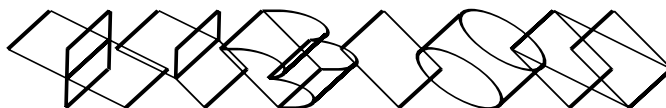


**ALGORITHMS USED IN
PATH/TOX SYSTEM
VERSION 4.2.2**

**INCLUDING ENHANCED
STATISTICS**



XYBION MEDICAL SYSTEMS

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1 INTRODUCTION

PATH/TOX SYSTEM provides an extensive list of pre-defined table report functions. This chapter summarizes the routine statistical analyses performed by PATH/TOX SYSTEM, as well as algorithms that are specific to individual Modules and to individual parameters within Modules.

Statistical analyses performed by PATH/TOX SYSTEM include:

- *descriptive* statistics;
- algorithms for use in defining *critical values*;
- algorithms for use in determining *numerical values*, for which the table functions utilize statistical procedures common to all System Modules, individual Modules and/or individual parameters within the Modules;
- algorithms for use in determining *observational statistics* for categorical data and tumor data analysis, for which the table functions generally utilize algorithms specific to individual Modules and to individual parameters within the Modules;
- algorithms for use in evaluating acute study data;
- algorithms for use in *partitioning/animal randomization*;
- algorithms for use in determining *food consumption/conversion efficiency* as related to body weight gain/loss;
- algorithms for use in determining *dosing data* as related to dosing via the feed; and
- algorithms for use in preparing *clinical signs summaries*.



2 STATISTICS

2.1 Rounding

The purpose of rounding is to adjust real numbers to the correct number of decimal places, as defined in the protocol for each measurement parameter and in option tables for calculated parameters.

If the digit after the least significant digit is 0,1,2,3 or 4, the number is rounded down (the least significant digit remains unchanged). If the digit after the least significant digit is 5,6,7,8 or 9, the number is rounded up (the least significant digit is incremented by one).

Routine FMTRDC is used for rounding prior to printouts or displays. Routine ROUND_REAL is used for internal rounding prior to calculations. Routine ADJUST_REAL is used for real value adjustment prior to calculations and rounding. Routine FMTRDC calls routine ROUND_REAL, and routine ROUND_REAL calls routine ADJUST_REAL. These three routines are further described, as follows:

2.1.1 Rounding Prior to Printouts or Displays

Routine FMTRDC is used to round data prior to printing or displaying data.

Input to routine: *x*, *Ndec*

Output from routine: *Buf*

Data types:

Real*4 *x*, *Val*

Integer *Ndec* (Number of decimals)

Character *(*) *Buf*

$$Val = Round_real(x, Ndec)$$

Final rounding is performed by a FORTRAN write statement. An example follows:



WRITE (UNIT=BUF, FMT='(F11.<Ndec>)') Val

If the number sent to FMTRDC is one digit larger than the buffer, the number is truncated prior to printing.

If the number sent to FMTRDC is two or more digits larger than the buffer, asterisks are printed.

2.1.2 Rounding Prior to Calculations

Rounding prior to calculations is performed by the routine ROUND_REAL.

Routine ROUND_REAL first calls routine ADJUST_REAL. If System Option 18 is set to FALSE, all data will be rounded to the prescribed number of decimal places. If System Option 18 is set to TRUE, rounding is not performed. Once the data, rounded or not rounded, are sent to statistical routines, all calculations are performed in double-precision, without further rounding.

Input to routine: x, Ndec

Output from routine: Val

Data types:

Real*4 x, Val

Double-precision y, Cf (Conversion Factor)

Integer Ndec (Number of Decimals)

$$y = x$$

Call Adjust_real (y)

$$Val = y$$

If System Option 18 is set to TRUE, ROUND_REAL returns the value, Val. If the number of decimals specified is less than -2 or greater than 6, ROUND_REAL returns the value, Val. If neither of these conditions is true, then the following steps are taken by routine ROUND_REAL:



set the Cf:

$$Cf = 10.0^{**Ndec}$$

shift the decimal point and round up:

$$\text{If } x \geq 0.0, \text{ then } y = y * Cf + 0.5$$

shift the decimal point and round down:

$$\text{If } x < 0.0, \text{ then } y = y * Cf - 0.5$$

use the Fortran intrinsic function Aint to truncate y:

$$y = \text{Aint}(y)$$

then shift the decimal point back:

$$Val = \frac{y}{Cf}$$

2.1.3 Real Value Adjustment Prior to Calculations and Rounding

Routine ADJUST_REAL is used to adjust the values of real numbers prior to calculations and rounding. This is done to compensate for the manner in which REAL*4 numbers are represented in the VAX and Alpha AXP computer systems and to ensure that subsequent manipulation does not result in loss of significant digits.

Input to routine: y

Output from routine: y

Data types:

Double-precision y, z, k



The Fortran intrinsic function `Abs` is used to obtain the absolute value of `y`:

$$z = \text{Abs}(y)$$

$$k = 16777216 \quad (2^{**}24)$$

$$z = z + \frac{z}{k}$$

$$y = z$$

2.2 Statistical Flow for Standard-format Reports

Standard Statistical Flow for Standard-format Reports

The following standard analysis will be performed if System Option number 9 is set false or is missing.

For summary reports using Dunnett's test:

- 1) Analysis of Variance is performed and may be reported.
- 2) Bartlett's test for homogeneity is performed. Homogeneity is determined at the 0.05 level.
- 3) If the data are homogeneous, Dunnett's test is performed. Significant differences at the 0.01 or 0.05 levels are indicated on the report.
- 4) If the data are not homogeneous, Cochran and Cox's t-test is performed. Significant differences at the 0.01 or 0.05 levels are indicated on the report.

For summary reports using Fisher's LSD test:

- 1) Analysis of Variance is performed and may be reported.
- 2) Bartlett's test for homogeneity is performed. Homogeneity is determined at the 0.05 level.
- 3) If the data are homogeneous, Fisher's LSD test is performed. Significant differences at the 0.01 or 0.05 levels are indicated on the report.



- 4) If the data are not homogeneous, Cochran and Cox's t-test is performed. Significant differences at the 0.01 or 0.05 levels are indicated on the report.

Alternate Statistical Flow for Standard-format Reports

The following alternate analysis will be performed if System Option number 9 is set true.

For summary reports using Dunnett's test:

- 1) Analysis of Variance is performed and may be reported.
- 2) Bartlett's test for homogeneity is performed. Homogeneity is determined at the 0.05 level.
- 3) If the Analysis of Variance test yields a P-value greater than 0.05, no unplanned test is performed.
- 4) If the data are homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.05 but greater than 0.01, Dunnett's test is performed at the 0.05 level. Significant differences at the 0.05 levels are indicated on the report.
- 5) If the data are homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.01, Dunnett's test is performed at the both the 0.05 level and the 0.01 level. Significant differences at the 0.01 or 0.05 levels are indicated on the report.
- 6) If the data are not homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.05 but greater than 0.01, Cox's test is performed at the 0.05 level. Significant differences at the 0.05 levels are indicated on the report.
- 7) If the data are not homogeneous and the Analysis of Variance test yields a P-value the is less than or equal to 0.01, Cox's test is performed at the both the 0.05 level and the 0.01 level. Significant differences at the 0.01 or 0.05 levels are indicated on the report.

For summary reports using Fisher's LSD test:

- 1) Analysis of Variance is performed and may be reported.
- 2) Bartlett's test for homogeneity is performed. Homogeneity is determined at the 0.05 level.
- 3) If the Analysis of Variance test yields a P-value greater than 0.05, no unplanned test is performed.



- 4) If the data are homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.05 but greater than 0.01, Fisher's test is performed at the 0.05 level. Significant differences at the 0.05 levels are indicated on the report.
- 5) If the data are homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.01, Fisher's test is performed at the both the 0.05 level and the 0.01 level. Significant differences at the 0.01 or 0.05 levels are indicated on the report.
- 6) If the data are not homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.05 but greater than 0.01, Cox's test is performed at the 0.05 level. Significant differences at the 0.05 levels are indicated on the report.
- 7) If the data are not homogeneous and the Analysis of Variance test yields a P-value that is less than or equal to 0.01, Cox's test is performed at the both the 0.05 level and the 0.01 level. Significant differences at the 0.01 or 0.05 levels are indicated on the report.



2.3 Descriptive Statistics

2.3.1 Algorithms for Calculating the Mean, Standard Deviation and Standard Error

The TOXLIB library routine COMP_MN_SD_SE, as well as the individual Module routines, perform the mean, standard deviation and standard error calculations using the following formulas:

$$M_i = \frac{1}{N_i} \sum_j X_{i,j}$$

In the individual routines:

$$VAR_i = \frac{N_i}{N_i - 1} \left[\frac{1}{N_i} \sum_j X_{i,j}^2 - M_i^2 \right]$$

In COMP_MN_SD_SE:

$$VAR_i = \frac{1}{N_i - 1} \sum_j [X_{i,j} - M_i]^2$$

$$Sd_i = \sqrt{VAR_i}$$

$$Se_i = \frac{Sd_i}{\sqrt{N_i}}$$

where:

- $X_{i,j}$ = j th observation for group i
- M_i = mean value of observations for group i
- N_i = number of observations in group i
- Sd_i = standard deviation of observations for group i
- Se_i = standard error of observations for group i
- VAR_i = variance of observations for group i



2.3.2 Algorithm for Calculating Percent Difference from the Mean

$$\text{Percent Difference} = \frac{(\text{Mean}_{\text{Control}} - \text{Mean}_{\text{Comparison}})}{\text{Mean}_{\text{Control}}} * 100.00$$

where:

M_{Control} = mean value of observations for the Control group
 $M_{\text{Comparison}}$ = mean value of observations for the Comparison group

2.3.3 Algorithm for Calculating the Minimum, Maximum and Median

Sort the observations in group i , X_i in ascending order.

$$\text{Sort}X_i = \text{Sort}(X_i)$$

$$\text{Min}_i = \text{Sort}X_1$$

$$\text{Max}_i = \text{Sort}X_n$$

$$\text{If } N_i \text{ is Odd: } MN = (N_i + 1) / 2 \text{ Med}_i = \text{Sort}X_{MN}$$

$$\text{If } N_i \text{ is even: } MN1 = N_i / 2 \text{ MN2} = N_i / 2 + 1 \text{ Med}_i = (\text{Sort}X_{MN1} + \text{Sort}X_{MN2}) / 2$$

where:

X_i = are the observations for group i

$\text{Sort}X_i$ = are the observations for group i
 sorted in ascending order.

Min_i = minimum value of observations for group i

Max_i = maximum value of observations for group i

Med_i = median value of observations for group i

N_i = number of observations in group i



2.3.4 Algorithm for Calculating Reverse Percent Difference from the Mean

$$\textit{Percent Difference} = \frac{(\textit{Mean}_{\textit{Comparison}} - \textit{Mean}_{\textit{Control}})}{\textit{Mean}_{\textit{Control}}} * 100.00$$

where:

M_{Control} = mean value of observations for the Control group

$M_{\text{Comparison}}$ = mean value of observations for the Comparison group



2.4 Critical Value Routines

The following routines, found in the source file ANOVA.FOR, calculate critical values that are used to assess the significance of the statistical tests.

- PNORM — calculates the cumulative probability $P(y < x)$ from the normal distribution for a standardized, normal variable.
- QNORM — calculates the inverse normal function for a given argument.
- FPROB - performs transformation of F statistic to a standardized, normal variable and computes F probability by calling the PNORM routine.
- CHI2 (X^2) — calculates the X^2 value for given degrees of freedom at the probability level 0.05.
- CRIT_STUDENT (DF, VAL05, VAL01) — for a given DF, degrees of freedom, the routine finds the critical value, VAL05, at the 0.05 level and the critical value, VAL01 at the 0.01 level for the Student-t statistic.

2.4.1 Cumulative Probability

Subroutine PNORM(X) calculates the cumulative probability $P(Y < X)$, assuming that Y is a standardized, normal variable. The parameter X is assumed to be a single-precision, positive variable; however, the internal calculations are performed in double-precision. The error $E(X)$ in the approximation used⁽¹⁾ is bounded by:

$$|E(X)| < 0.000000075$$

The algorithm is given by:

$$\text{If } X > 6, \text{ set } Z(X) = 0.0$$

$$\text{If } X \leq 6, \text{ set } Z(X) = \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}}$$

$$\text{Set } T = \frac{1.0}{(1.0 + 0.2316419 * X)}$$



$$P(Y < X) = 1.0 - Z(X)(B_1T + B_2T^2 + B_3T^3 + B_4T^4 + B_5T^5)$$

where the coefficients are given by:

$$B_1 = 0.319381530$$

$$B_2 = -0.356563782$$

$$B_3 = 1.781477937$$

$$B_4 = -1.821255978$$

$$B_5 = 1.330274429$$

2.4.2 F-Probability

Routine FPROB (FR, Dft, Dfe, PFR) calculates the probability PFR(Y<F) given the variance ratio, FR; the treatment degrees of freedom, Dft; and the error degrees of freedom, Dfe. The calculations are done by transforming FR to a standardized, normal variable⁽²⁾. The algorithm is given by:

$$X = \left[(FR^{1/3}) \left(1 - \frac{2}{9 * Dfe} \right) - \left(1 - \frac{2}{9 * Dft} \right) \right] / \sqrt{\left(\frac{2}{9 * Dft} + FR^{2/3} \frac{2}{9 * Dfe} \right)}$$

$$PFR = PNORM(|X|)$$

$$IF(X > 0), PFR = 1.0 - PFR$$



2.4.3 Inverse Normal

Routine QNORM(X) calculates the inverse normal for a given value of X. The variable X is assumed to be a double-precision, real value, and all calculations are performed in double-precision. The returned value QX is single-precision. Currently, only the LD₅₀ calculation from the acute Module calls the QNORM function. The error E(X) in the approximation used⁽³⁾ is bounded by:

$$|E(X)| < 0.00045$$

The algorithm is given by:

If X < 0.00000029, Set QX = -5.0 then exit

If X > 0.99999971, Set QX = 5.0 then exit

If X ≥ 0.5, Set PX = 1.0 - X

If X < 0.5, Set PX = X

$$T = \sqrt{\log \frac{1.0}{PX^2}}$$

$$QX = T - \frac{C_0 + C_1T + C_2T^2}{1.0 + D_1T + D_2T^2 + D_3T^3}$$



where the coefficients are given by:

$$C_0 = 2.515517$$

$$C_1 = 0.802853$$

$$C_2 = 0.010328$$

$$D_1 = 1.432788$$

$$D_2 = 0.189269$$

$$D_3 = 0.001308$$

If $X < 0.5$, then $QX = -QX$



2.4.4 Chi-Square

Routine CHI2(N) performs a table lookup for the X^2 value for the given degrees of freedom N at the 0.05 level. The number of degrees of freedom must be in the interval (1,15). The table values⁽⁴⁾ are:

Degrees of Freedom	X^2 Value
1	3.841
2	5.991
3	7.815
4	9.488
5	11.071
6	12.592
7	14.067
8	15.507
9	16.919
10	18.307
11	19.675
12	21.026
13	22.362
14	23.685
5	24.996



2.4.5 Student-t critical values

Routine CRIT_STUDENT(DF,T_05,T_01) performs a table lookup for the t-distribution critical values at the 0.01 and 0.05 levels for the given degrees of freedom DF. The table values⁽¹⁵⁾ are:

Degrees of Freedom	.01 Crit Value	.05 Crit Value
1	63.657	12.706
2	9.925	4.303
3	5.841	3.182
4	4.604	2.776
5	4.032	2.571
6	3.707	2.447
7	3.409	2.365
8	3.335	2.306
9	3.250	2.262
10	3.169	2.228
11	3.106	2.201
12	3.055	2.179
13	3.012	2.160
14	2.977	2.145
15	2.947	2.131
16	2.921	2.120



Degrees of Freedom	.01 Crit Value	.05 Crit Value
17	2.898	2.110
18	2.878	2.101
19	2.861	2.093
20	2.845	2.086
21	2.831	2.080
22	2.819	2.074
23	2.807	2.069
24	2.797	2.064
25	2.787	2.060
26	2.779	2.056
27	2.771	2.052
28	2.763	2.048
29	2.756	2.045
30	2.750	2.042
31-40	2.704	2.021
41-60	2.660	2.000
61-120	2.617	1.980
>120	2.576	1.960



2.5 Statistical Tests for Numerical Data

In this chapter, the calculations for each test are described, then the algorithms are presented that are used by System library functions to compute the appropriate test statistic. The library routines performing the calculations are in the STATLIB library.

2.5.1 Outlier Tests

2.5.1.1 3 Standard Deviation Test

The subroutine OUTLIER_3SD performs a three standard deviation test for outliers for each group in a study. This test is appropriate for studies with groups sizes of 30 or more.

Definitions:

- X_{ij} = the j th data point in the i th group
- M_i = mean of the i th group (see the *Descriptive Statistics* section in this chapter for the calculation)
- Std_i = standard deviation of the i th group (see the *Descriptive Statistics* section in this chapter for the calculation)
- $Cvlow_i$ = The lower critical value for the i th group
- $Cvup_i$ = The upper critical value for the i th group

Calculations:

$$Cvlow_i = M_i - 3 * Std_i$$

$$Cvup_i = M_i + 3 * Std_i$$

If X_{ij} is less than $Cvup_i$ or X_{ij} is greater than $Cvlow_i$, it is an outlier.



2.5.2 Homogeneity Tests

2.5.2.1 Bartlett's Test⁽⁵⁾

The subroutine BART_ROUTINE performs a study-specific test for homogeneity of variances which is appropriate for groups of equal or unequal sizes. This test is appropriate for normally distributed data. When there are fewer than 2 observations in any group, or the variance of any group equals zero, the test will not be performed and the result will default to declaring data nonhomogeneous.

Definitions:

- Ndg = number of groups
- VAR_i = variance of the i th group (see the *Descriptive Statistics* section in this chapter for the calculation)
- Dft = treatment degrees of freedom: Ndg-1
- N_i = number of observations in the i th group
- f_i = degrees of freedom for the i th group: N_i-1
- M = the numerator in Bartlett's test statistic
- C = Bartlett's correction factor



Calculations:

Preliminary calculations:

$$M = \sum_{i=1}^{Ndg} f_i * \log_e \left[\frac{\sum_{i=1}^{Ndg} f_i * VAR_i}{\sum_{i=1}^{Ndg} f_i} \right] - \sum_{i=1}^{Ndg} \left[f_i * \log_e VAR_i \right]$$

$$C = 1 + \frac{1}{3 * Dft} * \left[\sum_{i=1}^{Ndg} \frac{1}{f_i} - \frac{1}{\sum_{i=1}^{Ndg} f_i} \right]$$

The test statistic:

$$Test\ Statistic = \frac{M}{C}$$

The critical value is the chi-squared value for a given alpha level and DFT. This value can be calculated by the function Chi-Square (see the *Critical Value Routines* section in this chapter for the calculation). If the test statistic is greater than the critical value, reject that the groups are homogeneous.

If the test statistic is less than or equal to the critical value, accept that the groups are homogeneous.



2.5.3 Planned Comparisons

2.5.3.1 Parametric Test: One-Way Analysis Of Variance (ANOVA)

The subroutine AV_ROUTINE performs a standard one-way analysis of variance test. This test cannot be performed if there is only one dose group, if Dfe = 0, or if Dft = 0.

Definitions:

- Ndg = number of groups
- N_i = number of observations in the i th group
- T = total number of observations in the study
- M_i = mean of the i th group (see the *Descriptive Statistics* section in this chapter for the calculation)
- Var_i = variance of the i th group (see the *Descriptive Statistics* section in this chapter for the calculation)
- Dfe = error degrees of freedom: T-Ndg
- Dft = treatment degrees of freedom: Ndg-1

Calculations:

The correction factor (c):

$$C = \frac{\left(\sum_{i=1}^{Ndg} N_i * M_i \right)^2}{T}$$

The treatment sum of squares (TSS):

$$TSS = \sum_{i=1}^{Ndg} N_i * M_i^2 - C$$



The total sum of squares (TTLSS):

$$TTLSS = \sum_{i=1}^{Ndg} \left[(N_i - 1) * Var_i + N_i * M_i^2 \right] - C$$

The error sum of squares (ESS):

$$ESS = TTLSS - TSS$$

The F-Ratio (FR):

$$FR = \frac{TSS * Dfe}{ESS * Dft}$$

The probability value PFR is calculated by the subroutine FPROB (see the *Critical Value Routines* section in this chapter for the calculation). Values for FR, Dft and Dfe are sent to FPROB. FPROB returns with a value for PFR.

If PFR is greater than or equal to α , then there are no significant differences among the groups, at the α level.

If PFR is less than α , then there are significant differences among the groups, at the α level.



2.5.4 Parametric Unplanned Comparisons

2.5.4.1 Dunnett's t -Test^(6,7)

The subroutine DT_ROUTINE calculates the smallest difference between the means of the control and each comparison groups which is necessary to determine that the means of the two groups are significantly different. This test cannot be performed when Dfe is zero or when any group that has no observations. If the test cannot be performed, asterisks are printed in reports that display the LSD's.

Definitions:

- Ndg = number of groups
- N₁ = number of observations in the control group
- N_i = number of observations in the i th comparison group, where $i = 2, \text{Ndg}$
- T = total number of observations in the study
- Dfe = error degrees of freedom: $T - \text{Ndg}$
- Dft = treatment degrees of freedom: $\text{Ndg} - 1$
- ESS = error sum of squares (see the *Parametric Test: One-Way Analysis Of Variance (ANOVA)* section in this chapter for the calculation)
- M₁ = mean of observations for control group (see the *Descriptive Statistics* section in this chapter for the calculation)
- M_i = mean of the i th comparison group, where $i = 2, \text{Ndg}$ (see the *Descriptive Statistics* section in this chapter for the calculation)

Calculations:

The error mean square (EMS) or the mean square within:

$$EMS = \frac{ESS}{Dfe}$$

The critical t value for Dunnett's test, given Dfe and Dft, is approximated by:

$$t_{\alpha} = A_{\alpha, Dft, 0} + \frac{A_{\alpha, Dft, 1}}{Dfe} + \frac{A_{\alpha, Dft, 2}}{Dfe^2}$$

The coefficients $A_{\alpha, Dft, 0}$, $A_{\alpha, Dft, 1}$ and $A_{\alpha, Dft, 2}$ for 0.05 and 0.01 levels are given in the following table and produce t values accurate to 0.01 for error degrees of freedom greater than 5.



0.05 Level Coefficients				0.01 Level Coefficients			
Dft	$A_{05,Dft,0}$	$A_{05,Dft,1}$	$A_{05,Dft,2}$	Dft	$A_{01,Dft,0}$	$A_{01,Dft,1}$	$A_{01,Dft,2}$
1	1.960	2.450	3.000	1	2.580	4.689	12.81
2	2.210	3.167	4.667	2	2.790	5.932	16.34
3	2.350	3.500	6.000	3	2.920	6.257	20.22
4	2.440	3.867	6.667	4	3.000	6.700	22.02
5	2.510	4.017	7.667	5	3.060	7.146	23.02
6	2.570	4.200	8.000	6	3.110	7.419	24.15
7	2.610	4.650	7.000	7	3.150	7.713	24.93
8	2.650	4.583	8.333	8	3.190	7.808	26.21
9	2.690	4.800	8.000	9	3.220	8.128	26.11
10	2.720	4.750	9.000	10	3.250	8.225	27.13
11	2.740	5.217	7.667	11	3.270	8.576	26.62
12	2.770	5.183	8.333	12	3.290	8.675	27.38
13	2.790	5.238	8.560	13	3.310	8.726	28.12
14	2.810	5.293	8.786	14	3.330	8.774	28.88
15	2.830	5.350	9.000	15	3.350	8.825	29.63



The smallest difference (sd) at the 0.01($Dunn_{sd01}$) level:

$$Dunn_{sd01} = t_{01} \sqrt{\left(\frac{1}{N_I} + \frac{1}{N_i}\right) * EMS}$$

The smallest difference (sd) at the 0.05 level ($Dunn_{sd05}$) is:

$$Dunn_{sd05} = t_{05} \sqrt{\left(\frac{1}{N_I} + \frac{1}{N_i}\right) * EMS}$$

Critical Value test:

If the absolute value between two means is greater than the smallest difference at the given alpha level,

$$|M_I - M_i| > Dunn_{sd\alpha}$$

then the mean of the control group is significantly different from the mean of the comparison group.

If the absolute value between two means is less than or equal to the smallest difference at the given alpha level,

$$|M_I - M_i| \leq Dunn_{sd\alpha}$$

then the mean of the control group is not significantly different from the mean of the comparison group.



2.5.4.2 Fisher's LSD Pairwise Comparison Test⁽⁸⁾

The subroutine FISHER_ROUTINE calculates the smallest difference between the means of two groups in order to determine if the means of the two groups are significantly different. This is known as the least significant difference. The test is appropriate if the data are homogeneous and the sample sizes are unequal. This test cannot be performed when Dfe is zero or there are no observations in either of the two groups. If the test cannot be performed, asterisks printed are in reports that display the LSD's.

Definitions:

- Ndg = number of groups
- T = total number of observations in the study
- ESS = error sum of squares (see the *Parametric Test: One-Way Analysis Of Variance (ANOVA)* section in this chapter for the calculation)
- Dfe = error degrees of freedom: $T - N_{dg}$
- N_1 = number of observations in the first group
- N_2 = number of observations in the second group
- M_1 = mean of the first group (see the *Descriptive Statistics* section in this chapter for the calculation)
- M_2 = mean of the second group (see the *Descriptive Statistics* section in this chapter for the calculation)

Calculations:

The error mean square (EMS) or the mean square within:

$$EMS = \frac{ESS}{Dfe}$$

The critical t value at the 0.01 level, for a given error degrees of freedom, is approximated by:

$$t_{01} = 2.5760 + \frac{4.9938}{Dfe} + \frac{6.4255}{Dfe^2} + \frac{25.9657}{Dfe^3}$$



The critical t value at the 0.05 level, for a given error degrees of freedom, is approximated by:

$$t_{05} = 1.9600 + \frac{2.3802}{Dfe} + \frac{2.5950}{Dfe^2} + \frac{4.0333}{Dfe^3}$$

The smallest difference at the 0.01 level ($Fisher_{sd01}$) is:

$$Fisher_{sd01} = t_{01} * \sqrt{\left(\frac{1}{N_1} + \frac{1}{N_2}\right) * EMS}$$

The smallest difference at the 0.05 level ($Fisher_{sd05}$) is:

$$Fisher_{sd05} = t_{05} * \sqrt{\left(\frac{1}{N_1} + \frac{1}{N_2}\right) * EMS}$$

Critical Value test:

If the absolute difference between the means is greater than the smallest difference at a given alpha level,

$$|M_1 - M_2| > Fisher_{sd\alpha}$$

then the means of the two groups are significantly different.

If the absolute difference between the means is less than or equal to the smallest difference at a given alpha level,

$$|M_1 - M_2| \leq Fisher_{sd\alpha}$$

then the means of the two groups are not significantly different.



2.5.4.3 Cochran and Cox's Modified t-Test⁽⁹⁾

The subroutine COX_ROUTINE calculates the smallest difference between the means of two groups, which is necessary to determine that the means of the two groups are significantly different. The test is appropriate if the data are heterogeneous and the numbers of observations in each group are not equal. This test cannot be performed when Dfe is zero or there are fewer than 2 observations in either of the two groups. If the test cannot be performed, asterisks printed are in reports that display the LSD's.

Definitions:

Var_1 = variance of the first group (see the *Descriptive Statistics* section in this chapter for the calculation)

Var_2 = variance of the second group (see the *Descriptive Statistics* section in this chapter for the calculation)

N_1 = number of observations in the first group

N_2 = number of observations in the second group

Calculations:

The weighted variance for group (W_1) is:

$$W_1 = \frac{Var_1}{N_1}$$

The weighted variance for group (W_2) is:

$$W_2 = \frac{Var_2}{N_2}$$

The critical t value at the 0.01 level for group 1 is approximated by:

$$t_{1,01} = 2.5760 + \frac{4.9938}{N_1-1} + \frac{6.4255}{(N_1-1)^2} + \frac{25.9657}{(N_1-1)^3}$$

The critical t value at the 0.01 level for group 2 is approximated by:



$$t_{2,01} = 2.5760 + \frac{4.9938}{N_2-1} + \frac{6.4255}{(N_2-1)^2} + \frac{25.9657}{(N_2-1)^3}$$

The critical t value for the test at the 0.01 level is:

$$t_{01} = \frac{t_{1,01}W_1 + t_{2,01}W_2}{W_1 + W_2}$$

The smallest difference at the 0.01 level (Cox_{sd01}) is:

$$Cox_{sd01} = t_{01} * \sqrt{W_1 + W_2}$$

The critical t value at the 0.05 level for group 1 is approximated by:

$$t_{1,05} = 1.9600 + \frac{2.3802}{N_1-1} + \frac{2.5950}{(N_1-1)^2} + \frac{4.0333}{(N_1-1)^3}$$

The critical t value at the 0.05 level for group 2 is approximated by:

$$t_{2,05} = 1.9600 + \frac{2.3802}{N_2-1} + \frac{2.5950}{(N_2-1)^2} + \frac{4.0333}{(N_2-1)^3}$$

The critical t value for the test at the 0.05 level is:

$$t_{05} = \frac{t_{1,05}W_1 + t_{2,05}W_2}{W_1 + W_2}$$

The smallest difference at the 0.05 level (Cox_{sd05}):



$$Cox_{sd05} = t_{05} * \sqrt{W_1 + W_2}$$

Critical Value Test:

If the absolute difference between the two means is greater than the smallest difference at a given alpha level,

$$|M_1 - M_2| > Cox_{sd\alpha}$$

then the means of the two groups are significantly different.

If the absolute difference between the two means is less than or equal to the smallest difference at a given alpha level,

$$|M_1 - M_2| \leq Cox_{sd\alpha}$$

then the means of the two groups are not significantly different.



2.5.4.4 Welch's t-Test (16)

The subroutine WELCH_ROUTINE calculates the pairwise Student-t statistics for the control group versus a comparison group. It also calculates the appropriate degrees of freedom. The subroutine CRIT_STUDENT (see the *Critical Value Routines* section in this chapter for the calculation) is called to obtain the critical value at the 0.05 and 0.01 level, given Welch's degrees of freedom. For each pair, if the Student-t statistic is less than the critical value at the 0.05 level, the difference between the means of each pair are declared not to be statistically significant. If the Students-t statistic is greater or equal to the critical value at the 0.05 level, the difference between the means of the two groups are declared significantly different at the 0.05 level. If the Students-t statistic is greater than or equal to the critical value at the at the 0.01 level, then the means of the two groups are declared significantly different at the 0.01 level.

Definitions:

- M_i = mean value of observations for comparison group i
- N_i = number of observations in comparison group i
- VAR_i = variance of observations for comparison group i
- M_c = mean value of observations for the control group
- N_c = number of observations in the control group
- VAR_c = variance of observations for the control group
- $Stud-t_i$ = student-t statistic - the control versus comparison group i
- DFW_{Ni} = Numerator of the degrees of freedom calculation as defined by Welch - the control versus comparison group i
- DFW_{Di} = Denominator of the degrees of freedom calculation as defined by Welch - the control versus comparison group i
- DFW_i = The degrees of freedom as defined by Welch - the control versus comparison group i

Calculations:

Student-t

$$Stud-t_i = \frac{abs(M_c - M_i)}{\sqrt{\frac{VAR_c}{N_c} + \frac{VAR_i}{N_i}}}$$

Welch's Degrees of Freedom

Numerator:

$$DFWN_i = (VAR_c/N_c + VAR_i/N_i)^2$$



Denominator

$$DFWD_i = \frac{(VAR_c/N_c)^2}{N_c} + \frac{(VAR_i/N_i)^2}{N_i}$$

Degrees of Freedom:

$$DFW_i = \text{the closest integer value of } \frac{DFWN_i}{DFWD_i}$$



2.5.4.5 t-Test (19)

The subroutine T_ROUTINE calculates the pairwise Student-t statistics for the control group versus a comparison group. It also calculates the appropriate degrees of freedom. The PTS routine FPROB is called using the square of the calculated statistic and the calculated degrees of freedom. If the resulting P-value statistic is greater than 0.05, the difference between the means of each pair are declared not to be statistically significant. If the P-value is less than or equal to 0.05 but greater than 0.01 the difference between the means of the two groups are declared significantly different at the 0.05 level. If the Students-t statistic is less than or equal to 0.01 level, then the means of the two groups are declared significantly different at the 0.01 level.

Definitions:

- M_i = mean value of observations for comparison group i
- N_i = number of observations in comparison group i
- VAR_i = variance of observations for comparison group i
- M_c = mean value of observations for the control group
- N_c = number of observations in the control group
- VAR_c = variance of observations for the control group
- $Stud-t_i$ = student-t statistic - the control versus comparison group i
- DFW_i = The degrees of freedom - the control versus comparison group i

Calculations:

Student-t

$$Numerator_i = abs(M_c - M_i)$$

$$Denominator_i = \sqrt{\frac{(N_c - 1) * Var_c + (N_i - 1) * Var_i}{N_c + N_i - 2} * \frac{N_c + N_i}{N_c * N_i}}$$

$$Stud-t_i = \frac{Numerator_i}{Denominator_i}$$

Degrees of Freedom

$$DFW_i = N_c + N_i - 2$$



2.5.4.6 Modified t-Test⁽²⁰⁾

The subroutine MODT_ROUTINE calculates the pairwise Student-t statistics for the control group versus a comparison group. It also calculates the appropriate degrees of freedom. The PTS routine FPROB is called using the square of the calculated statistic and the calculated degrees of freedom. If the resulting P-value statistic is greater than 0.05, the difference between the means of each pair are declared not to be statistically significant. If the P-value is less than or equal to 0.05 but greater than 0.01 the difference between the means of the two groups are declared significantly different at the 0.05 level. If the Students-t statistic is less than or equal to 0.01 level, then the means of the two groups are declared significantly different at the 0.01 level.

Definitions:

- M_i = mean value of observations for comparison group i
- N_i = number of observations in comparison group i
- VAR_i = variance of observations for comparison group i
- M_c = mean value of observations for the control group
- N_c = number of observations in the control group
- VAR_c = variance of observations for the control group
- $Stud-t_i$ = student-t statistic - the control versus comparison group i
- $DFWN_i$ = Numerator of the degrees of freedom calculation as defined by Satterthwaite - the control versus comparison group i
- $DFWD_i$ = Denominator of the degrees of freedom calculation as defined by Satterthwaite - the control versus comparison group i
- DFW_i = The degrees of freedom as defined by Satterthwaite - the control versus comparison group i

Calculations:

Student-t

$$Stud-t_i = \frac{abs(M_c - M_i)}{\sqrt{\frac{VAR_c}{N_c} + \frac{VAR_i}{N_i}}}$$

Satterthwaite's Degrees of Freedom
Numerator:

$$DFWN_i = (VAR_c/N_c + VAR_i/N_i)^2$$



Denominator:

$$DFWD_i = \frac{(VAR_c/N_c)^2}{N_c-1} + \frac{(VAR_i/N_i)^2}{N_i-1}$$

Degrees of Freedom:

$$DFW_i = \text{the closest integer value of } \frac{DFWN_i}{DFWD_i}$$



2.5.4.7 Orthogonal Contrasts of Means⁽¹⁷⁾

The subroutine ORTH_ROUTINE calculates the Test, linear and quadratic contrasts and F-statistics. It uses the procedure FPROB to calculate the p-value of each F-statistic. The coefficients for orthogonal polynomials that are used are listed in the following table.

Definitions:

- Ndg = number of groups
- N₁ = number of observations in the control group
- N_i = number of observations in the *i*th comparison group, where *i* = 2,Ndg
- T = total number of observations in the study
- Dfe = error degrees of freedom: T-Ndg
- Dft = treatment degrees of freedom: Ndg-1
- ESS = error sum of squares (see the *Parametric Test: One-Way Analysis Of Variance (ANOVA)* section in this chapter for the calculation)
- Tot₁ = sum of observations for control group
- Tot_i = sum of observations in the *i*th comparison group, where *i* = 2,Ndg

- Coef1₁ = The Test Contrast Coefficient for the control group = -(Ndg-1).
- Coef1_i = The Test Contrast Coefficient for the *i*th comparison group, where *i* = 2,Ndg = 1

- Coef2₁ = The Linear Contrast Coefficient for the control group = 0.
- Coef2_i = The Linear Contrast Coefficient for the *i*th comparison group, where *i* = 2,Ndg - See the following table for values

- Coef3₁ = The Quadratic Contrast Coefficient for the control group = 0.
- Coef3_i = The Quadratic Contrast Coefficient for the *i*th comparison group, where *i* = 2,Ndg - See the following table for values.

Calculations:

The error mean square (EMS) or the mean square within:

$$EMS = \frac{ESS}{Dfe}$$



The Weighted Test Coefficients are:

$$WCoef1_1 = \frac{Coef1_1}{n_1}$$

$$WCoef1_i = \frac{Coef1_i}{n_i}$$

The Weighted Linear Coefficients are:

$$WCoef2_1 = 0$$

$$WCoef2_i = \frac{Coef2_i}{n_i}$$

The Weighted Quadratic Coefficients are:

$$WCoef3_1 = 0$$

$$WCoef3_i = \frac{Coef3_i}{n_i}$$

The Test Contrast is:

$$Test\ Contrast = (WCoef1_1)*(Tot_1) + \sum (WCoef1_i)*(Tot_i)$$

The Linear Contrast is:

$$Linear\ Contrast = \sum (WCoef2_i)*(Tot_i)$$

The Quadratic Contrast is:

$$Quadratic\ Contrast = \sum (WCoef3_i)*(Tot_i)$$



The Test Sum of Squares is:

$$\text{Test } S \text{ of } S = \frac{(\text{Test Contrast})^2}{(\text{WCoef1}_1 * \text{WCoef1}_1 * N_1) + \sum (\text{WCoef1}_i * \text{WCoef1}_i * N_i)}$$

The Linear Sum of Squares is:

$$\text{Linear } S \text{ of } S = \frac{(\text{Linear Contrast})^2}{\sum (\text{WCoef2}_i * \text{WCoef2}_i * N_i)}$$

The Quadratic Sum of Squares is:

$$\text{Quadratic } S \text{ of } S = \frac{(\text{Quadratic Contrast})^2}{\sum (\text{WCoef3}_i * \text{WCoef3}_i * N_i)}$$

The Test F-Statistic is:

$$\text{Test } F\text{-Statistic} = \frac{(\text{Test } S \text{ of } S)}{EMS}$$

The Linear F-Statistic is:

$$\text{Linear } F\text{-Statistic} = \frac{(\text{Linear } S \text{ of } S)}{EMS}$$

The Quadratic F-Statistic is:

$$\text{Quadratic } F\text{-Statistic} = \frac{(\text{Quadratic } S \text{ of } S)}{EMS}$$

Replace Coef1 with the Coefficients specified in the following table to calculate the Test Orthogonal Contrast of Means for a given number of dose groups. Replace Coef2 with the Coefficients specified in the following table to calculate the Linear Orthogonal Contrast of Means for a given number of dose groups. Replace Coef3 with the Coefficients specified in the following table to calculate the Quadratic Orthogonal Contrast of Means for a given number of dose groups. Note that Coef2₁ and Coef3₁ will always equal zero.



Orthogonal Contrasts of Means Coefficients (17-Table F)											
Group #	1	2	3	4	5	6	7	8	9	10	11
Test	-3	1	1	1							
Linear	0	-1	0	1							
Quadratic	0	1	-2	1							
Test	-4	1	1	1	1						
Linear	0	-3	-1	1	3						
Quadratic	0	1	-1	-1	1						
Test	-5	1	1	1	1	1					
Linear	0	-2	-1	0	1	2					
Quadratic	0	2	-1	-2	-1	2					
Test	-6	1	1	1	1	1	1				
Linear	0	-5	-3	-1	1	3	5				
Quadratic	0	5	-1	-4	-4	-1	5				
Test	-7	1	1	1	1	1	1	1			
Linear	0	-3	-2	-1	0	1	2	3			
Quadratic	0	5	0	-3	-4	-3	0	5			
Test	-8	1	1	1	1	1	1	1	1		
Linear	0	-7	-5	-3	-1	1	3	5	7		
Quadratic	0	7	1	-3	-5	-5	-3	-1	7		
Test	-9	1	1	1	1	1	1	1	1	1	
Linear	0	-4	-3	-2	-1	0	1	2	3	4	
Quadratic	0	28	7	-8	-17	-20	-17	-8	7	28	
Test	-10	1	1	1	1	1	1	1	1	1	1
Linear	0	-9	-7	-5	-3	-1	1	3	5	7	9



Orthogonal Contrasts of Means Coefficients (17-Table F)											
Group #	1	2	3	4	5	6	7	8	9	10	11
Quadratic	0	6	2	-1	-3	-4	-4	-3	-1	2	6



2.5.5 Transformations

2.5.5.1 Square Root Arc Sine

The subroutine TRANSFORM_ASIN uses the FORTRAN intrinsic functions ASIN and SQRT to transform each data point. The subroutine first checks to make sure that all data points are between 0 and 1. If any are not, the transformation is not performed.

Definitions:

x_{ij} = the j th data point in the i th group

When using a hand calculator, perform the following calculation:

$$Y_{ij} = \sin^{-1} (\sqrt{x_{ij}})$$

where \sin^{-1} means the inverse not the reciprocal.

2.5.5.2 Square Root X + 1

The subroutine TRANSFORM_XPL1 uses the FORTRAN intrinsic function SQRT to transform each data point. The subroutine first checks to make sure that all data points are greater than -1. If any are not, the transformation is not performed.

Definitions:

x_{ij} = the j th data point in the i th group

When using a hand calculator, perform the following calculation:

$$Y_{ij} = \sqrt{(x_{ij}+1)}$$



2.5.5.3 Probit⁽¹⁸⁾

The subroutine TRANSFORM_PRBT uses the PTS routine QNORM to calculate the probit transformation for each data point. The subroutine first ranks the study data and then uses the rank of each data point to determine the inverse normal value as defined in the routine QNORM.

Definitions:

x_{ij} = the *jth* data point in the *ith* group

r_{ij} = the *rank in the study of the jth* data point in the *ith* group

TOT = the total number of observations in the study

$$Y_{ij} = QNORM \left[\frac{r_{ij} - .5}{TOT} \right]$$



2.6 Categorical Data

2.6.1 Kolmogorov-Smirnov Two-Sample, One-Tailed Test⁽¹⁰⁾

Routine INS_SUM_KST uses the Kolmogorov-Smirnov Two-Sample One-Tailed Test to compare the cumulative distribution function of each comparison group (individually) with the cumulative distribution function of the control group.

The statistic, D , measures the greatest vertical distance between the graph lines of the two cumulative distribution functions. The user decides (by choosing which report to produce) whether to test if the comparison group has a greater severity than the control group or if the control group has a greater severity than the comparison group.

- n = maximum number of categories (severity levels)
- i = the i th category (severity level) being accumulated
- j = the j th category (severity level) being added to the accumulation
- fs_j = frequency of comparison entries at level j
- fc_j = frequency of control entries at level j

The value of the comparison cumulative distribution function Fs_i for level i is:

$$Fs_i = \frac{\sum_{j=1}^i fs_j}{\sum_{j=1}^n fs_j}$$

The value of the sample cumulative distribution function Fc_i for level i is:

$$Fc_i = \frac{\sum_{j=1}^i fc_j}{\sum_{j=1}^n fc_j}$$



To determine if the comparison group has a significantly greater severity level than the control group, compute for each finding:

$$D_i = Fs_i - Fc_i$$

To determine if the control group has a significantly greater severity level than the comparison group, compute for each finding:

$$D_i = Fc_i - Fs_i$$

The Kolmogorov-Smirnov Test statistic is:

$$D = \max (D_i)$$

At the 0.05 level, the Kolmogorov-Smirnov Critical Value (KS_{cv}):

$$KS_{cv} = \frac{5.99}{4} \left(\frac{\sum_{j=1}^n fs_j + \sum_{j=1}^n fc_j}{\sum_{j=1}^n fs_j * \sum_{j=1}^n fc_j} \right)$$

Therefore, if:

$$D > \sqrt{KS_{cv}}$$

the difference is significant at the 0.05 level.



2.6.2 Fisher's Exact Test

Fisher's Exact test is appropriate for analyzing two independent, random samples, X_1 and X_2 , drawn from a population of size N . The sample size for group 1 and group 2 is represented by N_1 and $N-N_1$, respectively. Each animal in each group either did or did not respond. Let p_1 be the proportion of animals in the first sample that responded, and let p_2 be the proportion of animals in the second sample that responded. Fisher's Exact test calculates the exact probability that p_1 equals p_2 for the observed data, as well as the probability that p_1 equals p_2 for all data, with the same margins, though more extreme.

Definitions:

A	=	number of animals in group 1 that responded
C	=	number of animals in group 2 that responded
A+C	=	total number of animals that responded
B	=	number of animals in group 1 that did not respond
D	=	number of animals in group 2 that did not respond
B+D	=	total number of animals that did not respond
A+B	=	total number of animals in group 1
C+D	=	total number of animals in group 2
A+B+C+D	=	total number of animals in group 1 and group 2
Nt1	=	number of tables used to calculate the right tail value when testing $p_1 < p_2$, where $A < B$
Ntg	=	number of tables used to calculate the right tail value when testing $p_1 > p_2$, where $A > B$
Nta	=	number of tables used to calculate the probability of the left tail value for the two-tailed test

The above designations can be summarized by the following table:

	Group 1	Group 2	Total
Responded	A	C	A+C
Not responded	B	D	B+D
Total	A+B	C+D	A+B+C+D



Calculations:

$$p_1 = \frac{A}{A+B}$$

$$p_2 = \frac{C}{C+D}$$

Pr, the probability that $p_1 = p_2$ for a given table, is calculated as follows:

$$Pr = \frac{(A+B)! (A+C)! (B+C)! (C+D)!}{A! B! C! D! (A+B+C+D)!}$$

Pr(observed) is calculated by the above equation, using the observed values.

Pr(more extreme) is the sum of the probabilities that $p_1 = p_2$ for each of the more extreme tables. It is also calculated using the above equation on the data from each of the more extreme tables.

The following method is used to calculate the values of each Pr:

If there are no zero cells, and the number responding (A) is greater than the number not responding (B), then:

$$Pr = \prod_{i=1}^C \frac{A+i}{A+B+i} \times \prod_{i=1}^D \frac{(B+i)(C+i)}{i(A+B+C+i)}$$

If there are no zero cells and the number responding (A) is less than or equal to the number not responding (B), then:

$$Pr = \prod_{i=1}^D \frac{B+i}{A+B+i} \times \prod_{i=1}^C \frac{(A+i)(D+i)}{i(A+B+D+i)}$$



The following are shortcut calculations:

If any margin equals 0, then:

$$Pr = 1.0$$

When either pair of diagonals both equals 0, then:

If A and D = 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^C \frac{i}{B+i}$$

If C and B = 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^D \frac{i}{A+i}$$

When only one cell equals 0, then:

If A equals 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^C \frac{D+i}{B+D+i}$$

If B equals 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^D \frac{C+i}{A+C+i}$$



If C equals 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^D \frac{B+i}{A+B+i}$$

If D equals 0, the probability can be calculated as:

$$Pr = \prod_{i=1}^C \frac{A+i}{A+B+i}$$

2.6.2.1 Fisher's Exact One-Tailed Test

If the alternative is $p_1 < p_2$, when $A < B$, the next more extreme table is determined by subtracting 1 from the first cell, then, keeping all margins fixed, adjusting all other cells accordingly. This process is followed until it is no longer possible to continue subtracting, while maintaining fixed margins. Note that no tables more extreme than the observed table may be possible. Let the probability for each of the possible Nt1 tables evaluated equal $P(< \text{extreme})_i$. For this alternative, calculate the total probability as:

$$Pr(\text{one-tail}) = Pr(\text{observed}) + \sum_{i=1}^{Nt1} Pr(< \text{extreme})_i$$

If the alternative is $p_1 > p_2$, when $A > B$, the next more extreme table is determined by adding 1 to the first cell, then, keeping the margins fixed, adjusting all other cells. This process is followed until it is impossible to continue added while maintaining fixed margins. Note that, in some cases, no tables more extreme than the observed are possible. Let the probability for each of the possible Nt2 tables evaluated equal $p(> \text{extreme})_i$. For this alternative, calculate the total probability as:

$$Pr(\text{one-tail}) = Pr(\text{observed}) + \sum_{i=1}^{Nt2} Pr(> \text{extreme})_i$$



2.6.2.2 Fisher's Exact Two-Tailed Test

The alternative is:

$$p_1 \neq p_2$$

If $A < B$, calculate the values of $\text{Pr}(\text{alternative})_i$ starting with $A = A+B$. Continue by decrementing A by 1 until $\text{Pr}(\text{alternative})_i = \text{Pr}(\text{observed})$.

check the following equation:

$$\text{Pr}(\text{two-tail}) = \sum_{i=1}^{Nta} \text{Pr}(\text{alternative})_i + \sum_{i=1}^{Nil} \text{Pr}(>\text{extreme})_i$$

If $A > B$, calculate the values of $\text{Pr}(\text{alternative})_i$ starting with $A = 0$. Continue by incrementing A by 1 until $\text{Pr}(\text{alternative})_i = \text{Pr}(\text{observed})$.

$$\text{Pr}(\text{two-tail}) = \sum_{i=1}^{Nta} \text{Pr}(\text{alternative})_i + \sum_{i=1}^{Nig} \text{Pr}(>\text{extreme})_i$$

2.6.3 Peto Analysis⁽¹¹⁾

The underlying method of analysis for a comparison of fatal and mortality independent tumors is described below.

If one or more animals die at each of the times $t_1 < t_2 < \dots < t_n$ of fatal (or probably fatal) tumors of the type of interest, then let the number of animals in all groups together just before t_k be R_k ; let the number of these dying of the tumor type of interest at time t_k in group i be o_{ik} (summing to $o_{.k}$); and let α_k be defined as unity if there were no ties at t_k , and as $o_{.k} / (R_k - o_{.k})$ otherwise. Let the proportion of the R_k at risk in group 1 be p_{1k} (so $\sum_i p_{ik} = 1$), and define $e_{ik} = o_{.k} * p_{ik}$ and $v_{ijk} = \alpha_k * p_{ik}(\delta_{ij} - p_{jk})$, where $\delta_{ij} = 1$ if $i = j$, and 0 otherwise. From the conditional contingency table of deaths from the tumor type of interest at time t_k , $(o_{ik} - e_{ik})$ and $(o_{jk} - e_{jk})$ are zero-expectation random variables with covariance v_{ijk} . Defining $O_i = \sum_k o_{ik}$, $E_i = \sum_k e_{ik}$ and $v_{ij} = \sum_k v_{ijk}$ yields the vector $\underline{O} - \underline{E}$ of zero-expectation mortality-standardized test statistics and their variance/covariance matrix, V , generally of rank r .



The prevalence method of analysis for incidental tumors must relate the likelihood of some incidental tumors being found to the number of autopsies in each group and generally proceed as above. Ignore autopsies of animals which died of the tumor of interest, or for which the organ of interest was not examined. Add together the Observed, Expected and Variance values from the death rate analysis and the prevalence analysis to get the pooled O , E , and V values.

For obvious reasons, some sort of a test for positive trend on only one degree of freedom is more likely to pick up any moderate carcinogenic effects that may exist than an r degrees of freedom test for heterogeneity between $(r+1)$ groups would be. Consequently we defined from our pooled O , E and V :

$$T = \sum_i D_i(O_i - E_i)$$

and

$$V = \sum_i \left(\sum_j D_i * D_j * V_{ij} \right)$$

where summation is over (Dose Group Numbers) 0 to r (or, if $D_0 = 0$, 1 to r). Under H_0 , $Z = T/V$ has mean zero and standard error unity, and analogy with the standard normal distribution allows a one-tailed P-value for positive trend to be estimated from Z .

If we let \underline{O} , \underline{E} and \underline{V} denote the r vectors and the rxr matrix derived from O , E and V by removal of row zero and/or column zero, then, if \underline{V} is of full rank, $X^2 = (\underline{O} - \underline{E})T * inv(\underline{V}) * (\underline{O} - \underline{E})$ will under *Hunderlino* be approximately X^2 on r degrees of freedom and may be used to test for heterogeneity between treatments in those few cases in which tests for positive trend with respect to dose are not more appropriate.

Maximum Likelihood estimation of the relative tumor on set rates in each group is a moderately complex procedure involving statistical principles well beyond the grasp of almost all experimentalists. However, the relative values of ML estimates can often be adequately approximated by the simple ratios O_i/E_i (unless there were high spontaneous prevalence of incidental tumors of the type being considered, or extreme differences in longevity between groups). The O_i/E_i are, in practice, usually useful descriptive statistics whenever treatment effects are only moderate (eg, 1, 2 or 3 standard deviations).



2.7 Acute Study Data

2.7.1 Litchfield and Wilcoxon's LD_{50} for Acute Studies⁽¹²⁾

Routine LDOUT estimates the parameters in the quantal response problem by the method of Litchfield and Wilcoxon. To perform the procedure, there must be at least three groups with non-infinite data. That is, there must be at least three groups in which all the animals are not all alive or all dead. The animals that respond in a group are those animals that died unscheduled deaths.

nr_i	=	number of animals responding in group i
pr_i	=	percent animals responding in group i
n_i	=	number of animals in group i
k	=	number of groups having respondents
N	=	total number of animals in responding groups
n_{av}	=	average number of animals in responding groups
n'	=	number of animals responding between LD_{16} and LD_{84} limits
A, B	=	line parameters (A intercept, B slope)
d_i	=	dose level for group i
ep_i	=	inverse normal of percent for group i
exp_i	=	expected percent for group i
Dgf	=	Degrees of Freedom



Table of Values at 0.05 Level of Significance

Dgf	CHI ₀₅	T ₀₅
1	3.84	12.71
2	5.99	4.30
3	7.81	3.18
4	9.49	2.78
5	11.1	2.57
6	12.6	2.45
7	14.1	2.87
8	15.5	2.31
9	16.9	2.26
10	18.3	2.23
11	19.7	2.20
12	21.0	2.18
13	22.4	2.16
14	23.7	2.15
15	25.0	2.13



Initial Values:

$$ep_i = QNORM(pr_i)$$

$$A = \frac{\sum_i ep_i * \sum_i \log_{10}^2 d_i - \sum_i \log_{10} d_i * \sum_i ep_i \log_{10} d_i}{k * \sum_i \log_{10}^2 d_i - (\sum_i \log_{10} d_i)^2}$$

$$B = \frac{k * \sum_i ep_i \log_{10} d_i - \sum_i \log_{10} d_i * \sum_i ep_i}{k * \sum_i \log_{10}^2 d_i - (\sum_i \log_{10} d_i)^2}$$

$$LD_{16} = 10 \frac{QNORM(.16) - A}{B}$$

$$LD_{50} = 10 \frac{QNORM(.50) - A}{B}$$

$$LD_{84} = 10 \frac{QNORM(.84) - A}{B}$$

$$slope = \frac{1}{2} \left(\frac{LD_{84}}{LD_{50}} + \frac{LD_{50}}{LD_{16}} \right)$$

$$lodose = \min_i(\log_{10} d_i)$$



$$hidose = \max_i(\log_{10}d_i)$$

$$R = 10.0^{(hidose - lodose)}$$

$$Dgf = k - 2$$

The expected percent dead for group i is:

$$\exp_i = PNORM (A + B * \log_{10}d_i), \text{ if } (A + B * \log_{10}d_i) \geq 0$$

$$\exp_i = 0, \text{ if } (A + B * \log_{10}d_i) < 0$$

The average number of animals in responding group is:

$$n_{av} = \frac{N}{k}$$

The chi-square dose effect value with (k-2) degrees of freedom is:

$$\chi^2 = n_{av} \sum_{i=1}^k \frac{(pr_i - \exp_i)^2}{\exp_i(1 - \exp_i)}, \text{ if } 0 < \exp_i < 1$$

$$\chi^2 = 0 \text{ if } \exp_i = 0 \text{ or } \exp_i = 1$$



This is a good fit if:

$$\chi^2 > \chi_{05}(k-2)$$

otherwise it is not a good fit.

The number of animals responding between LD₁₆ and LD₈₄ is:

$$n' = \sum_{i=1}^k n_i, \text{ if } .84 \geq \exp_i \geq .16$$

Compute the Confidence Intervals as follows if $n' > 0$:

The 95% Confidence Interval for Calculated LD₅₀ Value is:

$$\frac{LD_{50}}{FLD_{50}} \leq LD_{50} \leq LD_{50} * FLD_{50}$$

When a good fit:

$$FLD_{50} = slope \frac{2.77}{\sqrt{n'}}$$

When not a good fit:

$$FLD_{50} = slope \frac{1.4 * T_{05} Dgf * \sqrt{CHI_{05}(Dgf)}}{Dgf * n'}$$



The 95% Confidence Interval for Slope Value:

$$\frac{slope}{Fslope} \leq slope \leq slope * Fslope$$

First, calculate:

$$C = 10^{\frac{1.1 * \log_{10}^2 slope}{\log_{10} R}}$$

When a good fit:

$$Fslope = C^{\frac{10 * (k - 1)}{k * \sqrt{n'}}}$$

When not a good fit:

$$Fslope = C^{\frac{5.1 * T_{05}(Dgf) * (N-1) \sqrt{\frac{CHI_{05}(Dgf)}{Dgf * n'}}}{N}}$$



2.7.2 Finney's LD_{50} for Acute Studies⁽¹³⁾

Routine FINNEY_LDOUT estimates the parameters in the quantal response problem by the maximum likelihood method for an acute toxicology study (LD_{50}). The method used is a modified method of Finney. To perform the procedure, there must be at least three groups with non-infinite data.

nr_i	=	number of animals responding in group i
pr_i	=	percent animals responding in group i
n_i	=	number of animals in group i
k	=	number of groups having respondents
N	=	total number of animals in responding groups
A	=	intercept
B	=	slope
d_i	=	\log_{10} of the dose for group i
ep_i	=	empirical probit for group i
exp_i	=	expected probit for group i
wp_i	=	working probit for group i
w_i	=	weighing function for group i
σ^2	=	variance
exr_i	=	expected response (normalized) for group i
dr_i	=	difference response for group i
X^2	=	X^2 value with $(k-2)$ degrees of freedom



Calculations:

Step 1:

Initial Values:

$$t = 0$$

$$\textit{Previous slope}(t) = 0$$

$$ep_i = QNORM(pr_i) + 5$$

$$A(t) = \frac{\sum_i ep_i * \sum_i \log_{10}^2 d_i - \sum_i \log_{10} d_i * \sum_i ep_i \log_{10} d_i}{k * \sum_i \log_{10}^2 d_i - (\sum_i \log_{10} d_i)^2}$$

$$B(t) = \frac{N * \sum_i ep_i \log_{10} d_i - \sum_i \log_{10} d_i * \sum_i ep_i}{k * \sum_i \log_{10}^2 d_i - (\sum_i \log_{10} d_i)^2}$$



Step 2:

Begin Iteration:

$$t = t + 1$$

$$\text{exp}_i(t) = A (t - 1) + B (t - 1) * \log_{10} d_i$$

$$X(t) = \text{exp}_i(t) - 5$$

$$P_i(t) = PNORM (|X(t)|)$$

$$P_i(t) = 1 - P_i(t), \text{ if } X_i(t) < 0$$

$$Z_i(t) = -\frac{1}{\sqrt{2\pi}} e^{-\frac{X_i^2(t)}{2}}$$



$$wp_i(t) = \exp_i(t) + \frac{(pr_i - P_i(t))}{Z_i(t)} \text{ if } Z_i(t) > 0$$

$$wp_i(t) = 10 \text{ if } Z_i(t) \leq 0$$

$$w_i(t) = \frac{Z_i^2(t)}{(P_i(t)(1-P_i(t)))} \text{ if } 0 < P_i(t) < 1$$

$$w_i(t) = 0 \text{ if } P_i(t) = 1 \text{ or } P_i(t) = 0$$

$$\bar{x} = \frac{\sum_i n_i w_i(t) \log_{10} d_i}{\sum_i n_i w_i(t)}$$



$$\bar{y} = \frac{\sum_i n_i w_i(t) w p_i(t)}{\sum_i n_i w_i(t)}$$

$$sxx = \sum_i n_i w_i \log_{10}^2 d_i - \frac{\left(\sum_i n_i w_i(t) \log_{10} d_i \right)^2}{\sum_i n_i w_i(t)}$$

Step 3:

Calculate adjusted values for line constants:

$$B(t) = \frac{\sum_{i=1} n_i w_i(t) \left(\log_{10} d_i - \bar{x}(t) \right) \left(w p_i(t) - \bar{y}(t) \right)}{\sum_{i=1} n_i w_i(t) \left(\log_{10} d_i - \bar{x}(t) \right)^2}$$

$$A(t) = \bar{y}(t) - B(t) * \bar{x}(t)$$

$$\sigma^2(t) = \frac{1}{\sqrt{n_i w_i(t) \left(\log_{10} d_i - \bar{x}(t) \right)^2}}$$

$$Previous \ slope(t) = B(t - 1) - B(t)$$



Stop iterating if:

1. $B(t) = 0$
2. If $(t > 4)$ and
Previous Slope $(t-1) < \text{Previous Slope } (t)$
3. $t > 6$

Step 4:

Check if another iteration is indicated. If six iterations have been performed, stop iterating.

Calculate expected responses and X^2 values:

$$\text{exp}_i = A(t) + B(t) * \log_{10} d_i$$

$$X^2 = \text{exp}_i - 5$$

$$P_i(t) = \text{PNORM}(|X|)$$

$$P_i(t) = 1 - P(t) \text{ if } X < 0$$

$$\text{exp}_i = P_i(t)$$

$$dr_i = nr_i - P_i$$



The 95% Confidence Interval for the Slope = B(t) is:

$$B(t) - \sigma^2(t) \leq B(t) \leq B(t) + \sigma^2(t)$$

The LD values are:

$$LD_{16} = 10 \frac{4.0055 - A(t)}{B(t)}$$

$$LD_{50} = 10 \frac{5.0000 - A(t)}{B(t)}$$

$$LD_{84} = 10 \frac{5.9450 - A(t)}{B(t)}$$

The chi-square test statistic is:

$$X^2 = \sum \frac{(nr_i - n_i * P_i)^2}{n_i P_i (1 - P_i)}$$

If $x^2 \leq \text{CHI}_{05}(\text{Dgf})$, then homogeneous:

$$T = 1.96$$

$$H = 1.0$$

if $x^2 > \text{CHI}_{05}(\text{Dgf})$, then heterogenous:

$$T = T_{05} (K-2) \quad (\text{table value})$$

$$H = \text{CHI}_{05} (K-2) \quad (\text{table value})$$



Preliminary calculation for LD₅₀ Confidence Interval

$$m = \frac{5 - A(t)}{B(t)}$$

$$g = \frac{H * T^2}{B(t)^2 * SXX(t)}$$

$$v = m + \frac{g}{g - 1} (m - \bar{x}(t))$$

$$u = \frac{T}{B(t)(1-g)} \sqrt{H * \frac{1 - g}{\sum_{nl} w_i(t)} + \frac{m - \bar{x}(t)^2}{sxx(t)}}$$

The 95% confidence interval for calculated LD₅₀ is:

$$v - u \leq LD_{50} \leq v + u$$

2.7.3 Spearman-Kärber Estimator

Routine SPEARMAN_KARBER_LDOUT estimates the mean dose level. If the dose-response curve can be closely approximated by a logistic or a normal distribution, then the estimator can be used as an LD₅₀ estimate.



Definitions:

k = number of groups having respondents

d_i = dose level for group i

where:

$d_1 < d_2 < \dots < d_k$

n_i = number of animals in group i

nr_i = number of animals responding in group i

Calculations:

If nr_1 does not equal 0, then we must create a "zero" group. To do this, shift each value of i , starting with $i = k$.

Set:

$$nr_{i+1} = nr_i$$

$$n_{i+1} = n_i$$

$$d_{i+1} = d_i$$

Decrement i by 1 until $i = 1$.

$$\text{Set } nr_1 = 0$$

$$n_1 = n_2$$

$$d_1 = \frac{d_2}{2}$$

$$k = k + 1$$



If nr_k does not equal n_k , then we create an additional $k + 1$ group such that:

$$nr_{k+1} = n_k$$

$$n_{k+1} = n_k$$

$$d_{k+1} = d_1 + d_k$$

$$k = k + 1$$

The percent responding are:

$$pr_i = \frac{nr_i}{n_i}$$

where:

$i = 1, k$.

The Spearman-Kärber Estimator:

$$S-K \text{ Estimator} = \sum_{i=2}^k \frac{(pr_i - pr_{i-1})(d_i + d_{i-1})}{2}$$



The variance of the estimator:

$$Var_{SK} = \sum_{i=2}^{K-1} \frac{(d_{i+1} - d_{i-1})^2 (pr_i (1 - pr_i))}{4 \times n_i}$$

The standard error of the estimator:

$$Se_{SK} = \sqrt{Var_{SK}}$$

2.8 Printout of Raw Statistical Values

Output of raw statistical calculations can be obtained by assigning "X" privileges in the personnel table. The output will be appended to the selected summary output table. If ANOVA cannot be performed, this output will not be generated.

where:

NIDG	=	number of animals per group
MEAN	=	group means
SDEV	=	group standard deviations
MDIF	=	mean difference from control (compared to smallest difference)
VR	=	variance ratio (also called the F-Ratio, or FR, in this chapter)
FPR	=	F statistic probability
TSS	=	treatment sum of squares
DFT	=	treatment degrees of freedom
ESS	=	error sum of squares
DFE	=	error degrees of freedom
M	=	from Bartlett's test
C	=	from Bartlett's test
M/C	=	Bartlett's X^2 test statistic
DF	=	degrees of freedom (number of groups-1)
SD05	=	the smallest difference at the 0.05 level for given test
SD01	=	the smallest difference at the 0.01 level for given test
CHI ²	=	Chi-Square value for degrees of freedom, DF
VALUE	=	"TRUE" if variance is homogeneous, "FALSE" otherwise



The summary output table will look similar to the following example:

```
ANOVA STATISTICS
NIDG = nn      nn      nn      nn      nn
MEAN = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
SDEV = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
MDIF = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
      TSS  DFT  TSS/DFT      ESS  DFE  ESS/DFE      VR  FPR
STATS= xxxxxx  xx  xxxxxxxx xxxxxxx  xxx  xxxxxxxx xxxxxx xxxx

BARTLETT STATISTICS
      M      C      M/C DF CHI2 VALUE
STATS= xxxxxx xxxxxx xxxxxxx xx  xxxx true/false

DUNNETT STATISTICS
SD05 =          xxxxxxxx xxxxxxxx xxxxxxxx
SD01 =          xxxxxxxx xxxxxxxx xxxxxxxx

FISHER LSD STATISTICS
SD05 = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
SD01 = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx

COCHRAN AND COX MODIFIED T-TEST STATISTICS
SD05 = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
SD01 = xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxx
```



3 **PARTITIONING ALGORITHM FOR ANIMAL RANDOMIZATION INTO DOSE GROUPS**

3.1 **Overview**

The partitioning algorithm used by the AESLCT program provides an animal randomization procedure that avoids group mean body weight bias. This procedure is designed to perform well regardless of varying conditions of equal and unequal quantities of animals per group. The algorithm is executed once for each animal sex, and the starting point for each sex is the identification of an animal assignment list for that sex. The animal assignment list will consist of all animals of the appropriate sex that are currently alive and have not been removed by the user.



3.2 Definitions

In the algorithm for animal randomization, the following variables and functions are used:

NT	= total number of animals required by the protocol for assignment to dose groups
Ndg	= number of dose groups
NA(<i>i</i>)	= number of animals in the list of animals to be assigned. As each iteration of the partitioning algorithm is performed and animals are assigned, this list will shrink. There are 1 to NG iterations
ND	= number of animals to be discarded from the randomization process
N(<i>i</i>)	= dose group <i>i</i> , where <i>i</i> = 1...Ndg
CG(<i>i</i>)	= number of animals per dose group N(<i>i</i>)
K(<i>j</i>)	= number of animals in partition <i>j</i> during the assignment of group <i>i</i>
R(<i>i</i>)	= average partition size of animal list [of size NA(<i>i</i>)]
RAN(ISEED)	= standard FORTRAN intrinsic function to generate a random value in the open interval (0,1)
FLOAT(I)	= standard FORTRAN intrinsic function that converts an integer variable into a real (floating point) variable. The real variable is single precision with an accuracy of approximately 6.7 decimal digits
IFIX(R)	= standard FORTRAN intrinsic function that converts a variable into an integer variable. The IFIX function truncates the real variable to a value equal to the integer part of the real number. Examples of the conversion from real to integers by the IFIX function are:

<u>Real</u>	<u>IFIX(Real)</u>
1.99	1
3.01	3



3.3 A Textual Description of the Animal Partitioning Process

The algorithm performs preliminary steps to create the animal assignment list and the dose group list prior to executing an iterative procedure of partitioning, selecting and assigning animals to groups. The steps are as follows:

1. Sort the list of animals into descending order by body weight.
2. Animals without body weights are deleted from the assignment list first. They are deleted in descending animal number order, until either all zero-weight animals are deleted, or until the total number of animals to delete has been reached. Next, discard animals from the sorted list if the number of animals in the list is larger than the number of animals required for the study. The discard procedure is iterative, in that it discards the lightest animal first, the heaviest animal next, then the next lightest animal, the next heaviest animal, etc., until the list of animals has been reduced to the number of animals required for the study. The number of animals in the list to be assigned is $NA(i)$.
3. The dose groups, $N(i)$, are sorted into ascending order by the number of animals per dose group, $CG(i)$. That is: $CG(i) \leq CG(i + 1) \leq \dots \leq CG(Ndg)$.

4. Begin with the index $i = 1$.

Assign $CG(i)$ animals to dose group $N(i)$:

- Partition the animal list, of size $NA(i)$, into $CG(i)$ partitions (with an average of $R(i)$ animals per partition).
 - Randomly select an animal from each partition, and assign to dose group $N(i)$.
 - Remove these assigned animals from the animal list, leaving a shorter animal list, of size $NA(i + 1)$. If $NA(i + 1) = 0$, then all animals have been assigned; otherwise, continue to next step.
 - Increment the index ($i = i + 1$) and repeat this step.
5. When all groups have been assigned, the animal positions are then randomized within their assigned dose groups. This step avoids any weight bias in positioning the animals.



3.4 Discarding Animals from a Study

Animals must be discarded from the sorted list if the number of animals in the list is larger than the number of animals required for the study. There are two (2) discard procedures that can be used. The first is the menu option to discard animals uniformly by weight; the second is an automatic discard of animals if the first option is not exercised. The number of animals to be discarded, ND, is calculated for the current sex by subtracting the number of animals required in the groups from the number of animals in the assignment list (number of alive animals in pretest).

3.4.1 Option to Randomly Delete Animals Uniformly by Weight

This option uses a modification of the partitioning algorithm. First, the sorted list of animals is partitioned into ND partitions using the partitioning algorithm. Then, for each partition, an animal is randomly selected and marked for deletion. The random selection function is described in the algorithm calculations section, later in this chapter.

3.4.2 Automatic Discard

The automatic discard of animals is iterative, in that it discards the lightest animal first, the heaviest animal next, then the next lightest animal, the next heaviest, etc., until the list of animals has been reduced to the number of animals required for the study.



The partitioning algorithm is affected when the option to discard animals uniformly by weight is chosen first; the animals discarded are different. This would result in a different animal assignment list. However, either of these paths results in an unbiased animal assignment list.

If the option to discard animals uniformly by weight is chosen first, then the list of discarded animals is uniform. If this option is not chosen, and the animals are discarded automatically, then the discarded animals are outliers on both ends; only the heaviest and the lightest animals are discarded.

3.5 Calculation Algorithms

The average partition size, $R(i)$, is computed by:

$$R(i) = \frac{NA(i)}{CG(i)}$$

The number of animals in each partition, where $j = 1 \dots N(i)$, is computed by:

$$K(j) = IFIX \left(FLOAT(j) * R + \frac{1}{2} \right) - IFIX \left(FLOAT(j-1) * R + \frac{1}{2} \right)$$

For each partition, $j = 1 \dots N(i)$, set the starting animal index and ending animal index for each partition as:

$$\begin{aligned} \text{starting index } (j) &= \text{ending index } (j-1) + 1 \\ \text{ending index } (j) &= \text{starting index } (j) + K(j) - 1 \end{aligned}$$

where ending index (0) = 0.



For each partition, $j = 1 \dots N(i)$, the animals are selected randomly from the partition by computing the random index:

$$index = IFIX \left(RAN \left(ISEED \right) FLOAT \left(K \left(J \right) \right) \right) + starting\ index \left(j \right)$$

then the animals are assigned to group $N(i)$.

3.5.1 An Example of the Partitioning Algorithm Process

Presented here is an example of a partitioning algorithm for a study that requires a total of 9 animals. The animals are to be assigned to 3 dose groups, with dose group 1 requiring 2 animals, dose group 2 requiring 3 animals, and dose group 3 requiring 4 animals.

1. Animals are sorted in descending order by body weight. Dose groups are sorted in ascending order by number of animals.

The designation $NA(1)$ is used here to represent the first set of animals (namely, all of the animals) available for partitioning. The animals partitioned out of $NA(1)$ comprise the dose group denoted as $N(1)$. Similarly, the designation $NA(2)$ represents the second set of animals available for partitioning, and refers to the animals in $NA(1)$, less those assigned to $N(1)$. The animals partitioned out of $NA(2)$ comprise the dose group denoted as $N(2)$. The designation $NA(3)$ represents the third group of animals available for partitioning, and refers to the animals in $NA(2)$, less those assigned to $N(2)$. The animals partitioned out of $NA(3)$ comprise the dose group denoted as $N(3)$.



In this study, the body weights of the animals are:

All Animals Selected for Possible Use on Study

Animal I.D.	Body Weight	N(1) = 2	N(2) = 3	N(3) = 4
j	360.8			
a	358.3			
b	358.2			
c	356.0			
d	355.9			
e	355.8			
f	355.6			
g	355.0			
h	354.3			
i	353.1			
q	350.9			



2. The list of animals is then pared down to contain only as many animals as are needed for all dose groups. This is accomplished by discarding animals based on body weight: first the lightest, then the heaviest, then the next lightest, etc. In this case, two animals must be discarded, because we have 11 animals to partition, but need only 9 animals for the study. The lightest and heaviest animals, in this case animals q and j, are then discarded from the list of animal identifications. The following list represents the animals available for partitioning:

Animal List NA(1) — All Animals Available for Partitioning

Animal I.D.	Group N(1) CG(1) = 2	Group N(2) CG(2) = 3	Group N(3) CG(3) = 4
a	---	---	---
b	---	---	---
c		---	---
d			---
f			
g			
h			
i			



3. Partition $N(1)$ into 2 partitions, with the partitions being an average size of $K(j)$, as previously described in Section 3.5, *Algorithm Calculations*, in this chapter.

Animal I.D.
a
b
c
d
e
f
g
h
i

4. Randomly select an animal from each partition.

Animal I.D.	Random Selection
a	
b	←
c	
d	
e	
f	
g	
h	←
i	



5. Assign selected animals to dose group N(1), leaving the remaining animal list of size NA(2).

Dose Group N(1) — Animal Assignments

Animal List NA(2) — Animals Available for Partitioning

Animal I.D.	Group N(1) CG(1) = 2	Group N(2) CG(2) = 3	Group N(3) CG(3) = 4
a	b	---	---
c	h	---	---
d		---	---
e			---
f			
g			
i			

6. Partition animal list NA(2) into 3 partitions.

Animal I.D.
a
c
d
e
f
g
i



7. Randomly select an animal from each partition.

Animal I.D.	Random Selection
a	←
c	
d	
e	
f	←
g	←
i	

8. Assign selected animals to dose group N(2), leaving the remaining animal list of size NA(3).

Dose Groups N(1) and N(2) — Animal Assignments
Animal List NA(3) — Animals Available for Partitioning

Animal I.D.	Group N(1) CG(1) = 2	Group N(2) CG(2) = 3	Group N(3) CG(3) = 4
c	b	a	---
d	h	f	---
e		g	---
i			---



9. Partition animal list NA(3) into 4 partitions.

Animal I.D.
c
d
e
i

10. Randomly select an animal from each partition.

Animal I.D.	Random Selection
c	←
d	←
e	←
i	←

11. Assign selected animals to dose group N(3) (leaving no remaining animals, so partitioning stops).

Completed Dose Group Assignments

N(1) = 2	N(2) = 3	N(3) = 4
b	a	c
h	f	d
	g	e
		i



12. The animals are then randomized within each group.

Randomize Animals in Dose Groups N(1), N(2) and N(3)

N(1)	N(2)	N(3)
h	a	e
b	g	c
	f	i
		d

The option to randomly delete animals uniformly by weight utilizes a modification of the partitioning algorithm.



4 CLINICAL SIGNS AND CLINICAL OBSERVATIONS SUMMARIES

4.1 Number of Days the Sign is Present

The "number of days the sign is present" is used in a number of AOUTPT clinical signs reports, where the calculation is as follows:

- If the sign is present for consecutive examination sessions, the number of days present is the time between the two sessions, inclusive of the endpoints.
- If the sign is present on one session but not on the next session, the sign is counted as being present for only one day.

For example, if an observation is recorded on day 1, day 8, day 22, and day 29, and observations were also done on day 15, but the observation was not recorded on day 15, the adjusted algorithm will assume the observation was present only on days 1, 2, 3, 4, 5, 6, 7, 8, 22, 23, 24, 25, 26, 27, 28, and 29. No inference is made that the symptom may have been present on days 9, 10, 20, 21, etc. As a result, this calculation will most accurately reflect symptom persistence for studies where observations are made daily.



4.2 Mean Number of Animal Days with Clinical Sign or Observation

The "mean number of animal days with clinical sign" value designates the average number of days a sign was present for all animals in a group. The value is calculated as follows:

$$\text{Mean Number of Animal Days} = \frac{\sum_i DSP_i}{N}$$

where:

DSP_i = number of days the sign is present

N = number of animals affected by the sign

This number is then rounded to the number of decimal places required for the report.

4.3 Number of Animals Affected by the Sign

The "number of animals affected by the sign" is the total number of animals in the dose group which had the sign present on any day in the time interval chosen to be printed.



4.4 Time to Onset of Observation

The "time to onset of observation" is used in several VOUTPT summary tables.

The calculation is as follows:

$$\textit{Time to Onset of Observation} = \textit{DFSN} + 1 - \textit{ASD}$$

where:

DFSN = date an observation was first seen for the animal
ASD = animal start date

The animal start dates used when calculating "time to onset of observation" is determined in the following manner:

- If there is a randomization phase and the selected output date is after the randomization date, the animal start dates will be the animal start dates defined for the randomization phase.
- If there is no randomization phase or the selected output date is prior to the randomization date, the animal start dates will be the start dates for the earliest phase in which data was collected.



4.5 Mean Time to Onset of Observation

The "mean time to onset of observation" value designates the average time to onset of an observation for all animals in a group that have the observation. The value is calculated as follows and rounded to the nearest integer value:

$$\text{Mean Time Onset of observation} = \frac{\sum_i TTO_i}{N}$$

where:

TTO_i = the time to onset of the observation for animal i

N = the number of animals in the group with the observation

4.6 Median Time to Onset of Observation

The "median time to onset of observation" value designates the midpoint of the times the observation is seen for all animals in a group that have that observation. The value is calculated as follows:

$$\text{Median Time to Onset of the Observation} = MOA(N)$$

where:

MOA = array of animal observation onset times, sorted in ascending order

N = (the number of animals with observations in the group+1)/2

4.7 Percentage of Animals with Observation

The percentage of animals with the observation during an interval is calculated as:

$$\text{Percentage of Animals} = 100\% * \frac{N}{T}$$

where:

N = number of animals with observation

T = total number of animals in the group at the start of the observation period



5 FOOD AND WATER CONSUMPTION

5.1 Average Consumption/Animal/Day (Food and Water)

The following calculation is used when displaying this value during empty feeder/bottle weight entries in AINPUT, and when printing this value in food/water consumption reports in AOUTPT and AOTABS:

$$AC = \frac{(Tc - Sc)}{\sum_{i=1}^n Ndys(i)}$$

where:

- Ndys(i) = number of days between full and empty feeder/bottle weigh-outs for i-th animal in cage. If an animal dies during the interval, Ndys(i) = date of death minus full weigh-in date; otherwise, Ndys(i) = empty weigh-in date minus full weigh-in date. For outputs, if an animal is fasted prior to sacrifice, Ndys(i) = Ndys(i)-1 to adjust for the day of fasting.
- n = number animals alive in cage for any part of the weighing period
- Tc = total consumed for interval calculated as full weigh-ins less empty weigh-outs
- Sc = total feed spilled (zero for bottles)
- AC = average consumption per day per animal



5.2 Summary Food Conversion Efficiency

This algorithm assumes that food consumption and body weight gain are measured over a coincidental interval i and are calculated for each group and sex.

$$FE_i = \frac{\Delta BW_i}{FC_i} * 100.0$$

where:

FE_i = group food efficiency over interval i .

BW_i = group mean body weight gain over interval i .

FC_i = group mean food consumption over interval i . This may be calculated as a weighted or unweighted mean based on user selection.

Note that if any animals in a cage died during interval i , then the data for that cage is not used in the calculation.



5.3 Weighted Mean of Food/Water Consumed

The weighted means are calculated by summing the total food/water consumed per group, then dividing the group total by the number of animal days for the measurement period. The calculations are:

$$TOTFC = \sum_i FCC_i$$

$$TOTD = \sum_i TDC_i$$

$$MEAN = \frac{TOTFC}{TOTD}$$

$$SDEV = \sqrt{\frac{1}{TOTD - 1} \sum_i TDC_i \left(\frac{FCC_i}{TDC_i} - MEAN \right)^2}$$

where:

- FCC_i = total food/water consumed per cage i
- TDC_i = total alive animal days per cage i
- $TOTD$ = total animal days per group
- $TOTFC$ = total food/water consumption for group
- $MEAN$ = weighted group mean
- $SDEV$ = standard deviation of group mean



5.4 Unweighted Mean of Food/Water Consumed

The unweighted means are calculated by summing, for all cages in a group, the average food/water consumed per animal day per cage, and dividing that sum by the number of cages in the group. The average food/water consumed per animal day per cage is equal to the total food/water consumed per cage, divided by the total alive animal days per cage for the measurement period. The calculations are:

$$ACC_i = \frac{FCC_i}{TDC_i}$$

$$MEAN = \sum_i \frac{ACC_i}{NC}$$

$$SDEV = \sqrt{\frac{1}{NC - 1} \sum_i (ACC_i - MEAN)^2}$$

where:

FCC_i	=	total food/water consumed per cage i
TDC_i	=	total alive animal days per cage i
$AFCC_i$	=	average food/water consumed per animal day per cage
NC	=	number of cages for group
$MEAN$	=	unweighted group mean
$SDEV$	=	standard deviation of group mean



6 BODY WEIGHT LIMITS

6.1 Body Weight Limits

High and low tolerance factors, used in body weight range-checking, are extracted from the balance table for the calculation of body weight limits. The limits are calculated as follows:

$$R = BW_2 + \frac{BW_2 - BW_1}{T2 - T1} * (T - T2)$$

$$R = \text{MAX}(R, BW_2)$$

$$BWH = R * \left(1 + \frac{BWHI}{100}\right)$$

$$BWL = R * \left(1 - \frac{BWLO}{100}\right)$$

where:

- BW_1, BW_2 = two previous body weights
- BWH = high body weight range limit
- BWL = low body weight range limit
- $BWHI$ = high tolerance factor
- $BWLO$ = low tolerance factor
- T = current time point
- $T1, T2$ = two previous time points
- R = predicted weight for the current time point T , using $T1$ and $T2$ as the two previous time points

Values BWH and BWL are linearly extrapolated from the two (2) previous body weights, BW_1 and BW_2 , using $BWHI$ and $BWLO$. The default tolerance factor



is 5%. The relationship " $R = \text{MAX}(R, BW_2)$ " excludes the predicted weight when it is lower than the previously entered weight. In addition, a final low-weight limit is obtained from the following relationship:

$$BWL = \text{MIN} \left(BWL, BW_2 * \left(1 - \frac{BWLO}{100} \right) \right)$$

This algorithm calculates an upward range, even though there may be a sudden decrease in transient body weight and the tolerance factor is small, as in small animal studies.

6.2 Adjustment of Body Weight Limits for Pregnant Dams

In order to account for drastic loss of dam body weight during parturition, the lower limit of the body weight range is adjusted for all dam weights taken on the phase after the gestation phase.

After BWLO is calculated, as described in the previous section, for the phase after the gestation phase:

$$BWLO = BWLO * 2.5$$

6.3 Pup Body Weight Limits

Pup body weight limits are calculated based on the pup's most recent weight.

The default lower limit is calculated as 5% of the previous pup weight. The upper limit is calculated as 40% of the previous pup weight.



6.4 Terminal Body Weight Limits

Terminal body weight limits are calculated based on the last in-life body weight collected for the animal in A-Module.

The upper and lower limits are calculated as 5% of the last in-life body weight.



7 DOSING DATA CALCULATIONS

7.1 Least Squares Estimates

The least-squares calculation is used in the DOUTPT program to calculate the projected feed consumed and body weight values for a group, given three previous data values and associated dates when the data were collected.

X_i = date i (day of study on which data was collected)
 Y_i = data value i (mean feed consumed or mean body weight)
 N = total number of pairs of data values and associated dates
 A, B = estimated line parameters (A intercept, B slope)

The calculations are as follows:

$$B = \frac{\sum_i (X_i - \bar{X})(Y_i - \bar{Y})}{\sum_i (X_i - \bar{X})^2}$$

$$A = \bar{Y} - (B * \bar{X})$$

where:

$$\bar{X} = \frac{\sum_i X_i}{N}$$

$$\bar{Y} = \frac{\sum_i Y_i}{N}$$

Hence, the estimated value for the mean of X at Y_i is given by:

$$Y_i = A + (B * X_i)$$



7.2 Linear Extrapolation

Linear extrapolation is used in the DOUTPT program to calculate the projected feed consumed and body weight values for a group, given two previous data values and the associated dates when the data were collected.

X_i = data value i (mean feed consumed or mean body weight)

Y_i = date i (day of study on which data was collected)

D = duration of diet

A = estimated value

The calculation is as follows:

$$A = X_2 + \frac{X_2 - X_1}{Y_2 - Y_1} * \frac{D}{2}$$



7.3 Concentration of Test Article in Feed

This calculation, performed for each dose group and for each test article and control article, is performed by DOUTPT for diet concentration work sheets.

If the "disable expander ratio" parameter is added to the diet dosing determination, the DOUTPT calculation is changed to accommodate the use of this flag as it performs diet and drug consumption calculations.

The calculation of concentration in units/g of feed is as follows if the parameter is not added:

$$Conc = \frac{\left(\frac{dose_level * \overline{bw} * 0.001}{\overline{fc}} \right)}{(ratio * pre-mix)}$$

The calculation of concentration in units/g of feed is as follows if the parameter is added:

$$Conc = \frac{(dose_level * \overline{bw} * 0.001)}{\overline{fc}}$$

where:

$\overline{dose_level}$	=	group dose level in units/kg (from protocol)
\overline{bw}	=	dose group predicted mean body weight in g
\overline{fc}	=	dose group predicted mean feed consumed in g
ratio	=	article active ingredient ratio or purity (from protocol). If no purity is defined, the default value is 1.0
pre-mix	=	in cases where extra dilution takes place prior to diet work sheet generation. If no pre-mix is defined, the default value is 1.0 (controlled PRMX flag in DMODULE_OPTIONS.TBL)



7.4 Quantity of Feed Per Group to Use in Diet Admix Work Sheet

There are three different methods of generating the quantity of feed per group to be used in the diet.

1. The user may enter the quantity of feed for each group and sex if the flag GFWT is set to (TRUE) in the file DMODULE_OPTIONS.TBL.
2. The number of bags of feed per group and the weight of these bags may be key entered or obtained from the protocol if the flag GFWT is set to (FALSE) in the file DMODULE_OPTIONS.TBL.
3. If the flag PRED_QOD is set to (TRUE) in the file DMODULE_OPTIONS.TBL, then the quantity of feed for each group may be calculated based on the predicted feed consumed values. This flag is overridden by the setting of the flag GFWT.

The Quantity of Feed in g for each dose group is calculated as:

$$Feed_i = PFC_i * n_i$$

where:

PFC_i = each group's average predicted feed consumed for dose group i in g
 n_i = number of animals alive in dose group i at beginning of diet administration



7.5 Amount of Test Article to be Weighed

The total quantity of each test article to be weighed by group is first calculated, then these group totals are added to obtain the total amount to be weighed. The calculation to measure the quantity of each test article will differ based on whether the flag GFWT has been set to (TRUE) or (FALSE) in the DMODULE_OPTIONS.TBL.

If GFWT is (FALSE), then:

$$Chem = conc * feed$$

If GFWT is (TRUE), then:

$$x = \frac{1000 - conc}{1000}$$

$$Chem = \frac{(conc * feed)}{x}$$

Additionally, if the "disable expander ratio" parameter is in effect, the quantity of test article (chem) is divided by ratio.

where:

chem = total amount of test article to be weighed

conc = concentration of drug in feed (calculation shown previously) in units/g

feed = quantity of diet (obtained from protocol or key entry) in g

ratio = article active ingredient ratio or purity (from protocol)



7.6 Dose Based on Current Body Weight

When dose is dependent on body weight and:

dose_level	=	group dose level in unit/kg (from protocol)
bw	=	body weight of animal in g
ratio	=	article active ingredient ratio (from protocol)

individual doses, in units defined in the protocol, are calculated as:

$$Dose = \frac{(dose_level * bw * 0.001)}{ratio}$$



7.7 Dose Based on Body Surface

When dose calculation is dependent on body surface area:

dose_level = group dose level in units/m² (from protocol)
K = a constant value (from protocol)
bw = body weight of animal in g
ratio = article active ingredient ratio (from protocol)

Individual doses, in units defined in the protocol, are calculated as:

$$Dose = \frac{(dose_level * K * \sqrt[3]{bw^2}) * .0001}{ratio}$$

When dose calculation is dependent on body surface area and:

dose_level = group dose level in units/cm² (from protocol)
K = a constant value (from protocol)
bw = most recent body weight of animal in g
ratio = article active ingredient ratio (from protocol)

Individual doses, in units defined in the protocol, are calculated as:

$$Dose = \frac{dose_level * K * \sqrt[3]{bw^2}}{ratio}$$



7.8 Constant Dose

When dose is constant and independent of body weight and dose_level is defined in the protocol:

$$Dose = dose_level$$

7.9 Dose Based on Constant Body Weight

When a constant body weight is used to calculate dose throughout the study and:

dose_level = group dose level in units/kg (from protocol)
ratio = article active ingredient ratio (from protocol)
 bw_i = body weight of animal in g at date i

$$Dose = \frac{dose_level * bw_1 * 0.001}{ratio}$$



7.10 Up/Down Dosing

If the time critical observation approach in V-Module is chosen for use, there is an "up/down" dosing option which may be scheduled in D-Module; the up/down option must be chosen and the base dose, the dose increment, and the ratio of active ingredients to total material (expander ratio) entered.

The dose level is computed as follows:

$$dose_level = \frac{(base_dose * (increment)^{exponent})}{expander\ ratio}$$

then, if the animal body weight has been taken:

$$Dose = (dose_level * bw * 0.001)$$

When dosing using the up/down option, the Exponent (starting value 0) may be incremented or decremented with a corresponding increase or decrease in the dosing level. The new dosing level is applied to each animal. When the next animal is dosed, if it is alive, the Exponent is automatically increased by (1); if it is dead, the Exponent is decreased by one (1).



7.11 Time or Rate of Intravenous Administration

If the method of administration is designated as "infusion-speed" in the protocol, the user will also select the type of infusion pump to be used to administer the dose.

If a constant-rate pump is used, the amount of time to administer the given dose is calculated as:

$$Time = \frac{dose}{rate}$$

where:

dose = amount of drug (calculation shown previously)

rate = rate of administration as given in the protocol

If a variable-rate pump is used, the rate of administration for the given dose is calculated as:

$$Rate = \frac{dose}{time}$$

where:

dose = amount of drug (calculation shown previously)

time = time for administration as given in the protocol



7.12 Actual Drug Consumption

There are two methods of calculating actual drug consumption. Both use the following calculation of concentration:

$$Conc = \frac{(dose_level * \overline{bw} * 0.001)}{\overline{fc}}$$

where:

$\overline{dose_level}$ = group dose level in units/kg (from protocol)
 \overline{bw} = dose group predicted mean body weight in g
 \overline{fc} = dose group predicted mean feed consumed in g

The method used is determined by the setting of the flag ADC_FLG in the DMODULE_OPTIONS.TBL. If ADC_FLG is set to (FALSE), then drug consumption is based on a ratio of food consumption to body weight:

$$adc = conc \frac{(2000 * avfc)}{(bw(1) + bw(2))}$$

If ADC_FLG is (TRUE), then actual drug consumption is calculated as:

$$adc = conc * fc$$

If the "disable expander ratio" parameter is in the diet dosing determination, then no further calculation is done. If the "disable expander ratio" parameter is not in the diet dosing determination, then the ratio defined in the program is used to modify the drug consumed calculation:

$$adc = adc * (ratio * pre-mix)$$



where:

- adc = actual drug consumed in units
- fc = food consumed for animal over interval (in grams); calculated as
avfc * (number of cage days/number of animals in the cage)
- avfc = average food consumed/animal/day over interval (in grams)
- bw(1) = animal body weight at start of interval (in grams)
- bw(2) = animal body weight at end of interval (in grams)
- ratio = article active ingredient ratio (from protocol)
- pre-mix = in cases where extra dilution takes place prior to diet work sheet
generation. If no pre-mix is defined, the default value is 1.0
(controlled by the PRMX flag in DMODULE_OPTIONS.TBL).



7.13 Compound Consumption

This algorithm is used to calculate compound consumption for AOTABS/QOTABS reports when the .IDCV data command is used in the format file:

$$Consumption = \frac{(dose_level * \sum_{i=1}^n FC_i)}{BW * \sum_{i=1}^n Days_i}$$

where:

- dose_level** = group dose level in mg/kg or PPM (from protocol)
- FC** = food consumption which is the difference between the full and empty feeder weights for each consumption interval in grams. For "weeks across" reports each selected food consumption interval is summed.
- BW** = body weight in grams.
- Days** = Number of days for each included food consumption interval.

The resultant consumption is in either mg/kg/day or PPM.

Restrictions:

For "days across" compound consumption reports the set of dates presented for selection for the report are all days on which a scheduled empty feeder and scheduled body weight session exist on the same scheduled day.

For "weeks across" reports the set of dates are those on which a scheduled empty feeder session and a scheduled body weight session both exist on the following key days for the phase: 1,8,15,22,... etc. When the AMODULE_OPTIONS.TBL option DAYZ is FALSE these days are the first days of each week. When the DAYZ option is TRUE these are the last days of each week. If other body weight sessions exist during the chosen weeks, only the last body weight in the week that matches a food consumption scheduled day is used in the calculation for compound consumption. If other empty feeder sessions exist in the week the food consumed in those intervals are used in the summation of food consumption for the week.

Compound consumption intervals will be excluded from this report if a food consumption or body weight exclusion or both exist for that animal on the key day for that week (days 1,8,15,22,... etc.).

This algorithm will not account for sites with multiply housed animals, reporting "weeks across", with more than one food consumption scheduled during the week.



8 CELL COUNT AND CELL MORPHOLOGY

Program GOUTPT contains a Cell Count/Morphology Output Options menu of the cell count tables available within PATH/TOX SYSTEM. Calculations used by the System to prepare these tables are described as follows:

8.1 Corrected White Blood Count (WBC)

The correction of the WBC count consists of the following condition and calculation:

If there are five or more nucleated red blood counts (RBCs) per 100 white cells in a differential count, the WBC count will be corrected according to the following equation:

$$\text{Corrected WBC} = \frac{\text{Uncorrected WBC} * 100}{100 + \text{number of nucleated RBCs per 100 White Cells}}$$

Otherwise,

$$\text{Corrected WBC} = \text{WBC}$$

8.2 Absolute Differential Count

The calculation for the absolute differential count follows. If any value used in the calculation is blank, not taken (or "NT") or is a character string, the calculated value will be blank or contain "NT."

$$\frac{\text{Absolute Differential}}{\text{Cell Count}} = \frac{\text{Corrected WBC}}{100} * \frac{\text{percent of cells estimated}}{\text{by differential count}}$$



8.3 Percentage of Each Cell Type

The percentage of each cell type is calculated as follows:

$$\text{Percent of each cell type} = \frac{\text{number of cells of the selected type}}{\text{total number of cells counted}} * 100$$

The following algorithms are used to ensure that the percentages total 100 percent:

1. Truncate all values to a specified number of decimal places and save remainders.
2. Sum all truncated values.
3. If the sum does not equal 100, sort the remainders and calculate the number of entries to increase.

$$N = (100 - \sum \text{of truncated values}) * 10^{\text{number of decimal places}}$$

If sum equals 100, then go to Step 6.

5. The difference limit equals the N^{th} element of the sorted remainders.
6. Step through all values: if the difference between the actual and truncated value is greater than or equal to the difference in Step 5, increment the least significant decimal place of the truncated value.
7. Replace all percentages with new truncated value.

8.4 Miller Disk 111 Calculation of the Reticulocyte Count

Selecting the Miller Disk 111 table produces a reticulocyte count that is multiplied by 0.1 at the time of output.



9 PALPABLE MASS SUMMARIES

9.1 Mean Number of Masses Per Animal

The calculation for the mean number of masses per group for the group mean/median time to onset of tumors is different from the standard mean calculation used in the standard mass incidence summary tables. The calculation for mean number of masses per group is as follows:

$$\text{Mean Number Masses per Animal per Group} = \frac{\sum_i LMG_i}{N}$$

where:

LMG_i = for animal i largest number of masses over selected time interval

N = number of animals in the group



9.2 Time to Onset of Masses

The "time to onset of masses" is used in several MOUTPT summary tables.

The calculation is as follows:

$$\textit{Time to Onset of Masses} = \textit{DFSN} + 1 - \textit{ASD}$$

where:

DFSN = date a mass was first seen for the animal
ASD = animal start date

The animal start dates used when calculating "time to onset of masses" is determined in the following manner:

- If there is a randomization phase and the selected output date is after the randomization date, the animal start dates will be the animal start dates defined for the randomization phase.
- If there is no randomization phase or the selected output date is prior to the randomization date, the animal start dates will be the start dates for the earliest phase in which data was collected.



9.3 Mean Time to Onset of Masses

The "mean time to onset of masses" value designates the average time to onset of masses for all animals with masses in a group. The value is calculated as follows and rounded to the nearest integer value:

$$\textit{Mean Time, Onset of Masses} = \frac{\sum_i \textit{TTO}_i}{N}$$

where:

\textit{TTO}_i = the time to onset of first mass for animal i

N = the number of animals in the group with masses



9.4 Median Time to Onset of Masses

The "median time to onset of masses" value designates the midpoint of the time for the first mass to be seen for all animals with masses in a group. The value is calculated as follows:

$$\textit{Median Time to Onset of Masses} = \textit{MOA}(N)$$

where:

MOA = array of animal mass onset times, sorted in ascending order

N = (the number of animals with masses in the group+1)/2



10 SCHEDULED ANIMAL DEATHS

Algorithms used for determining scheduled animal deaths are described as follows, where the first two examples are based on pre-coded algorithms defined within the System, and the third example is for user-defined algorithms.

The processing screen for entering dead animal data follow these formats. An unscheduled death is indicated in this example by an "x."

```

                                SCHEDULED SACRIFICES
                                -----
                                Bottom of                Top of
                                Group                    Group

                                [ ] [ ] [x] [ ] [ ] [ ] [ ] [x] [ ] [ ]
                                1   2   3   4   5   6   7   8   9  10  <--- Animal Numbers
```

Example 1 — Select from top of group (pick 4 from group)

- a) Skip unscheduled dead (10,9,7,6)
- b) Do not skip unscheduled dead (10,9,7)

Example 2 — Select from bottom of group (pick 4 from group)

- a) Skip unscheduled dead (1,2,4,5)
- b) Do not skip unscheduled dead (1,2,4)

Example 3 — User algorithm

- a) Identify animals outside PATH/TOX SYSTEM
- b) Use A-Module or N-Module to select death status.



11 CONSUMABLE MATERIALS TRACKING

11.1 Solution/Suspension (mg/ml) Per Container

$$\begin{aligned}\text{VOL (ml)} &= \text{VEH (ml)} \\ \text{CNC (mg/ml)} &= (\text{CHEM (mg)}/\text{PUR})/\text{VEH (ml)} \\ \text{CHEM (mg)} &= \text{CNC (mg/ml)} * \text{VEH (ml)} \\ \text{VEH (ml)} &= \text{CHEM (mg)}/\text{CNC (mg/ml)} \\ \text{WGT (g)} &= (\text{VEH (ml)} * \text{SG (g/ml)}) + (\text{CHEM (mg)}/1000)\end{aligned}$$

where:

$$\begin{aligned}\text{CHEM} &= \text{total chemical} \\ \text{VEH} &= \text{total vehicle} \\ \text{CNC} &= \text{concentration of chemical in vehicle} \\ \text{PUR} &= \text{purity of chemical if not 100\%} \\ \text{SG} &= \text{specific gravity} \\ \text{VOL} &= \text{total volume of compound in a container} \\ \text{WGT} &= \text{total weight of compound in a container}\end{aligned}$$

11.2 Solution (ml/ml) Per Container

$$\begin{aligned}\text{CNC (ml/ml)} &= (\text{CHEM (ml)}/\text{PUR})/\text{VOL (ml)} \\ \text{VEH (ml)} &= \text{VOL (ml)} - (\text{CNC (ml/ml)} * \text{VOL (ml)}) \\ \text{CHEM (ml)} &= \text{CNC (ml/ml)} * \text{VOL (ml)} \\ \text{VOL (ml)} &= \text{CHEM (ml)} + \text{VEH (ml)} \\ \text{WGT (g)} &= (\text{VEH (ml)} * \text{SG (g/ml)}) + (\text{CHEM (ml)} * \text{SG (g/ml)})\end{aligned}$$

where:

$$\begin{aligned}\text{CHEM} &= \text{total chemical} \\ \text{VEH} &= \text{total vehicle} \\ \text{PUR} &= \text{purity of chemical if not 100\%} \\ \text{CNC} &= \text{concentration of chemical in vehicle} \\ \text{SG} &= \text{specific gravity} \\ \text{VOL} &= \text{total volume of compound in a container} \\ \text{WGT} &= \text{total weight of compound in a container}\end{aligned}$$



11.3 Solid (mg/mg) Per Container

$$\begin{aligned}\text{CNC (mg/mg)} &= (\text{CHEM (mg)/PUR})/\text{WGT (mg)} \\ \text{CHEM (mg)} &= \text{CNC (mg/mg)} * \text{WGT (mg)} \\ \text{FIL (mg)} &= \text{WGT (mg)} - (\text{CNC (mg/mg)} * \text{CHEM (mg)}) \\ \text{WGT (mg)} &= \text{CHEM (mg)} + \text{FIL (mg)}\end{aligned}$$

where:

CHEM = total chemical
FIL = total filler or vehicle
CNC = concentration of chemical to total weight
PUR = purity of chemical if not 100%
WGT = total weight of compound in a container

11.4 Diet Calculations (mg/kg)

$$\begin{aligned}\text{FEED (kg)} &= (\text{NDY} * \text{ANM} * \text{FC (g)})/1000 \\ \text{CNC (mg/g)} &= (\text{DOSE (mg/kg)} * (\text{BW (g)/1000}))/\text{FC (g)} * (\text{PUR} * \text{PMX}) \\ \text{CHEM (g)} &= (\text{CNC (mg/g)} * 1000) * \text{FEED (kg)}\end{aligned}$$

where:

CHEM = total chemical
FEED = total feed required for container
CNC = concentration of chemical to feed
BW = predicted group body weight average
FC = predicted group feed consumed average
PUR = purity of chemical if not 100%
PMX = pre-mix concentration if not 100%
DOSE = group dose level
NDY = number of days in dose period for formulation
ANM = number of animals alive in dose group at formulation time



12 **AUTOMATIC ASSIGNMENT OF ANIMALS TO STATIONS FOR NECROPSY**

The following algorithm is used by the program NIASSN for automatic assignment of animals to stations:

Given:

- The number of days over which necropsy is to occur (as defined in the protocol).
- The animals scheduled (determined during NINPUT initialization and from the protocol).
- The number of stations to assign (entered in NIASSN).

Assign so that the following occurs:

- Each station has an even distribution from each dose group.
- The order of the animals assigned to each station is such that the first animal on station 1 is assigned to dose group 1, the first animal on station 2 is assigned to dose group 2, etc.
- The order of the animals assigned to a station does not include two animals from the same dose group consecutively.
- Each prosector is to necropsy an equal number of animals from each dose group, and each dose group/prosector is evenly distributed over the day.

Separate each list of animals assigned to a station for necropsy on each defined sacrifice day:

- Determine the number n = animals per station/days for sacrifice.
- The first n animals for each station are to be done on the first day of necropsy, the second n animals for each station are to be done on the second day of necropsy, etc.



13 MICROPATHOLOGY MERGES

13.1 Tissue Merges

13.1.1 Destination Tissue Status

The following algorithm is used to determine the destination tissue's status. This algorithm is used once for each source tissue that is merged with a destination tissue.

Note that tissues with different sex masks cannot be combined. In addition, miscellaneous tissues are not allowed to be part of a tissue merge.

Sav_statd = Initial status of destination tissue.

Sav_stats = Initial status of source tissue.

Statd = Resulting status of destination tissue.

Statd = 0 (no status)

If either Sav_statd or Sav_stats has the status for the presence of a histological comment, Statd is set for the presence of a histological comment.

If either Sav_statd or Sav_stats has the status for the presence of a tissue recut request, the Tissue Recut Request status is added to the Statd status list field.

If either Sav_statd or Sav_stats has the status Tissue Missing, then

 this status is added to the Statd status list field.

Otherwise, if either Sav_statd or Sav_stats has the status Autolytic and Not-readable, then

 this status is added to the Statd status list field.

Otherwise, if either Sav_statd or Sav_stats has the status Inadequate, then

 this status is added to the Statd status list field.

Otherwise, if either Sav_statd or Sav_stats has the status Dashed, then

 this status is added to the Statd status list field.

Otherwise, if either Sav_statd or Sav_stats has the status Examined, then

 this status is added to the Statd status list field.



If either Sav_statd or Sav_stats has the status One of Pair Missing and Statd is not currently set for the Missing or Autolytic and Not-readable or Inadequate statuses, then

 this status is added to the Statd status list field.

If either Sav_statd or Sav_stats has the status Autolytic and Readable and Statd is not currently set for the Missing or Autolytic and Not-readable or Inadequate statuses, then

 this status is added to the Statd status list field.

13.1.2 Destination Tissue Required Flags

The following algorithm is used to determine the destination tissue's Required Flags:

For each source tissue involved in a tissue merge:

 For each group number on the study:

 If that group number flag has an unknown value for the destination tissue, the group number flag for the destination tissue is set to the corresponding value from the source tissue.

 If that group number flag is set to 'y' (required) for the source tissue, the group number flag for the destination tissue is set 'y'.

For each death code type:

 If that death code flag has an unknown value for the destination tissue, the death code flag for the destination tissue is set to the corresponding value from the source tissue.

 If that death code flag is set to 'y' (required) for the source tissue, the death code flag for the destination tissue is set 'y'.



13.2 Finding Merges

Findings are merged in the PATH/TOX SYSTEM in three cases:

- 1) When findings are merged permanently in PINPUT to remove a finding from the study.
- 2) When findings are explicitly merged using the finding merge utility in POUTPT.
- 3) When findings are implicitly merged when using the tissue merge utility in POUTPT.

In all three cases the following algorithm is followed to obtain the resultant finding data values:

If the destination finding has an entry (is not open and not dashed), no changes are made to the finding data for this animal's finding.

Otherwise:

The complete finding information including operator number and date are copied to the destination finding.



If the finding is a non-neoplasm or other-proliferative finding, and the severity grading schemes are different between the destination and source finding the following adjustment is done:

The original grading scheme of the destination finding will continue to be the grading scheme for the resultant destination finding.

DSL = Destination (final) severity level (integer value).

SSL = Source severity level (integer value)

NDSL = Number of severity levels defined for the destination severity grading scheme.

NSSL = Number of severity levels defined for the source severity grading scheme.

RDSL = Temporary severity level (floating point number).

$$RDSL = \text{FLOAT}(SSL) - 0.5$$

$$RDSL = (RDSL * \text{FLOAT}(NDSL)) / \text{FLOAT}(NSSL) + 0.5$$

$$DSL = RDSL(\text{truncated})$$

$$\text{IF } (DSL < 1) \text{ DSL} = 1$$

$$\text{IF } (DSL > NDSL) \text{ DSL} = NDSL$$



14 RANDOM SELECTION OF ANIMALS FOR PEER REVIEW

14.1 Overview

The program PIPEER is capable of producing a random list of animals for the Peer Review. The user selects the number (or percentage) of animals from each group and sex and (optionally) further conditions on the animals to be included such as death type and date of death range.

14.2 Definitions

In this algorithm the following variables and functions are used:

NT	=	total number of animals on the study
Ndg	=	number of dose groups
SEX	=	animal sex being processed
GROUP	=	animal group being processed
N(<i>SEX, GROUP</i>)	=	the number of animals to be selected from each dose group <i>GROUP</i> and sex <i>SEX</i>
RAN(ISEED)	=	standard FORTRAN intrinsic function to generate a random value in the open interval (0,1)
ISEED	=	The seed for the randomization function. The random seed is generated based on the protocol number, the number of randomizations during that run of the program, and the Peer Review number for that study. This results in the nth randomization attempt during the run of PIPEER for that study and Peer Review to be the same each time (for the same selection criteria).
Pnum	=	protocol number
PRnum	=	Peer Review number
Count	=	randomization interaction for this run of the program.
Animal_list(<i>L</i>)	=	list of the NT animals on the study
Selection_list(<i>M</i>)	=	list of the animals selected
Numsel	=	number of animals selected
Lastused	=	last animal used



14.3 A Textual Description of the Animal Selection Process

The steps are as follows:

1. Generate the starting seed number.

Seed = Pnum + (2 * Count) + (50 * PRnum)

2. Randomly order the entire list of animals.

For I = 1 to NT

J = RAN(ISEED) * (NT + 1 - I) * I

If J <= NT Then

Switch the values of Animal_list(I) and Animal_list(J)

End If

End For

3. Produce the selection list from this randomly ordered list.

Numsel = 0

For Sex = 1 to 2

For Group = 1 to Ndg

Lastused = 1

For K = 1 to N(Sex,Group)

For L = Lastused to NT

If Animal_list(L) is in group *Group* and sex *SEX* Then

Numsel = Numsel + 1

Select_list(Numsel) = Animal_list(L)

Lastused = L + 1

Break

Endif

End For

End For

End For

End For



15 REPRODUCTIVE DATA

15.1 Overview

The reproduction output functions use the following calculations when creating output reports. Several of these calculations are based on the number of females copulated. The criteria used to confirm copulation is based on the setting of the R-Module option flag CPLG. Please refer to the *PATH/TOX SYSTEM Customization Manual* for a complete description of the option flag CPLG and the determination of a female with "confirmed copulation".

15.2 Mating Index

The calculation for the Mating Index per group is as follows:

$$\text{Mating Index per Group} = \frac{MPVS}{NM} \times 100$$

where:

NPVS = number of Males resulting in a positive vaginal smear
NM = number of Males mated

15.3 Conception Index

The calculation for the Conception Index is as follows:

$$\text{Conception Index per Group} = \frac{MFPP}{MFCC} \times 100$$

where:

MFPP = Number of Male/Female pairs which resulted in a pregnant Female
MFCC = Number of Females with confirmed copulation



15.4 Fertility Index

The calculation for the Fertility Index is as follows:

$$\text{Fertility Index per Group} = \frac{MFPF}{MFC} \times 100$$

where:

MFPF = Number of Male/Female pairs which resulted in a pregnant Female
MFC = Number of Male/Female pairs cohabitated

15.5 Copulation Index

The calculation for the Copulation Index is as follows:

$$\text{Copulation Index per Group} = \frac{FCC}{MFC} \times 100$$

where:

FCC = Number of Females with confirmed copulation
MFC = Number of Male/Female pairs cohabitated

15.6 Pre-Implantation Loss (%)

The calculation for the Pre-implantation Loss is as follows:

$$\text{Pre-implantationloss}(\%) = \frac{CL-IMP}{CL} \times 100$$

where:

CL = Number of Corpora Lutea
IMP = Number of Implantations



15.7 Post-Implantation Loss (%)

The calculation for the Pre-implantation Loss is as follows:

$$\textit{Post-implantationloss}(\%) = \frac{\textit{IMP} - \textit{LF}}{\textit{IMP}} \times 100$$

where:

LF = Number of Live Fetuses/Embryos

IMP = Number of Implantations



16 TIME TO ONSET OF LESION

16.1 Overview

If an animal has at least one mass that correlates to a gross observation which in turn correlates to a finding; the program will determine the number of days from the start of the study the earliest mass that fits this criteria was palpated. Including the command `.DTON` in a P-Module POTABS raw report format file triggers this algorithm and causes the number of days to be displayed on a report. Including the command `.FIND` will cause the name of the finding to be displayed.

16.2 Time to Onset Algorithm

The algorithm first checks if an animal has any macroscopic findings. If the animal does, the algorithm scans each possible microscopic finding to see if it was recorded for the animal. If a recorded microscopic finding has no macroscopic finding correlation or has a macroscopic finding correlation, but no palpable mass correlation, it is ignored. If the recorded finding is correlated to more than one gross observation, the gross observation that correlates to the earliest mass will be used. If a palpated mass was formed by the convergence of two or more masses, the day of onset is calculated from the earliest palpated mass. If a palpated mass diverged from another mass, the day of onset is calculated from the first time the divergent mass was detected.

The algorithm may be checked by producing and analyzing four reports.

- 1) A POTABS report that includes the commands `.dton` and `.find`
 - 2) the POUTPT Individual Animal Report of Correlated Gross and Microscopic Diagnoses
 - 3) the NOUTPT Confirmed Raw Data Listing
 - 4) The MOUTPT raw data listing for Palpable Mass Tracking.
- A) Select an animal from report #1 and note the number of days to onset and the corresponding finding.
- B) For the animal selected, look at the data on report #2 and determine which findings for the animal are correlated to gross observations. Note this list of findings that are correlated to gross observations.
- C) Using the list of findings with correlated gross observations, note which observations on report #3 also have confirmed palpable masses and note the corresponding mass numbers. Select the mass with the lowest number. This mass should correspond to the finding listed on the POTABS report.
- D) On report #4 find the first occurrence of the mass number selected. This first occurrence should correspond to the day listed on the POTABS reported.



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