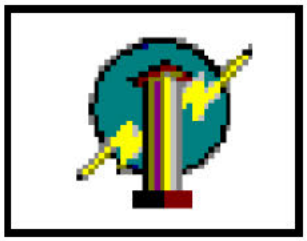
QUALITATIVE and QUANTITATIVE

STATISTICAL ANALYSES

SIMB Standard Operating Procedures (SOP)

including

User's Instructions for
STATOX and QRisk Module



April 6, 2016

Table of Contents

INTRODUCTION

The Health Effects Division (HED) evaluates long-term studies in mice, rats and hamsters conducted to assess a chemical's potential cancer risk to humans. If the toxicologist determines that there is a possible concern for carcinogenicity, a qualitative analyses of the tumor data is conducted. The Cancer Assessment Review Committee (CARC) uses this analysis when determining the classification of the carcinogenic potential and the need for quantification of human cancer risk. When quantification is considered appropriate, the quantitative statistical analysis estimates an upper-bound excess cancer risk estimate. Upper-bound cancer risk estimates may be calculated using models such as the one-hit, probit, logit, Weibull or Quantal multistage models.

Every HED risk assessment is based on the best science available and includes characterization of any uncertainties, either directly or by reference to fully vetted scientific guidance and standard operating procedures. Complex scientific decisions may be based on weight of the evidence (WOE) determinations that are discussed within the risk assessment teams and at peer review committees and documented in the risk assessment. Any changes to risk assessments as they progress through the regulatory process must be consistent with good science and science policy; significant deviations from policy or committee conclusions will be documented in the risk assessment or in follow-up committee documentation. Any committee that has previously reviewed a risk assessment that is subsequently changed will be informed of the rationale as to why the changes were made. Any dissenting opinions will be included in the record in accordance with the OCSPP Scientific Integrity Policy and elevated to management as appropriate for further review.

A general overview of the procedures to be followed by the Science Information Management Branch (SIMB)/HED when conducting these statistical analyses is presented in Chart 1.

HED currently uses STATOX (Fig. 1) to estimate the cancer risk. This program enable HED to perform survival (trend tests: the Kaplan-Meier Survival Curves, the Cox test, and a generalized K/W analysis; as well as pair-wise comparisons of the survival of each dose group versus the control), qualitative (Fisher's Exact and Trend Test, Fatal Tumor and Peto Prevalence) and quantitative analyses (QRisk module). A more detailed description of each of these methodologies is provided in separate sections.

See Appendix 3 for details on installing STATOX on your computer.

Figure 1. Main Window of STATOX

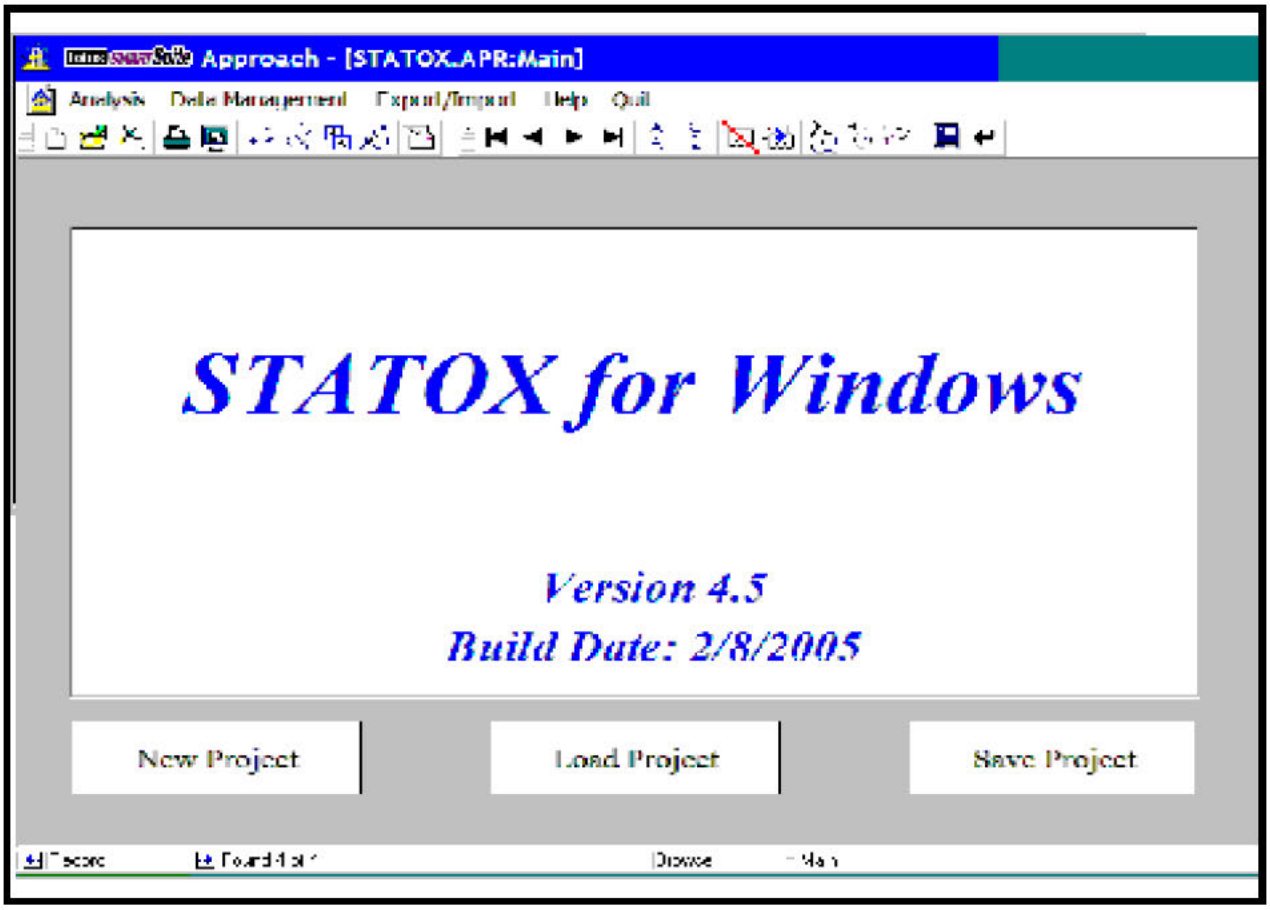
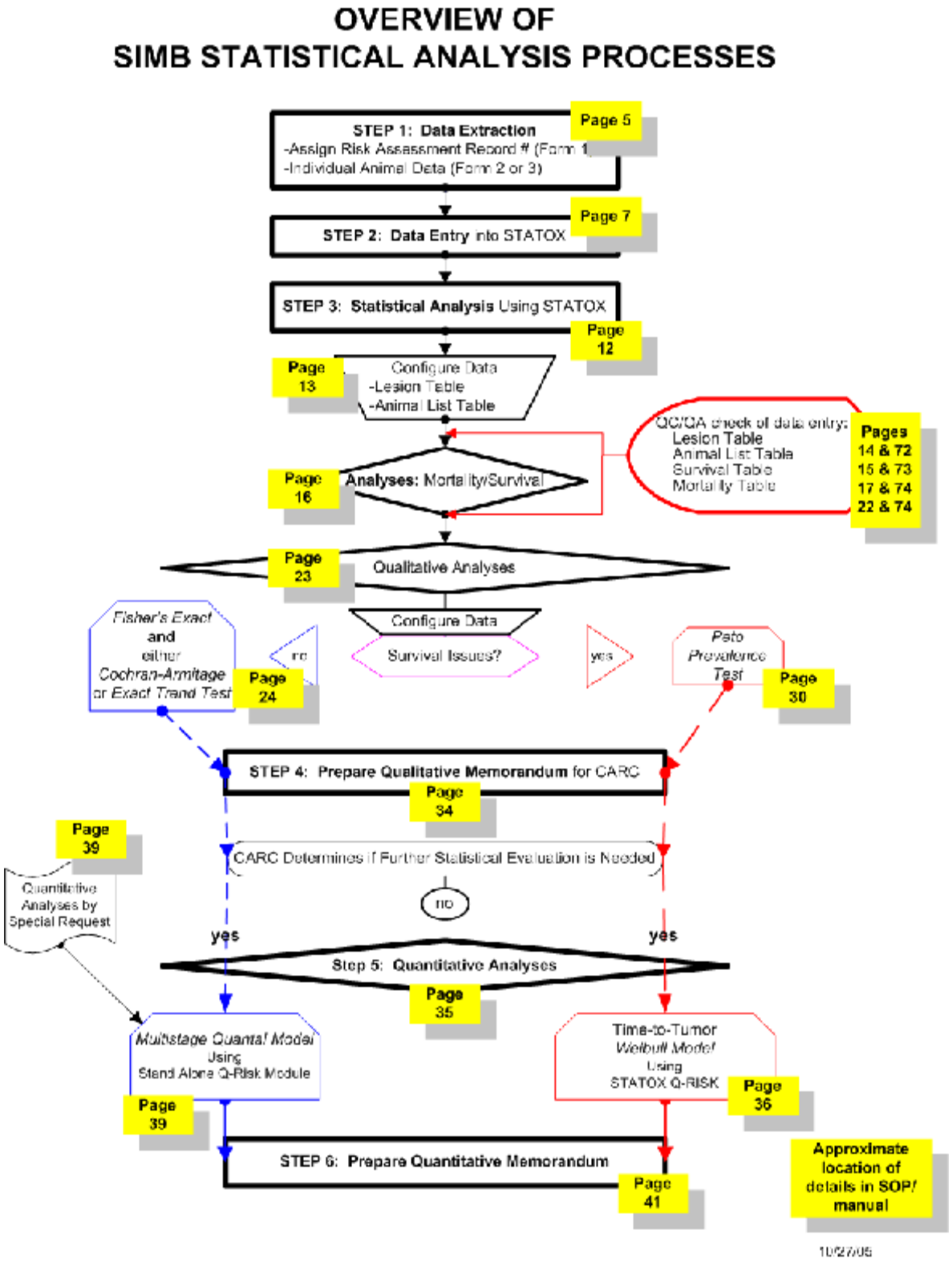


Chart 1:


OVERVIEW OF
SIMB STATISTICAL ANALYSIS PROCESSES
STEP 1: Data Extraction
-Assign Risk Assessment Record # (Form 1)
-Individual Animal Data (Form 2 or 3)
Page 5
STEP 2: Data Entry into STATOX
Page 7
STEP 3: Statistical Analysis Using STATOX
Page 12
Page 13
Configure Data
-Lesion Table
-Animal List Table
Page 16
Analyses: Mortality/Survival
QC/QA check of data entry:
Lesion Table
Animal List Table
Survival Table
Mortality Table
Pages
14 & 72
15 & 73
17 & 74
22 & 74
Page 23
Qualitative Analyses
Configure Data
Survival Issues?
no
yes
Fisher's Exact
and
either
Cochran-Armitage
or Exact Trend Test
Page 24
Peto
Prevalence
Test
Page 30
STEP 4: Prepare Qualitative Memorandum for CARC
Page 34
Page 39
Quantitative Analyses by Special Request
CARC Determines if Further Statistical Evaluation is Needed
no
yes
yes
Step 5: Quantitative Analyses
Page 35
Multistage Quantal Model
Using
Stand Alone Q-Risk Module
Page 39
Time-to-Tumor
Weibull Model
Using
STATOX Q-RISK
Page 36
STEP 6: Prepare Quantitative Memorandum
Page 41
Approximate location of details in SOP/ manual
10/27/05

Step 1: DATA EXTRACTION

The HED toxicologist provides the appropriate carcinogenicity studies to SIMB staff for statistical analyses by STATOX. A consultation between the toxicologist, the chairman of the Cancer Assessment Review Committee (CARC) and the statistician determines the tissues and tumors of concern to be extracted for statistical analyses. SIMB staff will review the study (which must include histopathology data for every animal) and the Data Evaluation Record (DER), especially those sections pertinent to survival and tumorigenicity, to ensure completeness of the data needed for analysis. These analyses will be presented to the CARC in a qualitative risk assessment memorandum.

When an analysis is requested for a chemical, the next unique sequential Risk Assessment Record Number will be assigned by the SIMB statistician to each sex of each species for each study. SIMB staff maintains the list of Risk Assessment Record Numbers used for STATOX. A separate Qualitative Analysis Cover Form (Appendix 1, Form 1) must be completed by the statistician for each Risk Assessment Record Number. The following study identifiers will be recorded:

chemical or trade name PC Code MRID number(s) study type study project number study laboratory study date duration of study	study number pathologist species strain sex registrant dose unit time unit	actual dosage route of administration dosage days/week tissue and tumor descriptions for each tumor morphology to be evaluated using the STATOX system

Tissues are assigned a unique code beginning with a "T" followed by a number (one code per tissue). Each tumor morphology (a.k.a. lesion) is also assigned a unique code beginning with an "M" followed by a number, one code per tumor morphology.

NOTE:	The "T" and "M" must be capitalized for the program to work correctly

The Individual Animal Data Entry Form (see Fig. 2 and Appendix 1, Form 2) is completed for each tissue/tumor morphology combination. This form accommodates up to four different tissue/tumor morphology combinations. The Risk Assessment Record Number must be recorded at the top of each Individual Animal Data Entry Form. Identifying information for each animal is recorded on the data forms and includes:

Figure 2. Heading for Form 2 - The Individual Animal Data Entry Form

unique animal number sex dose time of death disposition at death tissue and tumor information

ANIMAL NUMBER	SEX	DOSE	TIME OF DEATH	DISPOSITION	TUMOR MORPHOLOGY
_____	____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3

The disposition and tumor information in this form is coded as follows:

DISPOSITION at death for each animal	1 = death on study or moribund/humane sacrifice 2 = final sacrifice 3 = accidental death 4 = first interim sacrifice 5 = second interim sacrifice 6 = third interim sacrifice 7 = fourth interim sacrific
TUMOR MORPHOLOGY information for each animal	1 = animal did not have that tumor 2 = animal did have that tumor 3 = animal was not examined for that tissue and/or tumor morphology

On Form 2, the A, B, C, etc. correspond to the tumors of concern in Form 1. Following completion of Form 2, compare the tumor counts reported in the DER and study report to the tallies in Form 2. The staff performing data extraction should report any discrepancies to the statistician and toxicologist. This quality assurance step is repeated following data entry.

Form 3 (Appendix 1) is used when there are more than four tissue/ tumor morphology combinations. Follow the same steps and codes as for Form 2 listed above.

Step 2: DATA ENTRY

Getting Started:

Before opening STATOX: Create a new subdirectory for each chemical (project) identified by the name of the chemical (e.g., C:/STATOX4.5/chemical name).

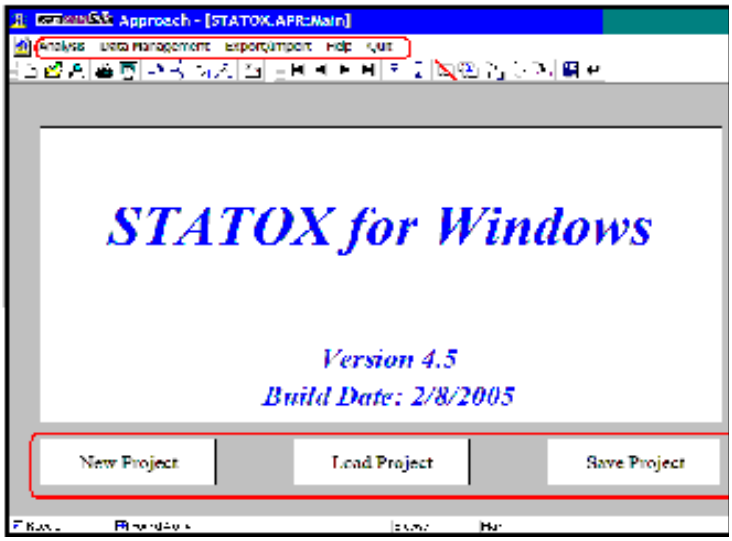
To open STATOX: The PC must have Notes Approach Version 9.5 or higher installed.

There are 2 alternative ways to open STATOX:

1. double-click the "STATOX.APR" icon on a Windows desktop **OR**
2. start Lotus Approach first, then open "STATOX.APR" from within Approach

A password dialog box will open. **Enter 1638 for the password.**

Figure 3: Main Window Buttons & Menu Bar



When STATOX is launched, the user begins at the Main STATOX Window (Fig. 3). This window contains three buttons located below the banner (New Project, Load Project, Save Project), and five drop-down menus at the top of the window (Analysis, Data Management, Export/Import, Help, Quit). The buttons perform project management functions. A project is defined as all Risk Assessment Record Numbers for a specific chemical. A project may contain more than one Risk Assessment Record Number. Project functions include: creating a New Project, Loading a saved Project, or Saving the current Project. The drop-down menus are used for editing and analyzing.

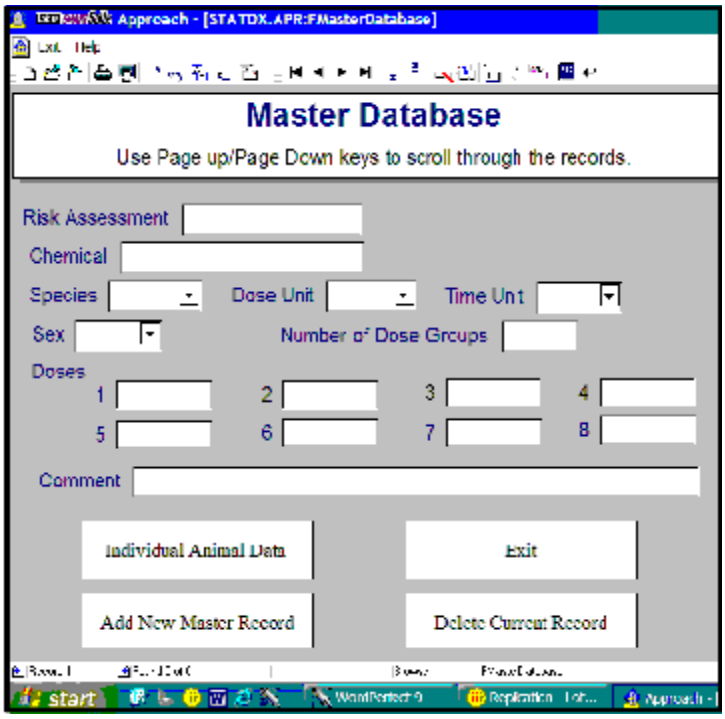
The "New Project" button creates a new project in the location specified by the user. Use of this

function requires only the name and location of a new project definition file, for which the user is prompted (.prj extention will be added automatically). User must have created a chemical project subdirectory prior to creating a new project, as previously stated (example: C:\STATOX4.5\Chemical name).

The "Load Project" button is used to open an existing, previously saved project.
The "Save Project" button is used to save the current project (as a chemical.prj file).

Master Database Form

Figure 4: Master Database Form



To enter data in a new project, click on the **New Project** button (Fig. 3). Once you choose the location for your new database, enter a file name–usually the chemical name–then click the **save** button (the .prj extension is automatically added). The Master Database form (Fig. 4) will open.

All fields are to be filled in with information obtained from the Qualitative Analysis Cover Form (Appendix 1, Form 1). The Master Database form is the editing and viewing interface for the master records. The "page-up" and "page-down" keys are used to scroll through the different Risk Assessment Record Numbers.

The Master Database Form requires input into the following fields:

- Risk Assessment (the Risk Assessment Record Number)
- Chemical
- Species
- Dose Unit
- Time Unit
- Sex
- Number of Dose Groups (including controls)
- Doses (including 0 for controls)
- The Comment Field, to be filled out as needed

The **Individual Animal Data** button in Figure 4 opens the **Animal Data Form** (Fig. 5) needed for entry of the animal and lesion information for the risk assessment currently displayed in the **Master Database** form (Fig. 4). See next page for details.

The **Add New Master Record** button opens a blank record allowing the creating of a new risk assessment in the project. As stated earlier, a unique Risk Assessment Record Number is required for each record.

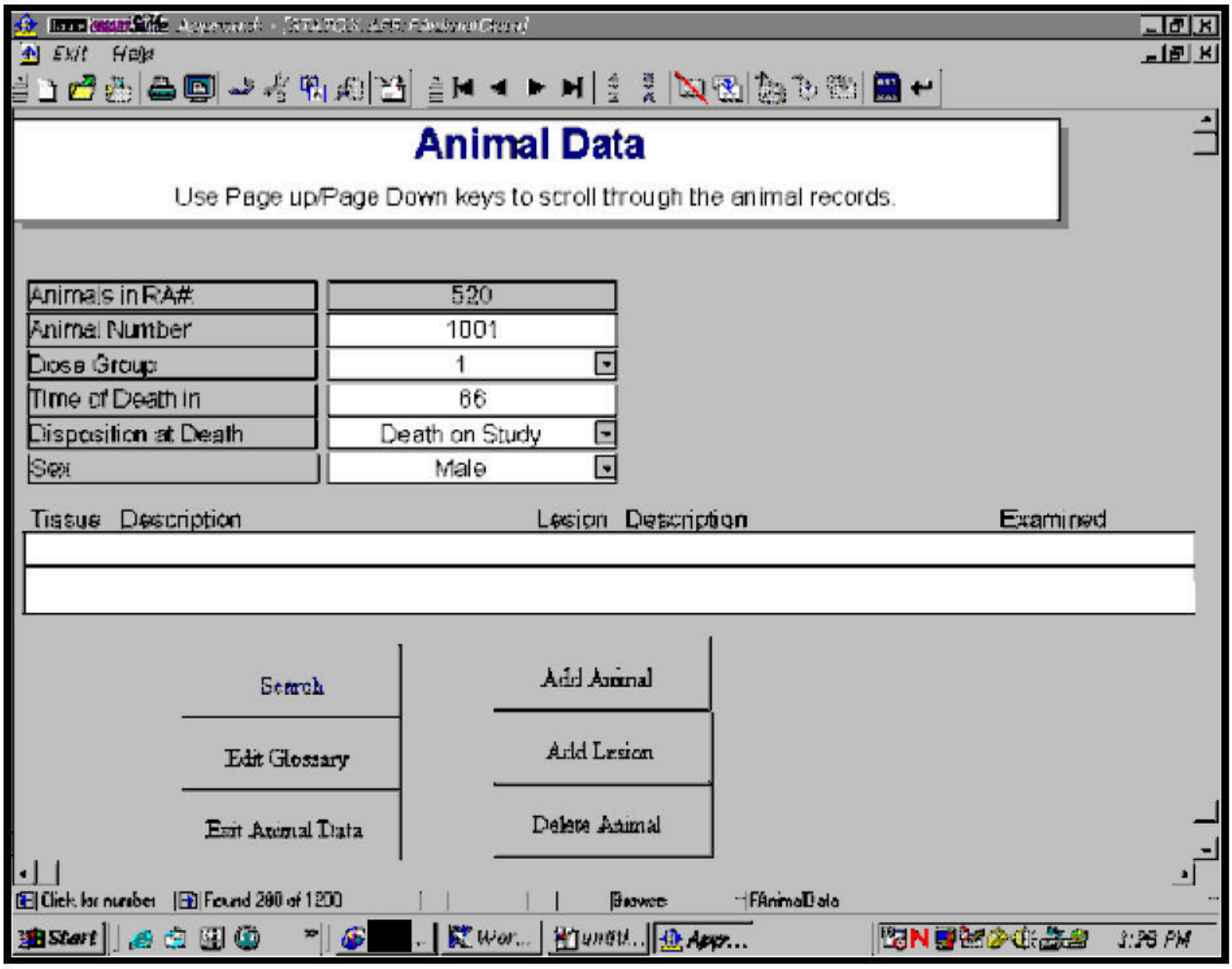
To return to the Main Window, use **Exit** on the menu bar (top left of the screen) or the **Exit** button at the bottom right of the **Master Database** form.

WARNING:	The **Delete Current Record** button (Fig. 4) deletes the current master record as well as all associated animal, lesion, and glossary data.

Entering Individual Animal Data

Click on the **Individual Animal Data** button and the Animal Data Form screen (Fig. 5) appears. Individual animal and lesion information is entered, deleted, changed, and viewed on the Animal Data Form screen. All fields are filled in with information obtained from the Individual Animal Data Entry Form (Form 2) or the Expanded Individual Animal Data Entry Form (Form 3).

Figure 5: Animal Data Form



New animals are added to the database by clicking the **Add Animal** button. Animals are deleted by clicking the **Delete Animal** button, which also deletes any lesions associated with that animal. The **Edit Glossary** button will bring up the Glossary Form screen (Fig. 6)

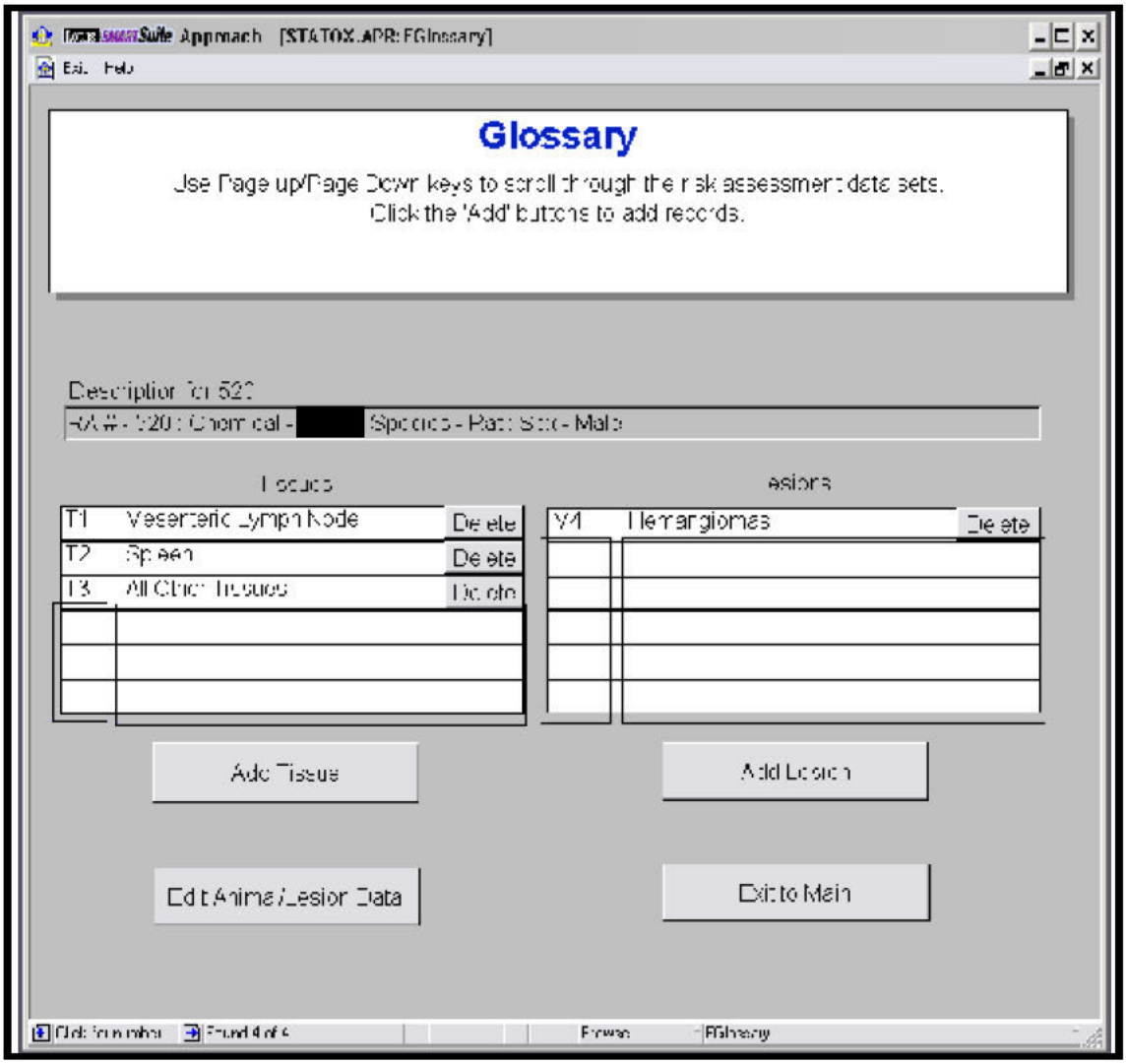
NOTE:	When using this method for glossary entry, at least one animal must have been entered before a glossary can be created.

Glossary

A glossary must be created for each unique Risk Assessment Record Number. Clicking the **Edit Glossary** button will bring up the Glossary Form screen (Fig. 6). The Glossary maintains descriptions of tissues and lesions. To scroll through the tissues and lesions for different risk assessments, use the "page-up" and "page-down" keys (located on your keyboard). All fields are filled in with information obtained from the Qualitative Analysis Cover Form (Form 1).

New tissues or lesions are added by clicking the **Add Tissue** or **Add Lesion** button at the bottom of the screen. Although it is not visible on the form, there are two columns for both tissues and lesions (see black outlined areas on Fig. 6). The first is for the tissue or lesion code (e.g., T1, T2 or M1, M2). The tissue or lesion name goes in the second column. Tissues and lesions are deleted by clicking the **Delete** button to the right of the tissue or lesion description. The **Edit Animal/Lesion Data** button returns the user to the Animal Data Form screen (Fig. 5). The **Exit** button on the menu bar (at the top of the screen) and the **Exit to Main** button on the bottom right of the form return the user to the Main Window.

Figure 6: Glossary Form



Entering Lesions

Individual animal lesions may be entered by either clicking in the blank row under the word **Tissue** which opens the drop-down menu or by clicking the **Add Lesion** button on the Animal Data Form (Fig. 7). The tissue, lesion and examined **(Yes/ No)** fields must be filled in.

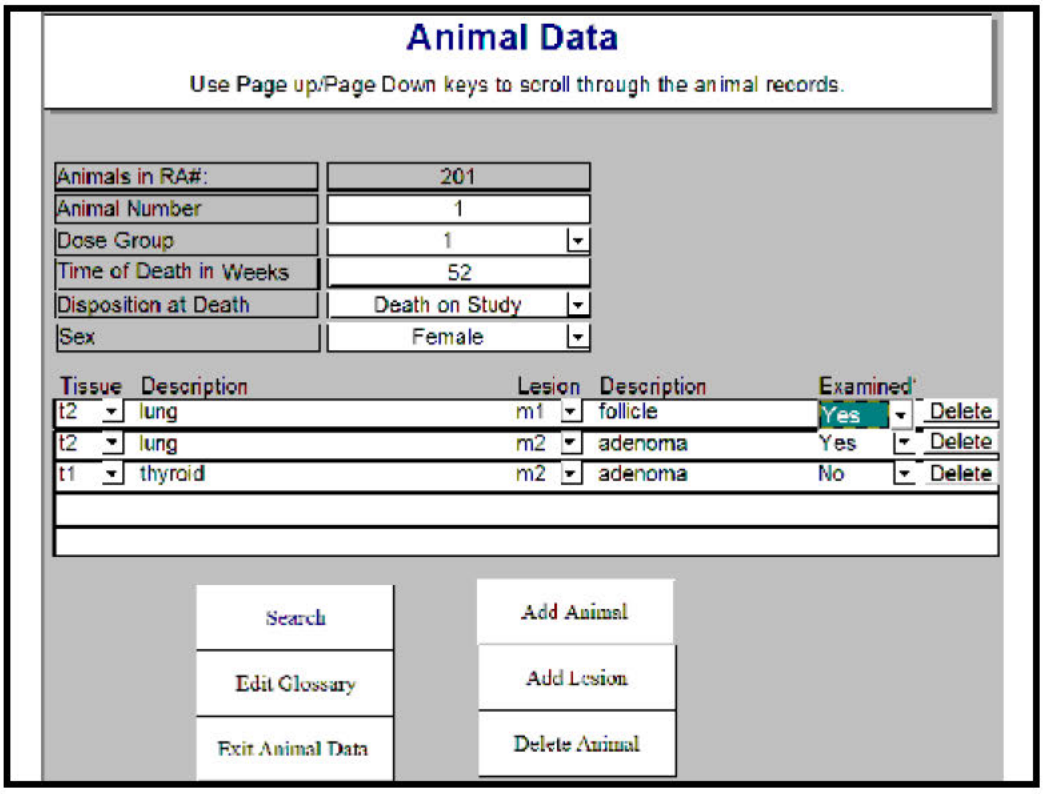
What to do when a tissue is not examined for some animals. When an animal is missing a tissue (listed under the **Tumors of Concern** on **Form 1. Qualitative Analysis Cover Form**), "**No**" must be selected from the drop down list in the "**Examined**" column for each lesion within that tissue (e.g., thyroid was not histologically examined for animal 1 in Fig. 7 below). This reduces the denominator in the Lesion Table by the number of animals not examined.

Tissues and lesions are deleted by clicking the **Delete** button to the right of the Tissue/Lesion Description.

If additional tissues/lesions, not already in the glossary, are identified at a later time, the user must click the **Edit Glossary** button to define each additional tissue/lesion.

WARNING:	Only the tissues and lesions present in the glossary for the selected Risk Assessment Record Number will be shown in the drop-down list.

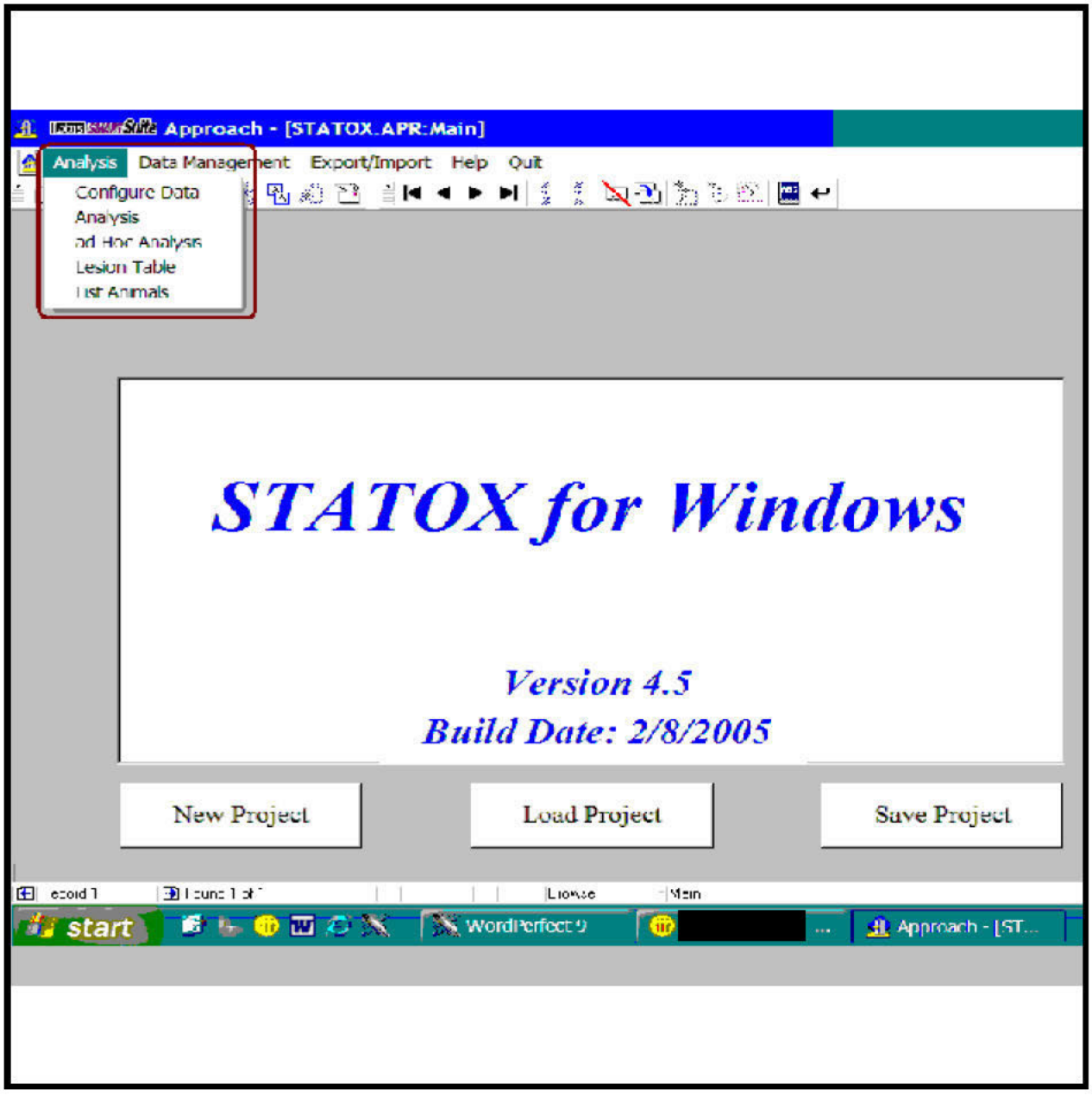
Figure 7: Entering Lesions in the Animal Data Form



Step 3: ANALYSIS MENU

Figure 8: Analysis Menu Located in the Main STATOX Window

The **Analysis** menu, located at the top left of the Main Window menu bar, contains options for **Configure Data**, **Analysis**, **ad Hoc Analysis**, **Lesion Table** and **List Animals** (Fig. 8).



Configure Data

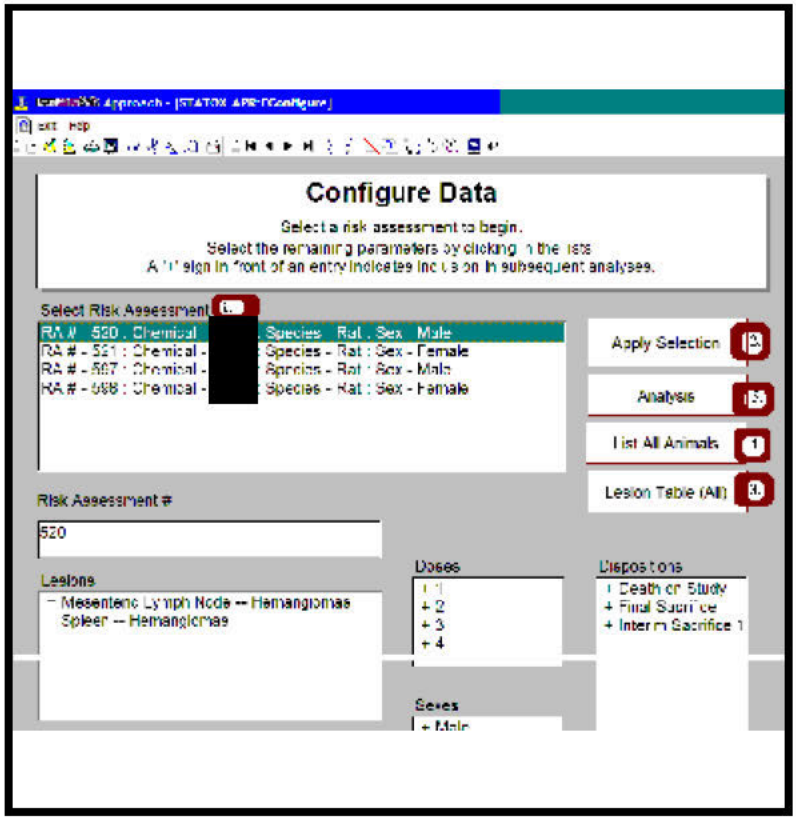
The **Configure Data** screen (Fig. 9) allows the user to choose subsets of each risk assessment for qualitative and quantitative analysis. Within each risk assessment, the data can be analyzed based upon **Lesions**, **Doses, Dispositions, Sexes,** and/or **Minimum TOD**. When a risk assessment is selected, all other fields are updated to reflect the possibilities for that specific risk assessment. Only one risk assessment can be analyzed at a time. Multiple elements for all other parameters can be chosen. Clicking the element makes the "+" mark beside the selection appear and disappear.

Before running any tumor analyses, the **Lesion Table**, **List All Animals**, **Mortality and Survival** analyses must be run as follows[1]:

1. Click on the desired **Risk Assessment** Record Number on the Configuration screen.

T The appropriate data for this Risk Assessment Record Number are displayed. All animals (Minimum TOD 0), all dispositions, all doses, all lesions are selected as indicated by a "+" mark.

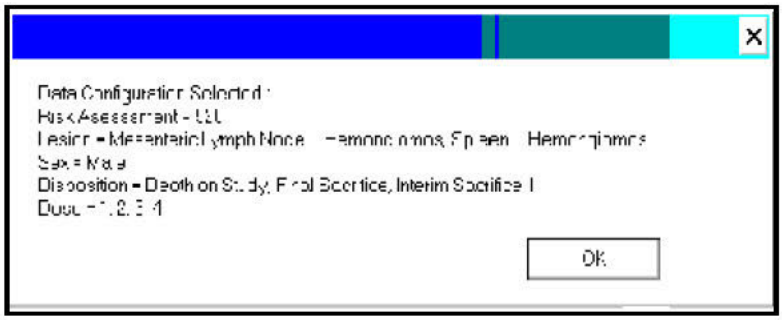
Figure 9: Configure Data Screen



Click the **Apply Selection** button. This insures that the chosen risk assessment is used for the analyses.

[1] The circled numbers (1-5) on the Configure Data Screen (Fig. 9) correspond to the numbered information in the following text.

Figure 10: Configuration Window



When the **Apply Selection** button is clicked, a pop-up window (Fig. 10) will appear with the current configuration selection listed. Check the information for accuracy. Click on the **OK** button to close this window.

If the information is not correct, changes can be made to the configuration. If the configuration is changed, click the **Apply Selection** button again to confirm changes.

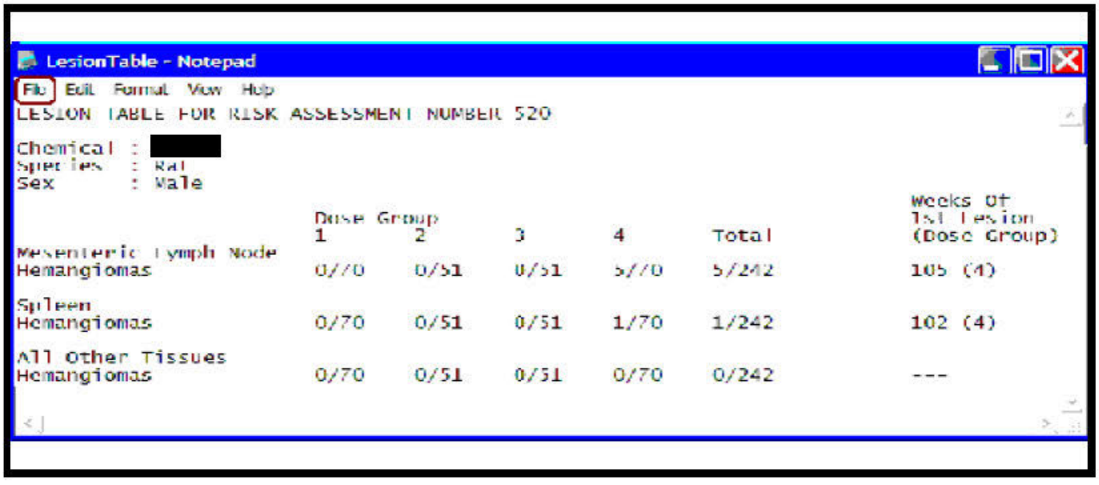
2.Click the **Lesion Table (All)** button on the **Configure Data** screen (Fig. 9) to run the **Lesion Table** (Fig. 11).

The Lesion Table is a pop-up window that displays all lesions with a ratio of tumors observed to animals at risk by dose group. This table is used as a QC/QA check of data entry (see Appendix 2). It should be compared to the tumor summaries in both the DER and the study report.

Once the Lesion Table has been created, it should be printed and saved electronically into the appropriate chemical subdirectory with the appropriate Risk Assessment Record Number in the file name (e.g., Fig. 11 output could be saved as **C:/Statox4.5/chemical name/520LesionTable.txt**). Since these are your working files, name them so you can distinguish them from each other.

TIP:	The Lesion Table is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly into Word. Click **OK/YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Figure 11: Lesion Table



Click the **List All Animals** button on the **Configure Data** screen (Fig. 9) to display the **Animal List Table** in a pop-up window (Fig. 12).

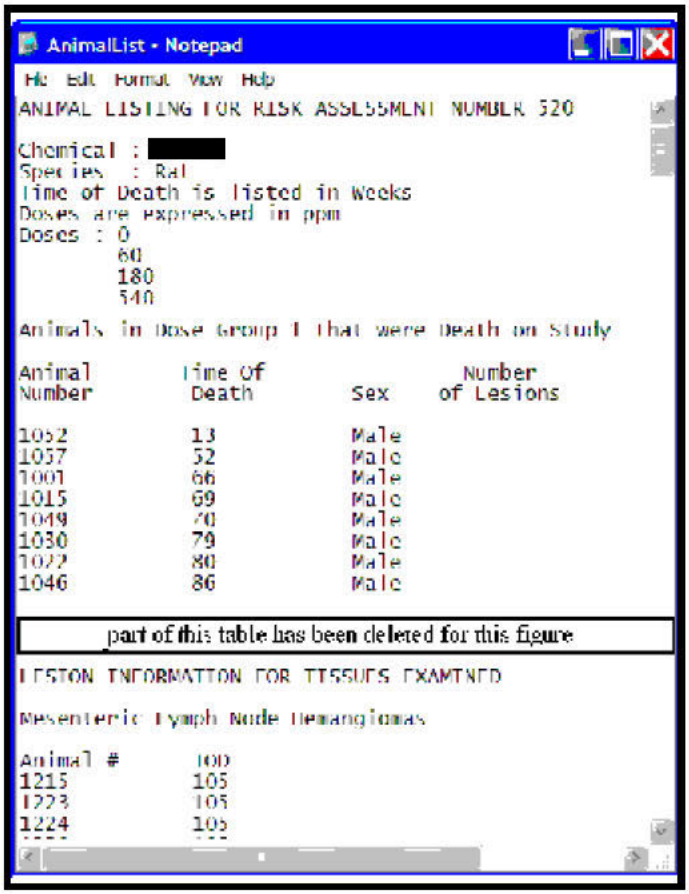
The **Animals List** table displays all of the animals in the dataset. It is used as a QC/QA check of data entry (see **Appendix 2**). The list is sorted by:

- disposition at death and time of death within each dose group
- lesions examined, listed by dose group
- animals/tissues that were not examined.

Once the **Animal List** table has been created, it should be printed and saved electronically into the appropriate chemical subdirectory with the appropriate Risk Assessment Record Number in the file name (e.g., Fig. 11 output could be saved as **C:/Statox4.5/Chemical Name/520AnimalList.txt**).

TIP:	The Animal List table is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **doc** extension allows it to be opened directly into Word. Click **OK/YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Figure 12: Animal List Table



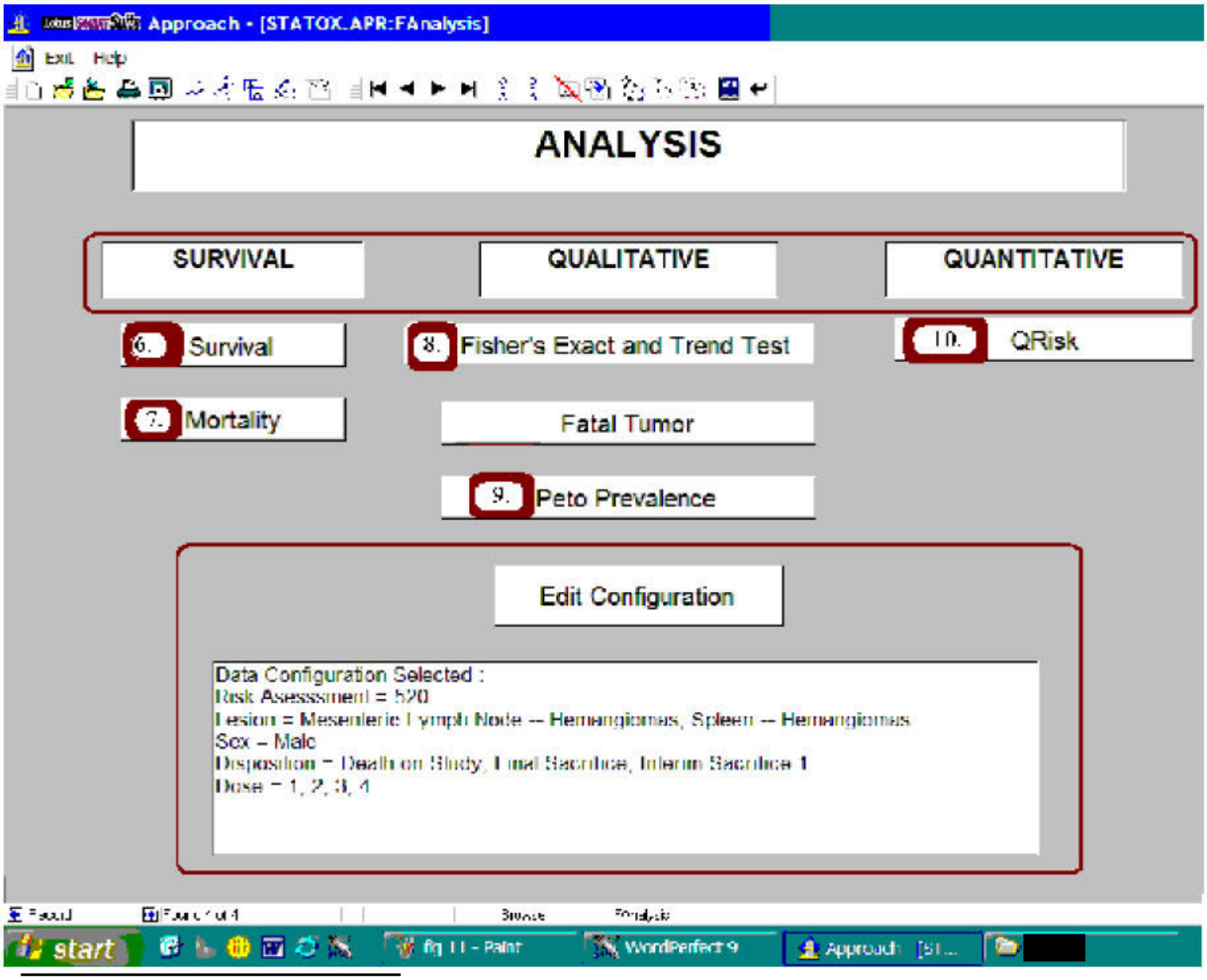
1. Click the **Analysis** button on the **Configure Data** screen (Fig. 9) to display the **Analysis selection** screen (Fig. 13).

Several statistical analyses are available on the Analysis Selection Screen.[2] These include **SURVIVAL**, **QUALITATIVE** and **QUANTITATIVE** analyses.

The current configuration information is displayed at the bottom of the screen. The displayed configuration will be used for any analyses chosen. Subsequent analyses will use this configuration until it is changed (use the **Edit Configuration** button just above the configuration display).

WARNING:	The same configuration may not be appropriate for all types of analyses.

Figure 13: Analysis Selection Screen



[2]The circled numbers (6-10) on the Analysis Selection Screen (Fig. 13) correspond to the numbered information in the following text.

Survival

1. To run the **Survival** analysis, click the **Survival** button on the Analysis selection screen (Fig. 13).

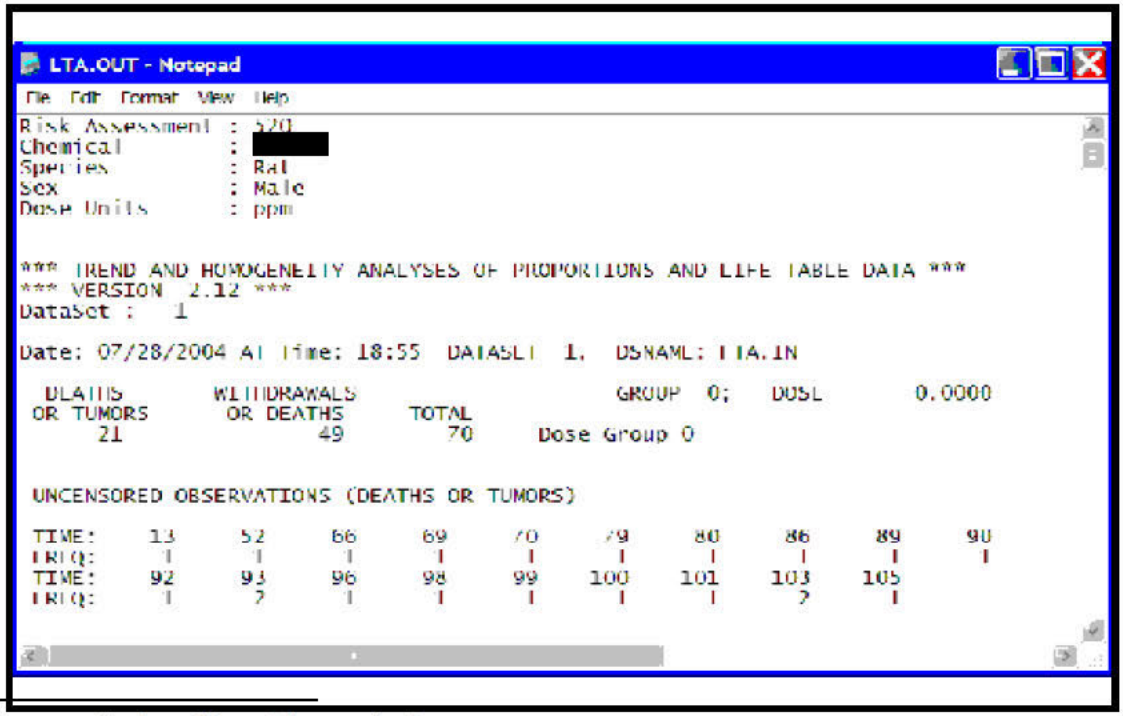
The **Survival Table** (called **LTA.OUT**[3]**)** is a pop-up window (Fig. 14) and provides three tests of the trend for survival, as well as pair-wise comparisons of the survival of each dose group versus the control.
The trend tests are:

- the Kaplan-Meier Survival Curve
- the Cox test
- the generalized K/W analysis (Gehan-Breslow)

Once the Survival table has been created, it should be printed and saved electronically into the appropriate chemical subdirectory with the appropriate Risk Assessment Record Number in the file name (e.g., Fig. 14 output could be saved as **C:/Statox4.5/Chemical Name/520Survival.txt**).

TIP:	The Survival table is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly into Word. Click **OK/YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Figure 14: Survival Table



[3] **LTA** stands for Life Table Analysis

If there are any significant probabilities for the Chi-square, the Lower Bound or the Upper Bound for the approximate tests for the unadjusted trend (Fig. 15), the adjusted trend (Fig. 16), or the Generalized K/W Analysis (Gehan-Breslow) (Fig. 17), then the trend is considered to be significant and this should be indicated on the Mortality Table (Fig. 20) in the Qualitative Risk Assessment Memorandum (to be completed in Step 4).

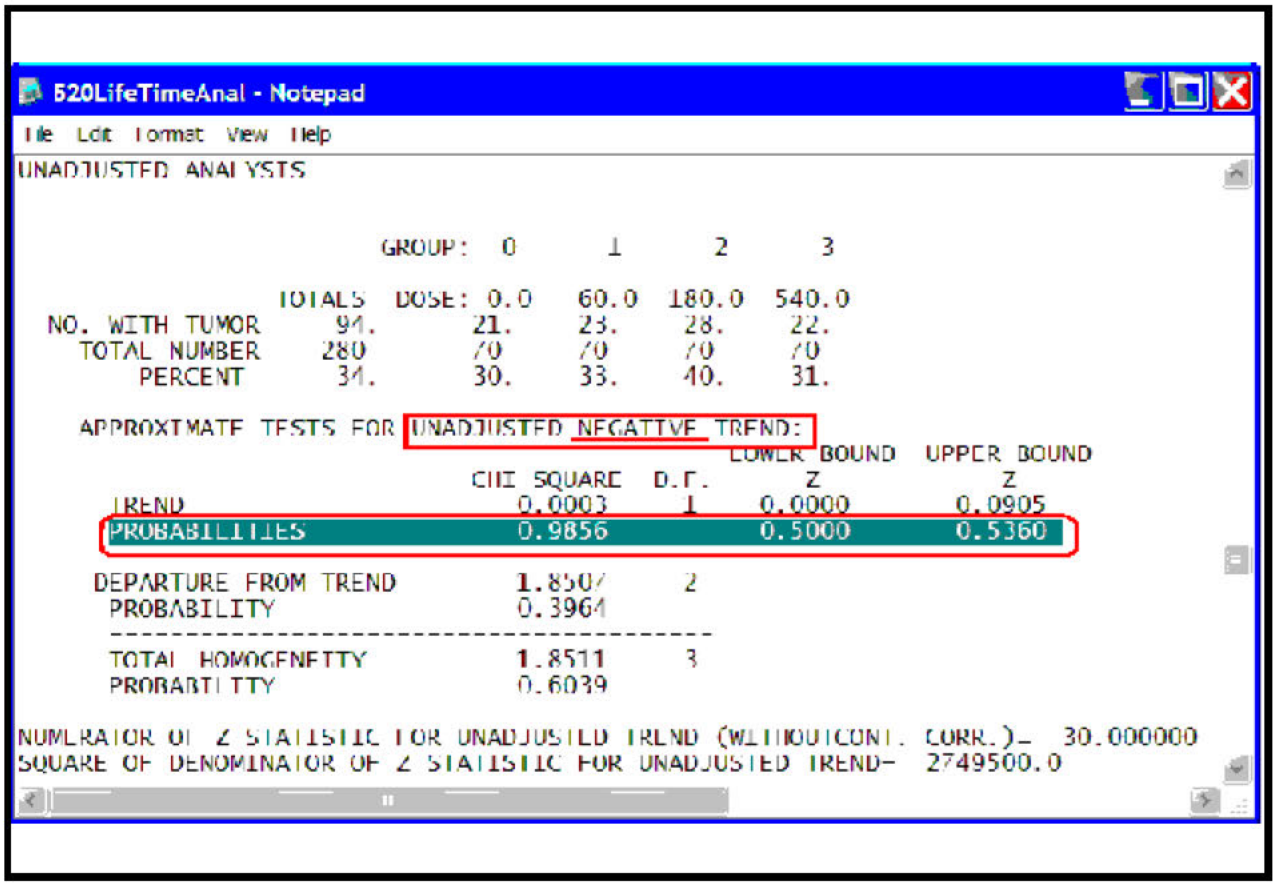
If there are any significant probabilities for the Cox's Test (Fig. 16) or the Generalized K/W Analysis (Fig. 17) for the control versus dosed groups, then the pair-wise comparison is considered to be significant and this should be indicated on the Mortality Table (Fig. 20) in the Qualitative Risk Assessment Memorandum.

Unadjusted Trend (Kaplan-Meier Survival Curve)

If any of these highlighted probabilities (Fig. 15) are less than or equal to 0.05, the survival test for trend is considered to be statistically significant.

This test indicates the direction of the trend between the words "unadjusted" and "trend" (in the example below, "negative").

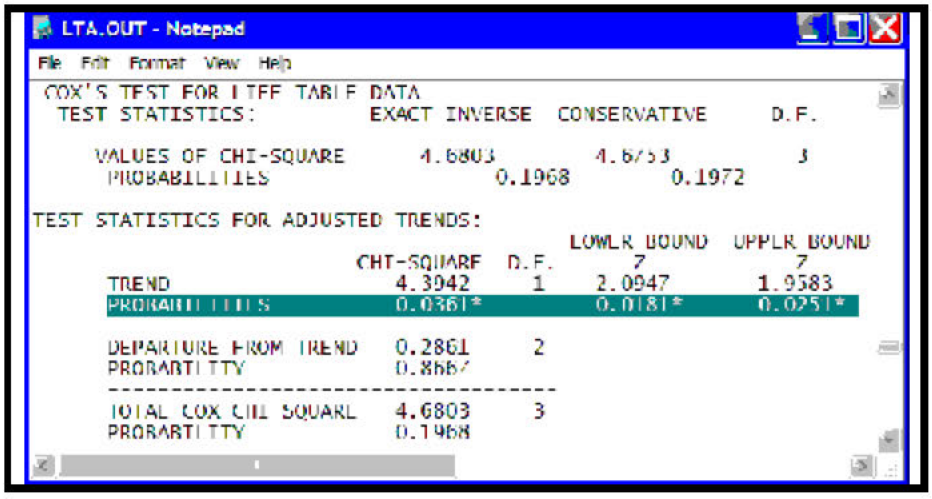
Figure 15: Unadjusted Trend from Survival Analysis Table



Adjusted Trend (Cox Test)

If any of these highlighted probabilities (Fig. 16) are less than or equal to 0.05, the survival test for trend is considered to be statistically significant.

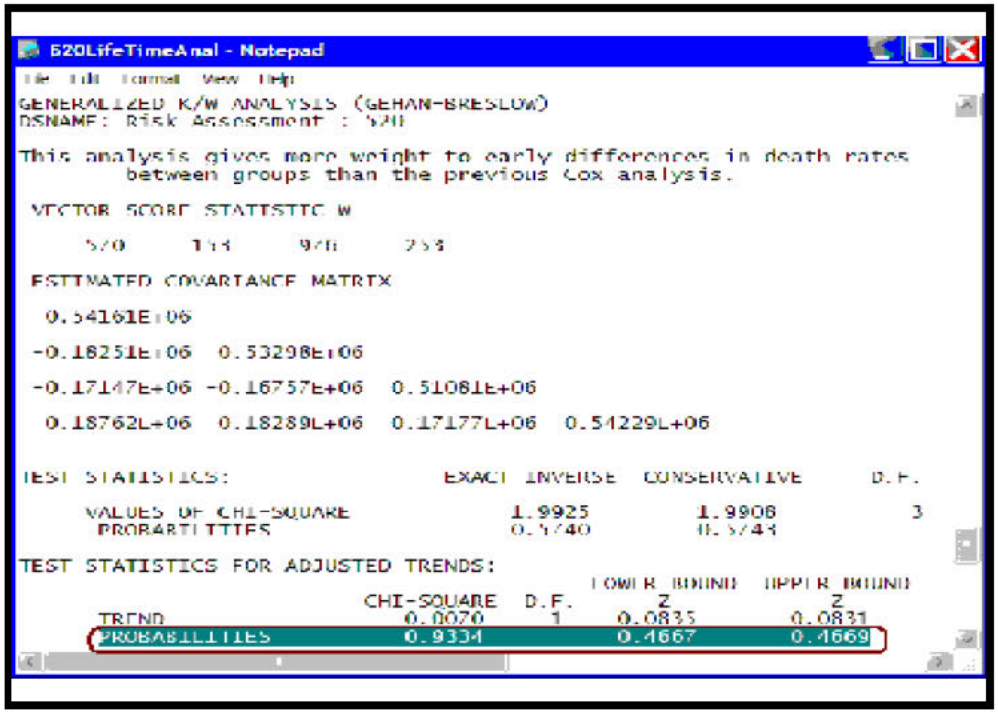
Figure 16: Adjusted Trend from Survival Analysis Table



Generalized K/W Analysis (Gehan-Breslow)

If any of these highlighted probabilities (Fig. 17) are less than or equal to 0.05, the survival test for trend is considered to be statistically significant.

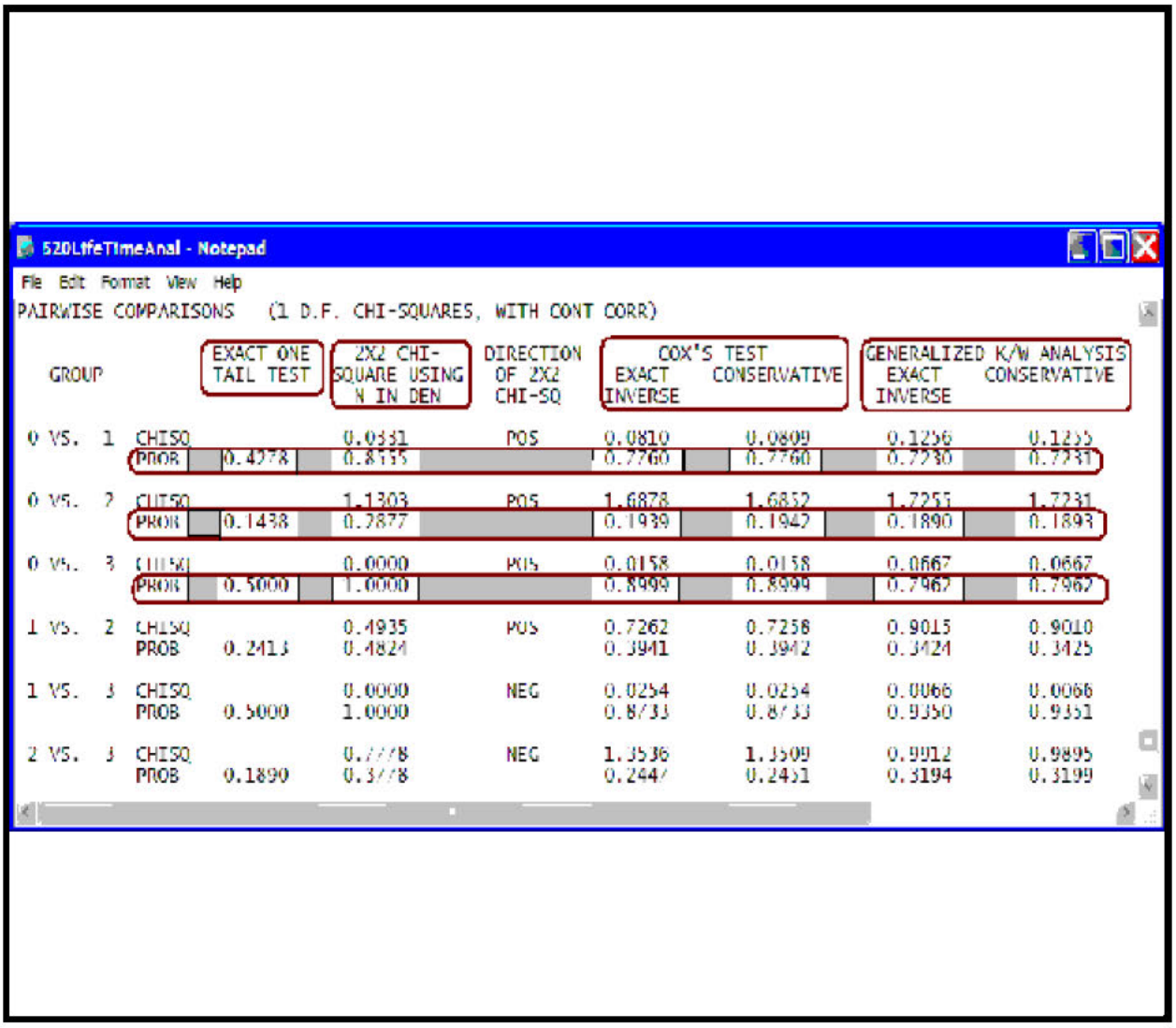
Figure 17: Generalized K/W Analysis-Survival Analysis Table



Survival Pair-Wise Comparisons

If any of the probabilities for the Exact One Tail Test, the 2X2 Chi-square, the Cox's Test (exact inverse or conservative) or the Generalized K/W Analysis (exact inverse or conservative) for the control versus dosed groups (0 vs. 1, 0 vs. 2, etc.) are less than or equal to 0.05, the pair-wise comparison of that particular dose group with the control group is considered to be statistically significant (Fig. 18).

Figure 18: Pair-Wise Comparisons from Survival Analysis Table



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PAIRWISE COMPARISONS (1 D.F. CHI-SQUARES, WITH CONT CORR)

GROUP		EXACT ONE TAIL TEST	2X2 CHI-SQUARE USING N IN DEN	DIRECTION OF 2X2 CHI-SQ	COX'S TEST EXACT INVERSE	COX'S TEST CONSERVATIVE	GENERALIZED K/W ANALYSIS EXACT INVERSE	GENERALIZED K/W ANALYSIS CONSERVATIVE
0 VS. 1	CHISQ		0.0331	POS	0.0810	0.0809	0.1256	0.1255
	PROB	0.4078	0.8555		0.7760	0.7760	0.7230	0.7231
0 VS. 2	CHISQ		1.1303	POS	1.6878	1.6852	1.7251	1.7231
	PROB	0.1438	0.2877		0.1939	0.1942	0.1890	0.1893
0 VS. 3	CHISQ		0.0000	POS	0.0158	0.0158	0.0667	0.0667
	PROB	0.5000	1.0000		0.8999	0.8999	0.7967	0.7967
1 VS. 2	CHISQ		0.4935	POS	0.7262	0.7258	0.9015	0.9010
	PROB	0.2413	0.4824		0.3941	0.3942	0.3424	0.3425
1 VS. 3	CHISQ		0.0000	NEG	0.0254	0.0254	0.0066	0.0066
	PROB	0.5000	1.0000		0.8733	0.8733	0.9350	0.9351
2 VS. 3	CHISQ		0.7778	NEG	1.3536	1.3509	0.9912	0.9895
	PROB	0.1890	0.3778		0.2447	0.2451	0.3194	0.3199

Mortality Analysis

To run the **Mortality** analysis, click the **Mortality** button on the Analysis selection screen (Fig. 13).

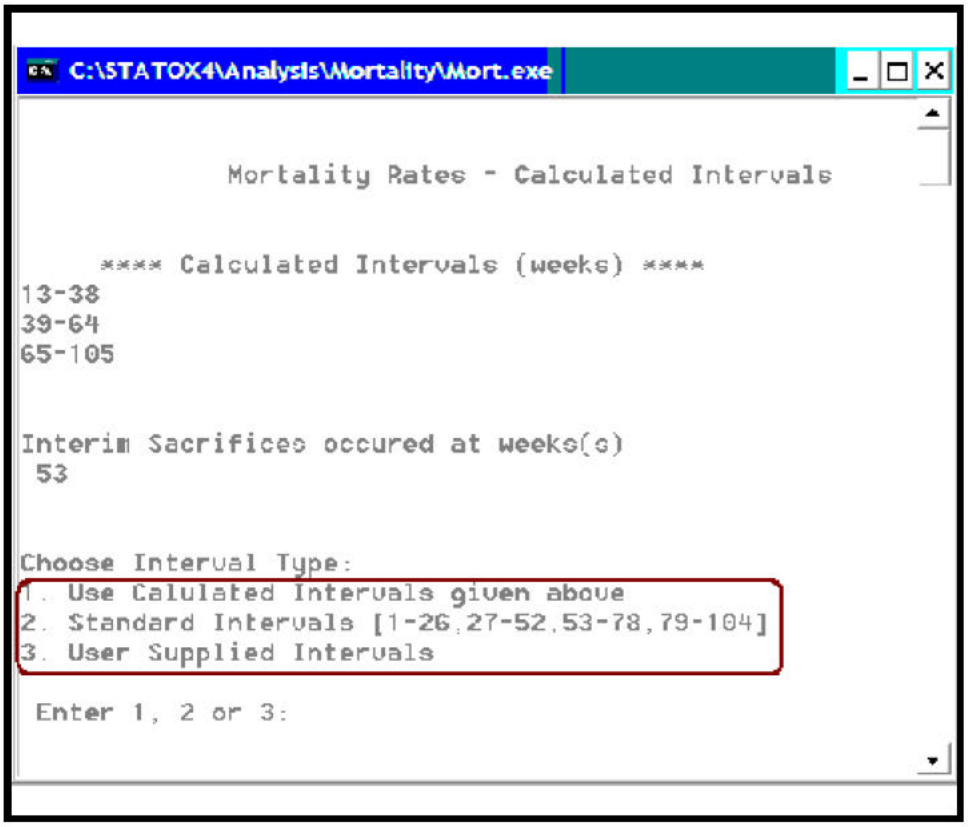
A series of prompts are displayed in a pop-up window (Fig. 19) that must be completed to continue with the Mortality Analysis.

There are 3 interval choices for the Mortality Analysis. Enter 1, 2, or 3 at the prompt and press **Enter**.

Option 1. Use Calculated Intervals given above (not used) Option 2. Standard Intervals (used most often, always assumes 104 weeks) Option 3. User Supplied Intervals (used when the standard sacrifice interval protocols are not followed– when the study duration is not 104 weeks or the study has more than one interim sacrifice)

Options 1 and 2 will calculate the mortality results using the indicated number and duration of intervals. For option 3, the user will be prompted to enter the number of intervals and the range for each.

Figure 19: Interval Options for Mortality Analysis

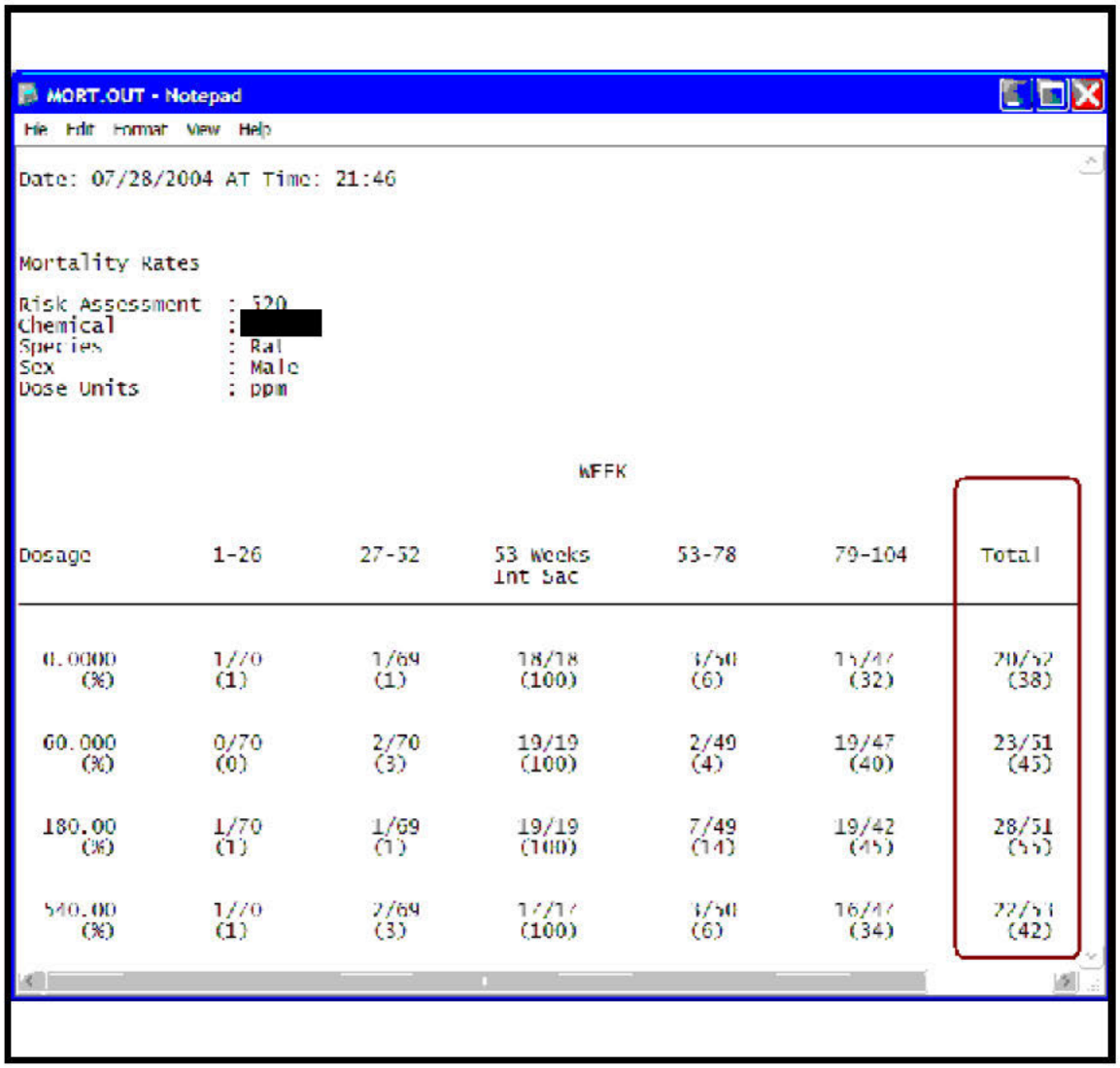


Mortality Results with Standard Intervals

Figure 20 is an example of a Mortality Analysis using Option 2, the Standard Intervals.

REMINDER:	When preparing the qualitative risk assessment memorandum, statistical significance of: □ **trend** (obtained from Survival Analysis) should be noted only at the control group in the Total column of the Mortality Table. □ **pair-wise comparisons** (obtained from Survival Analysis) should be noted at the appropriate dosage group in the Total column on the Mortality Table.

Figure 20: Mortality Analysis Using Standard Intervals



MORT.OUT - Notepad

File Edit Format View Help

Date: 07/28/2004 AT Time: 21:46

Mortality Rates

Risk Assessment : 520
Chemical : [redacted]
Species : Rat
Sex : Male
Dose Units : ppm

Dosage	WEEK 1-26	27-52	53 Weeks Int Sac	53-78	79-104	Total
0.0000 (%)	1/70 (1)	1/69 (1)	18/18 (100)	3/50 (6)	15/47 (32)	20/52 (38)
60.000 (%)	0/70 (0)	2/70 (3)	19/19 (100)	2/49 (4)	19/47 (40)	23/51 (45)
180.00 (%)	1/70 (1)	1/69 (1)	19/19 (100)	7/49 (14)	19/42 (45)	28/51 (55)
540.00 (%)	1/70 (1)	2/69 (3)	17/17 (100)	3/50 (6)	16/47 (34)	22/53 (42)

Qualitative Analysis

There are 3 **Qualitative Analysis** options in the Analysis Selection Screen (Fig. 13):

- Fisher's Exact and Trend Test
- Fatal Tumor
- Peto Prevalence

The **Fisher's Exact and Trend Test** is run when there are no significant survival disparities between the dosed groups and the control group (i.e., no statistical significance).

The **Fatal Tumor** is run when the tumor of interest is determined to have been fatal to the animals (this is EXTREMELY rare).

The **Peto Prevalence** test is run when there are significant survival disparities between the dosed groups and the control group.

IMPORTANT NOTE:	Before any qualitative analyses can be run, click the **Edit Configuration** button at the bottom of the **Analysis** Selection Screen to include only the information appropriate for the desired analysis.

Data Configuration for Fisher's Exact and Trend Test

To configure data for **Fisher's Exact and Trend Test** (refer to **Configure Data** screen, Fig. 9, or the **Analysis screen**, Fig. 13):

1. Select **Risk Assessment Record Number** of interest.
2. Select (with "+" mark) appropriate **Lesion(s),** separately and combined. For questions regarding appropriate combinations of tumor morphology, consult HED's pathologist.
3. Select **Doses** (all doses should be selected as the default)
4. Select **Dispositions**

   Death on Study and **Final Sacrifice** should always be selected ("+" mark)

   Accidental Kills before 52 Weeks: Animals that are accidentally killed (accidental kills) before 52 weeks, with or without tumors, are usually excluded from analysis. However, when the first tumor occurs (in a death on study animal) before the accidental kill, accidental kill animals are included in the analysis. The Qualitative Memorandum should contain a footnote listing the excluded accidental kills with tumors, indicating the week of death and dose group.

   Interim sacrifice animals and **Minimum Time of Death (TOD)**:

IF the tumor selected first occurs	**THEN**
before interim sacrifice: **or at** interim sacrifice **AND** the tumor is in a death on study animal:	Include interim sacrifice animals and adjust **Minimum TOD** (in weeks) to time of first lesion (refer to **Lesion** Table (Fig. 11) and **List All Animals** Table (Fig. 12) to determine time of first lesion and/or disposition).
after interim sacrifice: **or** **at** interim sacrifice **AND** the tumor is in an interim sacrifice animal:	Exclude interim sacrifice animals and adjust **Minimum TOD** (weeks) to interim sacrifice week plus one week (refer to **Lesion** Table (Fig. 11) and **List All Animals** Table (Fig. 12) to determine time of first lesion and/or disposition); make note on table in qualitative risk assessment memorandum indicating how many animals in interim sacrifice group had tumor and in which dose groups they occurred.

Click the **Apply Selection** button. When the **Apply Selection** button is clicked, a pop-up window (Fig. 10) will appear with the current configuration selection listed. Check the information for accuracy. Click on the **OK** button to close this window.

Click the **Analysis** button on the **Configure Data** screen (Fig. 9) to display the **Analysis selection** screen (Fig. 13).

Fisher's Exact and Trend Test

To run **Fisher's Exact and Trend Test**, click **Fisher's Exact and Trend Test** button on Analysis selection screen (Fig. 13).

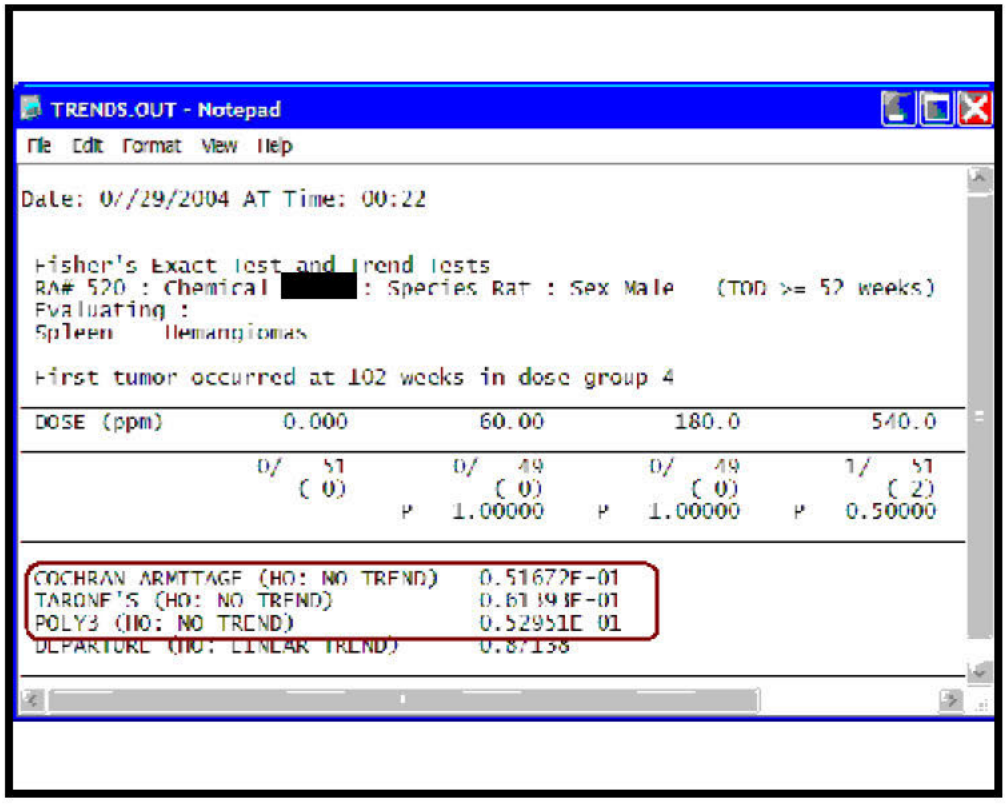
The resulting pop-up window (Fig. 21) provides a table containing pair-wise comparisons of the control versus dose groups and three tests of the trend.

Once the **Fisher's Exact and Trend Test** table has been created, it should be printed and saved electronically into the appropriate chemical subdirectory with the appropriate Risk Assessment Record Number and tissue/tumor morphology added to the file name (e.g., Fig. 21 output could be saved as **C:/Statox4.5/Chemical Name/520spleenhemanFishersTest.txt**).

Click **Edit Configuration** before running the next **Fisher's Exact and Trend Test** analysis. Repeat steps for **Data Configuration for Fisher's Exact and Trend Test** for each tissue/ tumor morphology combination.

TIP:	The **Fisher's Exact and Trend Test** is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly Word. Click **YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Figure 21: Fisher's Exact Test and Trend Test Table



Explanation of Fisher's Exact Test and Trend Tests Table (Fig. 21):

The **Fisher's Exact test** evaluates pair-wise comparisons of the treated dose groups with the controls. The output table consists of columns for the control and each treated dose group. The first two rows under each dose contain the tumor count and percentage of tumors (the number in parenthesis). The third row indicates the statistical significance (p-values) for each treated dose group versus the control (i.e., the statistical significance of the pair-wise comparison of the 540 ppm dose group with the controls is p = 0.50000).

There are 3 trend tests:

- The **Cochran-Armitage test** is the linear trend test usually used in HED reports. **See exception in the footnote.**[4]

- **Tarone's test** is also a test of linear trend, however, it adjusts for animals that died before the first occurrence of the tumor. Instead of using the total number of animals examined for the response of interest, Tarone's test uses only those animals that were examined for the response of interest and have a time of death greater than or equal to the time of first occurrence of that tumor.

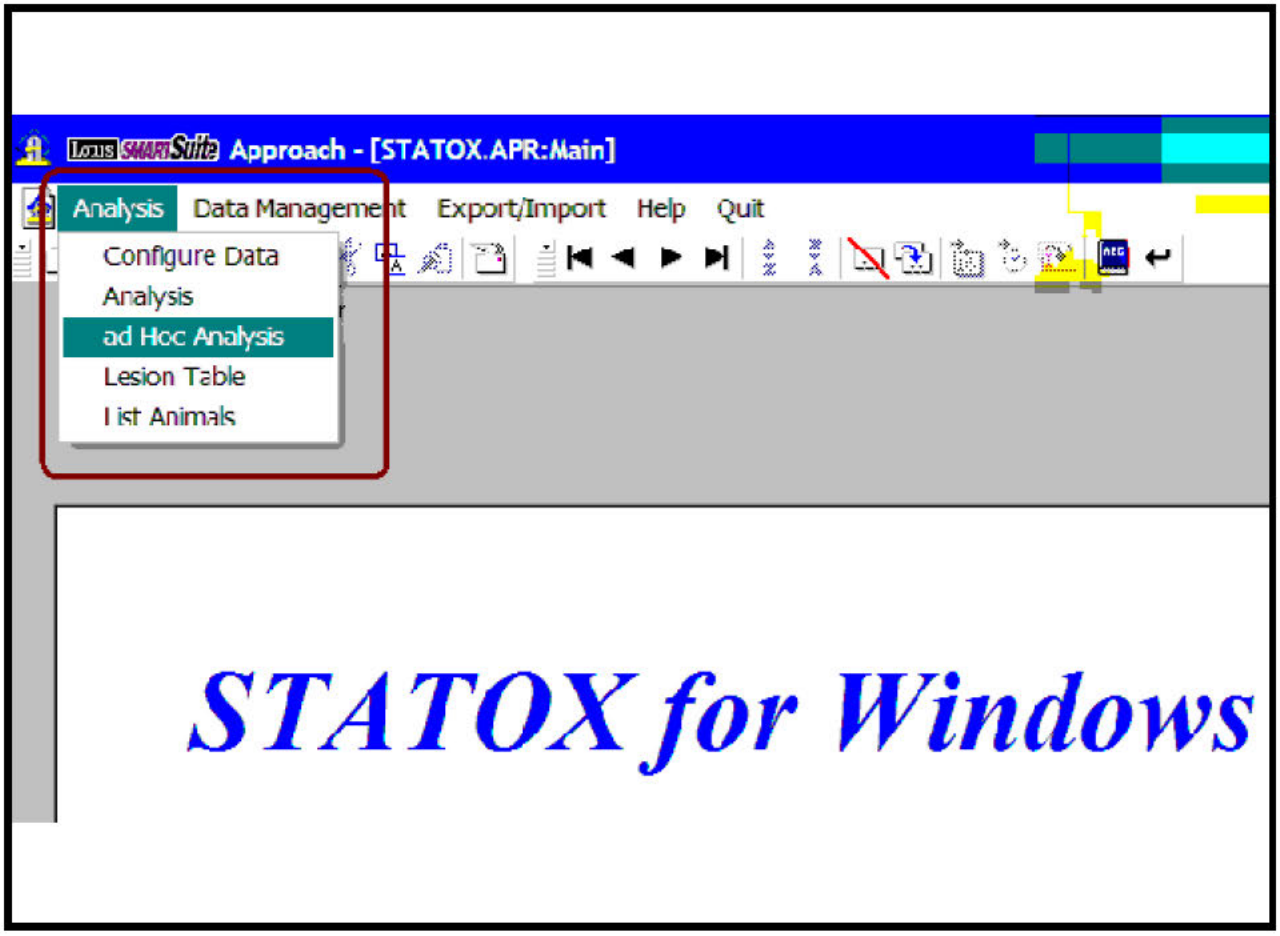
- The **Poly-3 test** is a test of trend and also makes an adjustment for animals that died before the first occurrence of tumor. However, instead of using the total number of animals examined for the response of interest as with Tarone's test, the Poly-3 test weights each animal and uses the sum of these weights in computing the test statistic.

[4] For "small" (< 10) numerators (tumor counts), the **Exact test for trend** is more precise than any of the trend analyses of the Fisher's Exact and Trend Test (Fig. 21). The **ad Hoc Analysis** (Fig. 23) should be run to determine the Exact test for trend.

ad Hoc Analysis

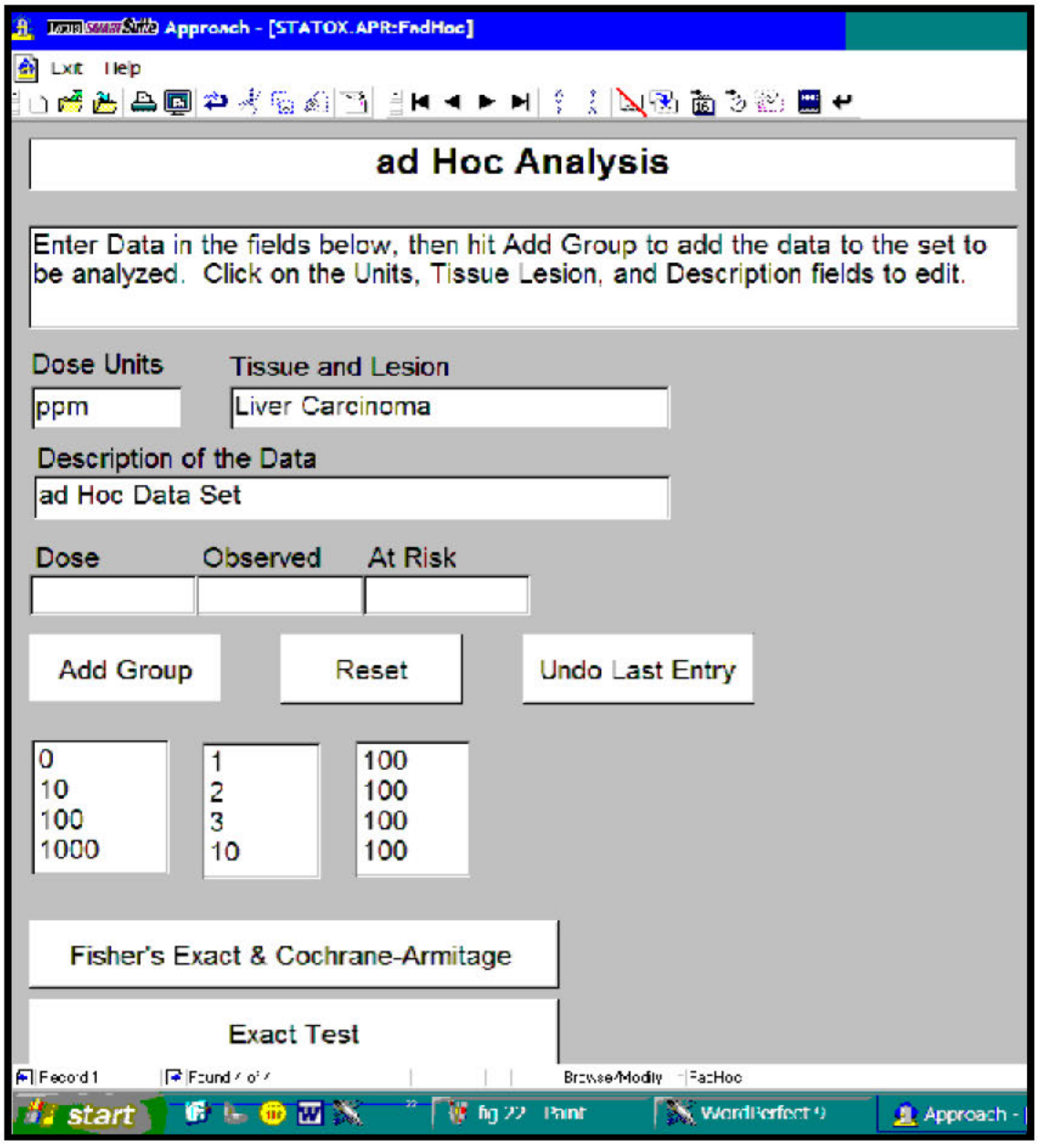
To run the **ad hoc Analysis**, exit to the Main STATOX Window (Fig. 22). Click the **Analysis** button from the Menu bar at the top left. Select **ad Hoc Analysis** from the drop down menu. This will retrieve the STATOX **ad Hoc Analysis** data entry screen (Fig. 23).

Figure 22: Main STATOX Window: ad Hoc Analysis Selection



Data for **ad Hoc Analyses** are entered in the **ad Hoc Analysis** data entry screen (Fig. 23). **Dose Units**, **Tissue and Lesion**, and **Description of the Data** are filled in or changed by clicking in the appropriate box, and then entering the new data. For each dose group fill in the **Dose**, **Observed** (# of tumor bearing animals), and **At Risk** (# of animals at risk) as follows. When the **Dose**, **Observed**, and **At Risk** data have been entered for a specific dose, click on the **Add Group** button to commit the tumor data to the analysis set. The tumor data can be cleared by clicking the **Reset** button (Click **NO** in the pop up box; clicking **YES** will clear all fields in this screen). Click the **Undo Last Entry** button to clear the last dose group entered. Only the **Fisher's Exact** & **Cochran-Armitage** test and the **Exact Test** for trend can be computed in the **ad Hoc Analysis**.

Figure 23: ad Hoc Analysis Data Entry Screen

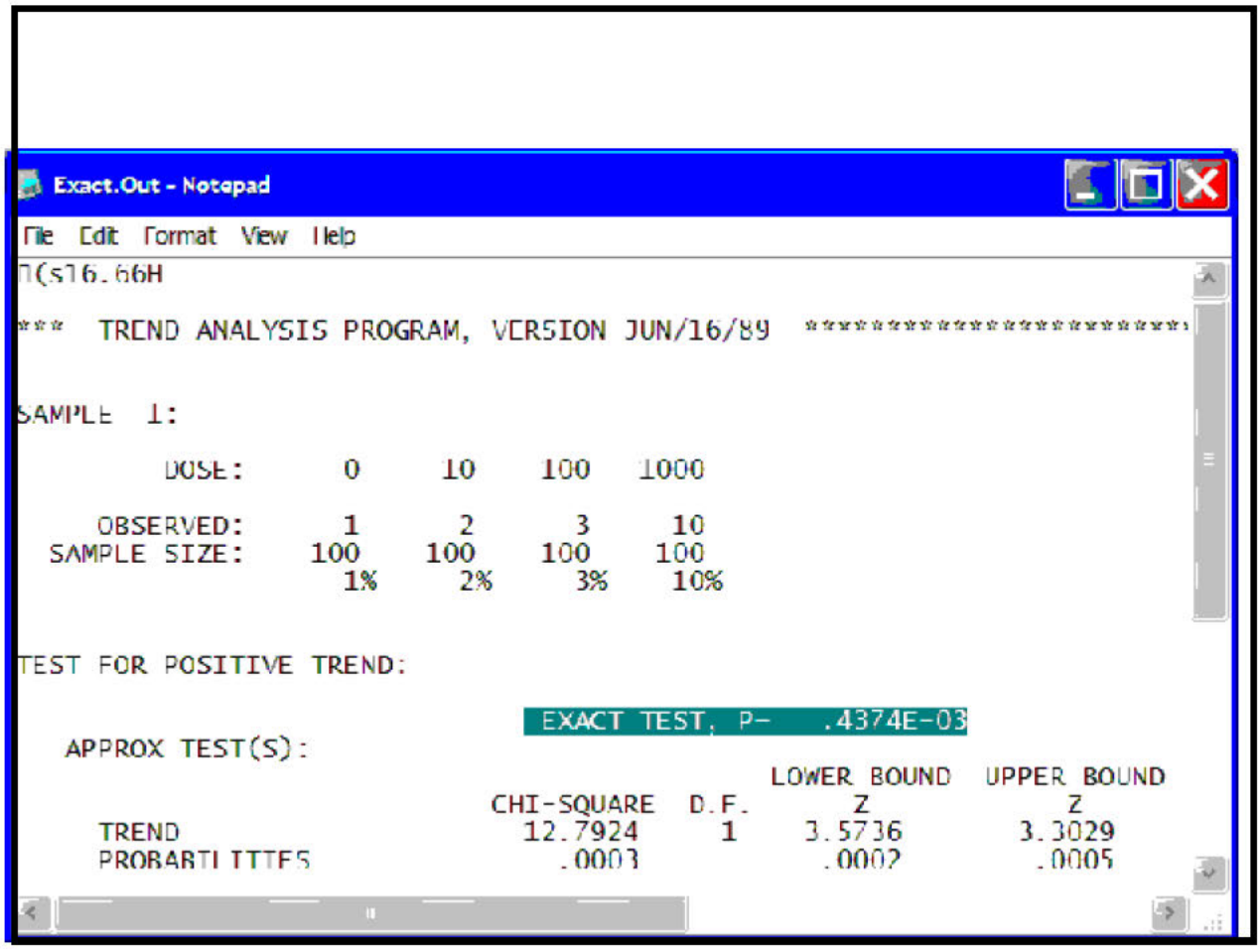


ad Hoc Analysis Output

Selecting the **Fisher's Exact & Cochrane-Armitage** button results in the same output table as shown in Figure 21.

Selecting the **Exact Test** button results in the output table as shown in Figure 24. If the p-value indicated in the highlighted area, "exact test, p = .4374E-03" (Fig. 24), is less than or equal to 0.05, then the exact test for trend is considered to be statistically significant. This value should be reported as the trend for this particular tumor morphology in the Qualitative Risk Assessment Memorandum.

Figure 24: Exact Test results



Fatal Tumor

This analysis is not currently included in OPP's battery of statistical tests.

Data Configuration for Peto Prevalence Test

To configure data for **Peto Prevalence Test** (refer to **Configure Data** screen, Fig. 9, or the Analysis screen, Fig. 13):

1. Select **Risk Assessment Record Number** of interest.

2. Select (with "+" mark) appropriate **Lesion(s),** separately and combined. For questions regarding appropriate combinations of tumor morphology, consult HED's pathologist.

3. Select **Doses** (all doses should be selected as the default)

4. Select **Dispositions**

   Death on Study and **Final Sacrifice** should always be selected ("+" mark)

   Accidental Kills: Animals that are accidentally killed (accidental kills), with or without tumors, are usually excluded from analysis. However, when the first tumor occurs (in a death on study animal) before the accidental kill, accidental kill animals are included in the analysis. The Qualitative Memorandum should contain a footnote listing the excluded accidental kills with tumors, indicating the week of death and dose group.

   Interim sacrifice animals and **Minimum Time of Death (TOD)**:

IF the tumor selected first occurs	**THEN**
before interim sacrifice: **or at** interim sacrifice **AND** the tumor is in a death on study animal:	Include interim sacrifice animals and adjust **Minimum TOD** (in weeks) to time of first lesion (refer to **Lesion** Table (Fig. 11) and **List All Animals** Table (Fig. 12) to determine time of first lesion and/or disposition).
after interim sacrifice: **or** **at** interim sacrifice **AND** the tumor is in an interim sacrifice animal:	Exclude interim sacrifice animals and adjust **Minimum TOD** (weeks) to time of first lesion in a death on study animal (refer to **Lesion** Table (Fig. 11) and **List All Animals** Table (Fig. 12) to determine time of first lesion and/or disposition); make note on table in qualitative risk assessment memorandum indicating how many animals in interim sacrifice group had tumor and in which dose groups they occurred.

Click the **Apply Selection** button. When the **Apply Selection** button is clicked, a pop-up window (Fig. 10) will appear with the current configuration selection listed. Check the information for accuracy. Click on the **OK** button to close this window.

To run the **Peto Prevalence test**, click the **Peto Prevalence** button on Analysis screen (Fig. 13). Fig. 25 will pop up.

Peto Prevalence without Accidental Kills:

1. Select **Do Not Use In Peto Analysis**.
2. Then click **Continue**.

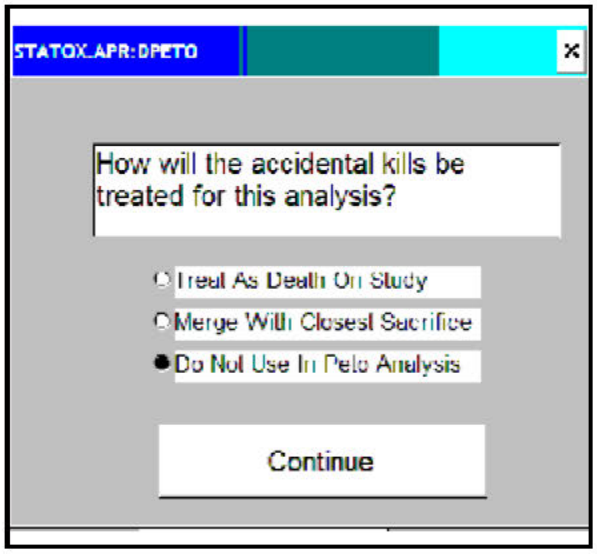
Peto Prevalence with Accidental Kills

If accidental kills are present in the experimental data being analyzed, the user is given three options for handling them before calculation of the intervals begins (Fig. 25):

1. merge them with the closest sacrifice period
2. exclude then from analysis
3. treat them as a death on study

Traditionally, accidental kills are treated as follows:

Figure 25: Options for Handling Accidental Kills



- Accidental kills WITH a tumor where the accidental kill occurred before the observation of the first tumor should be excluded from the analysis, but footnoted on the tumor table of the qualitative risk assessment memorandum.

- Accidental kills WITH a tumor where the accidental kill occurred during a sacrifice period (interim or final) should be merged with the closest sacrifice.

- Accidental kills WITH a tumor where the accidental kill occurred after the observation of the first tumor should be treated as a death on study unless the accidental kill occurred during a sacrifice period (interim or final), in which case the accidental kill should be merged with the closest sacrifice.

- Accidental kills WITHOUT tumors should always be excluded from the analysis.

NOTE:	Accidental kills with a tumor always take priority over accidental kills without a tumor.

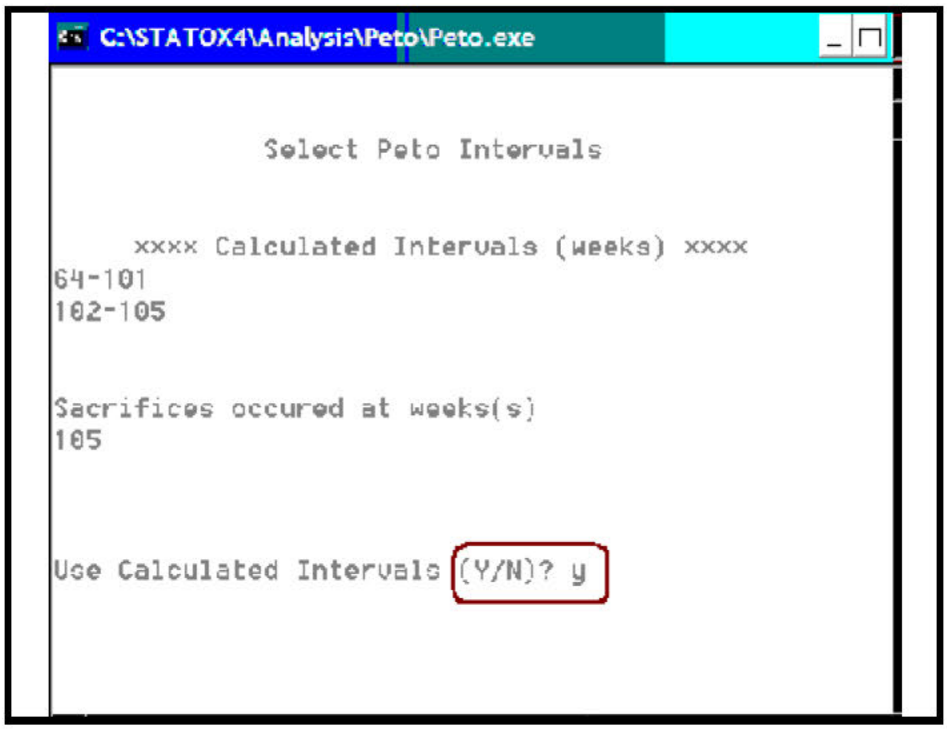
Peto Prevalence Intervals

The first portion of Figure 26 will pop-up when the **Continue** button is pressed (Fig. 25). A series of prompts must be completed in order to continue with the analyses.

The Calculated Intervals are used. The beginning of the first calculated interval should match the minimum TOD (weeks).

Type "Y" at the prompt, then click **Enter** to accept these intervals.

Figure 26: Peto Analysis - Accept Calculated Intervals



Peto Prevalence Test

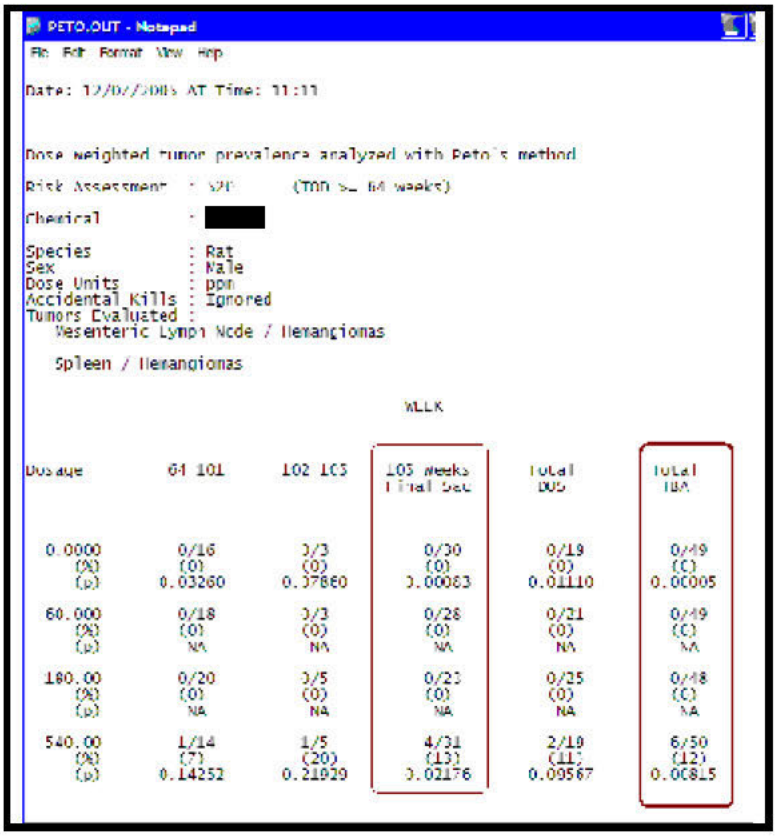
The main column of concern is the **Total TBA** (total tumor bearing animals) column. However, if the first tumor is observed in a final sacrifice animal, use only the **Final Sac** column. If the p-value for the control group is less than or equal to 0.05, then the trend is considered to be statistically significant for the Peto Prevalence test. If any of the p-values for the dosed groups are less than or equal to 0.05, then the pair-wise comparison of that dose group with the control group is considered to be statistically significant.

Once the Peto's Prevalence table has been created, it should be printed or saved electronically into the appropriate chemical subdirectory with the appropriate risk assessment number and tissue and tumor type added to the file name (e.g., Fig. 27 output could be saved as **C:/Statox4.5/Chemical Name/520spleenlnhemanPeto.txt**).

TIP:	The **Peto Prevalence Test** is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly into Word. Click **YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Click **Edit Configuration** before running next Peto Prevalence analysis. Repeat steps for **Data Configuration for Peto Prevalence Test** for each tissue and tumor type combination.

Figure 27: Peto Prevalence Test



PETO.OUT - Notepad

File Edit Format View Help

Date: 12/07/2005 AT Time: 11:11

Dose Weighted tumor prevalence analyzed with Peto's method

Risk Assessment : 520 (TDD >= 64 weeks)

Chemical : [redacted]

Species : Rat
Sex : Male
Dose Units : ppm
Accidental Kills : Ignored
Tumors Evaluated :
Mesenteric Lymph Node / Hemangiomas

Spleen / Hemangiomas

WLLK

Dosage	64 101	102 103	103 Weeks Final Sac	Total DOS	Total TBA
0.0000	0/16	3/3	0/30	0/19	0/49
(%)	(0)	(0)	(0)	(0)	(0)
(p)	0.03260	0.37860	0.00063	0.01110	0.00005
60.000	0/18	3/3	0/28	0/21	0/49
(%)	(0)	(0)	(0)	(0)	(0)
(p)	NA	NA	NA	NA	NA
180.00	0/20	3/5	0/23	0/25	0/48
(%)	(0)	(0)	(0)	(0)	(0)
(p)	NA	NA	NA	NA	NA
540.00	1/14	1/5	4/31	2/10	6/50
(%)	(7)	(20)	(13)	(11)	(12)
(p)	0.14252	0.21929	0.02176	0.09567	0.00815

Step 4: QUALITATIVE MEMORANDUM

After the Qualitative Statistical Analyses are completed, the statistician obtains a TXR number for the memorandum. The electronic copy of the final memorandum is saved in IHAD with the TXR number that is assigned to it. A signed hard copy of this memo is sent to the requesting toxicologist for inclusion in the CARC briefing package via the HED Plum Folder Process.

The CARC evaluates the carcinogenic potential of the pesticide in question and determines the need for additional statistical evaluation (i.e., Quantitative Statistical Analysis). Nothing additional is required unless requested by the CARC.

Step 5: QUANTITATIVE ANALYSIS

Quantitative analyses of the tumor data are conducted using the QRisk program. As input, QRisk uses either data specifically formulated for the program by the STATOX program or data entered on an ad Hoc basis (**StandAlone QRisk**). The key output of QRisk is the generation of the $\mathbf{Q_1}$*, the slope factor used to quantify the potential cancer risk of a chemical to humans.

There are **two models** for quantitative risk used in HED:

1. A **Quantal model** (**Multistage model**)
2. A **Time-to-Tumor model** (**Multistage Weibull model**)

The Multistage Quantal model is run when the following two conditions are met:

1. There are **no** significant **survival disparities** between treated and control groups, **and**
2. A Fisher's Exact Test and either Cochran-Armitage or Exact Trend test has been conducted for qualitative analysis.

The Time-to-Tumor Multistage Weibull model is run when:

1. there are significant survival disparities between treated and control groups, **and**
2. a Peto Prevalence test has been performed for qualitative analysis.

Mortality Disparities	Qualitative Analysis Test	Quantitative QRisk Model
NO	Fisher's Exact and either Cochran-Armitage or Exact Trend test	Multistage Quantal
YES	Peto Prevalence test	Time-to-Tumor (Multistage Weibull)

IMPORTANT NOTE:	Before any **quantitative analyses** can be run on STATOX, **the data must be configured** to include only the information appropriate for the desired analysis. The data configuration for **quantitative analyses** is the same as used for the **qualitative analyses**. Time-to-tumor analyses will include **ALL** animals, however, **accidental deaths MUST BE SELECTED AND EXCLUDED from time-to-tumor analyses.** Refer to the **Configure Data** screen, Fig. 9, or the Analysis screen, Fig. 13.

Time-to-Tumor (Multistage Weibull) Model

To run the **Time-to-Tumor (Multistage Weibull) Model**, click the **QRisk** button on Analysis screen (Fig. 13).

The **QRisk parameterization** screen (Fig. 28) uses data specifically formatted by STATOX.

Figure 28: Parameterization screen – Weibull

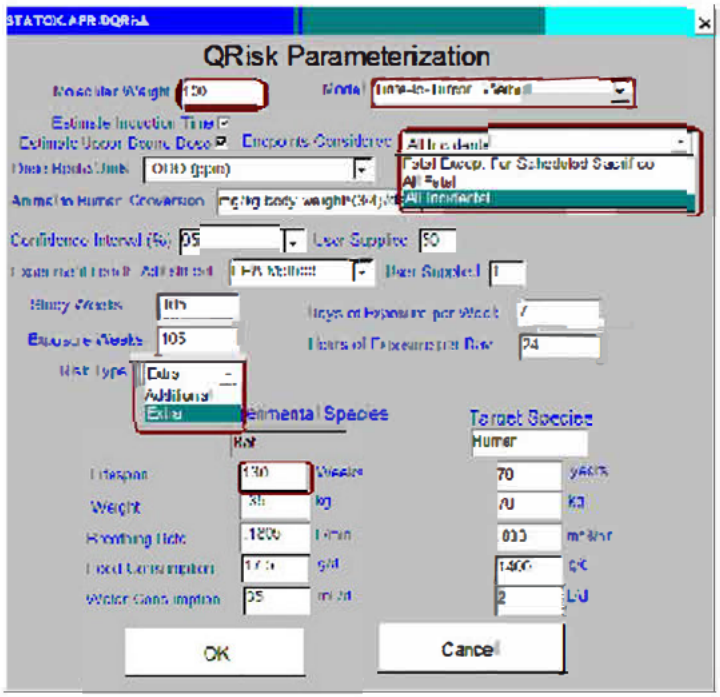
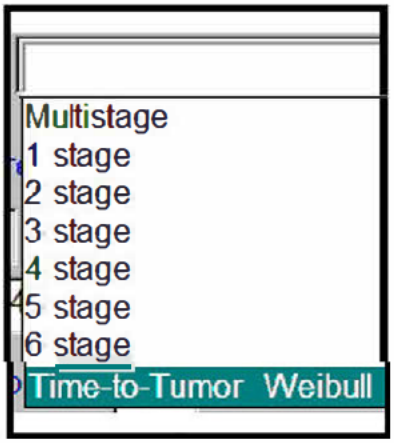


Figure 29: Drop-down List



Select the: **Time-to-Tumor Weibull** model from the drop-down list if it is not already selected (Fig. 29).

The fields are filled in based on the study parameters from the risk assessment and STATOX default values (Fig. 28). The following fields (circled in Fig. 28) may have to be modified as indicated.

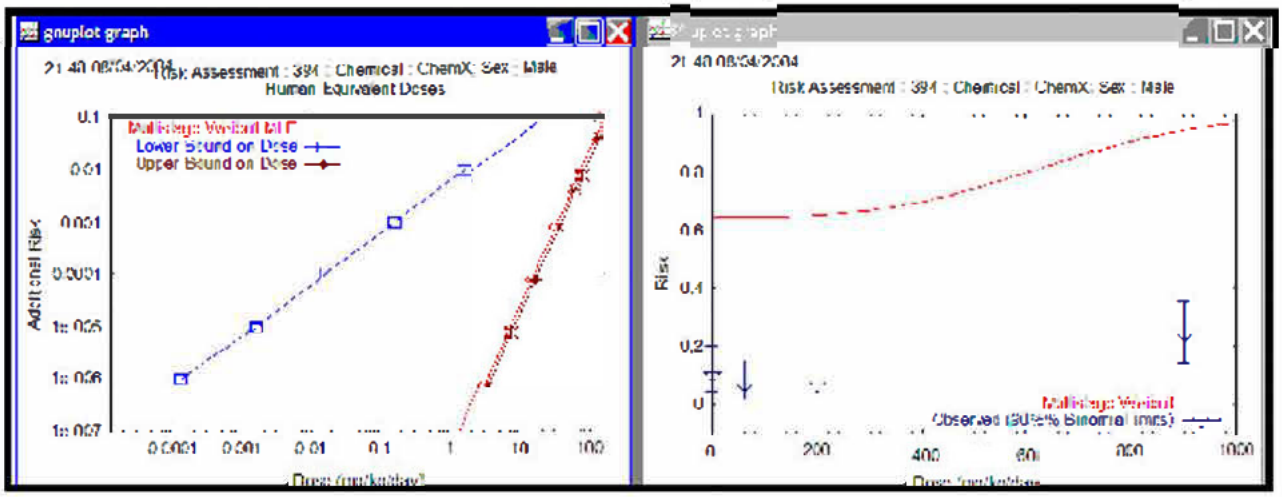
Field	Change needed
Molecular Weight	of test chemical
Model	**Time-to-tumor Weibull**
Endpoints Considered	**All Incidental**
Risk Type	**Extra**
Lifespan [5]	same as Study Weeks

[5]When you click in the Lifespan box, a window pops up for entry of the number of Study Weeks.

Confirm that the other fields have been filled in correctly by STATOX, then click the **OK** button on Figure 28 to complete the Weibull model analysis.

The output includes two graphs (Fig. 30) with model fit information and a table (Fig. 31) with the Q_1* value. The table should be attached to the end of the Quantitative Analysis memorandum.

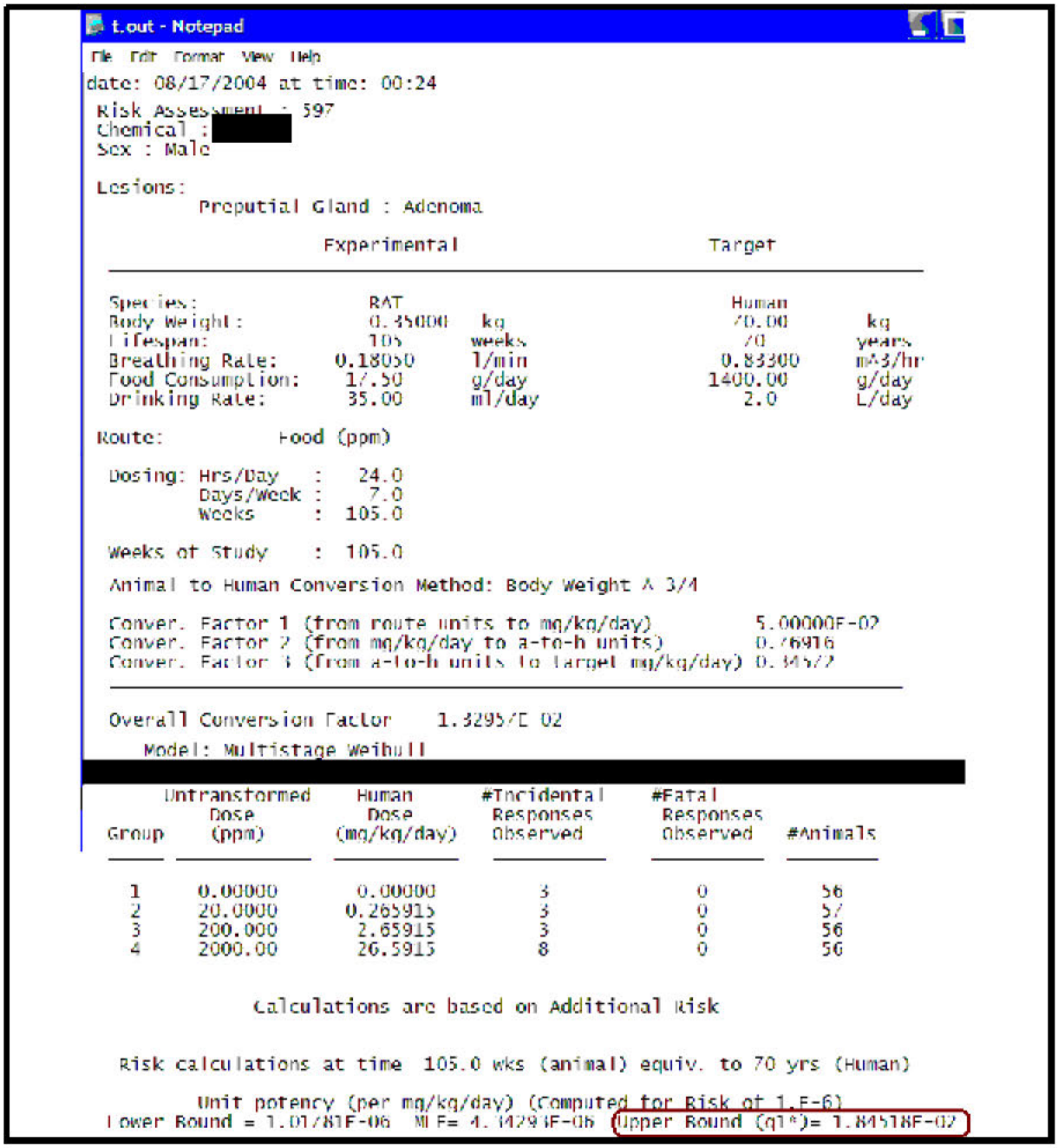
Figure 30: Graphs from Time-to-Tumor (Multistage Weibull) Model Analysis



Once the Time-to-Tumor (Multistage Weibull) table has been created, it should be printed or saved electronically into the appropriate chemical subdirectory with the appropriate risk assessment number and tissue and tumor type added to the file name (e.g., Fig. 31 output could be saved as **C:/Statox4.5/Chemical Name/597PreputialWeibull.txt**). Remember this table will be appended to the Quantitative Risk Assessment Memorandum, so choose a name that can be readily identified later.

TIP:	The **Weibull** table is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly into Word. Click **YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Figure 31: Time-to-Tumor (Multistage Weibull) Model Table with Q_1^* Value



t.out - Notepad

File Edit Format View Help

date: 08/17/2004 at time: 00:24

Risk Assessment : 597
Chemical : [redacted]
Sex : Male

Lesions:
Preputial Gland : Adenoma

	Experimental		Target	
Species:	RAT		Human	
Body Weight:	0.35000	kg	70.00	kg
Lifespan:	105	weeks	70	years
Breathing Rate:	0.18050	l/min	0.83300	m^3/hr
Food Consumption:	17.50	g/day	1400.00	g/day
Drinking Rate:	35.00	ml/day	2.0	L/day

Route: Food (ppm)

Dosing: Hrs/Day : 24.0
Days/Week : 7.0
Weeks : 105.0

Weeks of Study : 105.0

Animal to Human Conversion Method: Body Weight ^ 3/4

Conver. Factor 1 (from route units to mg/kg/day) 5.00000E-02
Conver. Factor 2 (from mg/kg/day to a-to-h units) 0.76916
Conver. Factor 3 (from a-to-h units to target mg/kg/day) 0.34572

Overall Conversion Factor 1.32957E 02

Model: Multistage Weibull

Group	Untransformed Dose (ppm)	Human Dose (mg/kg/day)	#Incidental Responses Observed	#Fatal Responses Observed	#Animals
1	0.00000	0.00000	3	0	56
2	20.0000	0.265915	3	0	57
3	200.000	2.65915	3	0	56
4	2000.00	26.5915	8	0	56

Calculations are based on Additional Risk

Risk calculations at time 105.0 wks (animal) equiv. to 70 yrs (Human)

Unit potency (per mg/kg/day) (Computed for Risk of 1.E-6)
Lower Bound = 1.01781E-06 MLE= 4.14298E-06 Upper Bound (q1*)= 1.84518E-02

Multistage Quantal Model

To run the Multistage (Quantal) Model, click the QRisk button on Analysis screen (Fig. 13). The QRisk parameterization screen (Fig. 32) uses data specifically formatted by STATOX.

Figure 32: Parameterization screen - Multistage Quantal

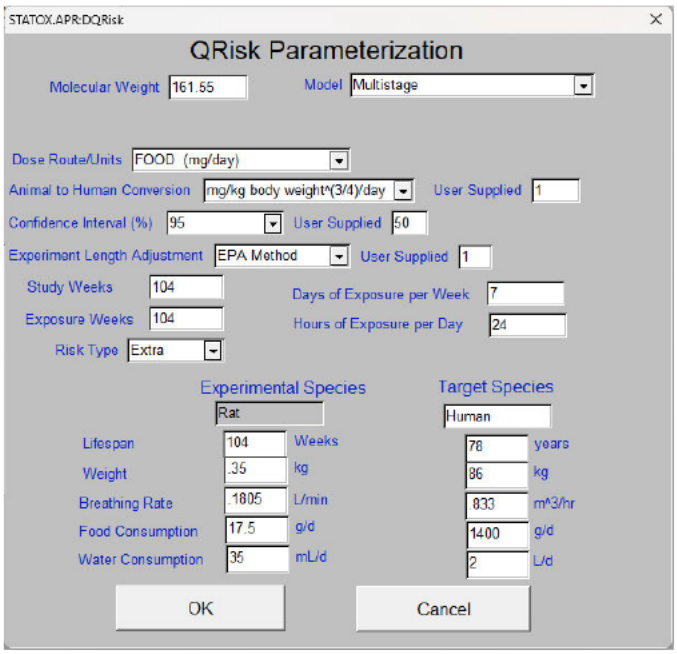
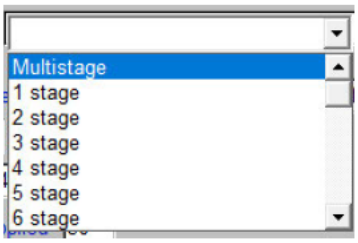


Figure 33: Drop-down List



Select the: **Multistage Quantal** model from the drop-down list if it is not already selected (Fig. 33).

The fields are filled in based on the study parameters from the risk assessment and STATOX default values (Fig. 32). The following fields (circled in Fig. 28) may have to be modified as indicated.

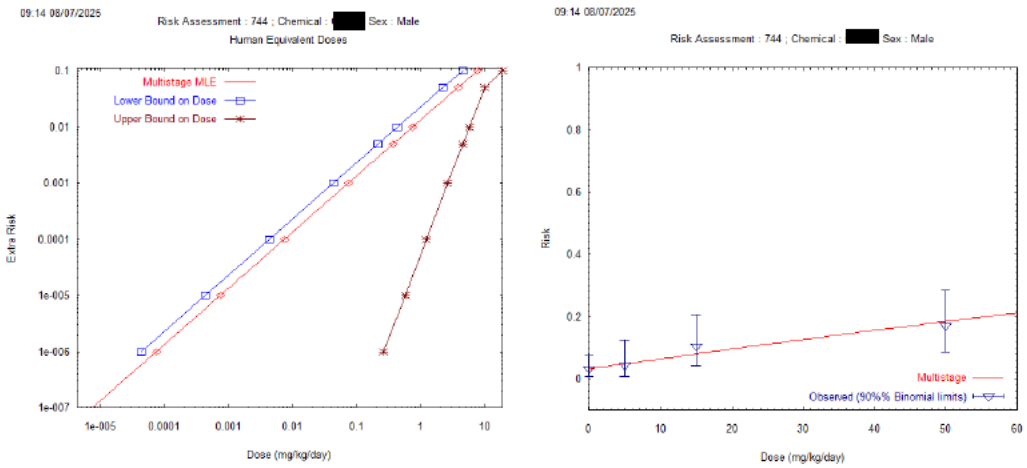
Field	Change needed
Molecular Weight	of test chemical
Model	**Time-to-tumor Weibull**
Endpoints Considered	**All Incidental**
Risk Type	**Extra**
Lifespan [5]	same as Study Weeks

[5]When you click in the Lifespan box, a window pops up for entry of the number of Study Weeks.

Confirm that the other fields have been filled in correctly by STATOX, then click the **OK** button on Figure 32 to complete the Multistage model analysis.

The output includes two graphs (Fig. 34) with model fit information and a table (Fig. 35) with the Q_1* value. The table should be attached to the end of the Quantitative Analysis memorandum.

Figure 34: Graphs from Multistage (Quantal) Model Analysis



Once the Multistage (Quantal) table has been created, it should be printed or saved electronically into the appropriate chemical subdirectory with the appropriate risk assessment number and tissue and tumor type added to the file name (e.g., Fig. 35 output could be saved as **C:/Statox4.5/Chemical_Name/744LiverMultistage.txt).** Remember this table will be appended to the Quantitative Risk Assessment Memorandum, so choose a name that can be readily identified later.

Figure 35: Multistage Table with Q_1* Value

File Edit View

```
Risk Assessment : 744
Chemical : [redacted]
Sex : Male
Lesions:
        Liver : Adenomas
        Liver : Carcinomas

                    Experimental                        Target
-------------------------------------------------------------------------
Species:                RAT                             Human
Body Weight:            0.35000   kg                    86.00       kg
Lifespan:               104       weeks                 78          years
Breathing Rate:         0.18050   l/min                 0.83300     m^3/hr
Food Consumption:       17.50     g/day                 1400.00     g/day
Drinking Rate:          35.00     ml/day                2.0         L/day

Route:          Food (mg/kg/day)

Dosing: Hrs/Day   :   24.0
        Days/Week :    7.0
        Weeks     :  104.0

Weeks of Study    :  104.0

Animal to Human Conversion Method: Body Weight ^ 3/4
-------------------------------------------------------------------------

   Model: Multistage
        p(d) = 1 - exp( -q0 - q1 * d - q2 * d^2 - q3 * d^3 )
-------------------------------------------------------------------------
             Calculations are based on Extra Risk

           Unit potency (per mg/kg/day) (Computed for Risk of 1.E-6)
Lower Bound = 3.79159E-06  MLE= 1.36033E-02  Upper Bound (q1*)= 2.35979E-02
```

TIP:	The **Multistage** table is displayed on the screen using Windows notepad. Go to **File** then **Save As** on the Notepad menu bar to save the file. Using the **.doc** extension allows it to be opened directly into Word. Click **YES** to convert file. Adjust font (**Courier New**) and text size as needed.

Step 6: QUANTITATIVE MEMORANDUM

After the Quantitative Statistical Analysis is completed, the statistician obtains a TXR number for the memorandum. The signed memorandum should be sent to the toxicologist of record via the HED Plum Folder Process. The final electronic copy of the memorandum is saved in IHAD with the TXR number that is assigned to it.

Appendix 1: Qualitative Analysis Forms: Study/Tumor Information, Individual and Expanded Data Entry

Form 1. Qualitative Analysis Study/Tumor Information

Form 2. Qualitative Analysis Individual Animal Data Entry Form

Form 3. Qualitative Analysis Expanded Individual Animal Data Entry Form

Form 1. QUALITATIVE ANALYSIS COVER FORM

Risk Assessment Record Number (Assigned by SIMB Statistician) _____

Chemical _____ P.C. Code _____ MRID Number _____

Project Number _____

Study Laboratory _____

Study Date _____ Duration (Months/Weeks/Days) _____

Study Number _____ Pathologist _____

Species _____ Strain _____ Sex _____

Registrant/Sponsor _____

Route of Administration _____

Dose Units: _____ ppm **OR** _____ mg/kg/day Time Units: _____ days **OR** _____ weeks

Actual Doses: _____ Dosage Days Per Week _____

HED Reviewer _____ Phone: _____

HED Statistician _____ Phone: _____

Tumors of Concern

Tissue/ Tumor Morphology Codes*		Tissue/Description of Tumor Morphology
A	_____/ _____	_____/_____
B	_____/ _____	_____/_____
C	_____/ _____	_____/_____
D	_____/ _____	_____/_____
E	_____/ _____	_____/_____
F	_____/ _____	_____/_____
G	_____/ _____	_____/_____
H	_____/ _____	_____/_____

* Tissue Code: T1, T2, etc.
Morphology Code: M1, M2, etc.

Form 2. QUALITATIVE ANALYSIS INDIVIDUAL ANIMAL DATA ENTRY FORM

RISK ASSESSMENT RECORD NUMBER _____

ANIMAL NUMBER	SEX	DOSE	TIME OF DEATH	DISPOSITION	TUMOR MORPHOLOGY
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3

Form 3: QUALITATIVE ANALYSIS EXPANDED INDIVIDUAL ANIMAL DATA ENTRY FORM
QUALITATIVE RISK ASSESSMENT RECORD NUMBER _____

ANIMAL NUMBER	SEX	DOSE	TIME OF DEATH	DISPOSITION AT DEATH	TUMOR MORPHOLOGY
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3
_____	_____	_____	_____	1 2 3 4 5 6 7	A 1 2 3 B 1 2 3 C 1 2 3 D 1 2 3 E 1 2 3 F 1 2 3 G 1 2 3 H 1 2 3 I 1 2 3 J 1 2 3

Appendix 2: QC/QA Data Entry Check Lists

QC/QA Check List 1. Lesion Table

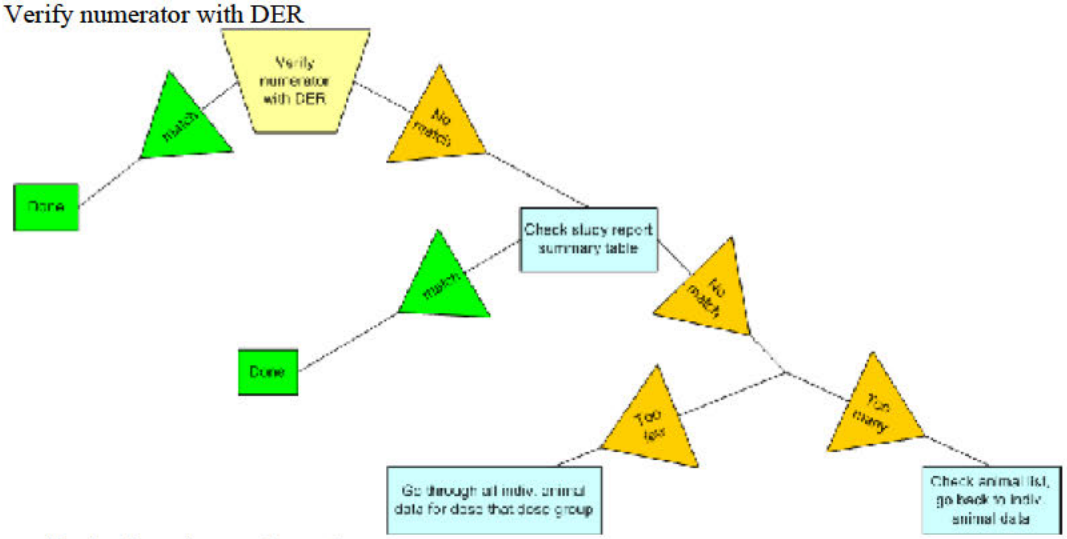
QC/QA Check List 2. Animal List Table

QC/QA Check List 3. Survival Table

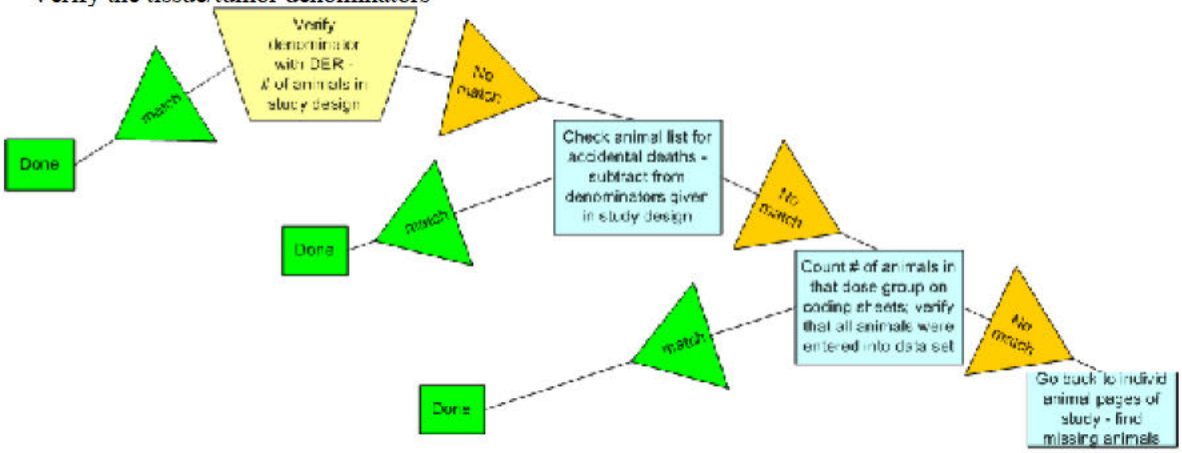
QC/QA Check List 4. Mortality Table

QC/QA Check List 1. Lesion Table Figure 11

- ☐ Verify that header information is correct (Risk Assessment Number, Chemical, Species, Sex).
- ☐ Verify that number of dose groups accurately reflect number of doses in the study.
- ☐ Verify that tissues and tumors listed accurately reflect tissues and tumors of concern discussed with reviewer and presented in the DER.
- ☐ Verify numerator with DER



- ☐ Verify the tissue/tumor denominators



QC/QA Check List 2. Animal List Table Figure 12

- ☐ Verify that header information is correct (Chemical, Species, time units (weeks or days) for Time of Death, dose units (ppm or mg/kg/day) for Doses, and actual doses).
- ☐ Add up Total Number of Animals for Death on Study and Final Sacrifice for each dose group. This should add up to the total number of animals designated for that particular dose group as indicated in the study design of the DER.
- ☐ Add up the Total Number of Animals for each Interim Sacrifice group for each dose group. This should add up to the total number of animals designated for interim sacrifice at that time point as indicated in the study design of the DER.
- ☐ Verify that there is a TOD entry for each DOS animal in the dataset and that it is within the designated time frame of the study (there shouldn't be a TOD of week 1040 in a 104 week study)
- ☐ Verify that there is a TOD entry for each Final Sacrifice animal in the dataset and that it is during the final sacrifice time indicated in the study design of the DER (there shouldn't be a final sacrifice TOD of week 115 in a 104 week study).
- ☐ Verify that there is a TOD entry for each Interim Sacrifice animal in the dataset and that it is at the appropriate interim sacrifice time indicated in the study design of the DER (there shouldn't be an interim sacrifice TOD of week 63 during an interim sacrifice at week 52).
- ☐ Verify that the sex of all animals is the same and appropriate for that risk assessment number
- ☐ Verify that there is animal data for each of the dose groups indicated in the header information (doses of 0, 10, 100 and 1000 should list animal data for dose groups 1, 2, 3 and 4).
- ☐ Verify that all lesions listed on the study cover form are also listed under Lesion Information for Tissues Examined.
- ☐ Under Lesion Information for Tissues Examined, verify that the Total Number of Animals in each Dose Group matches the tumor count for that particular tumor type for that dose group given in the DER and/or study report.

QC/QA Check List 3. Survival Table Figure 14

- ☐ Verify that header information is correct (Risk Assessment Number, Chemical, Species, Sex, Dose Units).
- ☐ Verify that the Doses accurately reflect the specific doses given in the study design of the DER.
- ☐ Verify that the Total for each dose group accurately reflects the total number of animals designated per dose group in the study design of the DER.
- ☐ Verify that the Censored Observations (Withdrawals or Deaths) or each dose group accurately reflects the time of the accidental deaths and the interim and final sacrifices designated in the study design of the DER.

QC/QA Check List 4. Mortality Table Figure 20

- ☐ Verify that header information is correct (Risk Assessment Number, Chemical, Species, Sex, Dose Units).
- ☐ Verify that Dosage accurately reflects number of doses and specific doses given in the study design of the DER.
- ☐ Verify that the interim sacrifice timepoint is accurate.
- ☐ Verify that the number of animals reported as being interim sacrifice animals is accurate.
- ☐ Verify that the last interval given accurately reflects the final sacrifice timepoint.
- ☐ Verify that the denominator of each dose group accurately reflects the number of animals in that dose as specified in the study design of the DER. If the denominator does not match that of the study design, check the Animal List for any animals in that dose group that were accidental deaths. The denominator in the Mortality Table should be the number of animals in each dose group as given in the study design of the DER minus the number of animals in each dose group that were accidental deaths.

Appendix 3: STATOX Installation and SQL File Trouble Shooting

1. First Time Installation Instructions
2. Trouble Shooting SQL File Problems

1. Installation Instructions

Steps for application installation:

1. Unzip **STATOX Installation.zip** to your hard drive (**C:** drive)
2. Install **Lotus SmartSuite** from **C:\STATOX Installation\Lotus SmartSuite** (setup.exe)
   Select "Custom" and install only **1-2-3** and **Approach**
3. Install **DOSBox** from **C:\STATOX Installation\DOSBOX\DOSBox0.74-win32-installer.exe**
   Accept all defaults
4. Grant the (authenticated) user full control rights to **C:\Program Files (x86)\DOSBox-0.74**
5. Copy the folder **C:\STATOX Installation\Statox4.5** to the root of the **C:** drive
6. Grant the (authenticated) user full control rights **C:\Statox4.5**
7. In the **C:\Users\<username>\AppData\Local\DOSBox** directory then rename **dosbox-0.74.conf** to **orig_dosbox-0.74.conf**
   [Note: In Windows the **AppData** folder is hidden. In **File Explorer**, select **View > Show > Hidden Items** to make the folder visible]
8. Copy **dosbox-0.74.conf** from **C:\STATOX Installation\DOSBOX\Win7_Statox_DOSBox** to **C:\Users\<username>\AppData\Local\DOSBox**
9. Place a shortcut to C:\Statox4.5\STATOX.APR on the user's desktop

2. Trouble Shooting SQL File Problems

1. Open the file Statox.ini (it's probably under C:\Statox4.5\Statox.ini).
2. Look at the path set in the Statox.ini file.
3. Check your hard drive and see that the designated path exists (the Statox.ini file probably points to a file folder that doesn't exist).
4. If, for example, Statox.ini points to C:\Statox4.5\ChemicalX\ChemicalX.prj, but no ChemicalX subdirectory exists and you're working on ChemicalY, that's your problem - the ChemicalY.prj files point to the correct subdirectory, but the Statox.ini file points to a different subdirectory (in this example, it points to ChemicalX).
5. In the above example, you should create a subdirectory for the chemical ChemicalX.
6. Copy all 11 of your ChemicalY files into it (doesn't matter what chemical you use to create this "dummy file").
7. Change the prj file name to ChemicalX.prj.
8. Launch STATOX with "user" password.
9. Load the ChemicalY project file (which is now called ChemicalX.prj).
10. Do your edits or run your stats.
11. Save the project as ChemicalY.prj.
12. The next time you launch STATOX, it will look at the Statox.ini which now points to the ChemicalY files.