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Sequential Design Calculator

A Tool for Use with

OECD TG 223: Avian Acute Oral Toxicity Test

User's Guide

SEDEC Version 1.3
October 5, 2009

Forward

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About SEDEC

SEDEC is an EXCEL workbook designed to aid in dose selection and analysis of avian acute oral toxicity studies performed in accordance with OECD TG 223. Facilities are provided for the user to enter basic study information and to guide the user through the sequential calculations and selection of doses. The user will be prompted to make various decisions regarding the conduct of the study. After a study is completed, SEDEC will calculate the LD50, 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, the identity of the individual performing the action and the time of the change. Major operations such as saving the workbook and printing results are also recorded. The menus and forms that SEDEC uses to guide the user mirror the procedures outlined in TG223, and proper use of SEDEC requires a thorough understanding of the contents of the guideline.

SEDEC requires that EXCEL 2000, or a later version, to be installed on the user's computer. SEDEC consists almost completely of VBA code that executes within the EXCEL application environment. As EXCEL macros are all recorded as VBA code, the term "VBA code" and "Excel macros" are essentially synonymous in this application.

No installation routine is needed to install SEDEC, as the workbook is completely self-contained. SEDEC is not an Excel add-in and no permanent modifications are made to your operating system or to Excel during installation. SEDEC is not a spreadsheet in the typical sense. All calculations are performed by locked VBA modules using code that cannot be accessed or changed by the user. Spreadsheet-like facilities are used only for data entry and no formulae for calculations exist in the spreadsheet cells. No part of a SEDEC workbook can be modified outside of control by SEDEC. For security reasons, SEDEC is not designed to be run in a window while other applications are visible. Other programs may be running at the same time as SEDEC, but they will be hidden behind SEDEC. SEDEC will use the entire screen.

Each time a new study is started, a uniquely named copy of SEDEC must be created to hold the study data. For example, if a study has been identified as project 100J-123, a copy of SEDEC could be saved as C:\projects\SED_100J-123.xls. Note that this means that the program, data, and audit trail for each study are saved as a single entity.

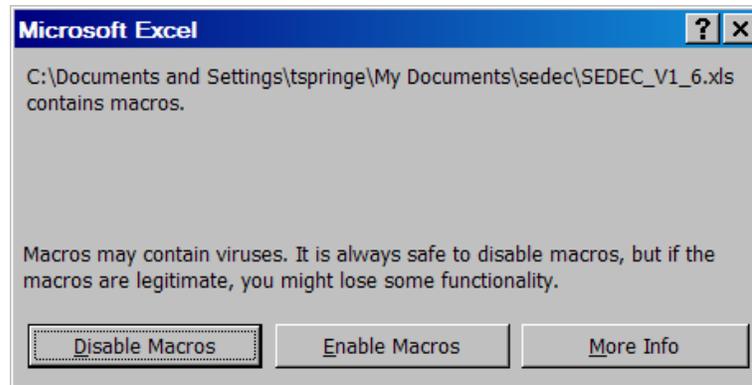
This version of SEDEC has undergone rigorous testing. *However, the final determination of the acceptability of results for a particular data set remains a matter for professional judgment and is the responsibility of the investigator.*

Note to EXCEL 2007 users: Due to incompatibilities between graphics routines in EXCEL2007 and earlier versions of EXCEL, dose response plots are not generated when SEDEC is executed using EXCEL2007.

Using SEDEC

Startup

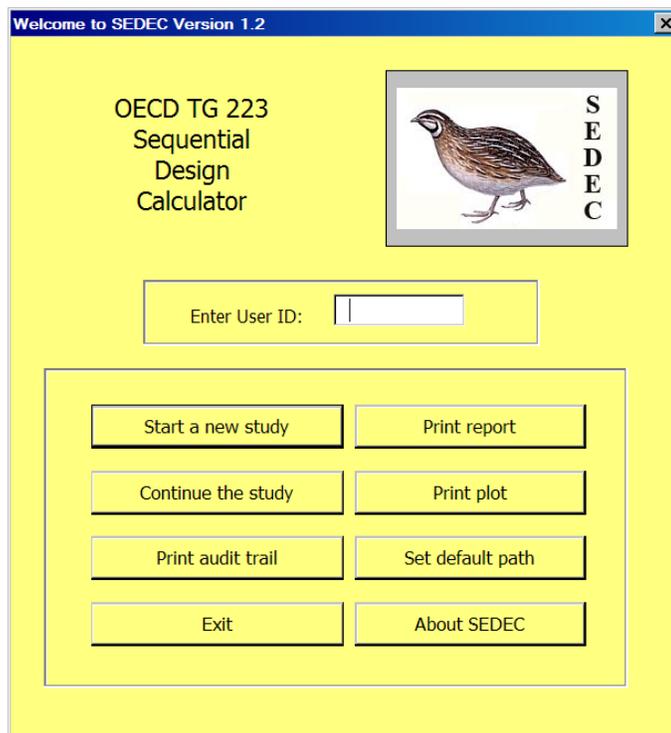
1. Download the file SEDEC_MASTER.XLS, and place a copy of this file in a directory on your hard drive or network file server. Be sure to put away a backup copy before proceeding.
2. Because SEDEC uses macros, EXCEL security settings must be set to allow execution of macros. This should be done prior to starting SEDEC, and is accomplished by opening EXCEL, and then selecting Tools > Macro > Security from the menu bar at the top of the screen. Make sure that the security level is set to medium (which allows macros to execute).
3. The easiest way to start SEDEC is by clicking on the filename or icon for SEDEC_MASTER.XLS, and it is best to start every new study by opening SEDEC_MASTER.XLS. As described below, after any information is entered into the master spreadsheet, it should be saved using a new name.
4. If EXCEL security is set to medium as described above, each time you open a SEDEC session, the following dialog form will appear.



When this dialog box opens, select “Enable Macros”, and EXCEL will continue to execute SEDEC. (Note: After you have familiarized yourself with the use of SEDEC, you should qualify your installation of the program according to the instructions in Appendix A.)

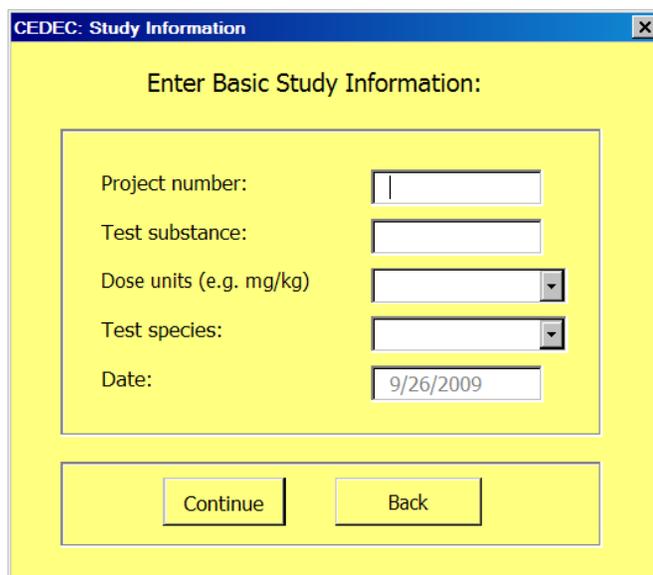
Note to EXCEL 2007 users: Security procedures changed substantially in Excel 2007. Two methods are available for allowing the execution of macros. The first is to click on the Microsoft Office button, and then select ‘Excel options’, ‘Trust center’, ‘macro settings’, and then ‘Enable all macros’. A more secure alternative is to click the Microsoft Office button, ‘Excel options’, ‘Trust center’, ‘Trust center settings’, ‘Trusted locations’, ‘Add new location’, and then select a location that is to be treated as a trusted source. If SEDEC is saved in this location, its macros will execute when it is started.

5. A menu providing various options will appear (see screenshot below). Enter a user ID consisting of at least three characters. The first action after this should be to choose “Set default path”. This is the default subdirectory for storage of the Excel workbook for a study (Note that each study is saved as a separate workbook.) This screen also allows access to the project report, a plot of the data and predicted dose response curve, and a project audit trail that tracks study data entry (e.g. allowing late mortalities to be identified).



The screenshot shows a window titled "Welcome to SEDEC Version 1.2". The background is yellow. On the left, the text reads "OECD TG 223 Sequential Design Calculator". On the right, there is a logo featuring a quail and the letters "SEDEC" stacked vertically. Below the logo is a text input field labeled "Enter User ID:". In the center, there is a large rectangular area containing several buttons: "Start a new study", "Print report", "Continue the study", "Print plot", "Print audit trail", "Set default path", "Exit", and "About SEDEC".

6. After the default path is set, then choose “Start a new study”, and the following screen appears. Note that the “Date” field is filled in automatically with the system date.

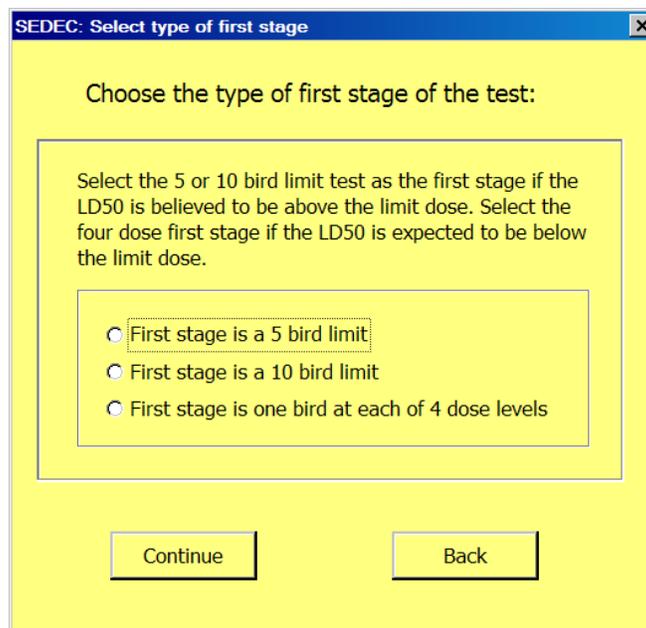


The screenshot shows a window titled "SEDEC: Study Information". The background is yellow. The text "Enter Basic Study Information:" is centered at the top. Below this, there are several input fields: "Project number:" (text input), "Test substance:" (text input), "Dose units (e.g. mg/kg)" (dropdown menu), "Test species:" (dropdown menu), and "Date:" (text input with the value "9/26/2009" pre-filled). At the bottom, there are two buttons: "Continue" and "Back".

7. Dose units used should be carefully considered, and whenever possible should be entered as mg/kg. Any units can be used, but the units selected can effect the operation of the program. The dependency on dose units arises because no dose greater than 3330 is allowed (assumed by the program to be 3330 mg formulation/kg) by the SEDEC. This limitation is included for animal welfare reasons. So, for example, expressing the doses as g formulation/kg could lead to doses greater than those considered humane being selected, and because different upper doses may be calculated by SEDEC because the upper limit is met in one case and not the other, slightly different LD50 estimates may be obtained.
8. After the data fields on this form are completed, select “Continue” to proceed to entering data describing the study design (see below), or select “Back” to return to the previous screen. Note that the “Back” option will not be available on all screens. Be sure that you are ready before selecting “Continue”.

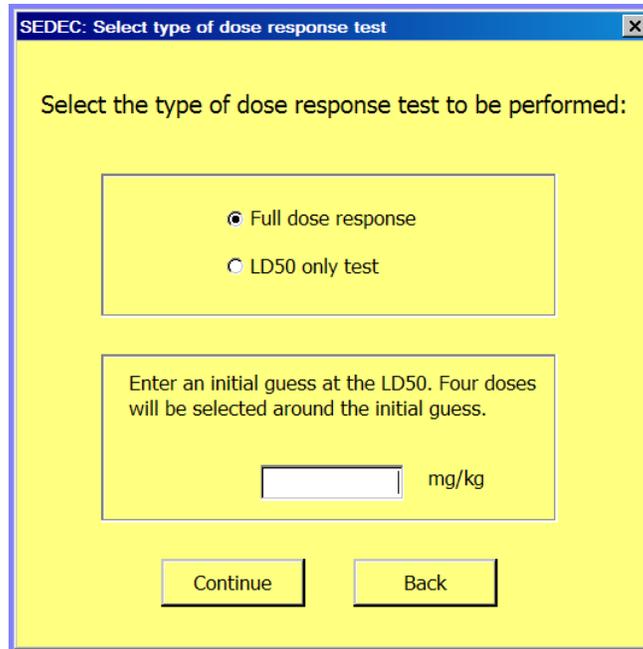
Four-Dose First Stage

9. For purposes of illustration we will follow the path that SEDEC follows after a 4-dose first stage is selected in the form displayed below. The program path followed if the study starts with a limit test is illustrated later (See section titled **Limit Test as First Stage**).



The screenshot shows a dialog box titled "SEDEC: Select type of first stage". The background is yellow. The text inside reads: "Choose the type of first stage of the test:". Below this is a larger yellow box containing the instruction: "Select the 5 or 10 bird limit test as the first stage if the LD50 is believed to be above the limit dose. Select the four dose first stage if the LD50 is expected to be below the limit dose." Underneath this instruction are three radio button options: "First stage is a 5 bird limit", "First stage is a 10 bird limit", and "First stage is one bird at each of 4 dose levels". At the bottom of the dialog box are two buttons: "Continue" and "Back".

10. Choose either full dose response (for estimation of the LD-50, slope of the dose response curve, and the confidence intervals for both) test or LD50-only test on the next form, and a guess at the LD50 based on best available information. Selecting 'LD50 only' has no effect on program operation except to record the initially selected study purpose in the audit trail.



The screenshot shows a dialog box titled "SEDEC: Select type of dose response test". The background is yellow. The text inside reads: "Select the type of dose response test to be performed:". Below this, there are two radio button options: "Full dose response" (which is selected) and "LD50 only test". Below the options, there is a text box with the instruction: "Enter an initial guess at the LD50. Four doses will be selected around the initial guess." To the right of the text box is a small input field followed by the unit "mg/kg". At the bottom of the dialog, there are two buttons: "Continue" and "Back".

11. SEDEC then will choose four doses, as described in the guideline, using the guessed LD50 from the previous stage, and display them in the following form.

SEDEC - Stage1 - Dose Selection (Multidose)

Stage 1 doses for an assumed LD50 of
100 mg formulation/kg

14.1	mg formulation/kg
52.1	
192	
707	

Accept suggested doses
 Over-ride maximum dose
 Over-ride minimum dose

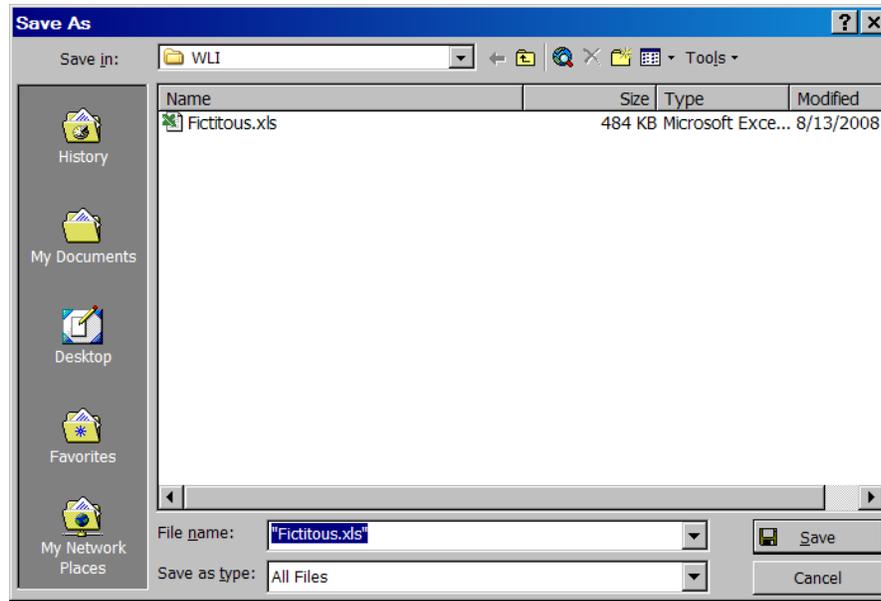
Note: Whenever possible, the suggested doses should be accepted. However, in some cases, the suggested maximum or minimum doses may not be feasible. Clicking the above over-ride buttons will allow new maximum or minimum dose values to be selected, and new dose levels will be calculated at equal intervals based on the new minimum or maximum.

-> Changing the maximum or minimum dose is likely to result in a less desirable distribution of doses around the LD50 than those initially calculated by SEDEC.

-> For animal welfare reasons, no dose above 3330 mg/kg is allowed.

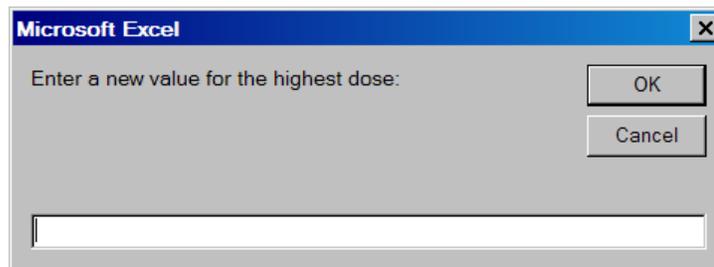
Save/Continue

12. Accept the doses (over-ride is provided for rare cases) and select “Save/Continue” and the following form will appear. Normally, the user would save the file at this point, as mortality data for choosing doses in stage 2 of the sequential design will not be available for 3 days (typically). Use a new name for the file (i.e. the workbook) so that the original file (SEDEC_MASTER.XLS) is not over-written. From this point forward, every time the project workbook is saved, the cumulative project record is written to the new workbook file.



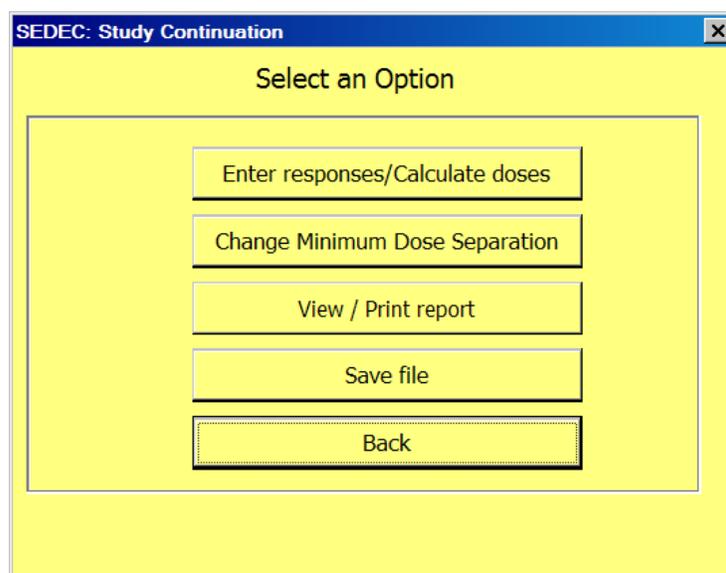
13. After saving the workbook, you will be returned to the startup menu. You may continue to work on the study file at this time, or you may exit the program.

If you select either ‘Over-ride maximum dose’ or ‘Over-ride minimum dose’ on the form following paragraph 10, SEDEC will open a dialog box (similar to the one below) that will prompt you to enter a new value. Entering a value and selecting OK will cause SEDEC to recalculate the dose levels using the new maximum or minimum. The new doses will be “pushed up or down” and equally spaced on the log scale. The default doses should be over-riden only as a last resort. *Note carefully the warnings displayed in the dose selection panel.* An example of a situation that might require a reduction in the maximum dose would be if it were necessary to reduce the the volume of the test material to allow it to be retained in the proventriculus. You are also given the opportunity to over-ride doses selected for stage 2.



14. If you chose to exit SEDEC in the previous step, you may reopen the study workbook by clicking on the file that you saved in the previous session. When EXCEL starts, you will again need to enable macros as described in step 7.

15. When SEDEC's startup form is displayed, you must re-enter your User ID, and then select "Continue the study" to proceed.
16. The following menu then appears. Among the options on this form are 'Enter responses/Calculate doses' and 'View/Print report'. Assuming that you now have the initial observations of the responses of the test birds to dosing, you will choose the first of these options. The responses (mortalities) of birds in stage 1 of the test are always entered after selecting "Enter responses/Calculate doses". After stage 1 is completed, mortality data can be entered and doses for subsequent stages calculated using this button or by selecting "View/Print report".



17. If 'Enter responses/Calculate doses' was selected in the previous step, a multidose first stage has been performed, and responses for stage 1 have not yet been entered, the following form will be displayed. Mortality for the multi-dose stage 1 is entered on this form. Stage 2 doses are calculated immediately after "Continue" is selected. Note that a different form will be displayed if stage 1 is a limit test (discussed below).

18. The option 'Change Minimum Dose Separation' from the previous (Study Continuation) menu requires explanation. As SEDEC accumulates data from different stages, it sometimes occurs that dose levels from two stages may be very similar. For example, due to rounding errors SEDEC may calculate the highest doses in each of two stages as 3330 and 3330.01 mg/kg. Clearly the responses of animals in these two groups should be combined and treated as occurring at a single treatment level when calculating the LD50, as the doses are not really different, and failing to do so can have undesirable effects on the calculation. By default, SEDEC combines dose levels that are within 1% of each other. This should usually be reasonable, but there may be circumstances when a different minimum dose separation must be used, and selecting 'Change Minimum Dose Separation' allows a different minimum dose separation to be specified. Caution should be applied in increasing the minimum dose separation. 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.

The screenshot shows a window titled "SEDEC: First stage responses" with a yellow background. The main heading is "Enter 1st Stage Responses". Below this is a table with three columns: "Dose (mg/kg)", "Dead", and "Alive". The "Dose (mg/kg)" column contains a list of four values: 14.1, 52.1, 192, and 707. The "Dead" and "Alive" columns contain radio buttons for each dose level. In the "Alive" column, the radio button for the first dose (14.1) is selected. Below the table is a label "Gussed LD50" (note the typo) followed by a text input field containing the value "100". At the bottom of the window are two buttons: "Continue" and "Back".

Dose (mg/kg)	Dead	Alive
14.1	<input type="radio"/>	<input checked="" type="radio"/>
52.1	<input type="radio"/>	<input checked="" type="radio"/>
192	<input type="radio"/>	<input checked="" type="radio"/>
707	<input type="radio"/>	<input checked="" type="radio"/>

Gussed LD50
100

Continue Back

Second Stage

19. The 10 doses for the second stage are then displayed (form below). Once again, the user has the option to over-ride dose maximum or minimum. Generally, the suggested doses should be accepted, unless they are physically impractical. *Note carefully the warnings displayed in the dose selection panel.* When you are satisfied with dose calculations, select Save/Continue. You will be prompted for a file name. Normally, you should select the same file name previously used.

SEDEC: Verify Doses

Stage 2 doses following a four-bird multidose first stage

34.3	
43.5	
55.2	
70.0	
88.8	mg
113	formulation/kg
143	
181	
230	
292	

Accept suggested doses
 Over-ride maximum dose
 Over-ride minimum dose

Note: Whenever possible, the suggested doses should be accepted. However, in some cases, the suggested maximum or minimum doses may not be feasible. Clicking the above over-ride buttons will allow new maximum or minimum dose values to be selected, and new dose levels will be calculated at equal intervals based on the new minimum or maximum.

-> Changing the maximum or minimum dose is likely to result in a less desirable distribution of doses around the LD50 than those initially calculated by SEDEC.

-> For animal welfare reasons, no dose above 3330 mg/kg is allowed.

Save/Continue

20. After the doses are accepted for stage 2, a spreadsheet-style display (see below) of all study information collected up to this time is displayed. Note that the spreadsheet view can be printed and other functions performed from the floating menu bar. However, as stage two doses have just been selected, you will have no new mortality data to enter, and you would normally select the 'Back' button on this screen, 'Back' on the next screen, and finally 'Exit'. All mortality information for the study that is collected from this point forward will be entered onto the spreadsheet layout.

SEDEC V1.2 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 LD50 Guess =	100	Step Size =	1.2687702
Date =	5-Oct-09		
Initials =	TAS		
Study Status Code =	140		
Min % Dose Sep =	1	(Combine doses differing by < Min% for analysis)	
Stage 3 Type =			

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding									
14.1	1	0	34.3	1	1						
52.1	1	0	43.5	1	1						
192	1	1	55.2	1	1						
707	1	1	70	1	1						
			88.8	1	1						
			113	1	1						
			143	1	1						
			181	1	1						
			230	1	1						
			292	1	1						

Analysis

Working LD50 Estimate From 4-Bird Multidose 1st Stage =	100.0159987
Over-rides =	None
Notes =	

SEDEC: Stage Report

Print Back Calculate LD50 View plot

- When the initial mortality data from stage 2 are in hand, you will need to re-open the project workbook, and after entering your initials, select “Continue the study” and then “Enter responses/calculate doses”. The spreadsheet view shown above will again be displayed, and will be ready to accept the stage 2 mortality data. This view can also be reached by selecting the “View/Print Report” menu choice. At this time you can also enter new mortalities due to delayed mortality that might have occurred among stage 1 birds. Note that doses cannot be changed by the user.

SEDEC V1.2 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 LD50 Guess = 100 Step Size = 1.2694297
 Date = 5-Oct-09
 Initials = TAS
 Study Status Code = 24
 Min % Dose Sep = 1 (Combine doses differing by < Min% for analysis)
 Stage 3 Type = B

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
Dose	N Tested	N Responding									
14.1	1	0	34.3	1	0	62.1	2				
52.1	1	0	43.5	1	0	78.9	2				
192	1	1	55.2	1	0	100	2				
707	1	1	70	1	0	127	2				
			88.8	1	1	161	2				
			113	1	0						
			143	1	1						
			181	1	1						
			230	1	1						
			292	1	1						

SEDEC: Stage Report

Print Back Calculate LD50 View plot

Analysis

Probit Analysis Results

Iterations	Chi-square	Probability	G	N
7	4.222261298	0.979083455	1.089009205	14

Slope = 7.964159639
 95% Confidence Limits = -0.34688724 and 16.27520652
 LD50 = 100.111524
 95% Confidence Limits = 0 and + Infinity

22. When stage 2 mortality data has been entered into this spreadsheet layout, selecting “Calculate LD50” on the floating command bar causes the LD50 based on the mortality data (from all dosing stages combined) to be calculated, and also causes the doses for the next study stage to be automatically calculated. SEDEC will examine the mortality pattern and for stage 3 will select either 2 or 5 doses around the working LD50 estimate, according to the number of reversals observed in the combined stage 1 and 2 data. (See the guideline for further explanation). The LD50, confidence intervals, slope and measures of curve fit will appear in the spreadsheet view. A note will appear below the LD50 if the algorithm fails to obtain a sound LD50 estimate. Note that a plot of the data can be viewed by selecting “View plot” from the floating menu bar. Finally, select the ‘Back’ button on this screen, and on the next screen, click the ‘Save file’ button, and save the workbook when prompted by SEDEC. You will return to this screen view when you are ready to enter the stage 3 mortality data. If at the end of stage 2, all birds that have been included in the test have died, there is insufficient information to calculate a working LD50. If this condition is obtained, SEDEC will direct the researcher to return to stage 1 and re-initiate the study using lower doses. If at the end of stage 2 all birds tested have survived the researcher will be directed to re-initiate the test using higher doses or to perform a limit test. TG223 does not provide clear guidance for the very rare situation of having a calculated negative slope estimate after stage 2. If this occurs, SEDEC assumes a working slope of 1 (consistent with the guideline), estimates the working LD50 as the geometric mean of the doses where mortalities have occurred (not described in the guideline), and then calculates doses for stage 3b using the assumed slope and working LD50. Note that if the calculated slope is negative at the end of stage 2, SEDEC will proceed to stage 3b regardless of the number of reversals and partial mortalities have occurred.

Stage 3

23. When the mortality data from stage 3 are in hand, you will need to re-open the project workbook, and after entering your initials, select “Continue the study” and then “Enter responses/calculate doses”. The spreadsheet view shown above will again be displayed, and will be ready to accept the stage 3 mortality data. This view also can be reached by selecting the “View/Print Report” menu choice. At this time you can also enter new mortalities due to delayed mortality among stage 1 and 2 birds.
24. When stage 3 mortality data has been entered into this spreadsheet layout, selecting “Calculate LD50” on the floating command bar causes the LD50 based on the mortality data (from all dosing stages combined) to be calculated, and also causes the doses for the study stage 4 to be automatically calculated (if needed). The new LD50, confidence intervals, slope and measures of curve fit will then appear in the spreadsheet view. A note will appear below the LD50 if the algorithm fails to obtain a sound LD50 estimate. Several pieces of information are provided to assist the user in evaluation of the study. At the bottom of the ‘Analysis’ section of the display, the number of reversals and partial group mortalities are printed. Below the ‘Analysis’ section, the combined data from all study stages is listed in dose order.

SEDEC V1.2 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 LD50 Guess = 100 Step Size = 1.2694297
 Date = 5-Oct-09
 Initials = TAS
 Study Status Code = 43
 Min % Dose Sep = 1 (Combine doses differing by < Min% for analysis)
 Stage 3 Type = B

Doses / Responses

Stage 1			Stage 2			Stage 3			Stage 4		
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

Iterations	Chi-square	Probability	G	N
8	6.087097931	0.992581075	0.710195894	24

Slope = 11.89865035
 95% Confidence Limits = 1.871286328 and 21.92601438
 LD50 = 100.0872194
 95% Confidence Limits = 71.5067982 and 139.9416652

SEDEC: Stage Report
 Print Back Calculate LD50 View plot

25. Finally, select the ‘Back’ button on this screen, and on the next screen, click the ‘Save file’ button, and save the workbook when prompted by SEDEC.

Stage 4

26. Note that doses for a fourth stage are calculated automatically when the LD50 is calculated for stage 3. You will return to this form view if you need to perform a fourth study stage. However, according to TG223, stage four is needed only if there are less than two reversals of the mortality pattern and less than two dose groups with partial mortality. Stage 4 procedures are almost identical to stage 3, except that no new doses are calculated after stage 4 mortality data has been entered.

Limit Test as First Stage

27. If a limit test is performed as the first stage of the test, steps 1 to 8 (above) are the same as for the four-dose first stage. However, when the form shown below is displayed, select “First stage is a 5 bird limit test” or “First stage is a 10 bird limit test”.

The screenshot shows a dialog box titled "SEDEC: Select type of first stage" with a yellow background. The text inside reads: "Choose the type of first stage of the test: Select the 5 or 10 bird limit test as the first stage if the LD50 is believed to be above the limit dose. Select the four dose first stage if the LD50 is expected to be below the limit dose." Below this text are three radio button options: "First stage is a 5 bird limit" (selected), "First stage is a 10 bird limit", and "First stage is one bird at each of 4 dose levels". At the bottom of the dialog are two buttons: "Continue" and "Back".

28. The next form (below) allows the limit dose to be set. The default limit dose is 2000 mg a.i./kg (where a.i. = active ingredient), and should be used in almost all cases. If a different limit dose is to be used, enter it here. Otherwise, click continues. Consult regulatory authorities before using a different limit dose, as other dose levels may not be acceptable. Select ‘Save/Continue’ to proceed. After the file is saved, you will be returned to the startup form, and typically you will exit the program at this time to perform dosing.

The screenshot shows a dialog box titled "SEDEC: Confirm Limit Dose". The main heading is "Set the limit dose to be used:". Below this, there is a text box containing the text: "The default limit dose is 2000 mg a.i./kg. If a different limit dose is to be used, enter it here. Otherwise, click continue. Consult regulatory authorities before using a different limit dose." To the left of this text is a text input field containing the number "2000" followed by the text "mg a.i./kg". At the bottom of the dialog, there are two buttons: "Save/Continue" and "Back".

29. When the mortality data from the limit test is in hand, restart the project workbook, enter your user ID, and select "Continue the study". On the next form that appears, select "Enter responses/calculate doses", and the following form appears. Select the number of birds used for the limit test (both 5 bird and 10 bird limit tests are allowed). Be sure to enter the number of mortalities and to click on the buttons indicating whether signs of toxicity were observed among survivors. Based on the information entered on this form SEDEC will choose the appropriate options for the next study stage.

The screenshot shows a dialog box titled "SEDEC: Limit Test Responses". The main heading is "Enter Limit Test Responses". Below this, there are two spinners. The first spinner is labeled "Number of birds in limit test" and has the value "5". The second spinner is labeled "Number of mortalities" and has the value "0". Below the spinners, there are two radio buttons. The first radio button is labeled "Signs of toxicity were observed among survivors" and is unselected. The second radio button is labeled "No signs of toxicity were observed among survivors" and is selected. At the bottom of the dialog, there are two buttons: "Continue" and "Back".

30. If the limit test is passed, no other action is needed. If it is failed, one of several routes may be taken. If there is more than one mortality in the limit test, but mortality is not complete, or there is one mortality and signs of toxicity among survivors, then the program will proceed directly to stage 2 of the test. If the limit test had one mortality, and there were no signs of toxicity among survivors, then the following form is displayed. The investigator is free to choose one of two options for the next stage. He/she may choose to proceed directly to perform a multi-dose stage two, or, if the first stage was a five-bird limit test, given the observed mortality pattern and absence of signs of toxicity, an additional five birds can be tested at the limit dose. This decision must be made based on professional judgment concerning the bird's responses, but may also take time limitations into account. If the user selects "Proceed to multi-dose stage 2, the program will proceed to the form displayed below paragraph 17, and the same procedures already described for the multidose steps in paragraphs 17 to 24 will be followed to study completion. If mortality is complete, the form below is not displayed, and the user is directed to reinitiate the study using a multi-dose first stage.

SEDEC: Continue following failed limit test with 1 mortality

Choose Step Following a Failed Limit Test

The limit test failed. One mortality occurred, and there were no signs of toxicity among survivors. Therefore, the test may proceed to the multi-dose stage 2 of the sequential design, or an additional 5 birds may be tested at the limit.

Note: If you choose to test an additional 5 birds at the limit, you must re-initiate the study as a new project, treating it as a 10-bird limit test.

Proceed to multi-dose stage 2

Test an additional 5 birds at the limit

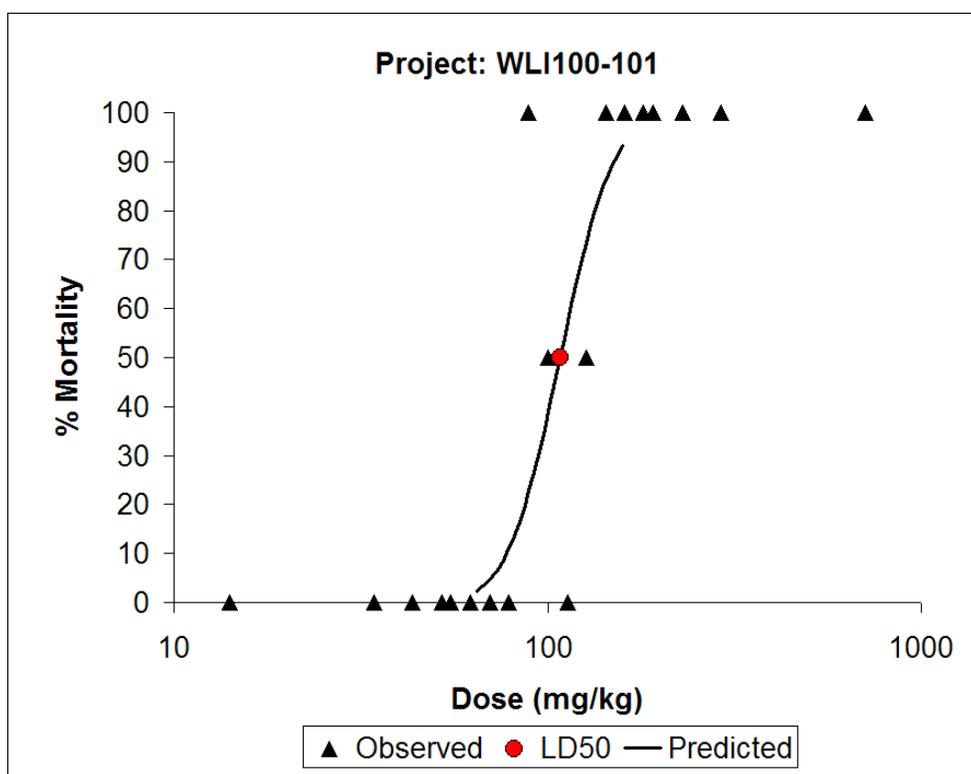
Back

Assessing the Study Data and the Final Estimate of the LD50

31. The acceptability of data from an Avian Acute Oral Toxicity Test depends on many factors not addressed in SEDEC. For example, the condition of control birds must be considered. Neither SEDEC nor this user's guide attempt to incorporate full support for decisions on the quality of study results, and the investigator should refer to TG223 for guidance. However, SEDEC does supply some support for assessing the statistical aspects of the quality of estimates derived from the study. *The final determination of the acceptability of results for a particular data set remains a matter for professional judgment and is the responsibility of the investigator.*
32. The Probit method algorithms used to estimate the LD50 assume that there is no background mortality (that is, no death due to disease or mishandling of birds), and no correction for background mortality is provided. TG223 provides guidance on appropriate actions to be taken should appreciable mortality occur among control birds.
33. A note will appear below the LD50 if the algorithm fails to obtain a sound LD50 estimate. However, SEDEC cannot identify all problematic conditions, and LD50 values, confidence intervals, slopes and measures of curve fit should be carefully examined. In very rare instances additional dosing stages may improve the quality of the LD50 estimate, but ordinarily, little will be gained by performing more than four stages. SEDEC does not support calculations for studies that incorporate more than four stages.
34. If there are less than 2 partial group mortalities or less than one reversal, maximum likelihood methods cannot provide accurate estimates of the slope, or of confidence intervals for the LD50 or the slope. When these conditions occur SEDEC reports these values as "Undefined". It is also possible for the dose response curve to have a negative slope after stage two (i.e. mortality tends to be higher at low doses than at higher doses). If this occurs, the working LD50 for calculation of stage 3 doses is set to the geometric mean of all the doses where mortality has occurred. Since the slope is less than 1, it is set to 1, in accordance with the instructions in the guideline. The occurrence of a negative slope at any step in the study should trigger a careful examination of observations and dose selection.
35. If a study is initiated as a limit test, and there is 10 or 20% mortality, and in the second stage, numerous birds die at concentrations below the limit dose, LD50 and or slope and confidence limit estimates made by SEDEC may be completely unusable. Failures of the computational algorithm can be easily recognized by examining the estimate of the slope, which will be a very large number, usually greater than 1×10^6 . The conditions leading to this failure should be extremely rare in practice. But if such a failure does occur, it may be necessary to use an alternate means for performing the calculations to select doses for the next stage and to estimate the LD50.
36. At the bottom of the 'Analysis' section of the display, the number of reversals and partial group mortalities are printed. At the end of the third stage of dosing, this information can

be used by the investigator to decide whether a fourth dosing stage is required. Below the 'Analysis' section, the combined data from all study stages is listed in dose order. The rightmost column of the information displayed indicates whether the responses at the dose level represent groups that were combined because they differ less than the value assigned to "minimum dose separation" (default > 1%).

37. A Chi-square goodness of fit statistic is calculated to aid in evaluation of the fit of the probit dose response model to the data. This statistic is a measure of how far the observed data fall from the best-fit probit model, with large Chi square values indicating a poor correspondence between the model and the data. SEDEC calculates the probability that random variability could produce a larger Chi-square if the probit model is the correct model. If this probability is less than 0.05, SEDEC will alert the user that the data do not fit the model well, and that the LD50 may not be dependable. This does not mean that another model will necessarily fit the data better, but may instead mean that, due to random variation, no reasonable model that describes a simple curved line can fit the data.
38. The investigator should further evaluate the fit of the model by examining a plot of the fit of the dose response curve to the data. This can be done by selecting 'View Plot' from the spreadsheet view. Notice that on the plot that is displayed (see example below), if a single bird was assigned a dose, mortality can only be 0 or 100%. Thus, the dose response plot might at first appear odd, but this odd appearance simply reflects the nature of the study design. *The 'View Plot' function is not available if SEDEC is executed using EXCEL2007.*



39. The G statistic, a measure of numerical stability of maximum likelihood estimates, is also calculated. When the G statistic is between 0.96 and 1.00, the confidence limits on the LC_{50} are approximate. Only one confidence limit can be calculated when G is greater than 1.0.
40. The algorithms fitting the probit model to the data may not converge to a unique result under some conditions. SEDEC warns the user of this condition. Results from the probit analysis may not provide dependable LD50 estimates.

Audit Trail

41. SEDEC maintains an audit trail that lists significant data handling activities. The audit trail cannot be modified by the user or viewed on-screen. If 'Print audit trail' is selected from SEDEC's start-up form, a listing similar to the one below will be obtained. All mortality entries made after the initial entry of stage 1 data are logged individually, which allows identification of any delayed mortalities that are entered after calculation of doses and LD50 estimates.

SEDEC Version 1.2 Audit Trail

Project: WLI100-101
 Test Subst.: Fictitious
 Last Entry: 48

Date	ID	Entry
9/27/2009	TAS	Study initialized
9/27/2009	TAS	Four dose 1st stage chosen
9/27/2009	TAS	Guessed LD50 100
9/27/2009	TAS	Chose Dose-Response: Full
9/27/2009	TAS	Stage 1 Doses Calculated
9/27/2009	RED	Mortality entered for dose 14.1 = 0
9/27/2009	RED	Mortality entered for dose 52.1 = 0
9/27/2009	RED	Mortality entered for dose 192 = 1
9/27/2009	RED	Mortality entered for dose 707 = 1
9/27/2009	RED	Stage1 LD50 calculated: 100.015998720205
9/27/2009	RED	Stage 2 doses calculated
9/27/2009	RED	Mortality entered for dose 34.3 = 0
9/27/2009	RED	Mortality entered for dose 43.5 = 1
9/27/2009	RED	Mortality entered for dose 55.2 = 1
9/27/2009	RED	Mortality entered for dose 43.5 = 0
9/27/2009	RED	Mortality entered for dose 55.2 = 0
9/27/2009	RED	Mortality entered for dose 70 = 1
9/27/2009	RED	Mortality entered for dose 88.8 = 0
9/27/2009	RED	Mortality entered for dose 113 = 1
9/27/2009	RED	Mortality entered for dose 143 = 1
9/27/2009	RED	Mortality entered for dose 181 = 1
9/27/2009	RED	Mortality entered for dose 230 = 1
9/27/2009	RED	Mortality entered for dose 292 = 1
9/27/2009	RED	Stage 3 type = B
9/27/2009	RED	LD50 calculated: 80.1543118706598
9/27/2009	RED	Mortality entered for dose 49.7 = 0
9/27/2009	RED	Mortality entered for dose 63.1 = 1
9/27/2009	RED	Mortality entered for dose 80.1 = 0
9/27/2009	RED	Mortality entered for dose 102 = 2
9/27/2009	RED	Mortality entered for dose 129 = 2
9/27/2009	RED	LD50 calculated: 79.4771661560073
9/27/2009	RED	File Saved
9/27/2009	RED	File saved
9/27/2009	RED	Audit Trail Printed
9/27/2009	TAS	Mortality entered for dose 80.1 = 1
9/27/2009	TAS	LD50 calculated: 74.2232230299749
9/27/2009	TAS	Audit Trail Printed
9/27/2009	TAAS	Audit Trail Printed

Exporting or Printing a Report

42. The SEDEC report can be made available for use in several ways. Several SEDEC menus provide an opportunity to print the report. It is possible to send the report to a PDF file instead of a printer, if an appropriate print driver has been installed. Many facilities will already have PDF print drivers installed on their computer systems, but if none have been installed, a number of excellent free-of-cost drivers exist. One such driver is PDFcreator, which can be downloaded from <http://sourceforge.net/projects/pdfcreator>, and is made available under a GNU general public license. Using PDFcreator as an example, the steps in creating a PDF file containing an image of SEDEC's report are as follow:
- a. Before starting the SEDEC session, download the PDFcreator software, and run the installation program that comes with it. When the installation program asks you to choose between a local and network installation, use the local installation if possible. After the installation is completed, a printer named PDFcreator will appear in the Windows printer list. This is a print driver rather than a physical printer, and serves to route any printed output to a PDF file.
 - b. Click the Windows Start button, select "Printers and Faxes", and the list of available printers should appear.
 - c. SEDEC always prints using the Windows default printer, so you must right click on PDFcreator and make it the default printer.
 - d. Now start the SEDEC session, and print as needed. When a print command is issued a window will pop up that allows entry of a file name and location to save the report image, and the PDF file will be created immediately. This file can then be emailed or manipulated as desired.
 - e. When the SEDEC session is ended, it may be desirable to return to the Windows "Printers and Faxes" control panel to set the default printer back to a physical printer.
43. The easiest way to incorporate a SEDEC report into a Microsoft Word document is to use simple cut and paste procedures. One approach that works well is as follows:
- a. Open a Word document and then start SEDEC.
 - b. Anytime that SEDEC's report window is visible, a region of the report can be selected by clicking and dragging with the mouse.
 - c. The selected region can be copied to the Windows Clipboard by pressing Control-C.
 - d. After the region is copied, click on the icon for the open Word document (visible at the bottom of the display screen) to display the document.
 - e. Click inside a blank space in the Word document, and then select 'Edit' > 'Paste Special' > 'Picture'. Other methods of pasting may produce erratic results.
 - f. Note that the information in the pasted image cannot be edited as text.

Exporting a Plot of the Dose Response Curve

44. SEDEC allows a plot of the dose response curve to be exported. After the LD50 has been calculated, choose 'View Plot' from the spreadsheet style view. A form will appear that includes the option 'Export plot image'. Clicking this button causes SEDEC to open a dialog box that asks for a file name. After the file name is entered, SEDEC will write a bitmapped representation in .GIF format to the same directory where the spreadsheet is saved. The image can be inserted into a Microsoft Word document by selecting 'Insert' on the Word menu bar, then 'Picture', and then 'From File'. Navigate to the subdirectory where the .GIF file resides and select it. The image will be brought into Word, where it can be resized and positioned as needed. Microsoft Word text boxes can be added to the figure as annotation. Note that a triangle symbol on the plot can represent one or more individuals, and that annotation can be added to clarify this as needed. *The plot export function is not available if SEDEC is executed using EXCEL2007.*

SEDEC TECHNICAL REFERENCE

45. SEDEC is an EXCEL workbook designed to aid in dose selection and analysis of studies performed in accordance with OECD TG 223. SEDEC requires that EXCEL 2000 or a later version to be installed on the user's computer. SEDEC consists almost completely of VBA code that executes within the EXCEL application environment. As EXCEL macros are all recorded as VBA (Visual Basic for Applications) code, the term "VBA code" and "Excel macros" are essentially synonymous in this application. SEDEC can be thought of as consisting of two major functional blocks. Block 1 guides the user through setting up a study, estimating working LD50 values using heuristic methods (but not using maximum likelihood estimation) and selecting doses. Block 2 of SEDEC consists of the algorithms that estimate probit model parameters and the LD50 by maximum likelihood methods, and that calculate confidence intervals for the LD50 and slope of the dose response curve. Block 1 code was written by T.A. Springer of Wildlife International, Ltd., (Easton, Maryland USA) with the assistance of the Avian Acute Working group.
46. Block 2 algorithms were originally developed by Charles Stephan of the US EPA Environmental Research Laboratory, Duluth, Minnesota, based on methods proposed by Finney 1964. A printed listing of the computer code for the algorithms used in SEDEC was originally acquired by Wildlife International in a personal communication from Charles Stephan (Stephan 1985). This code was later distributed in the EPA document "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms." (Peltier and Weber 1985 -- EPA/600/4-85/013) and the Probit method used is discussed in detail in the Environmental Assessment Technical Assistance Handbook, Document 5.01, published by the Food and Drug Administration (Eirkson et. al.) in 1987. The LD50 and slope and confidence limits are estimated using classical methods described by Finney (1962) and many others. These methods are summarized below.
47. The original code for calculating maximum likelihood estimates of the LD50 that was obtained from C.E. Stephan was written in a subset of the BASIC programming language that was available for PDP 11/70 and Cyber 171 minicomputers. This code was converted to VBA by T.A. Springer. Computational algorithms were slightly modified to suit the needs of TG223, but the code used in SEDEC produces results that are essentially identical to the program originally created by Stephan. The error trapping in the original code that prevented calculation of an LD50 unless at least two treatment groups had partial mortality was relaxed to be consistent with TG 223, allowing the calculation if a 'reversal' is detected. The maximum likelihood method is used to determine regression parameters for the relationship between the probits of the proportion of treated animals that die and the log of test substance concentration. The calculation of the 95% confidence limits for the LC₅₀ are based on Fieller's theorem (See Finney 1964, Pg. 114) and are described in detail in table A-6 of the FDA Environmental Assessment Technical

Assistance Document. A Chi-square goodness of fit statistic is also calculated to aid in evaluation of the fit of the regression line to the data. The G statistic, a measure of numerical stability of maximum likelihood estimates, is also calculated. When G is between 0.96 and 1.00 the confidence limits on the LC_{50} are approximate.

48. The algorithms used in Block 2 of SEDEC have been used for over two decades, and it is believed that the results are accurate. **However, the final determination of the acceptability of results for a particular data set remains a matter for professional judgment and is the responsibility of the investigator.**

49. *SEDEC implementation of the probit model*

SEDEC fits the mortality data to a standard probit model. The probit model is based on the cumulative normal distribution function. In particular, the proportion, y , of subjects responding is related to the log of the dose, d , by means of Φ (the standard normal distribution) as follows:

$$y = \Phi(a + b d).$$

This can be rewritten as

$$\Phi^{-1}(y) = a + b d,$$

where Φ^{-1} is the function-inverse of Φ . This inverse is called the probit of y and can be seen to be a linearizing transform of y .

Maximum Likelihood (ML)

Maximum likelihood methods are used to estimate the parameters a and b of the dose response and their variances. If n_i birds are given a dose d_i and m_i birds die, then the probability of this outcome is equal to $C_{n_i}^{m_i} p_i^{m_i} (1 - p_i)^{n_i - m_i}$ where

$p_i = I(a + b \log_{10}(d_i)) = I(a + b x_i)$. The likelihood is expressed as

$L = \prod_i^k C_{n_i}^{m_i} p_i^{m_i} (1 - p_i)^{n_i - m_i}$, and the log likelihood is expressed as:

$$\text{Log}(L) = \sum_{i=1}^k \left\{ \log \binom{n_i}{m_i} + m_i \log_{10}(p_i) + (n_i - m_i) \log_{10}(1 - p_i) \right\}.$$

Maximizing this function results in estimates \hat{a} for a and \hat{b} for b .

Estimating the log(LD₅₀)

Once parameter estimates \hat{a} and \hat{b} have been derived, the estimate of the log(LD₅₀) is simply calculated as $\log_{10}(LD_{50}) = -\hat{a} / \hat{b}$.

Estimating the Confidence Limits of the LD50 (Fieller's Theorem)

Fieller's Theorem (Finney 1962) is used to estimate the confidence limits around estimates of the LD₅₀.

$$CI(\log LD_{50}) = m + \left(\frac{g}{1-g}\right)\left(m - \frac{v_{12}}{v_{22}}\right) \pm \frac{Z_{\alpha/2}}{b(1-g)} \left[v_{11} - 2m v_{12} + m^2 v_{22} - g\left(v_{11} - \frac{v_{12}^2}{v_{22}}\right) \right]^{1/2}$$

$$m = -a/b = \log(LD_{50})$$

$$g = z_{\alpha/2}^2 v_{22} / \beta^2$$

a = estimate of intercept

b = estimate of slope

v₁₁ = variance estimate of intercept

v₁₂ = covariance estimate between numerator and denominator of m
(i.e. between -a and b)

v₂₂ = variance estimate of slope

Estimating Confidence Limits for the Slope

The 95% confidence intervals on the slope are computed as:

$$\hat{b} \pm 1.96 * SQRT(\text{variance of } \hat{b})$$

REFERENCES

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Appendix A.

Qualification of SEDEC On Your Computer

After SEDEC has been installed, the following test should be performed to ensure the program's integrity and its functionality on your computer. The test consists of stepping through the stages of a study using a pre-specified data set, and checking your results against the outcomes listed below. Simply follow these instructions:

- (1) Initiate a new study. Any study ID or species can be selected. Dose units should be mg a.i. / kg.
- (2) For the first stage of the test, select a five bird limit test, and accept the default limit dose of 2000 mg a.i. / kg..
- (3) Enter a stage 1 mortality of 2 birds.
- (4) SEDEC should calculate stage 2 doses that are listed in the table below.
- (5) Enter the stage 2 mortalities indicated in the table.

Dose	Morality
392	0
497	1
630	0
800	0
1014	0
1286	1
1632	1
2070	1
2625	1
3330	1

- (6) Calculate the stage 3 doses (doses that should be obtained are in the following table).
- (7) Enter the stage 3 mortalities indicated in the following table.

Dose	Morality
285	1
3330	5

- (8) Calculate the LD50 and its confidence limits. The following values should be obtained:

Parameter	Value
LD50	979.0655422
Lower 95% Confidence Limit	307.1547224
Upper 95% Confidence Limit	2012.671476

- (9) Your installation of SEDEC is considered to be qualified if the computed values match the table values. The worksheet should be printed out, signed, dated, and retained for future reference.