1	100
1	863	1	1(R)	100
2	972	1	1	100
2	1150	1	1	100

Example 7. 2 Stage LD_{50} (n=12). LD_{50} = 166mg/kg.
R = Partial regurgitation of the dose by some individuals
No reversal mortalities

Stage	Dose	No. tested	No. dead	% mortality
1	24	1	0	0
2	53	1	0	0
2	75	1	0	0
1	85	1	0	0
2	106	1	0	0
2	140	1	0	0
2	192	1	1	100
2	265	1	1	100
1	311	1	1(R)	100
2	355	1	1	100
2	485	1	1	100
1	1150	1	1	100

Example 8. 2 Stage LD_{50} (n=12). LD_{50} = 2381mg/kg
2 reversal mortalities

Stage	Dose	No. tested	No. dead	% mortality
1	67	1	0	0
1	247	1	0	0
2	598	1	0	0
2	763	1	0	0
1	909	1	0	0
2	974	1	0	0
2	1243	1	0	0
2	1587	1	1	100
2	2026	1	0	0[1]
2	2585	1	1	100
2	3300	1	0	0[1]
1	3350	1	1	100

[1]Clinical signs of toxicity.

Example 9. 2 Stage LD_{50} (n=12). LD_{50} = 348mg/kg.
No reversal mortalities

Stage	Dose	No. tested	No. dead	% mortality
1	67	1	0	0
2	162	1	0	0
2	220	1	0	0
1	247	1	0	0
2	299	1	0	0
2	406	1	1	100
2	552	1	1	100
2	750	1	1	100
1	909	1	1	100
2	1019	1	1	100
2	1384	1	1	100
1	3350	1	1	100