Statistical Tool for Data Analysis and Elaboration of Draft Maximum Residue Limits for Veterinary Drugs (MRLVDs) in Edible Tissues

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Summary

This paper describes a statistical tool for experts who prepare working papers for the FAO/WHO Joint Expert Committee on Food Additives. It may be used in cases where a statistical approach appears to be the method of first choice when elaborating MRLs.

The proposed approach includes:

(1) Linear regression analysis of kinetic data describing the terminal depletion of a suitable marker residue in edible tissues following the (last) administration of the drug under approved conditions of use;
(2) Subsequent use of the results of the regression analysis for the estimation of upper limits of the 95% (alternatively 99%) confidence interval for the upper one-sided tolerance limit on the 95th (alternatively 99th) percentile of the population sampled;
(3) Iterative calculation of such statistical limits as a function of time over the whole phase of terminal elimination of the marker residue;
(4) Proposal of Maximum Residue Limits for Veterinary Drugs derived from a set of such statistical limits for the standard edible tissues. The proposed limits have to meet the following requirements:
  a. The time point selected to derive the MRLVDs is compatible with (established) practical withdrawal times of the veterinary drug.
  b. Observation of the proposed MRLVDs does not result in an excess over the ADI of the daily intake of consumers averaged over lifetime of residues of toxicological / microbiological concern.

The main advantages of the suggested approach are: The MRL- values derived by the methods described take account of both, the ADI and the kinetic behaviour of the residues in the standard edible tissues. A link between proposed MRLs and the necessary depletion times to reach these MRLs is created and the numerical values of the MRLs reflect the proportions in which the residues occur in different tissues following treatment of the animals according to good practices in the use of veterinary drugs.

Basic Assumptions and Requirements

According to the well known compartment model of pharmacokinetics, the time course of the concentration of residues in tissues following administration of veterinary drugs to animals and through all phases of absorption, distribution and excretion can typically be described by the sum of a suitable number of exponential terms.

$$C_t = \sum_1^n C_{0,i}\, e^{-k_i\, t} \qquad [1]$$

where:

C_t is the concentration at time t,
$C_{0,i}$ is the fictitious concentration corresponding to t = 0 and the i th exponential term,
k_i is the rate constant corresponding to the i th exponential term.

For the description of the terminal phase of elimination the use of a single exponential term is most frequently fully adequate. Equation [1] then reduces to:

$$C_t = C_0 e^{-kt} \qquad [2]$$

or:

$$\log C_t = \ln C_0 - kt = \log C_0 - \frac{k}{2.303} t \qquad [3]$$

From equation [3] it follows that there exists a linear relationship between the logarithmically transformed concentration data (expressed as dimensionless "mass fractions", e.g. ppm, ppb, ppt) and time. Equation [3] can be re-written in the more general form of a straight regression line:

$$y = a_{yx} + b_{yx} x \qquad [4]$$

where a_{yx} and b_{yx} are estimates of log (C_0) and of –k/2.303, respectively. The method of the "least squares" can be applied for calculating the regression line, i.e. the straight line which fits best to the given data. The parameters of the regression line can be calculated according to standard procedures. Formulas used are given below.

Table 1: Calculation of the regression line*

$y = \bar{y} + b_{yx}(x - \bar{x}) = a_{yx} + b_{yx} x$	$b_{yx} = \frac{S_{xy}}{S_x}$ $a_{yx} = \bar{y} - b_{yx}\bar{x}$	$S_x = \sum x^2 - \frac{(\sum x)^2}{n}$ $S_y = \sum y^2 - \frac{(\sum y)^2}{n}$ $S_{xy} = \sum xy - \frac{\sum x \sum y}{n}$
	$r = \frac{S_{xy}}{\sqrt{S_x S_y}}$	
	$s_{y.x} = \sqrt{\frac{S_{y.x}}{n-2}}$	$S_{y.x} = S_y - b_{yx} S_{xy}$

*see reference (1)

Confidence intervals for the upper one-sided tolerance limit on given higher percentiles of the population can easily be calculated using the results of the regression analysis:

$$\hat{y} = a_{yx} + b_{yx} x + k_T s_{yx} \qquad [5]$$

y "hat" ($\hat{y}$) of equation [5] in this context represents the upper limit of the 1-α confidence interval for the one-sided upper tolerance limit of the fraction 1-γ of the residue concentrations predicted for the population of animals treated in the same way. Its significance is that at least the fraction 1-γ of the residue concentrations found in the animals of this population at the given time points can be expected to be below this numerical value with a confidence of 1-α. A convenient procedure for the calculation of k_T has been published by Stange (3). The equations are as follows:

$$k_T = \frac{\sqrt{2n-4}}{(2n-4)-u_{1-\alpha}^2}\left(\sqrt{2n-4}u_{1-\gamma} + u_{1-\alpha}W_n\right) \qquad [6]$$

$$W_n = \sqrt{u_{1-\gamma}^2 + ((2n-4)-u_{1-\alpha}^2)\left(\frac{1}{n} + \frac{(x-\bar{x})^2}{S_x}\right)}$$

It is suggested that normally 0.95 should be used for both, 1-γ and 1-α (i.e. 95% of the population and 95% statistical confidence). The respective percentage points of the standard normal distribution $u_{1-\gamma}$ and $u_{1-\alpha}$ which are used in the Stange-equation are 1.6448 if 0.95 is chosen for 1-α (1-γ) and 2.3264 if 1-α (1-γ) is 0.99.

Regression Analysis

Independent variable: Linear regression analysis requires that the independent ("predictor") variable chosen is not subject to random variation. The errors of the independent variable should also not be correlated to the errors of the dependent variable. In residue depletion studies with slaughter animals the time after treatment of the animals is typically the **only** useful independent variable which meets the conditions for regression analysis.

Dependent variable: The data used as estimates of the dependent ("response") variable for regression analysis should be independent from each other. This requirement is normally met since at every time point of tissue sampling another animal is sacrificed. However, when replicate analyses of the same tissue are performed, averages should be used instead of the single results of the individual replicate analysis. The number of replicate analyses should be similar for all samples used in the same statistical evaluation. If data are obtained from similar tissues sampled from different sites of the body (e.g. subcutaneous fat, perirenal fat, omental fat) averages should also be calculated before performing statistical calculations.

Homogeneity of variances of the log-transformed data on each time point: It should be tested whether or not the variances of the logarithmically transformed concentration data vary as a function of time, i.e. whether or not the hypothesis of in-homogeneity of variances can be rejected. Depending on the results either equi-weighted (homogeneity) or weighted (heterogeneity) linear regression has to be performed. National (e.g., the Food and Drug Administration of the United States of America (FDA)
[see: http://www.fda.gov/cvm/guidance/guideline3pt6.html]
as well as international institutions, such as the Committee for Veterinary Medicinal Products of the European Agency for the evaluation of Medicines (CVMP/EMEA)
[see: http://www.emea.eu.int/pdfs/vet/swp/003695en.pdf] recommend certain tests. The EMEA offers a programme for the calculation of withdrawal times which uses the recommended tests and which can also be downloaded from the Internet
[see: http://www.emea.eu.int/index/indexv1.htm]

Linear relationship between time of slaughter and the log-transformed data and log-normality of the distribution of the errors: The logarithmically transformed concentration data plotted against the slaughter times should at least be visually inspected for linearity of the relationship between the two variables. Deviation may be due to, e.g., continued influence on the concentration values of the distribution phase (at earlier time points) or closeness to the limit of quantification/detection of the analytical method (at late time points). FDA guidelines as well as EU guidelines recommend to carry out an analysis of variance for further statistical assurance of the linearity.

Furthermore a graph of the ordered normalised residuals (ordered differences between the experimental data and the values predicted by the regression model divided by the "residual error" $s_{y..x}$) versus their cumulative frequency should be prepared on a normal probability scale. The results should ideally lie on a straight line if the errors of the logarithmically transformed concentration data are normally distributed.

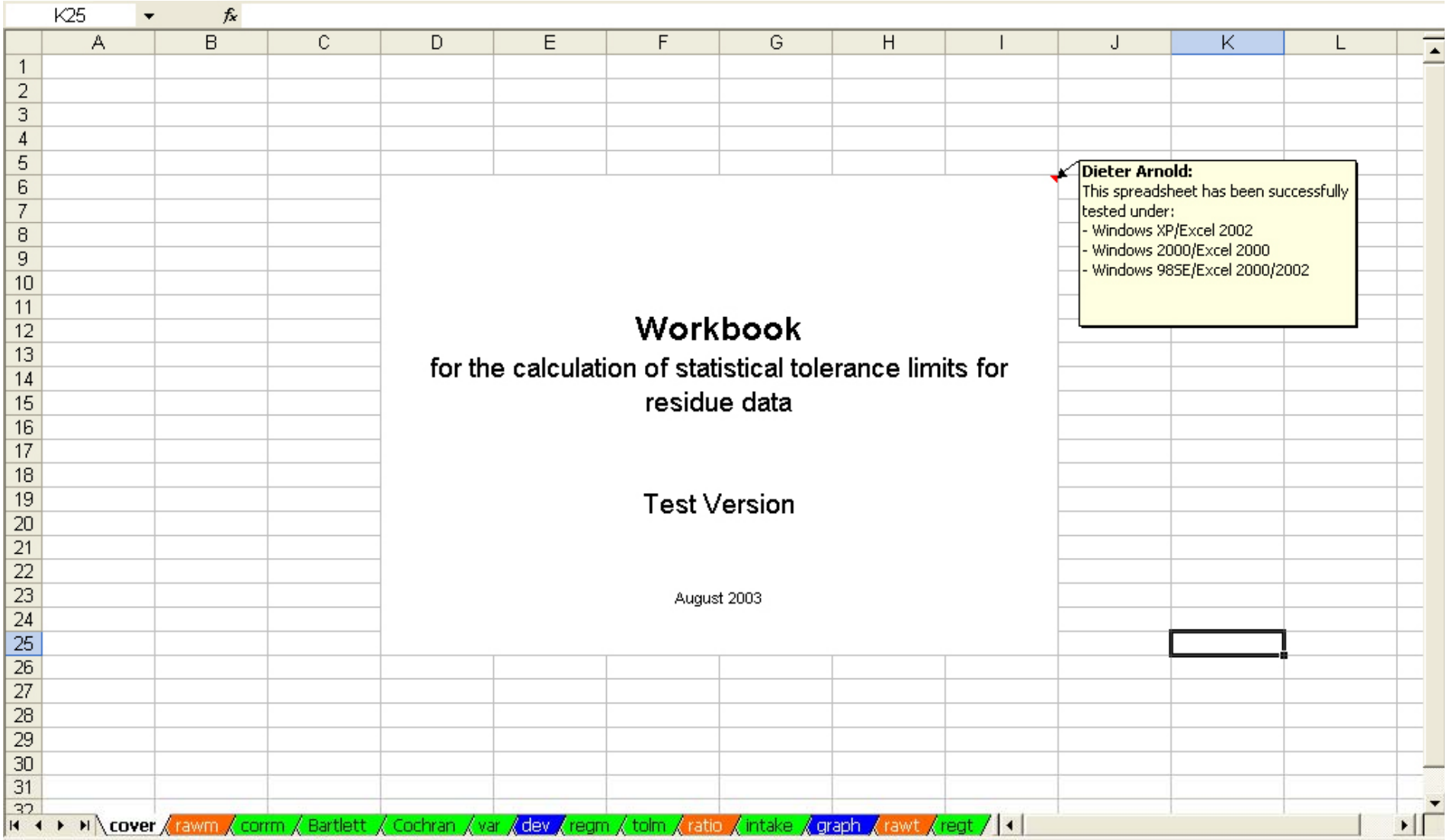
References

(1) Wissenschaftliche Tabellen Geigy, Teilband Statistik, 8. Auflage, Basel 1980, p 211.
(2) Kurt Stange, Angewandte Statistik, Vol.III, pp 141-143, Springer Verlag, Berlin, Heidelberg, New York, 1971.
(3) Handbook of Mathematical Functions, pp 932-933; edited by M. Abramowitz and A. Stegun. Dover Publications, Inc., New York. Ninth Printing 1970.
(4) Lothar Sachs, Angewandte Statistik, Springer Verlag Berlin, Heidelberg, New York, Tokyo 1992

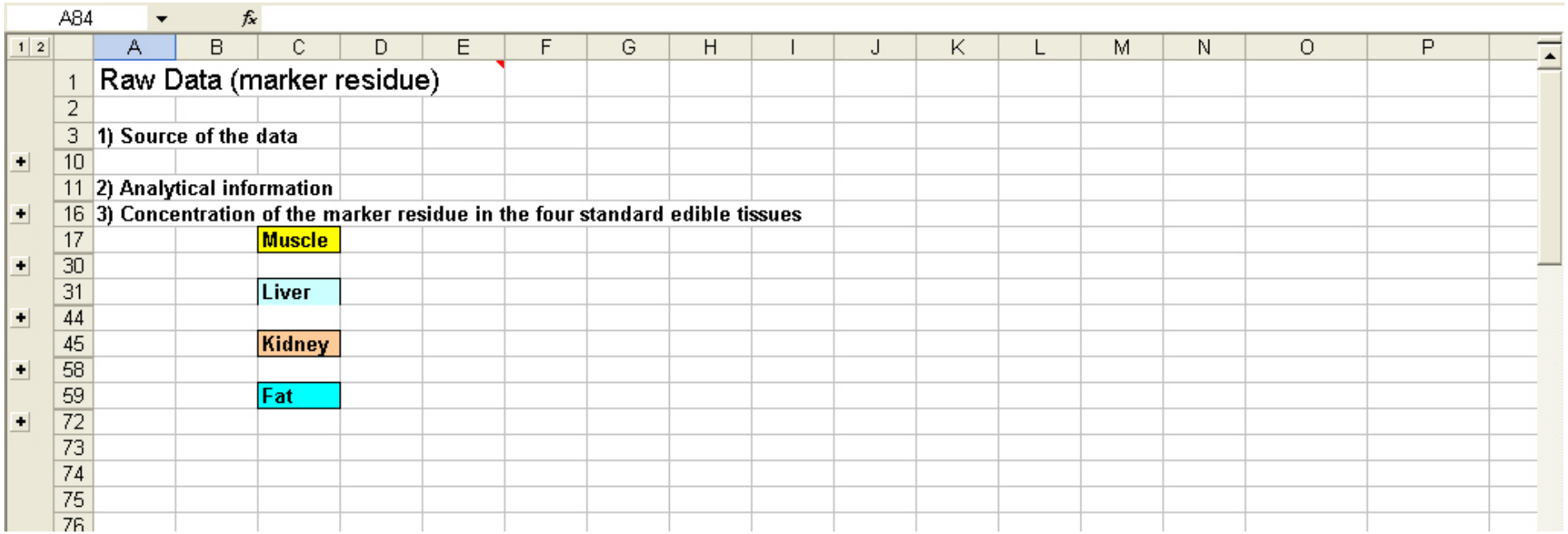
Excel workbook

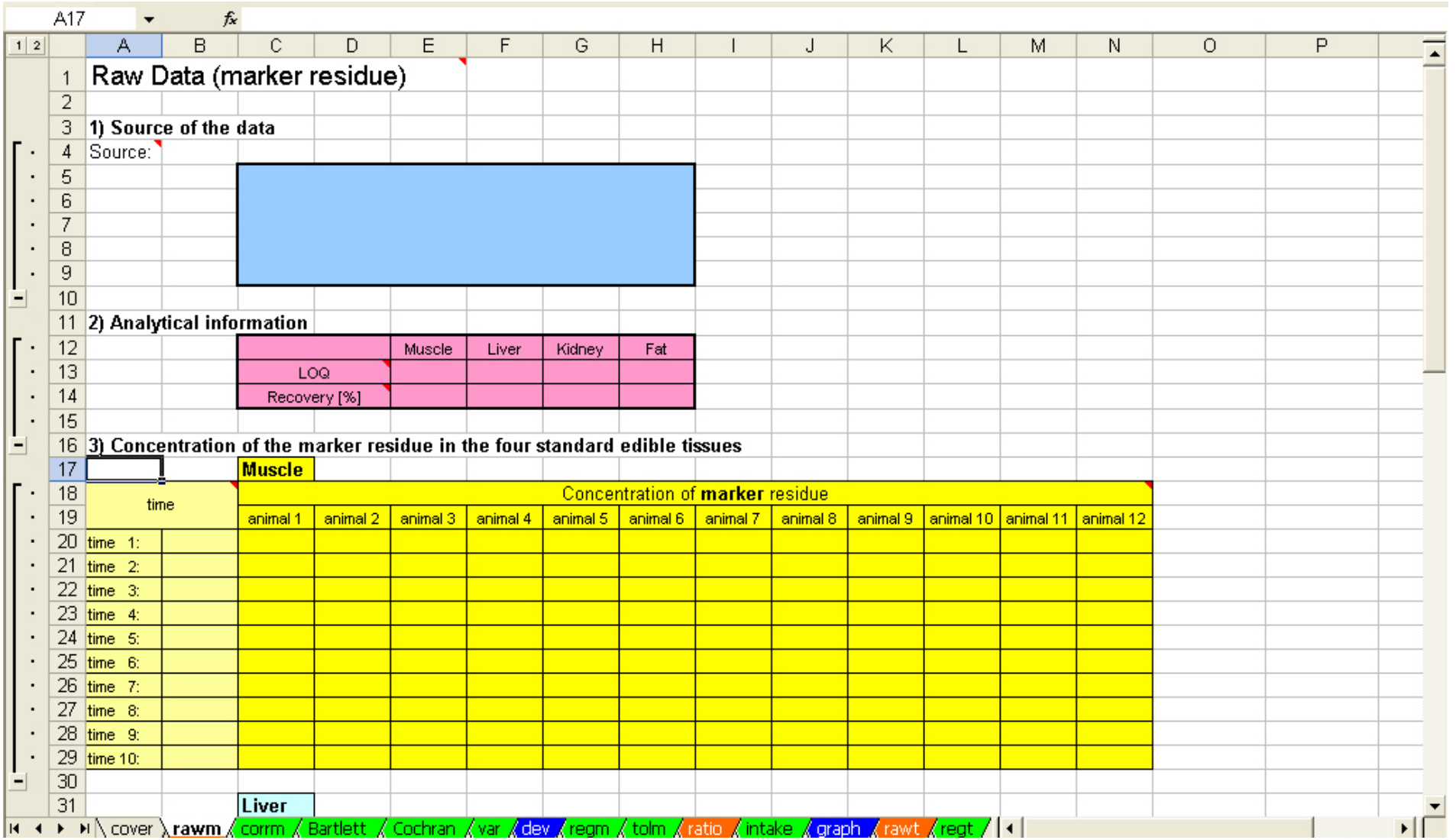
To facilitate the application of statistical tests to kinetic residue depletion data and the calculation of statistical tolerance limits a workbook has been developed which is based on Microsoft EXCEL 2002. The workbook uses simple EXCEL instructions only. Intentionally no use of more sophisticated techniques, e.g., VBA programming has been made in order to allow the user to check every individual number calculated, make the procedures fully transparent, and ensure compatibility with as many as possible of the earlier versions of EXCEL The workbook contains 13 worksheets which serve the following purposes:

“cover”	
Description of the function of the worksheet	Comments found on the worksheet
Cover of the workbook The “empty” workbook has been saved as template (extension .xlt). It is recommended to rename and save it (now with extension .xls) shortly after opening it and entering the first data. Windows 98 tends to be relatively unstable when working with large Excel workbooks (this has never been observed with Windows 2000 and Windows XP). Therefore, it is recommended to save the progress of the ongoing work frequently. The workbook contains a huge number of active cells. They will not necessarily contain meaningful results before all the required data have been filled into the appropriate tables. Red tabs indicate that the user has to fill in data into the tables of the sheet. Blue tabs indicate that the user has to sort and copy certain data for graphical purposes on these sheets. Green tabs indicate that no changes have to be made by the user. Users should read and follow the comments and instructions given on certain worksheets.	This spreadsheet has been successfully tested under: - Windows XP/Excel 2002 - Windows 2000/Excel 2000 - Windows 98SE/Excel 2000/2002


K25
Dieter Arnold:
This spreadsheet has been successfully tested under:
- Windows XP/Excel 2002
- Windows 2000/Excel 2000
- Windows 98SE/Excel 2000/2002
Workbook
for the calculation of statistical tolerance limits for residue data
Test Version
August 2003
cover
rawm
corrm
Bartlett
Cochran
var
dev
regm
tolm
ratio
intake
graph
rawt
regt

"rawm"	
Description of the function of the worksheet	Comments found on the worksheet
This worksheet has to be used to enter raw data relating to the marker residue! The individual tables of the worksheet are hidden in the template. Only the basic structure of the sheet is visible. The user has to klick on the "+" buttons in column 1 (rows 10, 16, 30, 44, 58, and 72) to unfold the tables and enter the data.	Cell E1: Do not use this spreadsheet for the calculation of statistical tolerance limits if the number of time points is lower than 3 and the number of animals per time point is lower than 4 to 5. (Certain statistical tests require a minimum number of 5 animals per time point). Cell E4: Please provide a full reference to the study. Cell D13: The LOQ must be given in the same units of concentrations as in the below tables! Cell D14: Recoveries must be given in %. Cell B18: All time points must be given in the same dimensions (e.g. days, hours, etc.). The number of time points and animals can differ for all four tissues as long as it remains <=10 for the time points and <=12 for the animals. Do not delete empty rows since this could disturb some subsequent calculations. Cells N18, N32, N46 and N60: For this table the worksheet sheet will accept: - either a numerical value, - or <LOQ, - or an empty cell.


A84
Raw Data (marker residue)
1) Source of the data
2) Analytical information
3) Concentration of the marker residue in the four standard edible tissues
Muscle
Liver
Kidney
Fat



Raw Data (marker residue)

1) Source of the data

Source:

2) Analytical information

	Muscle	Liver	Kidney	Fat
LOQ				
Recovery [%]				

3) Concentration of the marker residue in the four standard edible tissues

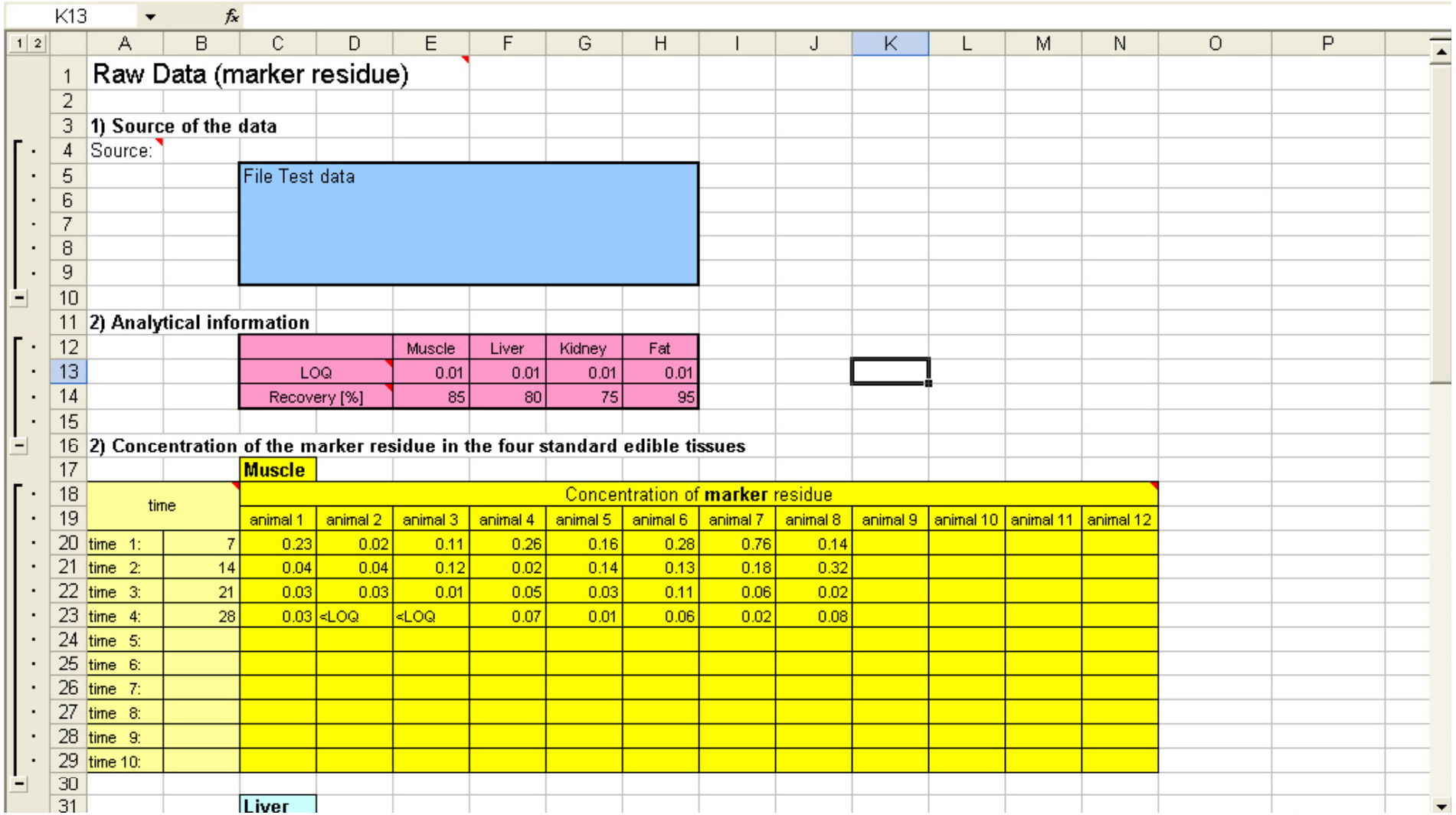
Muscle

time	Concentration of **marker** residue											
	animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	animal 11	animal 12
time 1:												
time 2:												
time 3:												
time 4:												
time 5:												
time 6:												
time 7:												
time 8:												
time 9:												
time 10:												

Liver

cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

Example of a table containing raw data



Raw Data (marker residue)

1) Source of the data

Source:

File Test data

2) Analytical information

	Muscle	Liver	Kidney	Fat
LOQ	0.01	0.01	0.01	0.01
Recovery [%]	85	80	75	95

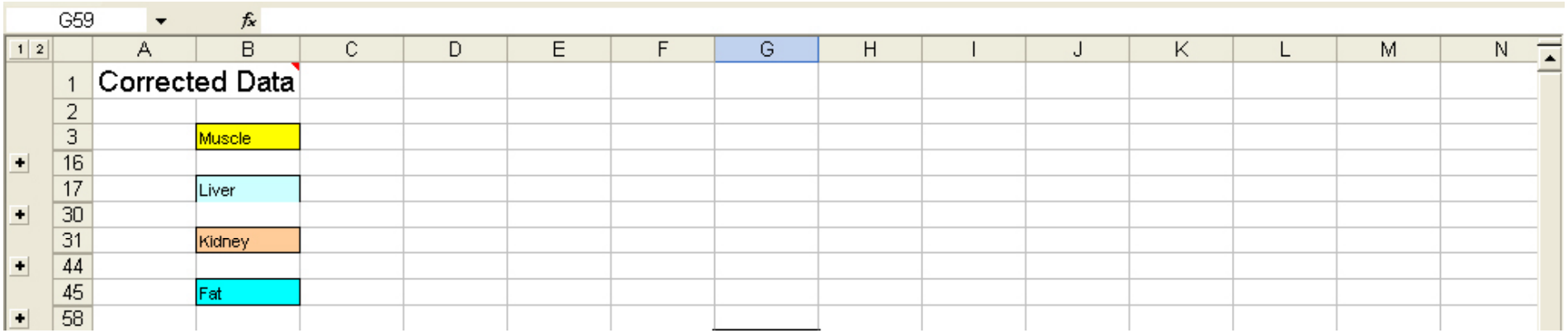
2) Concentration of the marker residue in the four standard edible tissues

Muscle

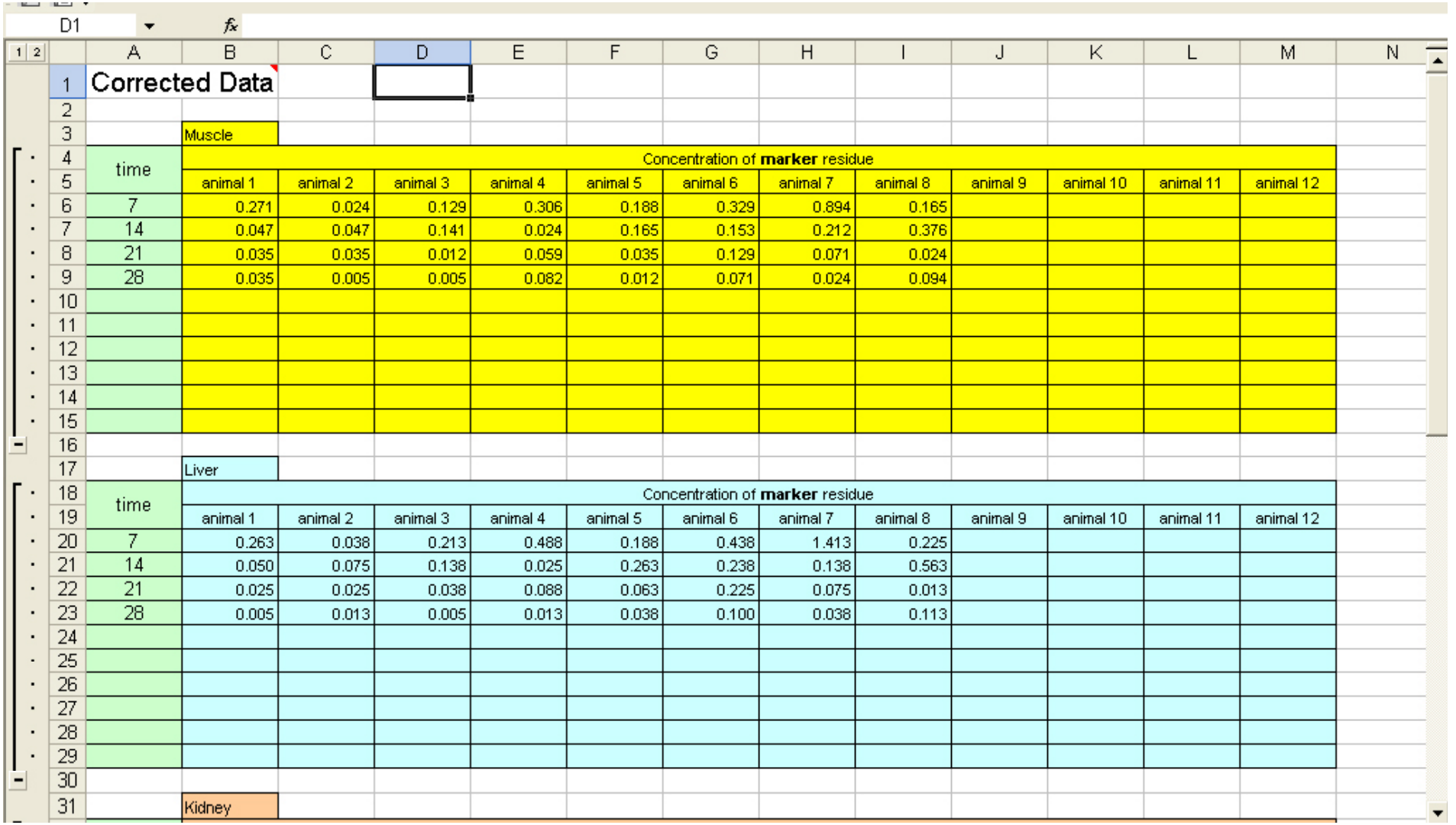
time		Concentration of **marker** residue											
		animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	animal 11	animal 12
time 1:	7	0.23	0.02	0.11	0.26	0.16	0.28	0.76	0.14				
time 2:	14	0.04	0.04	0.12	0.02	0.14	0.13	0.18	0.32				
time 3:	21	0.03	0.03	0.01	0.05	0.03	0.11	0.06	0.02				
time 4:	28	0.03	<LOQ	<LOQ	0.07	0.01	0.06	0.02	0.08				
time 5:													
time 6:													
time 7:													
time 8:													
time 9:													
time 10:													

Liver

“corm”	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet assigns LOQ/2 to all cells corresponding to raw data cells containing the text <LOQ. It also corrects the raw data for recoveries. When opening the template the individual tables are hidden. Only the basic structure of the work-sheet can be seen. The user should unfold the tables and view the data. The tables have to re-main open because otherwise the data points would not be visible in the worksheet “graph”	Cell B1: All calculations are carried out automatically. The user has no possibility to change the results. Necessary changes should be made in the worksheet "rawm" which contains the raw data.



Example of tables containing corrected data



Corrected Data

Muscle

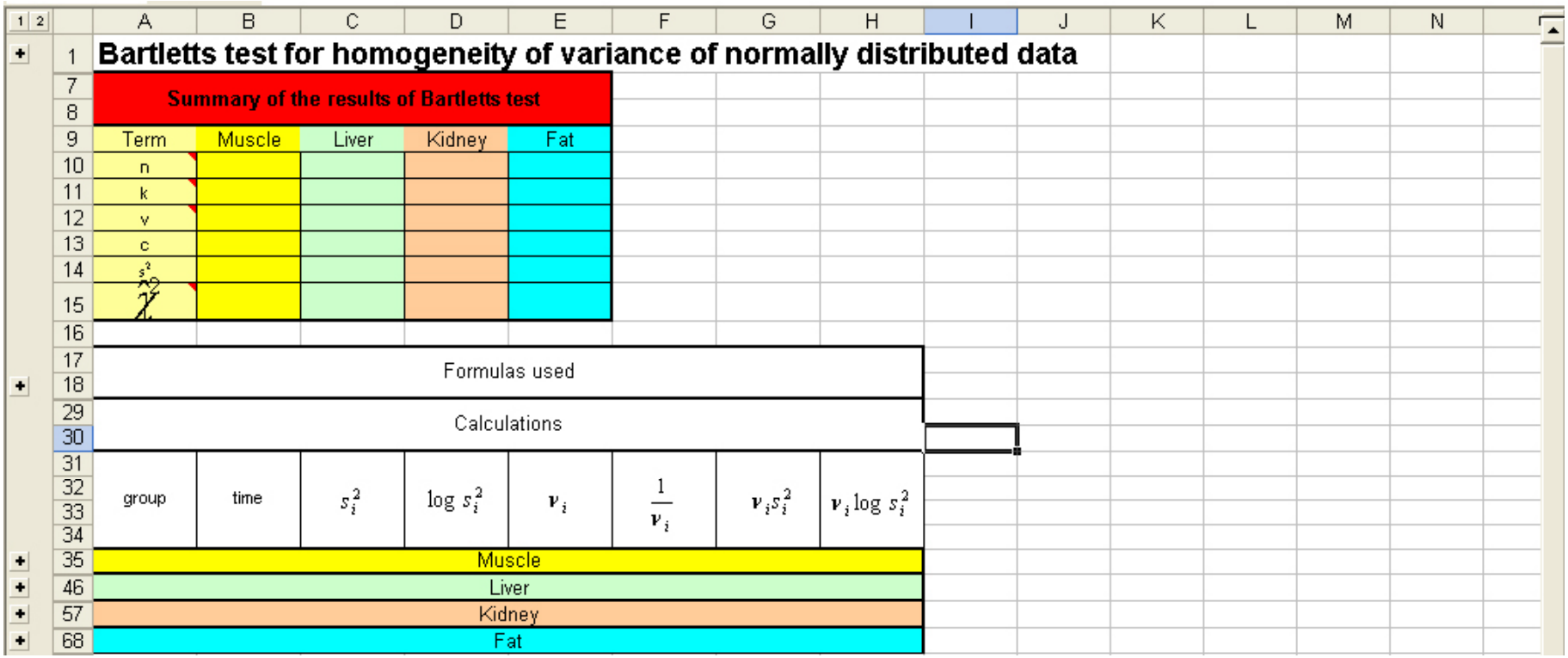
time	Concentration of **marker** residue											
	animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	animal 11	animal 12
7	0.271	0.024	0.129	0.306	0.188	0.329	0.894	0.165				
14	0.047	0.047	0.141	0.024	0.165	0.153	0.212	0.376				
21	0.035	0.035	0.012	0.059	0.035	0.129	0.071	0.024				
28	0.035	0.005	0.005	0.082	0.012	0.071	0.024	0.094				

Liver

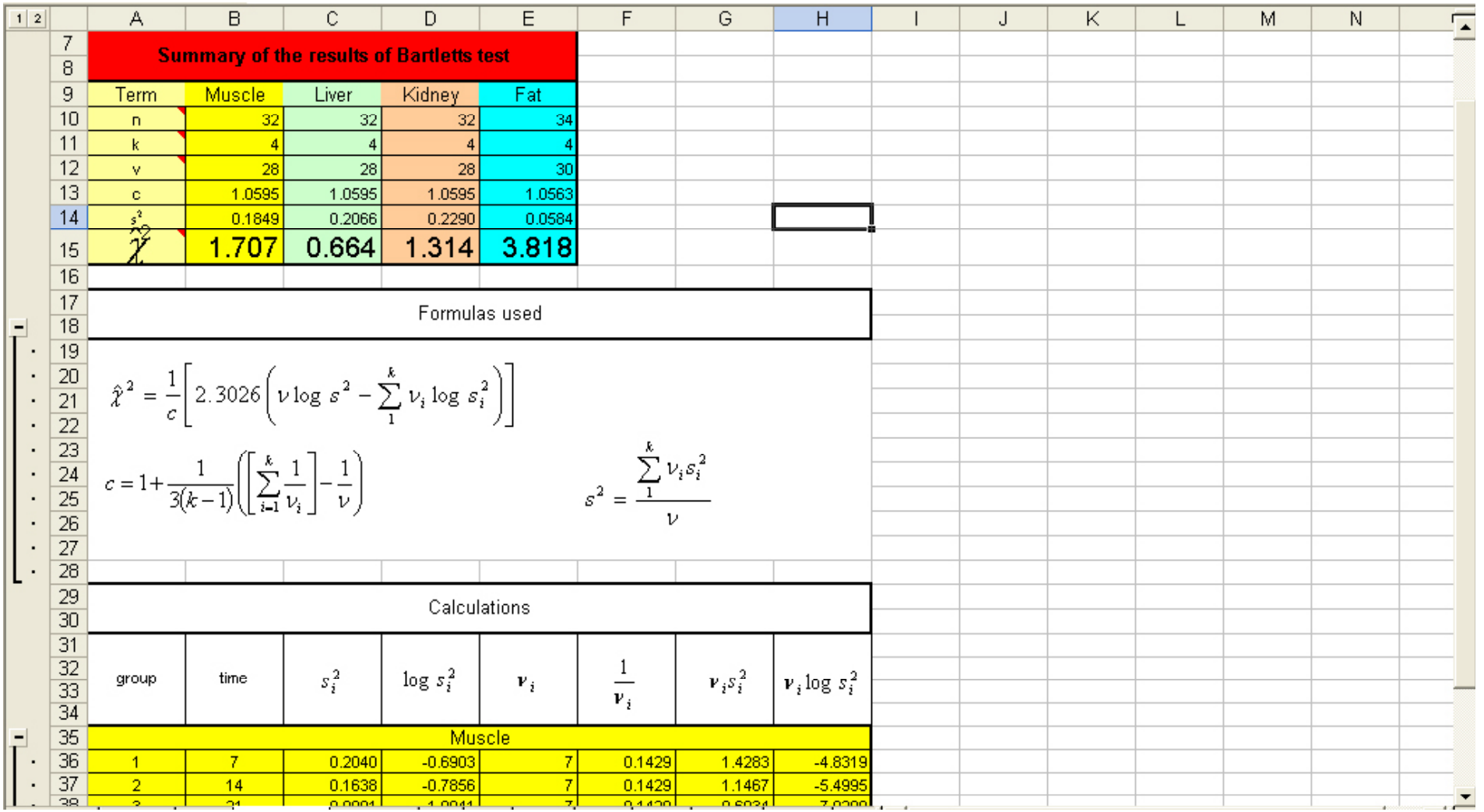
time	Concentration of **marker** residue											
	animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	animal 11	animal 12
7	0.263	0.038	0.213	0.488	0.188	0.438	1.413	0.225				
14	0.050	0.075	0.138	0.025	0.263	0.238	0.138	0.563				
21	0.025	0.025	0.038	0.088	0.063	0.225	0.075	0.013				
28	0.005	0.013	0.005	0.013	0.038	0.100	0.038	0.113				

Kidney

"Bartlett"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet calculates the test statistics of Bartletts test for homogeneity of variance of normally distributed data. The template shows only the basic structure of the worksheet. The results of the calculations can immediately be seen in the summary table as soon as the raw data have been filled into the tables of worksheet "rawm". The user may unfold the tables in order to view the detailed calculations and to obtain further information. Unfolding rows 2-5 provides hyperlinks with information on Bartletts test and a table with critical values of the chi-square distribution	Cell A10: total number of observations Cell A11: number of groups Cell A12: total number of degrees of freedom Cell 15A: In this row you will find the value which has to be compared with the critical values of the chi-square distribution.



Partial view of the worksheet after completion of data entry in worksheet "rawm"
Unfolding of rows 19-27 results in a display of the formulas used.



Summary of the results of Bartletts test

Term	Muscle	Liver	Kidney	Fat
n	32	32	32	34
k	4	4	4	4
v	28	28	28	30
c	1.0595	1.0595	1.0595	1.0563
s^2	0.1849	0.2066	0.2290	0.0584
χ^2	1.707	0.664	1.314	3.818

Formulas used

$$\hat{\chi}^2 = \frac{1}{c}\left[2.3026\left(\nu \log s^2 - \sum_1^k \nu_i \log s_i^2\right)\right]$$

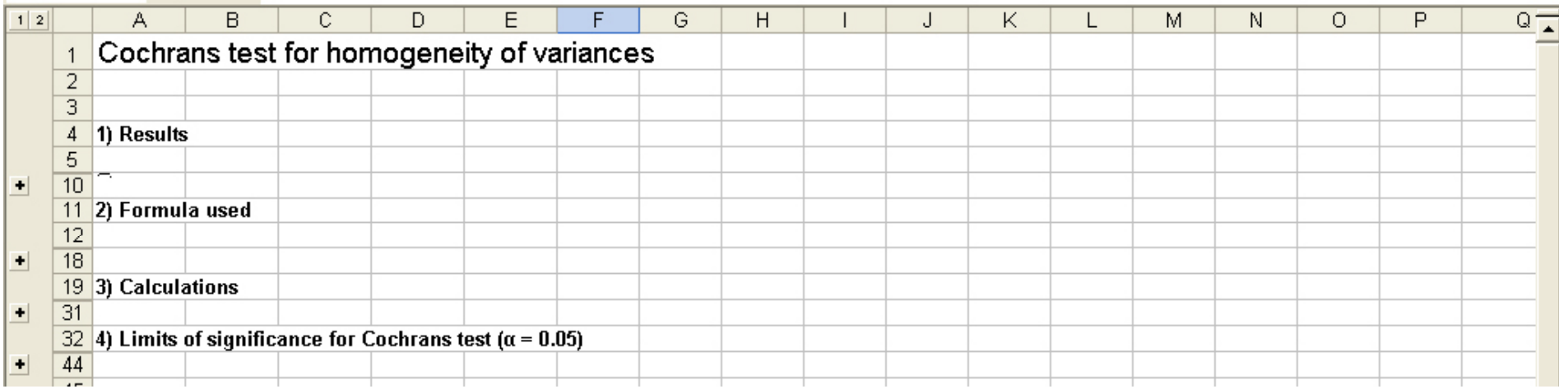
$$c = 1 + \frac{1}{3(k-1)}\left(\left[\sum_{i=1}^{k}\frac{1}{\nu_i}\right] - \frac{1}{\nu}\right)$$

$$s^2 = \frac{\sum_1^k \nu_i s_i^2}{\nu}$$

Calculations

group	time	s_i^2	$\log s_i^2$	ν_i	$\frac{1}{\nu_i}$	$\nu_i s_i^2$	$\nu_i \log s_i^2$
Muscle							
1	7	0.2040	-0.6903	7	0.1429	1.4283	-4.8319
2	14	0.1638	-0.7856	7	0.1429	1.1467	-5.4995

"Cochran"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet calculates the test statistics for Cochrans test for homogeneity of variance. The template shows only the basic structure of the worksheet. The user has to unfold the tables by clicking on the "+" buttons in order to view results, formulas used, the calculations performed, and a table of limits of significance of Cochrans test.	



Example of a worksheet containing test data

Cochrans test for homogeneity of variances

1) Results

Term	Muscle	Liver	Kidney	Fat
$\hat{G}_{max}$	0.3688	0.3441	0.3365	0.3926
k	3	3	3	3
$\bar{x}_H$	7	7	7	7

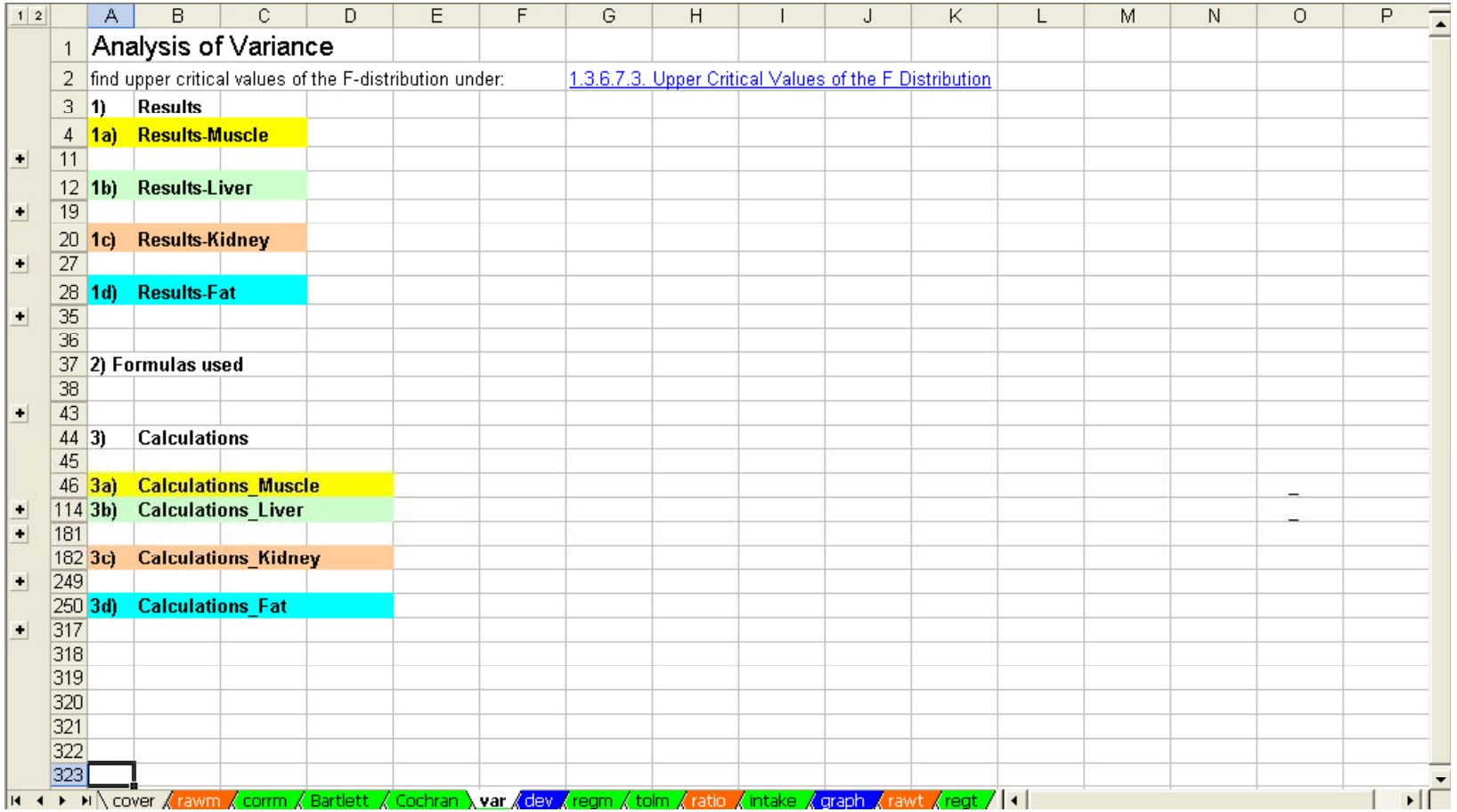
2) Formula used

$$\hat{G}_{max} = \frac{s^2_{max}}{\sum_1^k s_i^2}$$

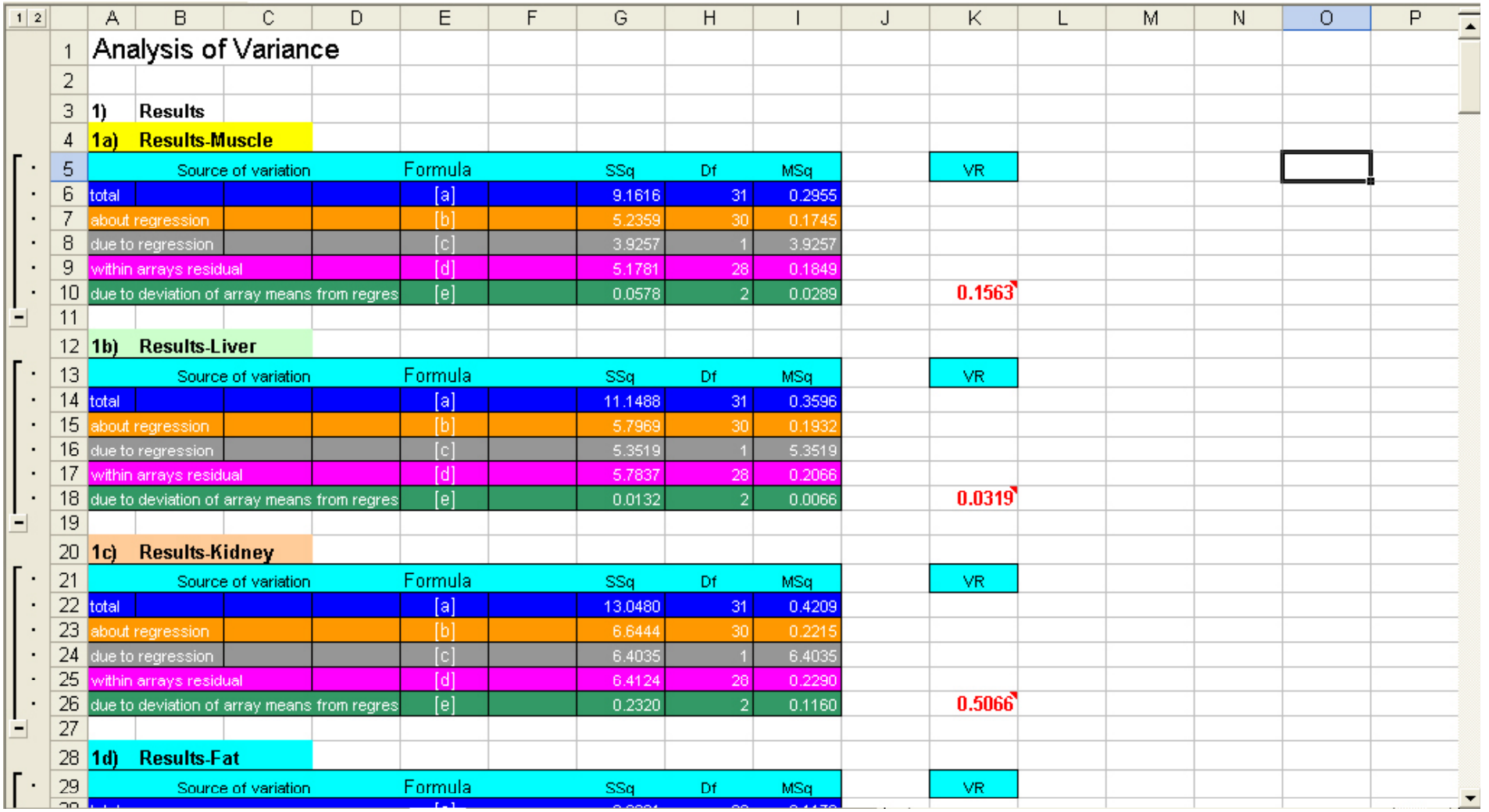
3) Calculations

i	time	s_i^2	ν_i	s_i^2	ν_i	s_i^2	ν_i	s_i^2	ν_i
1	7	0.2040	7	0.2005	7	0.1976	7	0.0892	7
2	14	0.1638	7	0.1868	7	0.1340	7	0.0292	7
3	21	0.0991	7	0.1546	7	0.3083	7	0.0284	7
4	28	0.2728	7	0.2843	7	0.2762	7	0.0804	9

"var"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet performs a one-way analysis of variance. The more than 300 rows of active cells are hidden in the template. The user has to unfold the tables by clicking on the "+" buttons in order to view the results of the calculations. The worksheet includes a hyperlink to view upper critical limits of the F-distribution	Cells K10, K18, K26, K34: Find there the variance ratio which has to be compared with the corresponding upper critical value of the F-distribution.



Partial view of the results of the analysis of variance (example)



Analysis of Variance

1) Results

1a) Results-Muscle

Source of variation	Formula	SSq	Df	MSq	VR
total	[a]	9.1616	31	0.2955	
about regression	[b]	5.2359	30	0.1745	
due to regression	[c]	3.9257	1	3.9257	
within arrays residual	[d]	5.1781	28	0.1849	
due to deviation of array means from regres	[e]	0.0578	2	0.0289	0.1563

1b) Results-Liver

Source of variation	Formula	SSq	Df	MSq	VR
total	[a]	11.1488	31	0.3596	
about regression	[b]	5.7969	30	0.1932	
due to regression	[c]	5.3519	1	5.3519	
within arrays residual	[d]	5.7837	28	0.2066	
due to deviation of array means from regres	[e]	0.0132	2	0.0066	0.0319

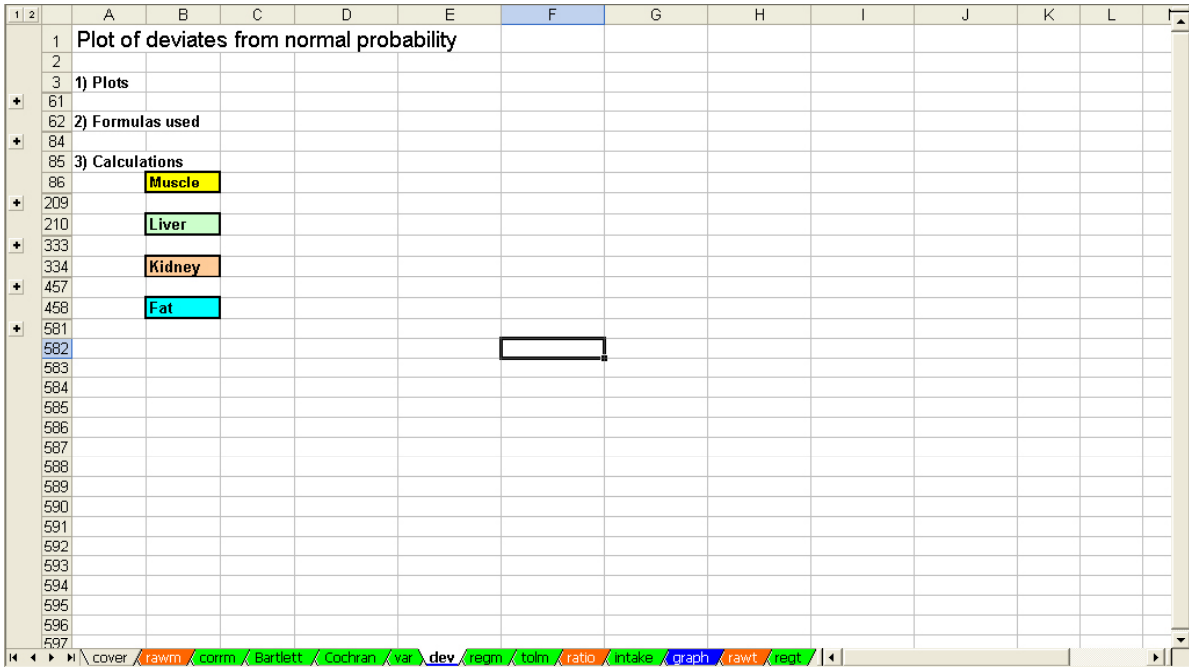
1c) Results-Kidney

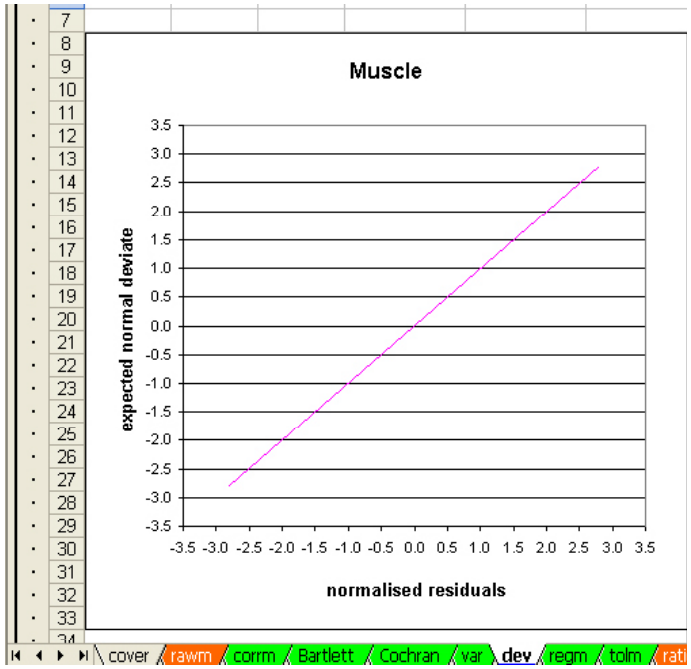
Source of variation	Formula	SSq	Df	MSq	VR
total	[a]	13.0480	31	0.4209	
about regression	[b]	6.6444	30	0.2215	
due to regression	[c]	6.4035	1	6.4035	
within arrays residual	[d]	6.4124	28	0.2290	
due to deviation of array means from regres	[e]	0.2320	2	0.1160	0.5066

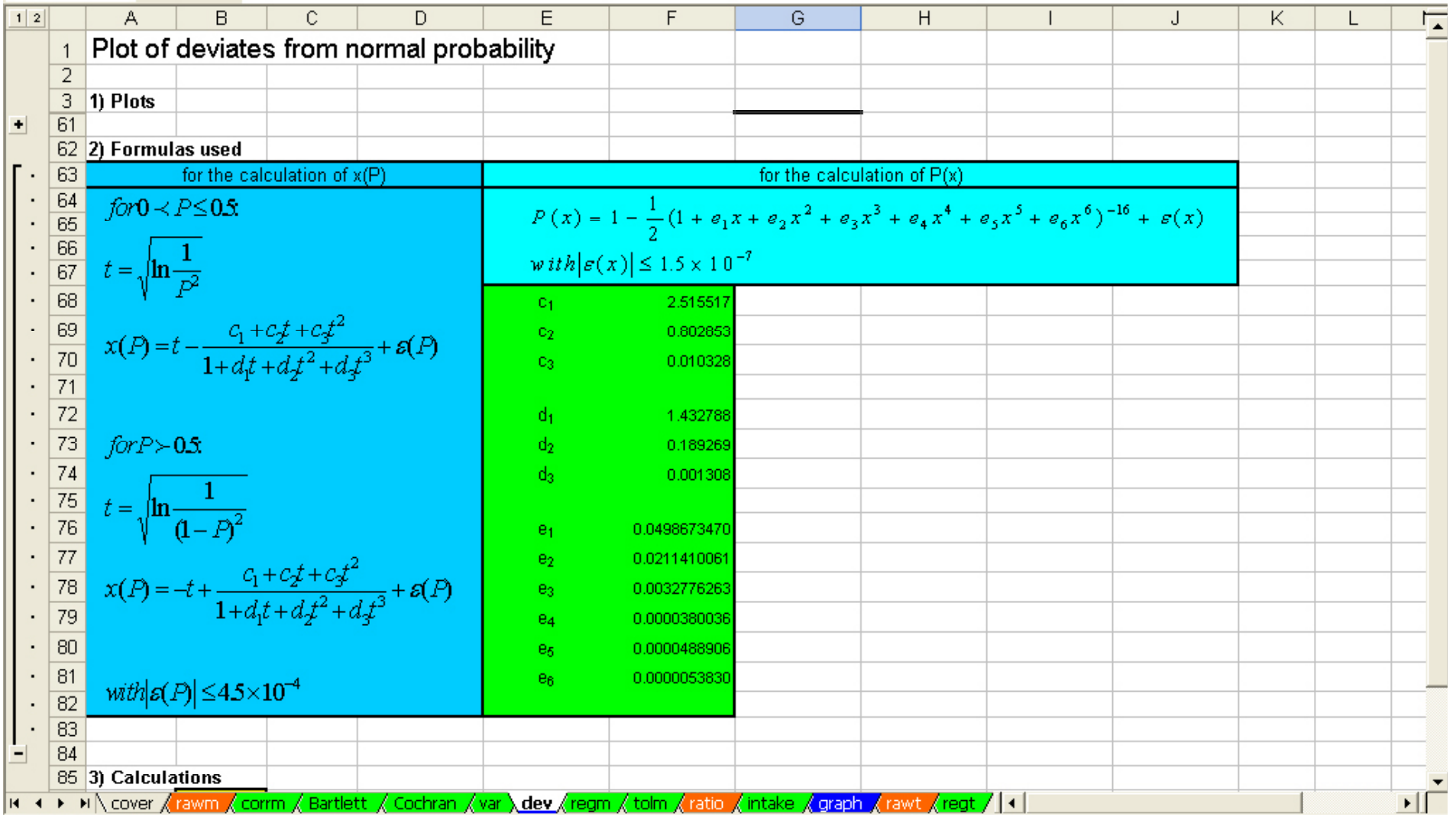
1d) Results-Fat

Source of variation	Formula	SSq	Df	MSq	VR

"dev"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet assists the user to create plots of deviations from normal probability. The "empty" template shows only the basic structure of the worksheet. In the "empty" template the four plots (example given below for muscle) show only the theoretical straight line. Clicking on the "+" in row 84 unfolds rows 63-84 enabling a view on the formulas used (see reference 3) to calculate x(P), the standard deviation for a given cumulative probability P(x) and vice versa.	







Plot of deviates from normal probability

1) Plots

2) Formulas used

for the calculation of x(P)	for the calculation of P(x)
$for\ 0 < P \le 0.5$: $t = \sqrt{\ln\frac{1}{P^2}}$ $x(P) = t - \frac{c_1 + c_2 t + c_3 t^2}{1 + d_1 t + d_2 t^2 + d_3 t^3} + \varepsilon(P)$	$P(x) = 1 - \frac{1}{2}(1 + e_1 x + e_2 x^2 + e_3 x^3 + e_4 x^4 + e_5 x^5 + e_6 x^6)^{-16} + \varepsilon(x)$
$for\ P > 0.5$: $t = \sqrt{\ln\frac{1}{(1-P)^2}}$ $x(P) = -t + \frac{c_1 + c_2 t + c_3 t^2}{1 + d_1 t + d_2 t^2 + d_3 t^3} + \varepsilon(P)$	$with\ \lvert\varepsilon(x)\rvert \le 1.5 \times 10^{-7}$
$with\ \lvert\varepsilon(P)\rvert \le 4.5 \times 10^{-4}$	

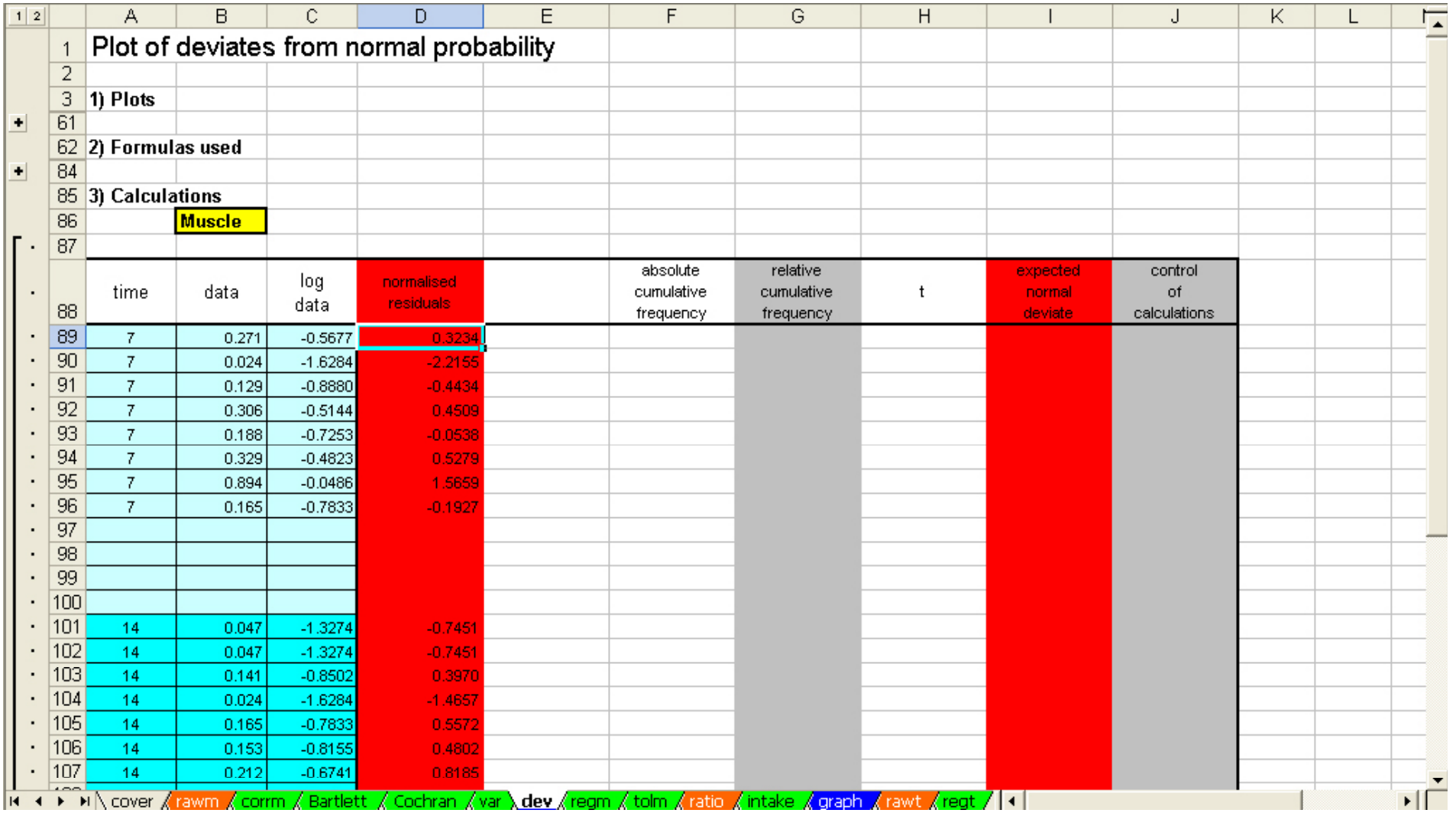
c_1	2.515517
c_2	0.802853
c_3	0.010328
d_1	1.432788
d_2	0.189269
d_3	0.001308
e_1	0.0498673470
e_2	0.0211410061
e_3	0.0032776263
e_4	0.0000380036
e_5	0.0000488906
e_6	0.0000053830

3) Calculations

cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

"dev" (continuation)	
Description of the function of the worksheet	Comments found on the worksheet
In order to create the desired plots, the user should now perform (tissue by tissue; example given her for muscle) the following steps: **Step 1:** Unfold "3) Calculations"! In the columns A to C (under "time", "data", and "log data"), the worksheets shows the corrected residue concentrations and their logarithms. The groups of data for each time point show a different background colour. Column D shows the corresponding "normalised residuals". **Step 2:** Select the first cell under "normalised residuals" (here cell D89) and sort the data in ascending order by clicking on the corresponding icon in the tool bar. Check that the values in column D now start with the lowest normalised residual (a negative value!) and that in columns A-C all values of the same row have the same colour (see the next screenshot). **Step 3:** Start entering the absolute cumulative frequencies by typing 1 and 2 into the first two cells of the corresponding column (The worksheet will automatically calculate the corresponding values of the remaining four columns. If every thing is o.k. you will see almost identical numbers in the corresponding cells of the columns "relative cumulative frequency" and "control of calculations". Complete entering absolute cumulative frequentcies by selecting the first two cells and dragging down the fill handle. **Stop as soon as there appears the first empty cell in the column with the normalised residuals!** **Step 4:** Click on the "empty" plot for the corresponding tissue (here: Muscle). The menu "chart" will appear in Excels menu bar. Select "add data" from the menu. The usual box appears. Now select first all data including the headline from the column "normalised residuals" as x-values. Then, while pressing the control key, select all values including the headline from the column "expected normal deviate" as y-values. Fill in the checkbox and click "ok". View the plot and proceed to the next tissue, but do not attempt to hide the tables with the calculations because otherwise the data points will also disappear from the plots!	

Illustration of steps 1 and 2 of the table "dev"



Plot of deviates from normal probability

1) Plots

2) Formulas used

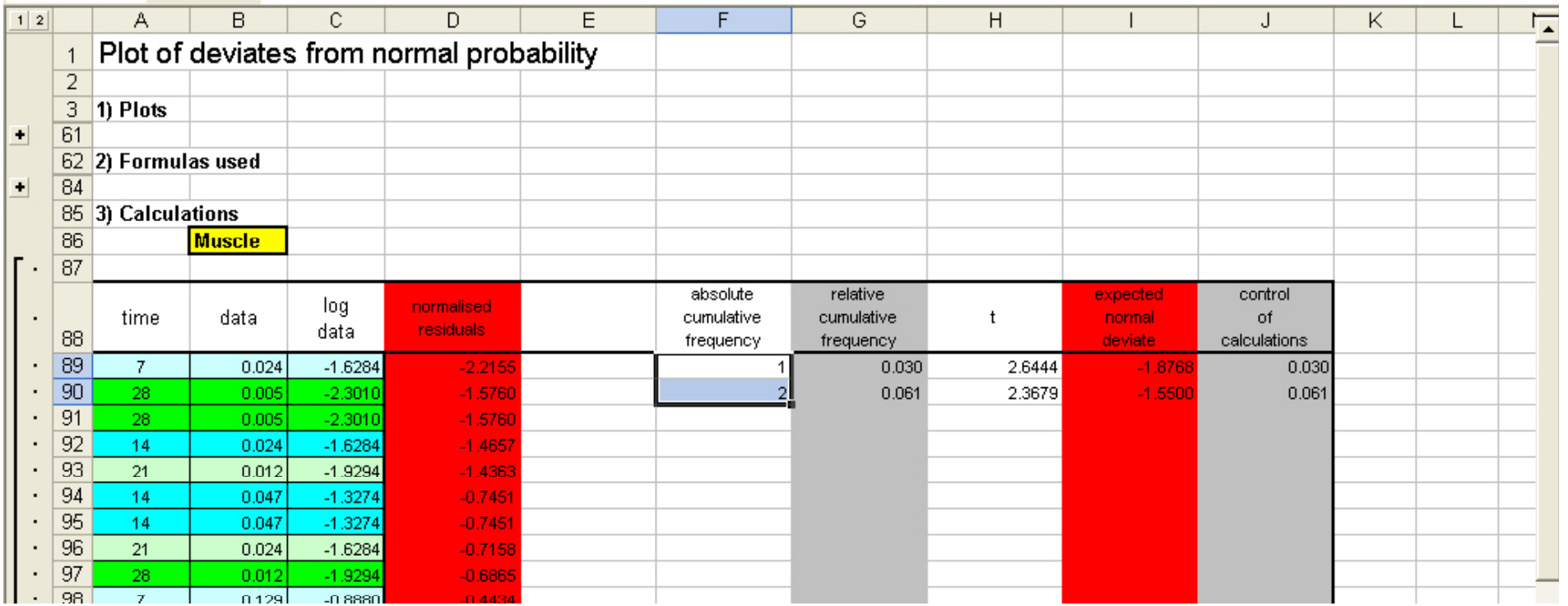
3) Calculations

Muscle

time	data	log data	normalised residuals		absolute cumulative frequency	relative cumulative frequency	t	expected normal deviate	control of calculations
7	0.271	-0.5677	0.3234						
7	0.024	-1.6284	-2.2155						
7	0.129	-0.8880	-0.4434						
7	0.306	-0.5144	0.4509						
7	0.188	-0.7253	-0.0538						
7	0.329	-0.4823	0.5279						
7	0.894	-0.0486	1.5659						
7	0.165	-0.7833	-0.1927						
14	0.047	-1.3274	-0.7451						
14	0.047	-1.3274	-0.7451						
14	0.141	-0.8502	0.3970						
14	0.024	-1.6284	-1.4657						
14	0.165	-0.7833	0.5572						
14	0.153	-0.8155	0.4802						
14	0.212	-0.6741	0.8185						

cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

Illustration of step 3 of the table "dev"



Plot of deviates from normal probability

1) Plots

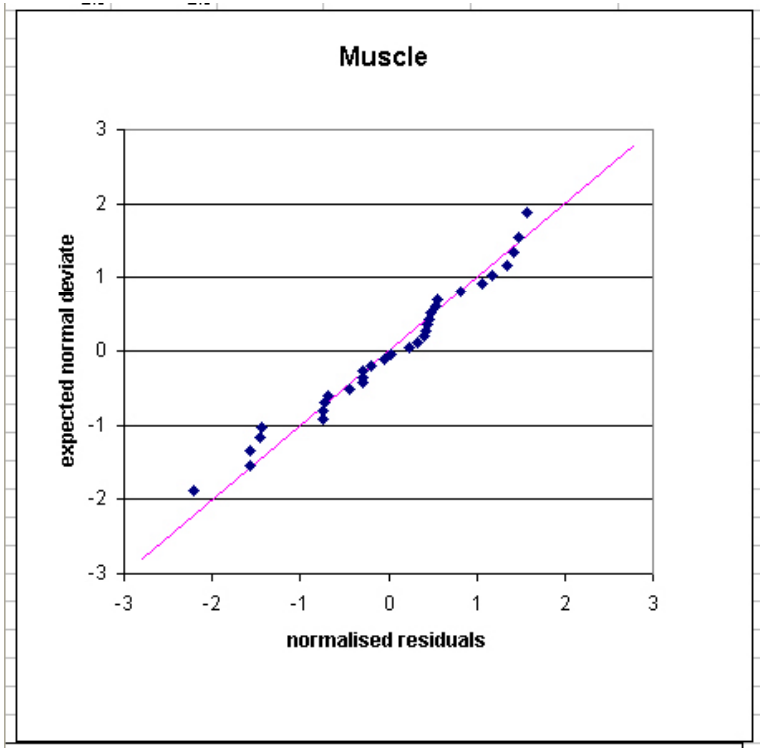
2) Formulas used

3) Calculations

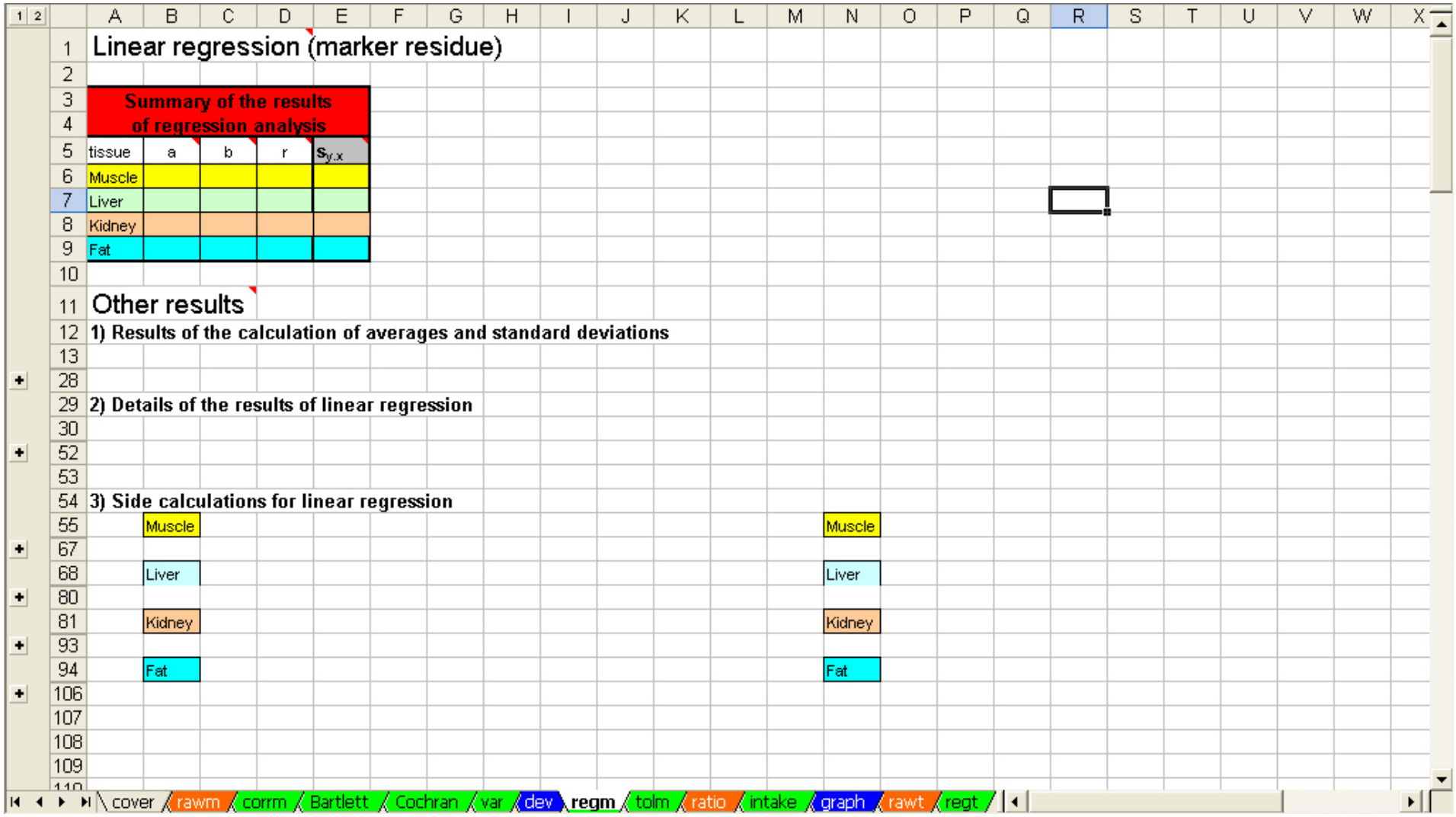
Muscle

time	data	log data	normalised residuals		absolute cumulative frequency	relative cumulative frequency	t	expected normal deviate	control of calculations
7	0.024	-1.6284	-2.2155		1	0.030	2.6444	-1.8768	0.030
28	0.005	-2.3010	-1.5760		2	0.061	2.3679	-1.5500	0.061
28	0.005	-2.3010	-1.5760						
14	0.024	-1.6284	-1.4657						
21	0.012	-1.9294	-1.4363						
14	0.047	-1.3274	-0.7451						
14	0.047	-1.3274	-0.7451						
21	0.024	-1.6284	-0.7158						
28	0.012	-1.9294	-0.6865						
7	0.129	-0.8860	-0.4434						

Example of a plot created with worksheet “dev”



"regm"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet performs linear regression analysis on the logarithms of the corrected data. It returns the parameters of the straight line. In addition it calculates other useful characteristics of the data, such as geometric mean. The "empty" template shows only the summary of the results and the basic structure of the worksheet. In order to view all details the user has to click on the "+" buttons in order to unfold the tables.	Cell D1: The user of this spreadsheet has no influence on the calculations performed on this worksheet. All calculations are carried out automatically. Any necessary changes to the data have to be made on the worksheet "rawm" Cell B5: intercept of the straight line Cell C5: slope of the straight line Cell D5: coefficient of correlation Cell E5: residual variance Cell C11: The results of this table might be used as a basis for the calculation of MRLs in cases where the "tolerance limits approach" yields unsatisfactory results. Cells F16, K16, P16, U16: The results given in this column could be used for the "mean plus three standard deviations approach" sometimes followed by experts. Cell M56: The below figures represent the log10-values of the corrected results. Cell Z56 These are intermediate calculations needed for the final calculation of the sum of x in cases where the number of animals differs for individual rows. Cell AA56: These are intermediate calculations needed for the final calculation of the sum of squared x in cases where the number of animals differs for individual rows


Linear regression (marker residue)
Summary of the results of regression analysis
tissue
a
b
r
$s_{y.x}$
Muscle
Liver
Kidney
Fat
Other results
1) Results of the calculation of averages and standard deviations
2) Details of the results of linear regression
3) Side calculations for linear regression
Muscle
Liver
Kidney
Fat
Muscle
Liver
Kidney
Fat
cover
rawm
corrm
Bartlett
Cochran
var
dev
regm
tolm
ratio
intake
graph
rawt
regt

Partial view of a worksheet containing test data

Linear regression (marker residue)

Summary of the results of regression analysis

tissue	a	b	r	$s_{y,x}$
Muscle	-0.390	-0.045	-0.655	0.418
Liver	-0.197	-0.052	-0.693	0.440
Kidney	-0.231	-0.057	-0.701	0.471
Fat	-0.267	-0.031	-0.730	0.238

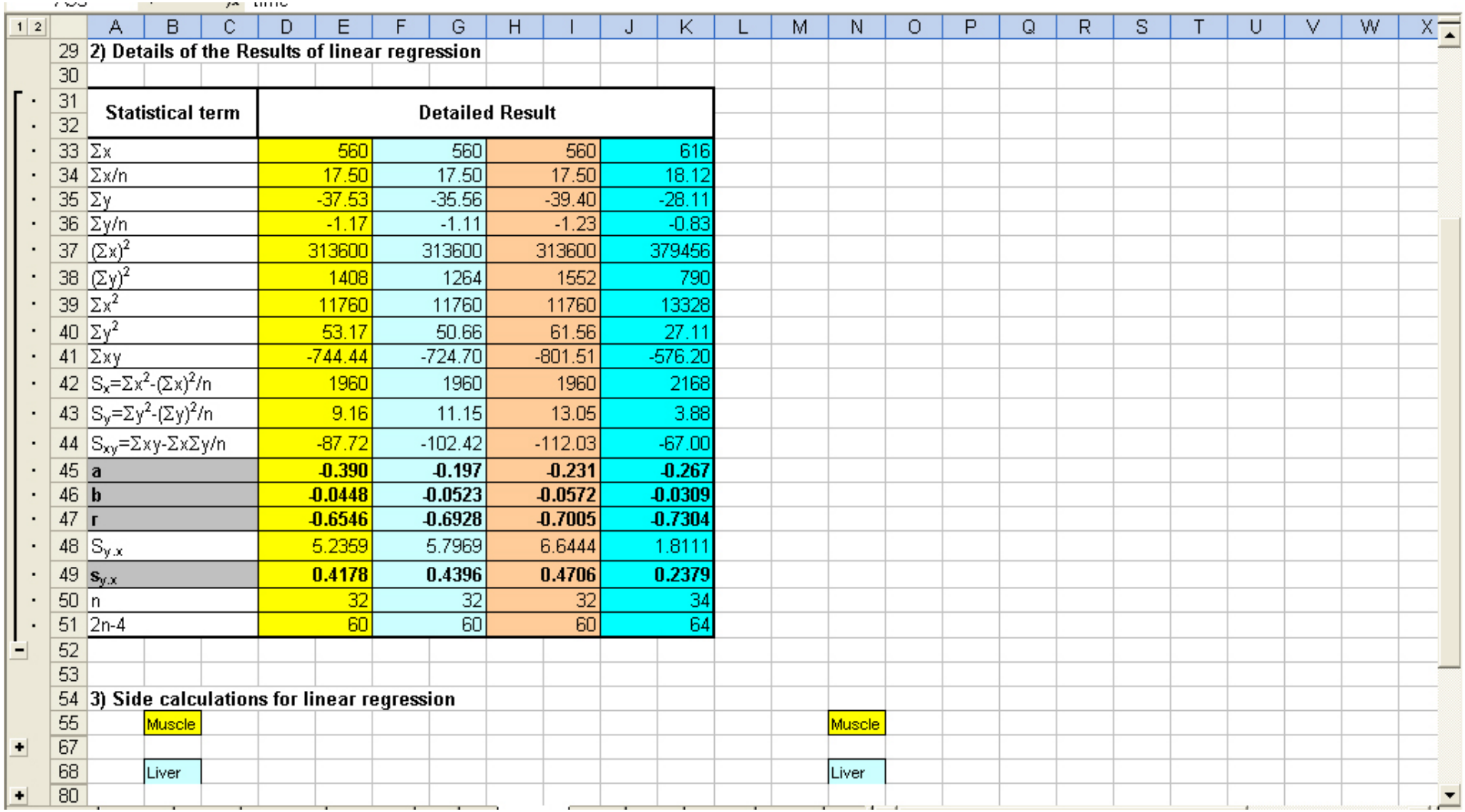
Other results

1) Results of the calculation of averages and standard deviations

	Muscle					Liver					Kidney					Fat				
	logarithms			antilogs		logarithms			antilogs		logarithms			antilogs		logarithms			antilogs	
x	$\bar{y}$	s_y	$y+3s_y$	$\bar{y}$	$y+3s_y$	$\bar{y}$	s_y	$y+3s_y$	$\bar{y}$	$y+3s_y$	$\bar{y}$	s_y	$y+3s_y$	$\bar{y}$	$y+3s_y$	$\bar{y}$	s_y	$y+3s_y$	$\bar{y}$	$y+3s_y$
7	-0.705	0.452	0.65	0.197	4.471	-0.572	0.448	0.771	0.268	5.907	-0.623	0.445	0.711	0.238	5.138	-0.514	0.299	0.382	0.306	2.408
14	-0.979	0.405	0.235	0.105	1.72	-0.901	0.432	0.396	0.126	2.488	-0.973	0.366	0.125	0.106	1.335	-0.631	0.171	-0.118	0.234	0.762
21	-1.398	0.315	-0.454	0.04	0.352	-1.321	0.393	-0.141	0.048	0.722	-1.573	0.555	0.093	0.027	1.239	-0.957	0.169	-0.452	0.11	0.354
28	-1.609	0.522	-0.042	0.025	0.907	-1.651	0.533	-0.051	0.022	0.888	-1.757	0.526	-0.18	0.018	0.661	-1.128	0.284	-0.277	0.074	0.528

2) Details of the Results of linear regression

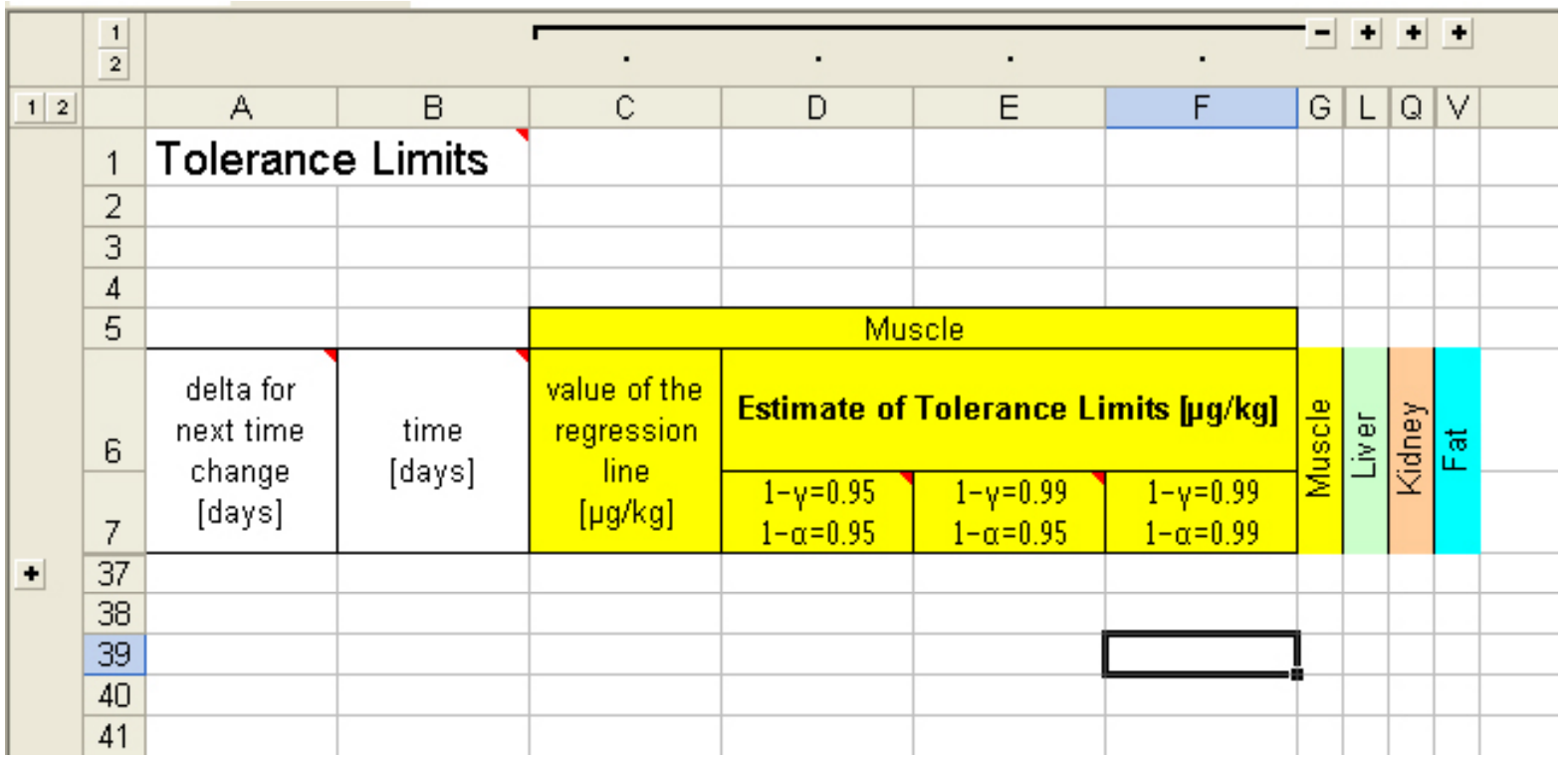
Partial view of a worksheet containing test data



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
29	**2) Details of the Results of linear regression**																							
30																								
31	**Statistical term**			**Detailed Result**																				
32																								
33	Σx			560		560		560		616														
34	$\Sigma x/n$			17.50		17.50		17.50		18.12														
35	Σy			-37.53		-35.56		-39.40		-28.11														
36	$\Sigma y/n$			-1.17		-1.11		-1.23		-0.83														
37	$(\Sigma x)^2$			313600		313600		313600		379456														
38	$(\Sigma y)^2$			1408		1264		1552		790														
39	Σx^2			11760		11760		11760		13328														
40	Σy^2			53.17		50.66		61.56		27.11														
41	Σxy			-744.44		-724.70		-801.51		-576.20														
42	$S_x=\Sigma x^2-(\Sigma x)^2/n$			1960		1960		1960		2168														
43	$S_y=\Sigma y^2-(\Sigma y)^2/n$			9.16		11.15		13.05		3.88														
44	$S_{xy}=\Sigma xy-\Sigma x\Sigma y/n$			-87.72		-102.42		-112.03		-67.00														
45	**a**			**-0.390**		**-0.197**		**-0.231**		**-0.267**														
46	**b**			**-0.0448**		**-0.0523**		**-0.0572**		**-0.0309**														
47	**r**			**-0.6546**		**-0.6928**		**-0.7005**		**-0.7304**														
48	$S_{y.x}$			5.2359		5.7969		6.6444		1.8111														
49	$\mathbf{s_{y.x}}$			**0.4178**		**0.4396**		**0.4706**		**0.2379**														
50	n			32		32		32		34														
51	2n-4			60		60		60		64														
52																								
53																								
54	**3) Side calculations for linear regression**																							
55		Muscle												Muscle										
67																								
68		Liver												Liver										
80																								

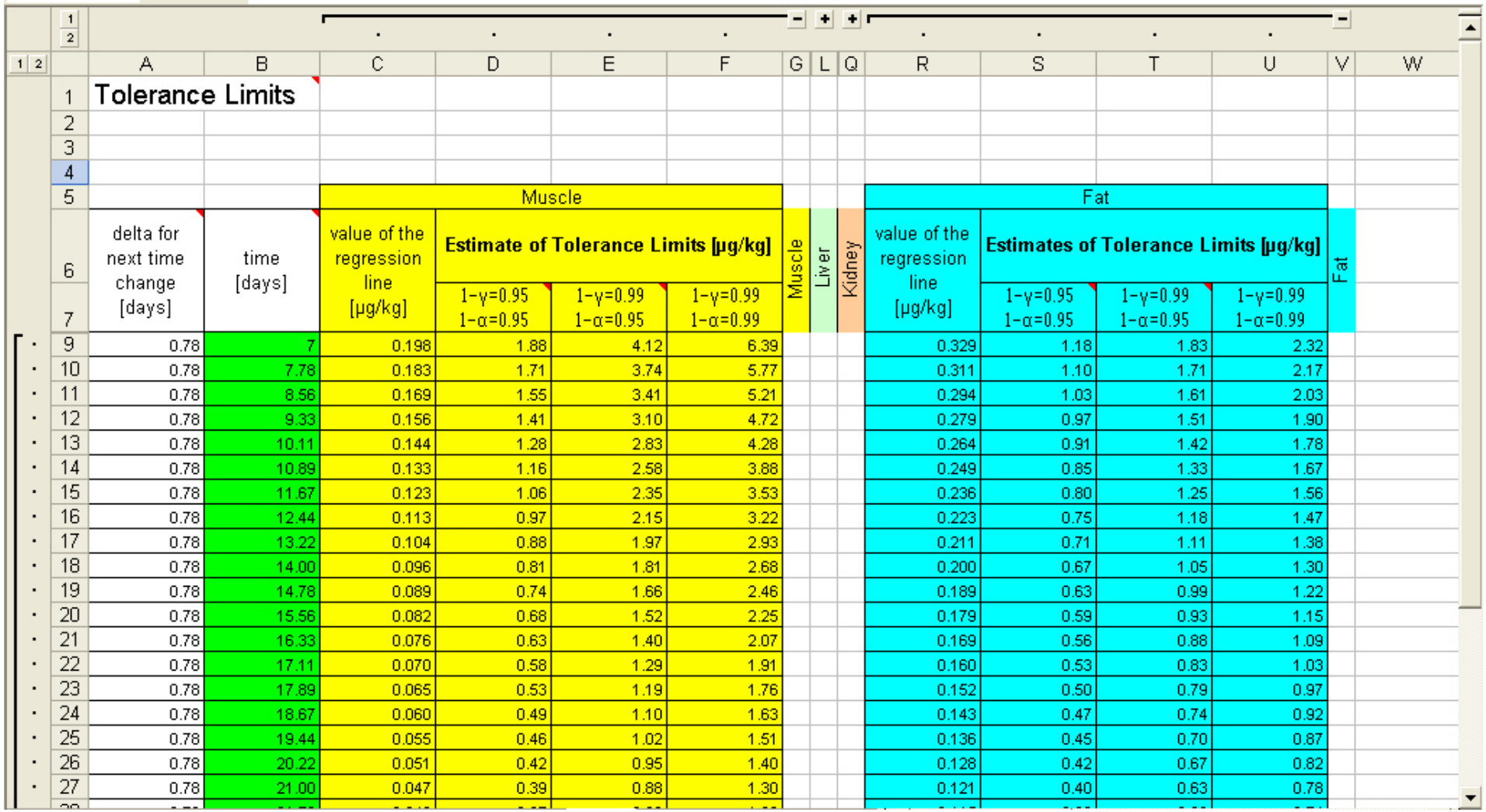
"tolm"	
Description of the function of the worksheet	Comments found on the worksheet
The worksheet calculates tolerance limits on the basis of the results of linear regression. It also provides the corresponding values of the regression line. The "empty" template shows only the basic structure of the worksheet. The user has to click on the "+" buttons in order to view all data points of all tissues	Cell B1:The results of this worksheet are used to obtain initial estimates of MRLs. Cell A6 and B6: This sheet calculates automatically statistical tolerance limits for 28 equally spaced time points including the first and the last time point of the experimental data.

Partial



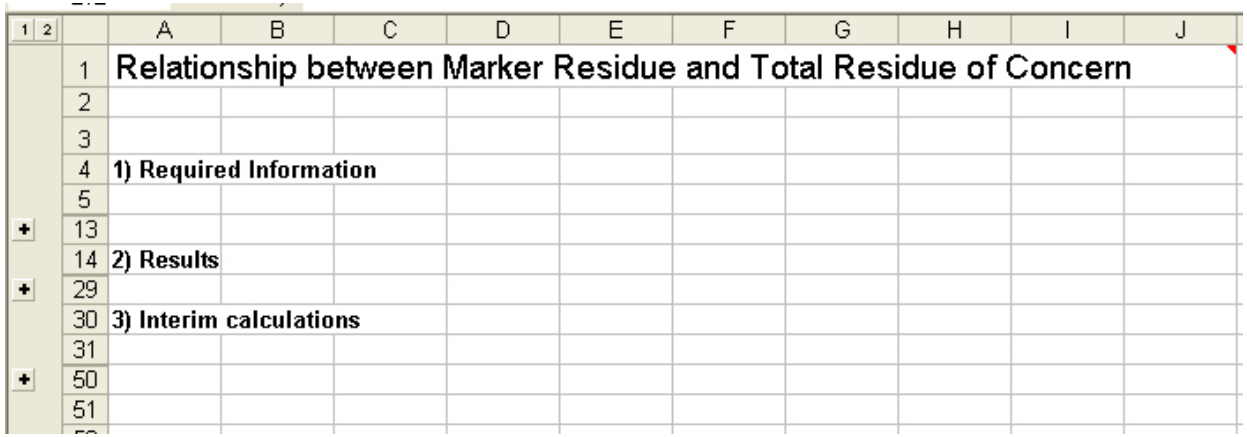
view of a

worksheet containing test data

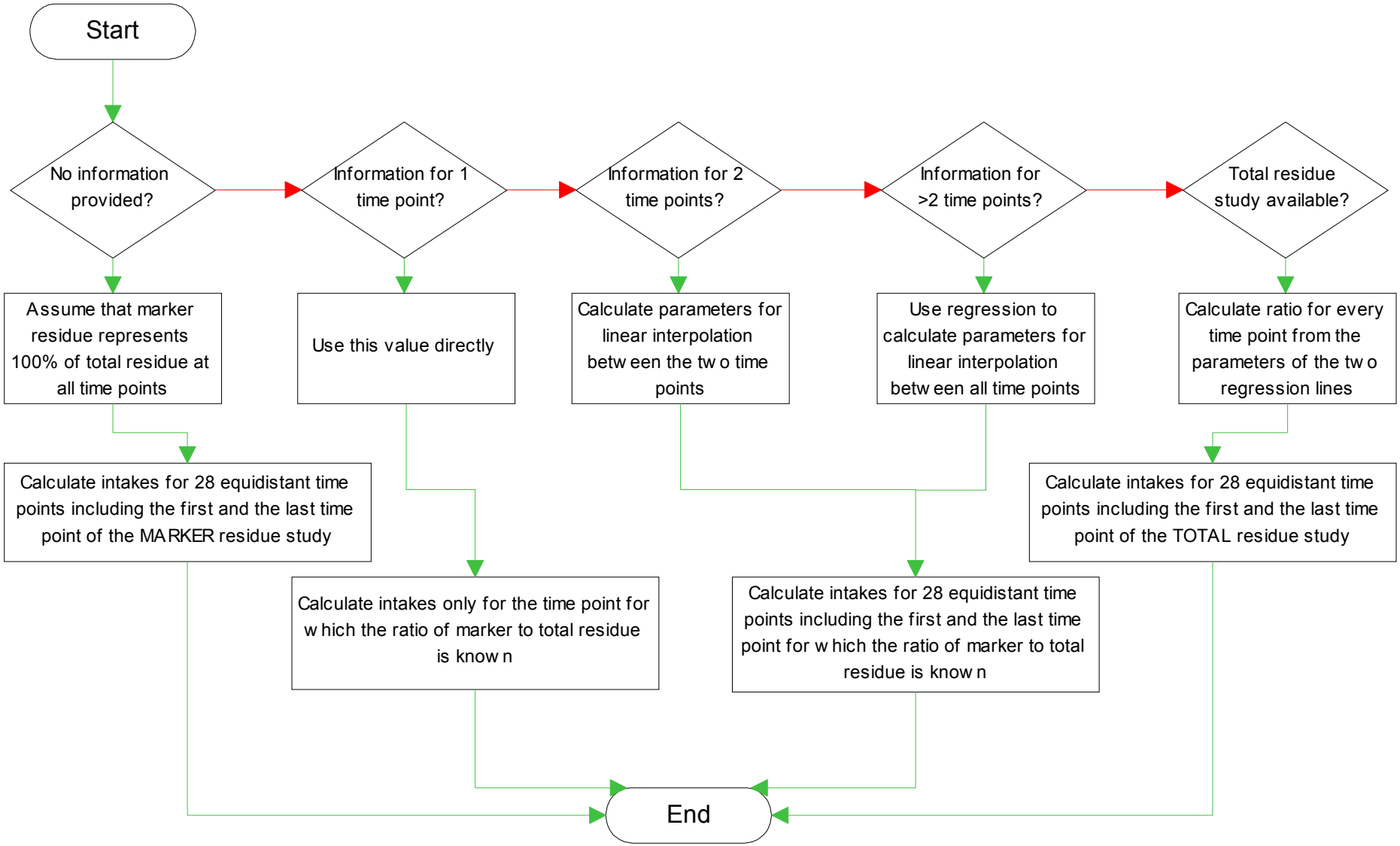


Tolerance Limits

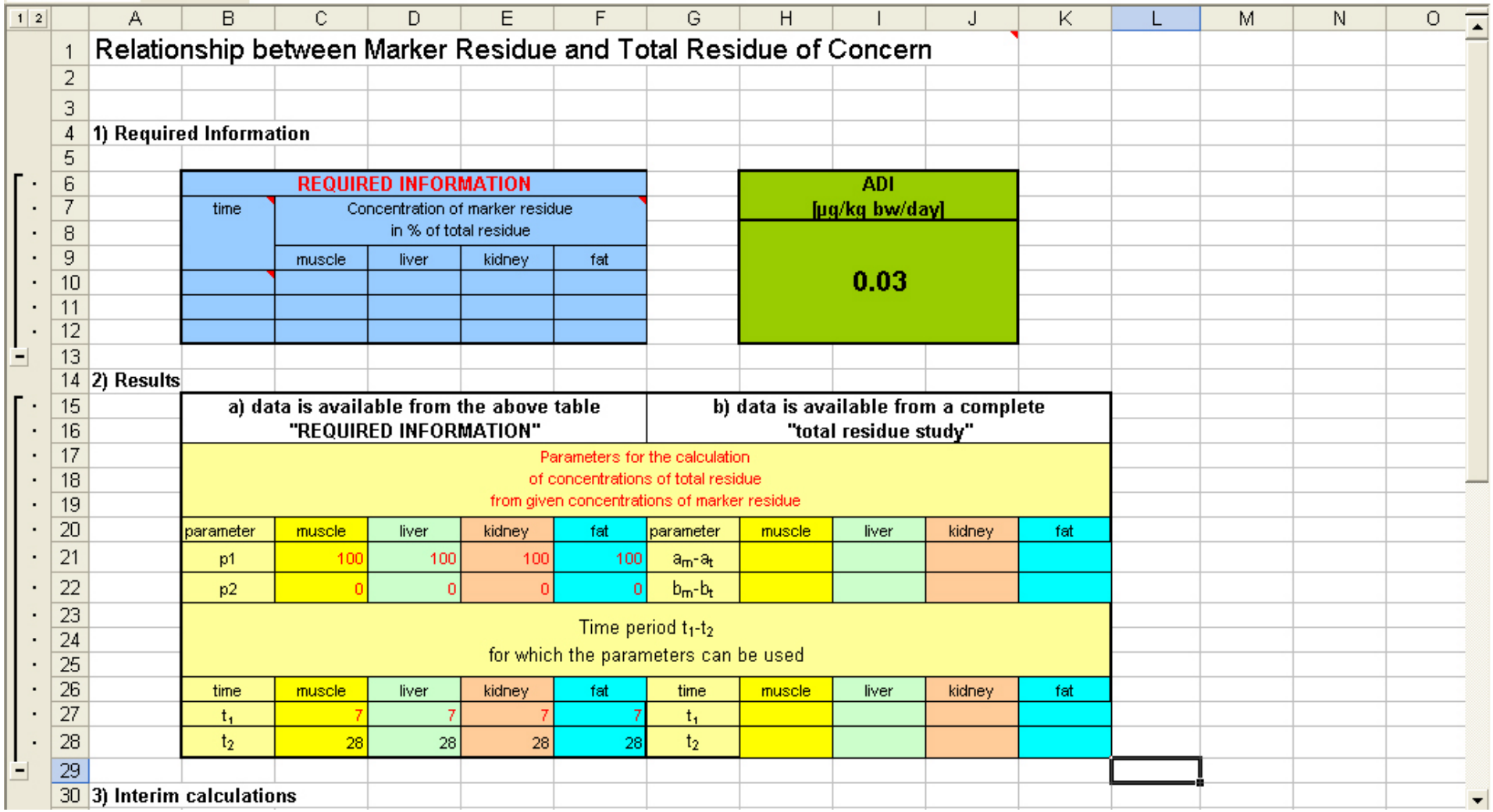
	delta for next time change [days]	time [days]	Muscle: value of the regression line [µg/kg]	Muscle: Estimate of Tolerance Limits [µg/kg] 1-γ=0.95 1-α=0.95	Muscle: 1-γ=0.99 1-α=0.95	Muscle: 1-γ=0.99 1-α=0.99	Muscle	Liver	Kidney	Fat: value of the regression line [µg/kg]	Fat: Estimates of Tolerance Limits [µg/kg] 1-γ=0.95 1-α=0.95	Fat: 1-γ=0.99 1-α=0.95	Fat: 1-γ=0.99 1-α=0.99	Fat
9	0.78	7	0.198	1.88	4.12	6.39				0.329	1.18	1.83	2.32	
10	0.78	7.78	0.183	1.71	3.74	5.77				0.311	1.10	1.71	2.17	
11	0.78	8.56	0.169	1.55	3.41	5.21				0.294	1.03	1.61	2.03	
12	0.78	9.33	0.156	1.41	3.10	4.72				0.279	0.97	1.51	1.90	
13	0.78	10.11	0.144	1.28	2.83	4.28				0.264	0.91	1.42	1.78	
14	0.78	10.89	0.133	1.16	2.58	3.88				0.249	0.85	1.33	1.67	
15	0.78	11.67	0.123	1.06	2.35	3.53				0.236	0.80	1.25	1.56	
16	0.78	12.44	0.113	0.97	2.15	3.22				0.223	0.75	1.18	1.47	
17	0.78	13.22	0.104	0.88	1.97	2.93				0.211	0.71	1.11	1.38	
18	0.78	14.00	0.096	0.81	1.81	2.68				0.200	0.67	1.05	1.30	
19	0.78	14.78	0.089	0.74	1.66	2.46				0.189	0.63	0.99	1.22	
20	0.78	15.56	0.082	0.68	1.52	2.25				0.179	0.59	0.93	1.15	
21	0.78	16.33	0.076	0.63	1.40	2.07				0.169	0.56	0.88	1.09	
22	0.78	17.11	0.070	0.58	1.29	1.91				0.160	0.53	0.83	1.03	
23	0.78	17.89	0.065	0.53	1.19	1.76				0.152	0.50	0.79	0.97	
24	0.78	18.67	0.060	0.49	1.10	1.63				0.143	0.47	0.74	0.92	
25	0.78	19.44	0.055	0.46	1.02	1.51				0.136	0.45	0.70	0.87	
26	0.78	20.22	0.051	0.42	0.95	1.40				0.128	0.42	0.67	0.82	
27	0.78	21.00	0.047	0.39	0.88	1.30				0.121	0.40	0.63	0.78	

“ratio”	
Description of the function of the worksheet	Comments found on the worksheet
This worksheet establishes the ratio between the concentrations of marker residue and total residue. The results are required for intake calculations. The “empty” template shows only the basic structure of the worksheet. In order to enter the necessary information and to view the results the user has to unfold the tables by clicking on the “+” buttons. Depending on the nature of the information available five different options for the calculation of total residue of concern have been programmed and will be explained by a flowchart on the next page and five examples. 	Cell J1: This worksheet provides factors necessary for the calculation of concentrations of "total residue" of concern for given data on the concentrations of the marker residue.The user has to fill in the necessary information in table "1. REQUIRED INFORMATION" and in the field “ADI” (see below on this worksheet!) Cell B7: Please use the identical units for the time scale throughout the worksheets of this workbook. Cell B10: 1) If there is a constant ratio between marker residue and total residue which is valid for a single time point only fill in: time point percent value 2) If a constant factor is valid for a given time period please fill in: time point 1 percent value time point 2 percent value 3) If the factor varies over time and you have information for two or more time points please fill in: time point 1 percent value 1 time point 2 percent value 2 time point 3 percent value 3 .... time point n percent value n 4) If a complete kinetic study of the depletion of the total residuei is available use worksheet "rawt" to enter the data and leave the cells of this table empty. Cell F7: All date filled into the below four columns must express the percentage of total residue which consists of marker residue.

Flowchart for the calculation of total residue from marker residue



Option 1



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat

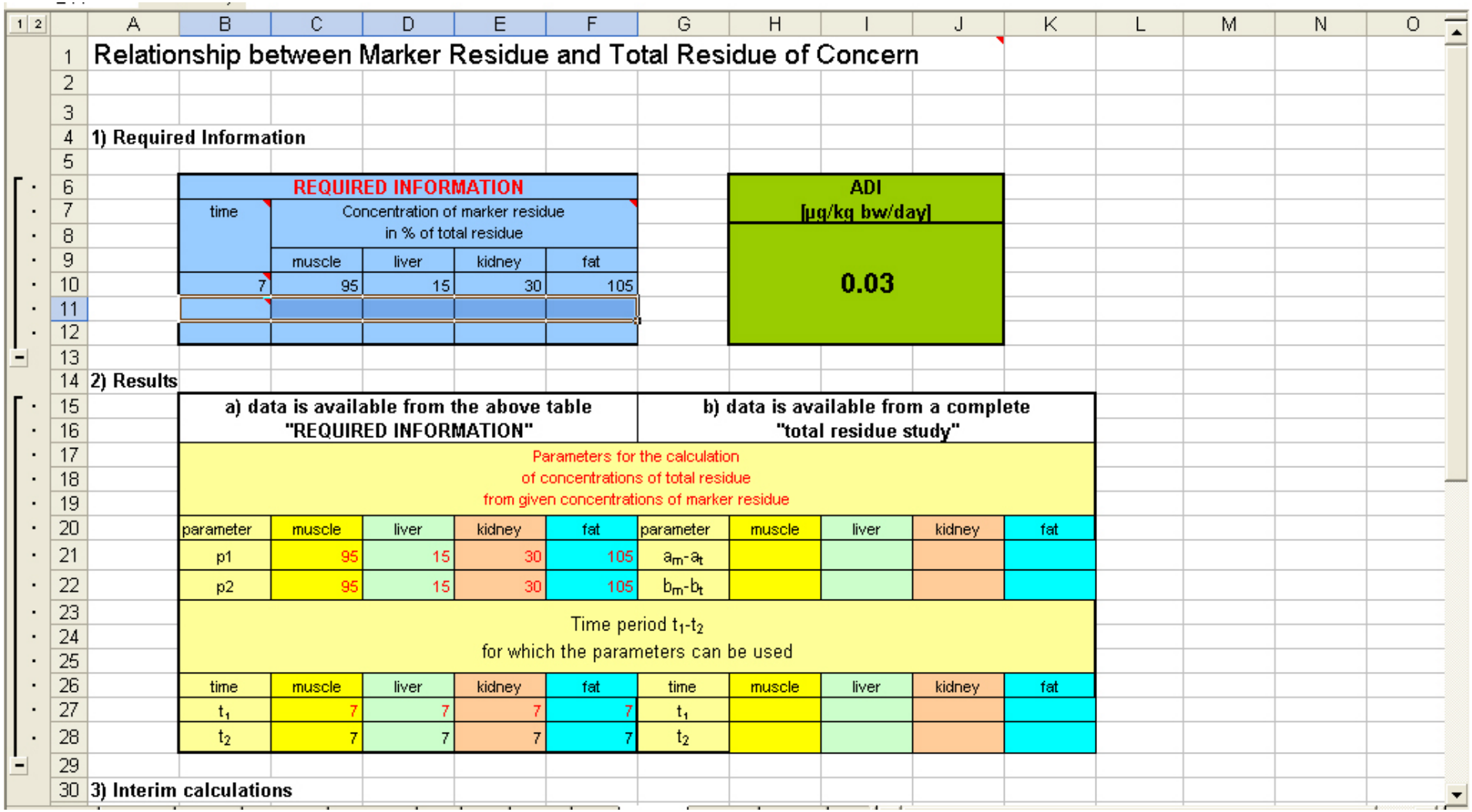
ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"					b) data is available from a complete "total residue study"				
Parameters for the calculation of concentrations of total residue from given concentrations of marker residue									
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1	100	100	100	100	a_m-a_t				
p2	0	0	0	0	b_m-b_t				
Time period t_1-t_2 for which the parameters can be used									
time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1	7	7	7	7	t_1				
t_2	28	28	28	28	t_2				

3) Interim calculations

Option 2



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat
7	95	15	30	105

ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"	b) data is available from a complete "total residue study"

Parameters for the calculation of concentrations of total residue from given concentrations of marker residue

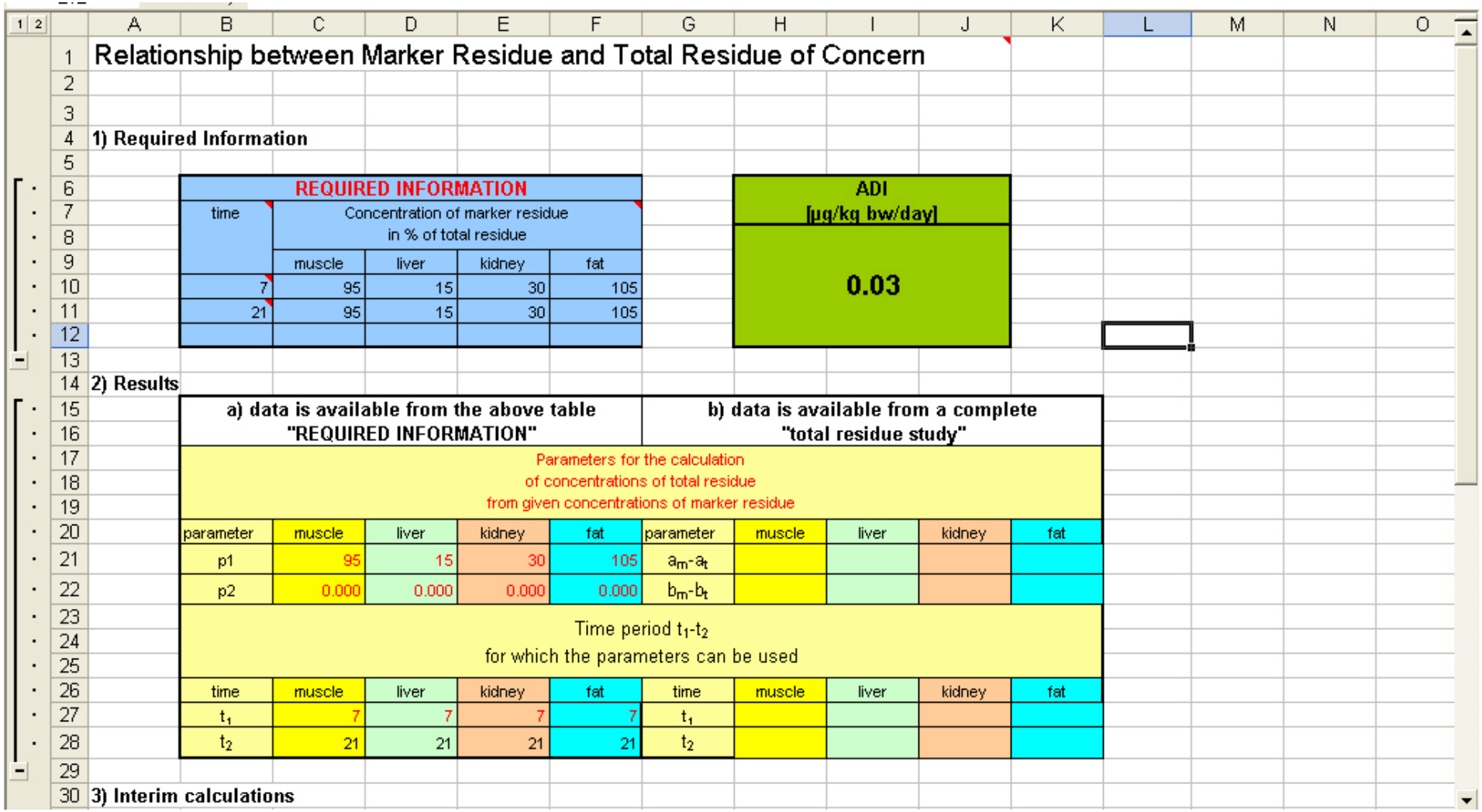
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1	95	15	30	105	a_m-a_t				
p2	95	15	30	105	b_m-b_t				

Time period t_1-t_2 for which the parameters can be used

time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1	7	7	7	7	t_1				
t_2	7	7	7	7	t_2				

3) Interim calculations

Option 3 case a



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat
7	95	15	30	105
21	95	15	30	105

ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"

b) data is available from a complete "total residue study"

Parameters for the calculation of concentrations of total residue from given concentrations of marker residue

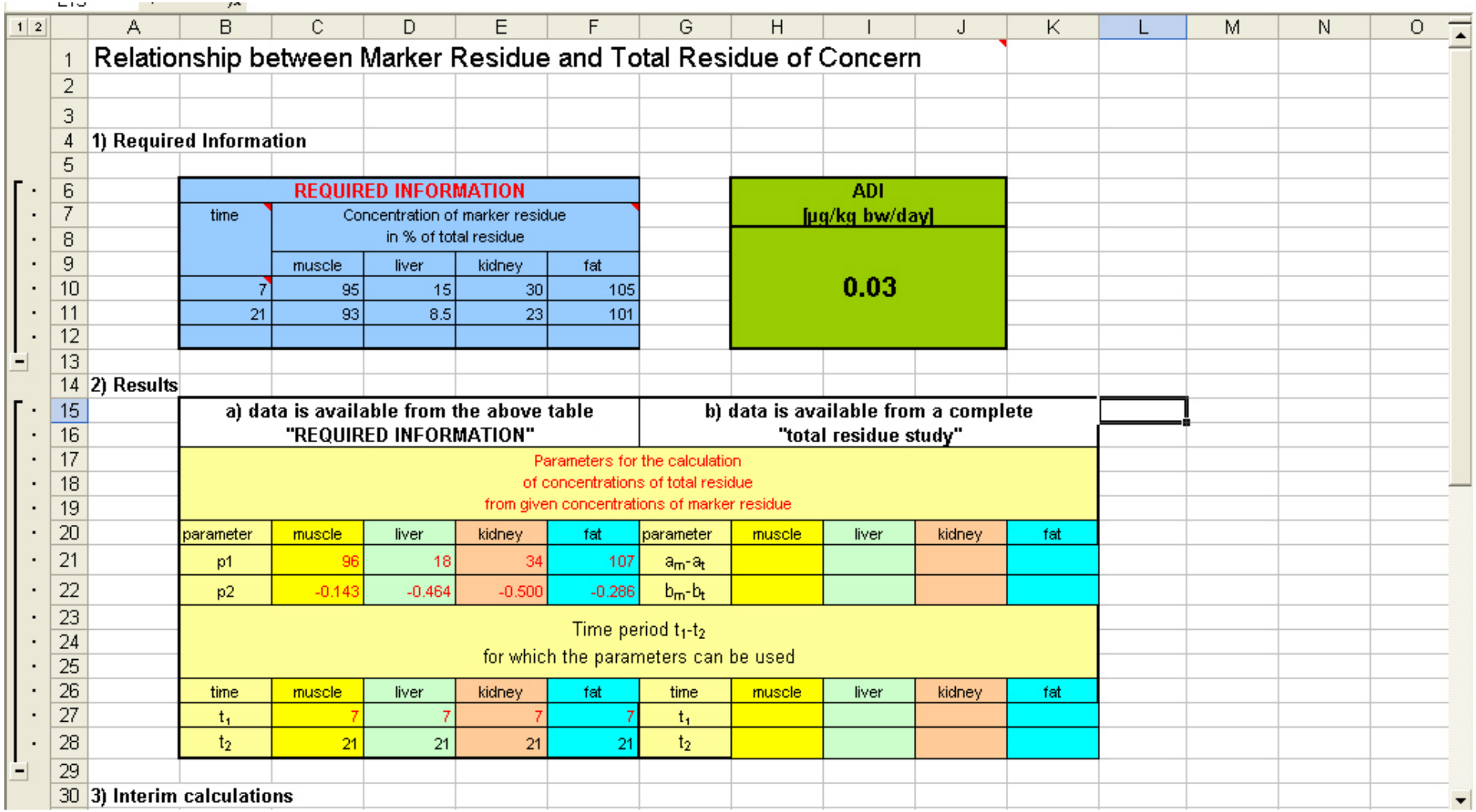
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1	95	15	30	105	a_m-a_t				
p2	0.000	0.000	0.000	0.000	b_m-b_t				

Time period t_1-t_2 for which the parameters can be used

time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1	7	7	7	7	t_1				
t_2	21	21	21	21	t_2				

3) Interim calculations

Option 3 case b



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat
7	95	15	30	105
21	93	8.5	23	101

ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"

b) data is available from a complete "total residue study"

Parameters for the calculation of concentrations of total residue from given concentrations of marker residue

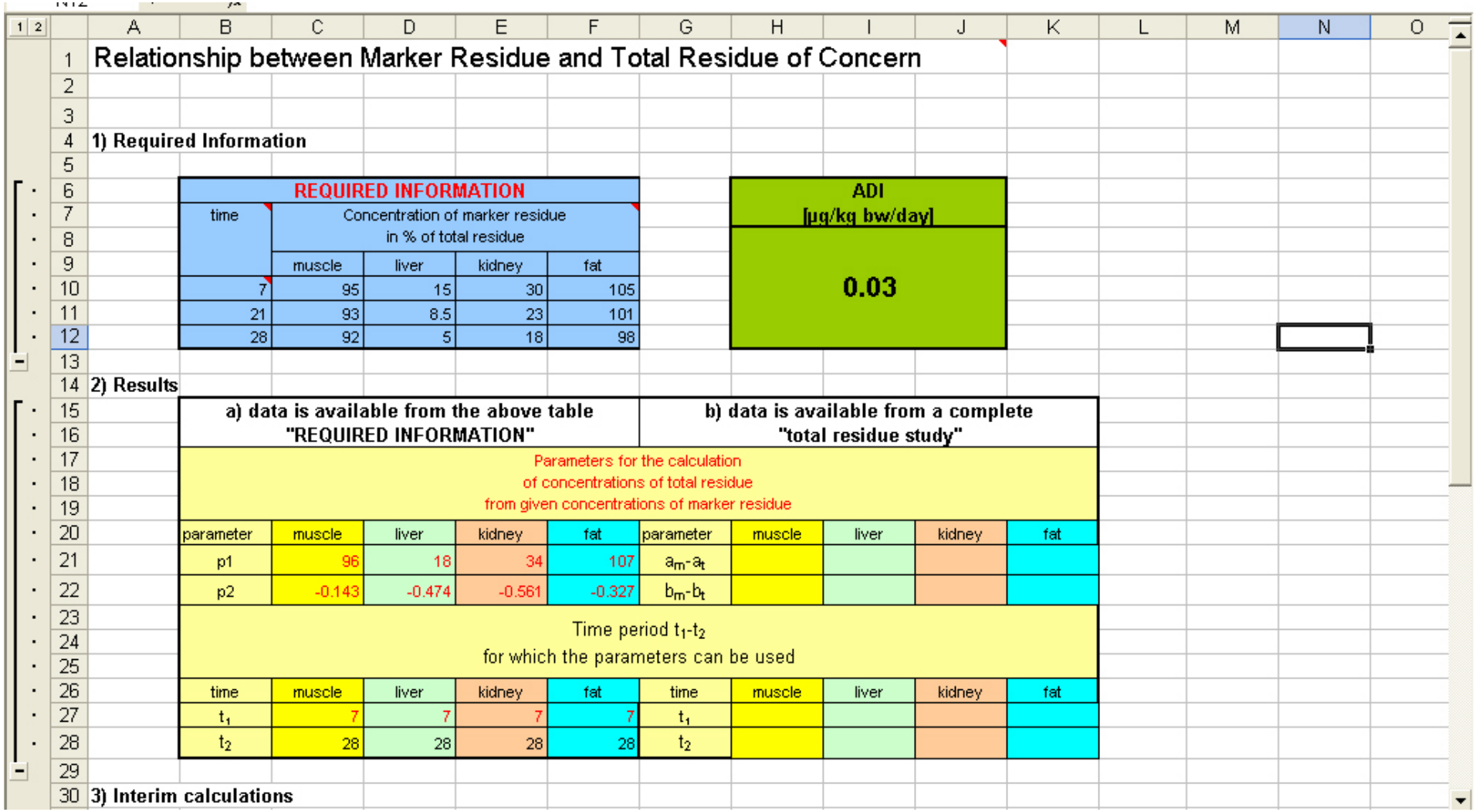
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1	96	18	34	107	a_m-a_t				
p2	-0.143	-0.464	-0.500	-0.286	b_m-b_t				

Time period t_1-t_2 for which the parameters can be used

time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1	7	7	7	7	t_1				
t_2	21	21	21	21	t_2				

3) Interim calculations

Option 4



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat
7	95	15	30	105
21	93	8.5	23	101
28	92	5	18	98

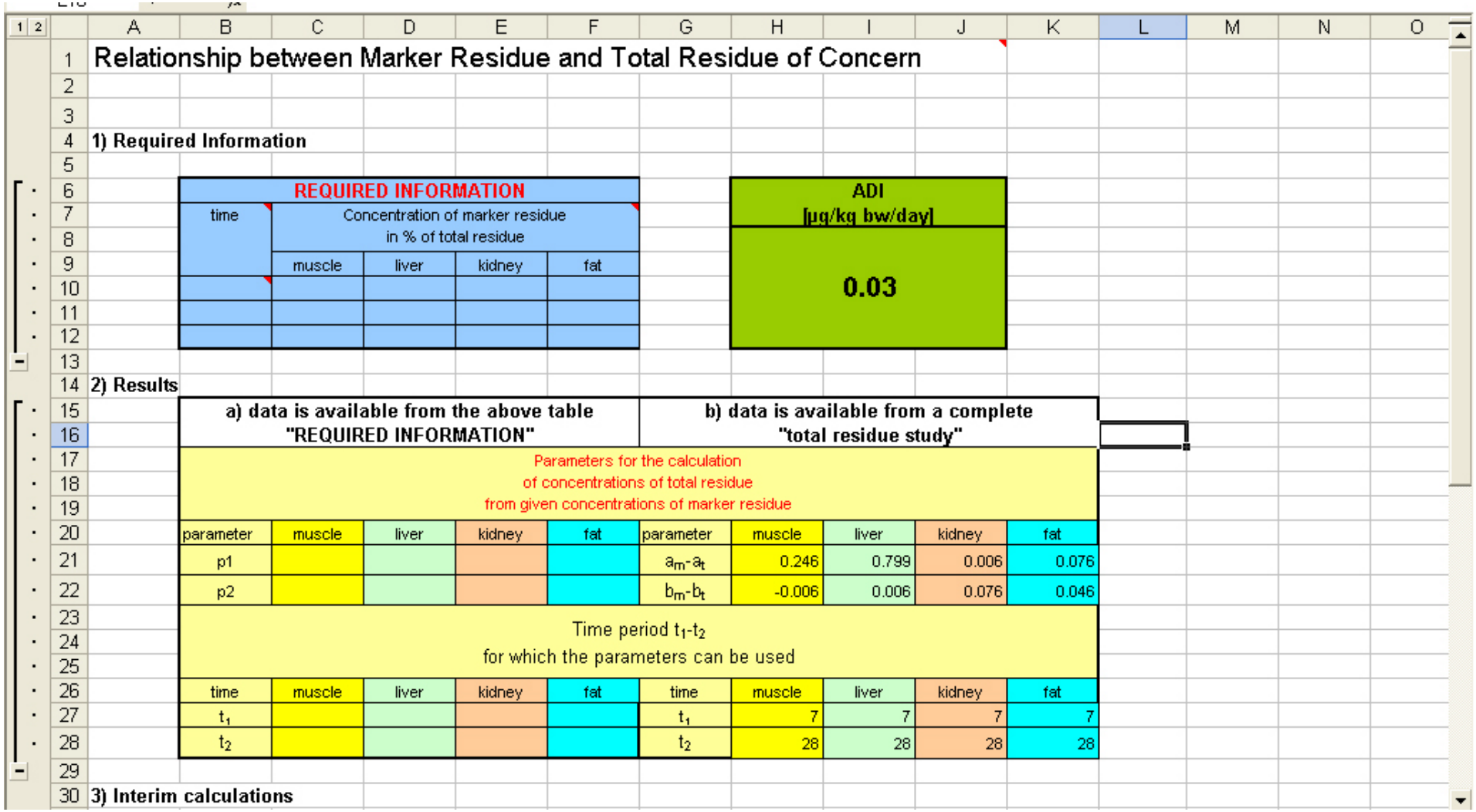
ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"					b) data is available from a complete "total residue study"				
Parameters for the calculation of concentrations of total residue from given concentrations of marker residue									
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1	96	18	34	107	a_m-a_t				
p2	-0.143	-0.474	-0.561	-0.327	b_m-b_t				
Time period t_1-t_2 for which the parameters can be used									
time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1	7	7	7	7	t_1				
t_2	28	28	28	28	t_2				

3) Interim calculations

Option 5



Relationship between Marker Residue and Total Residue of Concern

1) Required Information

REQUIRED INFORMATION				
time	Concentration of marker residue in % of total residue			
	muscle	liver	kidney	fat

ADI [µg/kg bw/day]
0.03

2) Results

a) data is available from the above table "REQUIRED INFORMATION"	b) data is available from a complete "total residue study"

Parameters for the calculation of concentrations of total residue from given concentrations of marker residue

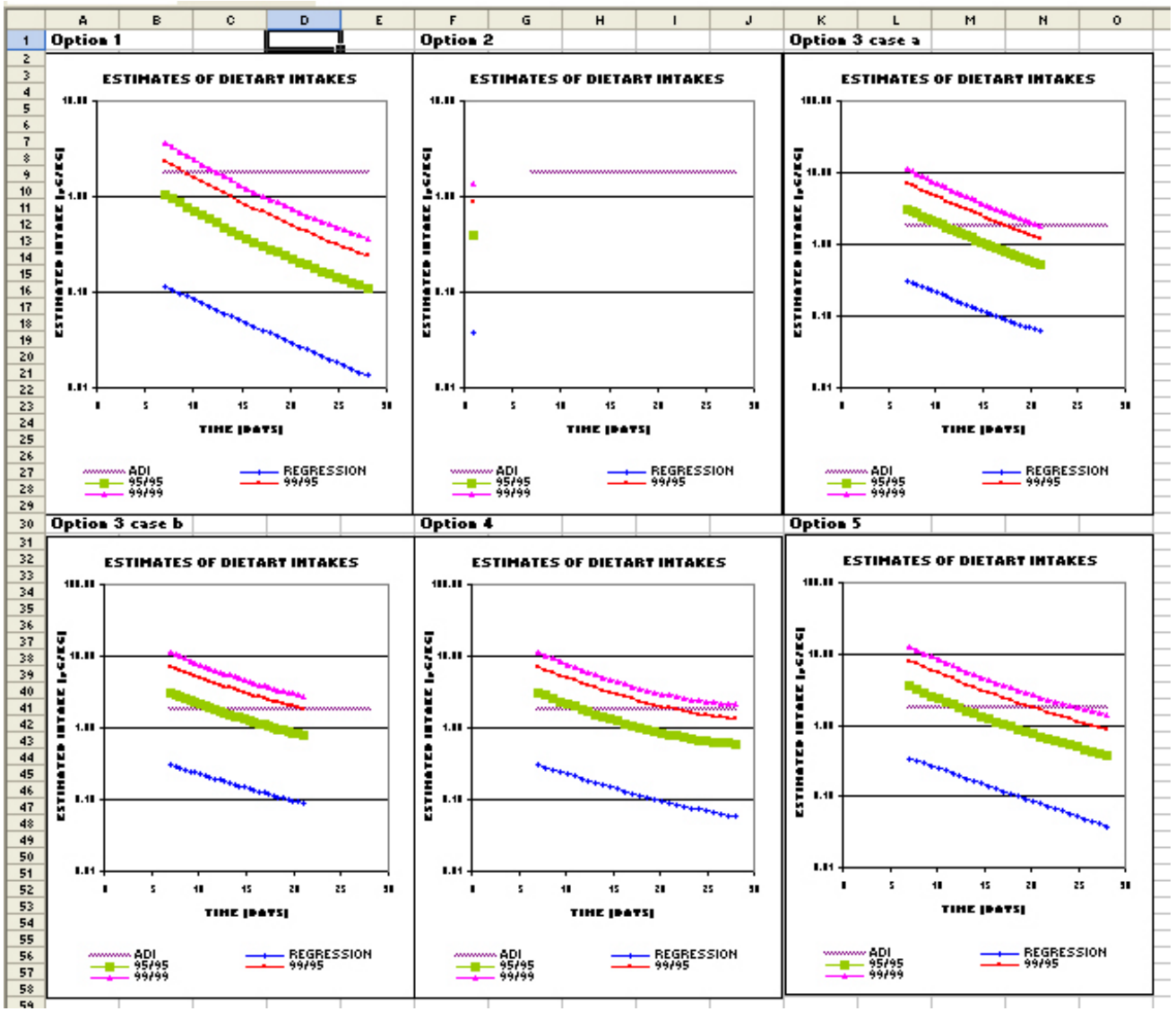
parameter	muscle	liver	kidney	fat	parameter	muscle	liver	kidney	fat
p1					a_m-a_t	0.246	0.799	0.006	0.076
p2					b_m-b_t	-0.006	0.006	0.076	0.046

Time period t_1-t_2 for which the parameters can be used

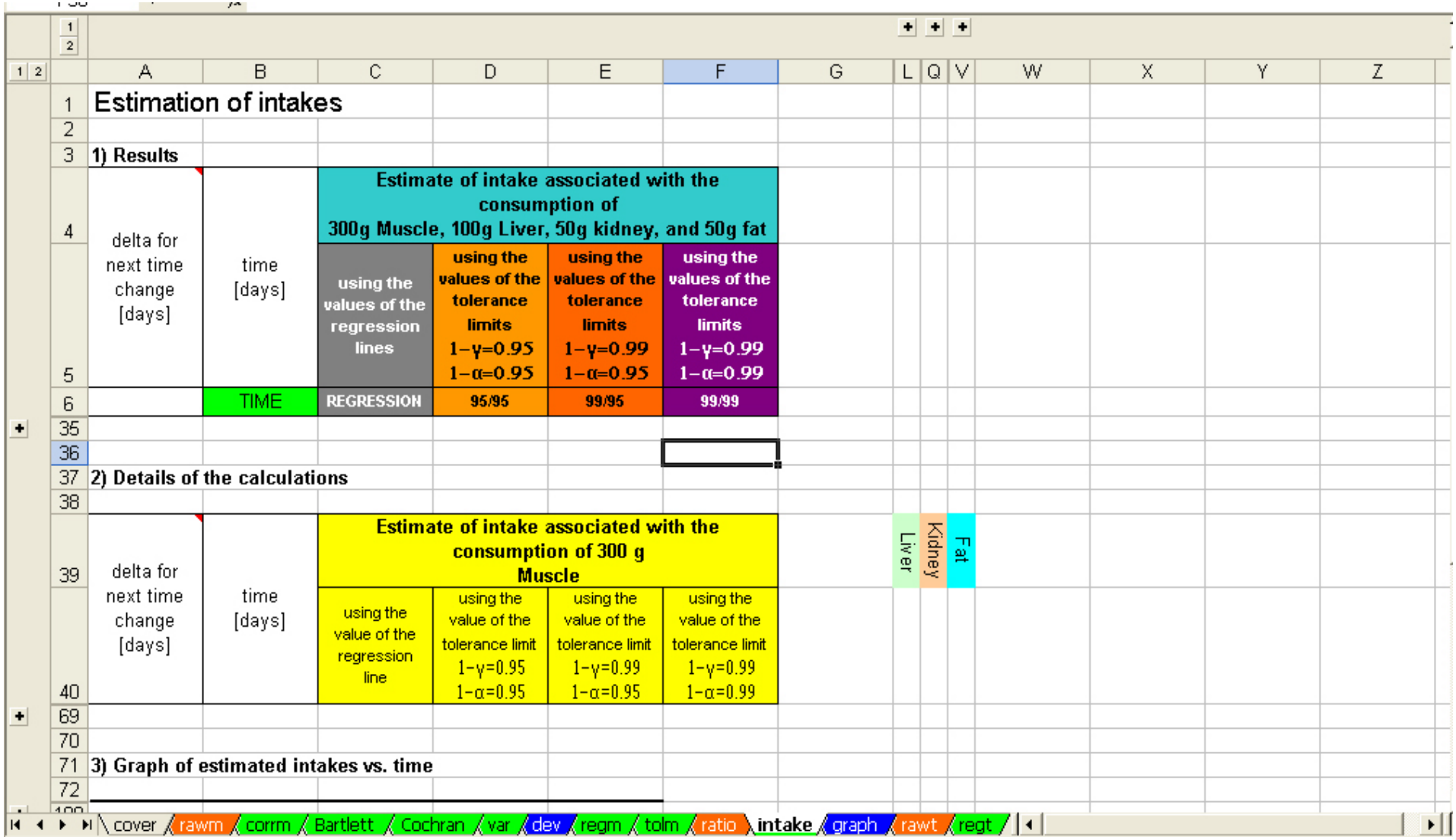
time	muscle	liver	kidney	fat	time	muscle	liver	kidney	fat
t_1					t_1	7	7	7	7
t_2					t_2	28	28	28	28

3) Interim calculations

The influence of different options for the calculation of concentrations of total residue on intake estimates using the same data set for the marker residue



"intake"	
Description of the function of the worksheet	Comments found on the worksheet
This worksheet calculates estimates of daily intakes of "total residue of concern" as a function of the depletion time and assuming that a person of 60 kg consumes 300g of muscle, 100g of liver, 50 g of kidney, and 50g of fat. For the calculation it uses the parameters of the regression line established in worksheet "regm", the tolerance limits calculated in worksheet "tolm" and the relationship between marker and total residue developed in worksheet "ratio" The "empty" template shows only the basic structure of the worksheet. In order to view the results and the plots which are automatically generated by the worksheet the user has to unfold the tables by clicking on the "+" buttons. Depending on the range of the data it might also be necessary to rescale the graphs.	Cells $4 and A39: This woeksheet calculates estimates of dietary intakes for 28 equally spaced time points including the first and the last time point of the experimental data for which the ratio of marker to total residue is known. If that ratio is known for only one time point, calculations are carried out only for that time point. The worksheet uses estimates of concentrations of residues obtained from the regression line or, alternatively from calculated statistical tolerance limits.



Estimation of intakes

1) Results

delta for next time change [days]	time [days]	Estimate of intake associated with the consumption of 300g Muscle, 100g Liver, 50g kidney, and 50g fat			
		using the values of the regression lines	using the values of the tolerance limits 1−γ=0.95 1−α=0.95	using the values of the tolerance limits 1−γ=0.99 1−α=0.95	using the values of the tolerance limits 1−γ=0.99 1−α=0.99
	TIME	REGRESSION	95/95	99/95	99/99

2) Details of the calculations

delta for next time change [days]	time [days]	Estimate of intake associated with the consumption of 300 g Muscle			
		using the value of the regression line	using the value of the tolerance limit 1−γ=0.95 1−α=0.95	using the value of the tolerance limit 1−γ=0.99 1−α=0.95	using the value of the tolerance limit 1−γ=0.99 1−α=0.99

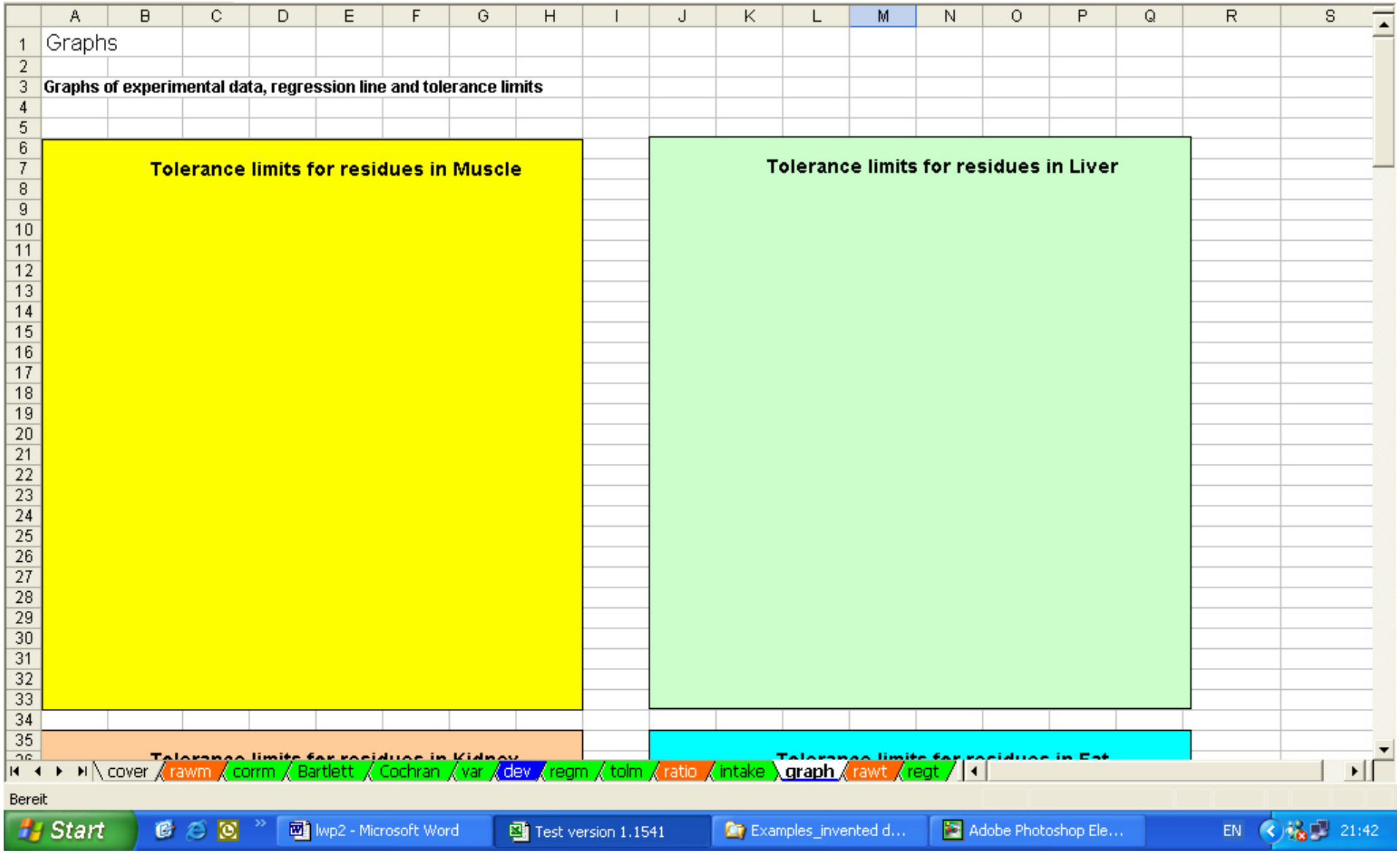
Liver Kidney Fat

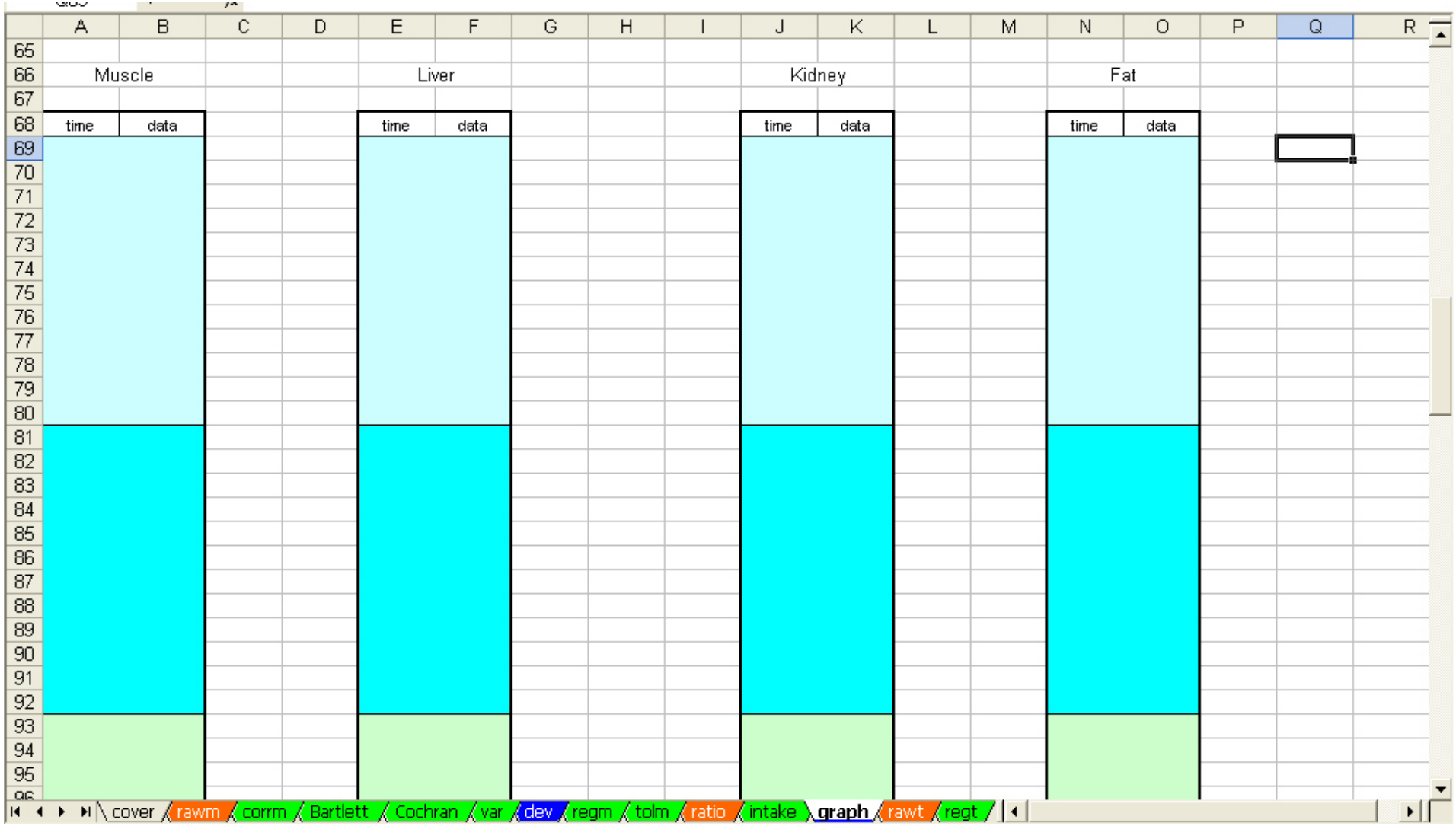
3) Graph of estimated intakes vs. time

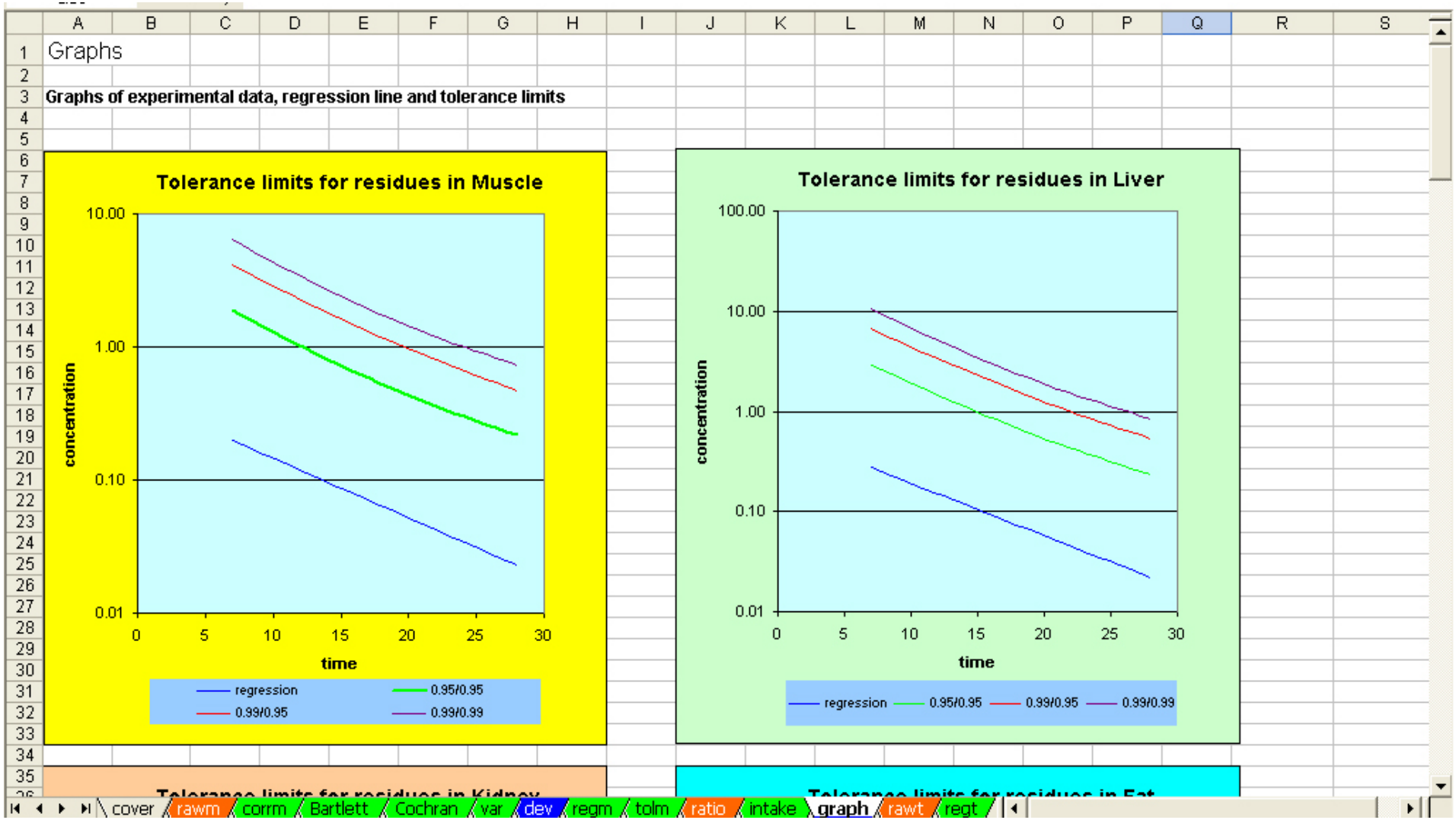
cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

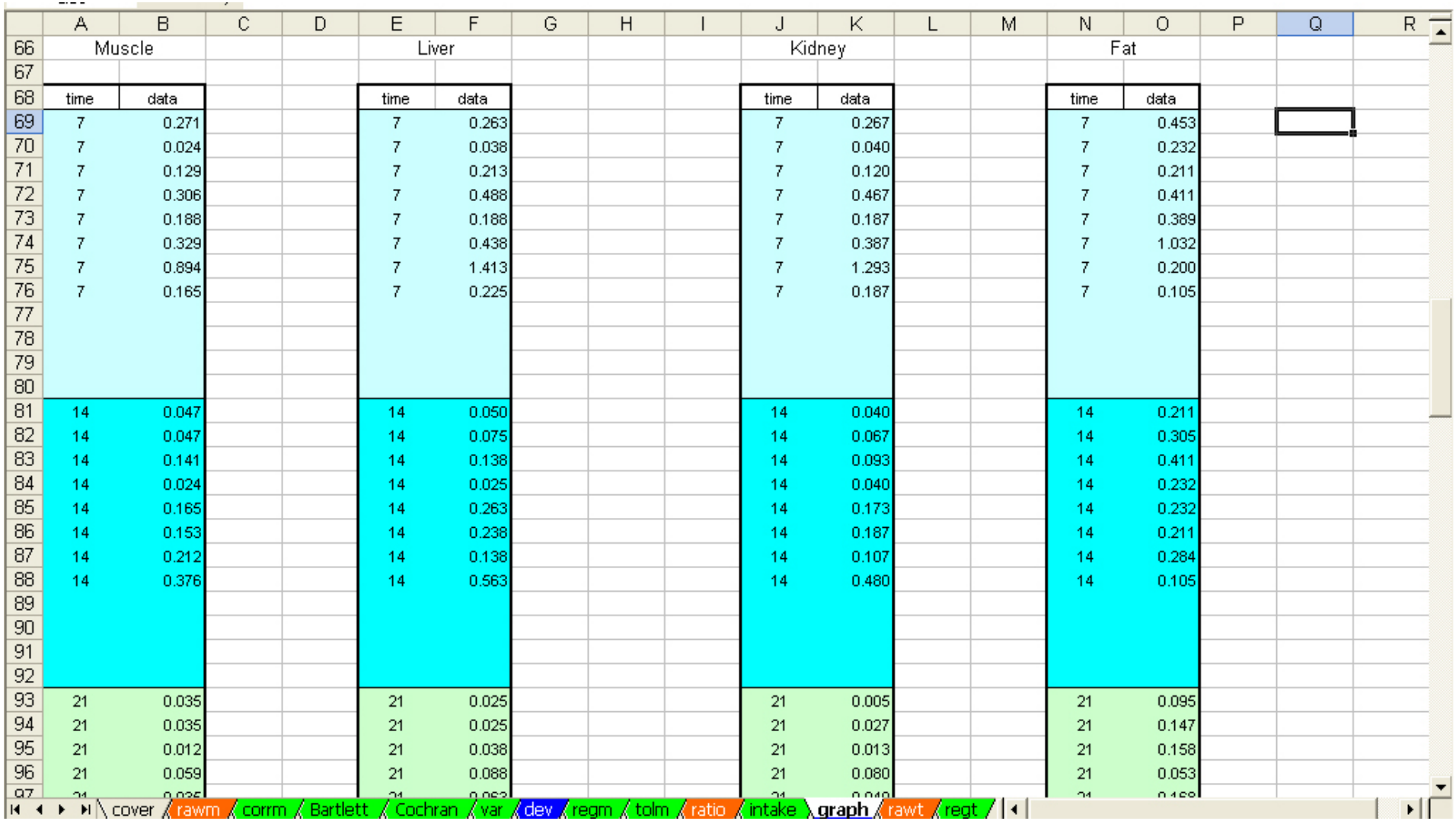
“graph”

Description of the function of the worksheet	Comments found on the worksheet
This worksheet assists the user to generate graphs containing the corrected concentrations of the residues as individual data points plus the regression line and the tolerance limits as continuous lines. The user has to apply a stepwise procedure similar to the one followed in worksheet “dev” to obtain the graphs. Depending on the range of the data it might be necessary to rescale the graphs. The first two of the following screenshots show parts of the “empty” template. The next two screenshots show what happens when the user has worked through the previous worksheets and has made sure that no data are hidden on worksheets “corm” and “tolm”. The worksheet “graph” then has already automatically plotted the regression line and the tolerance limits and columns with the individual data points have been prepared.	


Graphs
Graphs of experimental data, regression line and tolerance limits
Tolerance limits for residues in Muscle
Tolerance limits for residues in Liver
cover
rawm
corrm
Bartlett
Cochran
var
dev
regm
tolm
ratio
intake
graph
rawt
regt
Bereit
Start
lwp2 - Microsoft Word
Test version 1.1541
Examples_invented d...
Adobe Photoshop Ele...
EN
21:42


Muscle
Liver
Kidney
Fat
time
data
cover
rawm
corrm
Bartlett
Cochran
var
dev
regm
tolm
ratio
intake
graph
rawt
regt

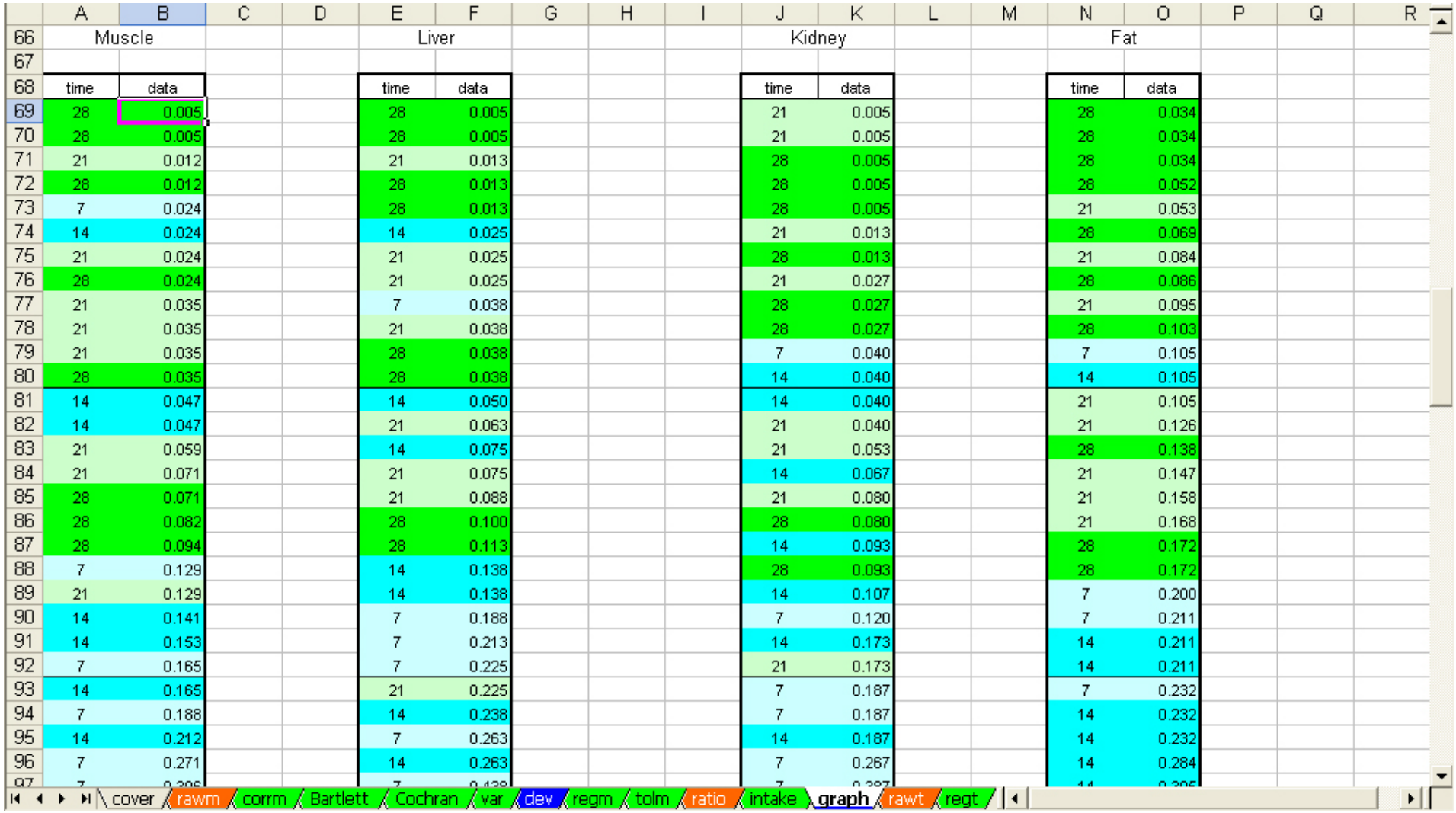

Graphs
Graphs of experimental data, regression line and tolerance limits
Tolerance limits for residues in Muscle
concentration
10.00
1.00
0.10
0.01
0 5 10 15 20 25 30
time
regression
0.95/0.95
0.99/0.95
0.99/0.99
Tolerance limits for residues in Liver
concentration
100.00
10.00
1.00
0.10
0.01
0 5 10 15 20 25 30
time
regression
0.95/0.95
0.99/0.95
0.99/0.99
cover
rawm
corrm
Bartlett
Cochran
var
dev
regm
tolm
ratio
intake
graph
rawt
regt



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
66	Muscle				Liver					Kidney				Fat				
67																		
68	time	data			time	data				time	data			time	data			
69	7	0.271			7	0.263				7	0.267			7	0.453			
70	7	0.024			7	0.038				7	0.040			7	0.232			
71	7	0.129			7	0.213				7	0.120			7	0.211			
72	7	0.306			7	0.488				7	0.467			7	0.411			
73	7	0.188			7	0.188				7	0.187			7	0.389			
74	7	0.329			7	0.438				7	0.387			7	1.032			
75	7	0.894			7	1.413				7	1.293			7	0.200			
76	7	0.165			7	0.225				7	0.187			7	0.105			
77																		
78																		
79																		
80																		
81	14	0.047			14	0.050				14	0.040			14	0.211			
82	14	0.047			14	0.075				14	0.067			14	0.305			
83	14	0.141			14	0.138				14	0.093			14	0.411			
84	14	0.024			14	0.025				14	0.040			14	0.232			
85	14	0.165			14	0.263				14	0.173			14	0.232			
86	14	0.153			14	0.238				14	0.187			14	0.211			
87	14	0.212			14	0.138				14	0.107			14	0.284			
88	14	0.376			14	0.563				14	0.480			14	0.105			
89																		
90																		
91																		
92																		
93	21	0.035			21	0.025				21	0.005			21	0.095			
94	21	0.035			21	0.025				21	0.027			21	0.147			
95	21	0.012			21	0.038				21	0.013			21	0.158			
96	21	0.059			21	0.088				21	0.080			21	0.053			
97	21	0.035			21	0.063				21	0.040			21	0.168			

cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

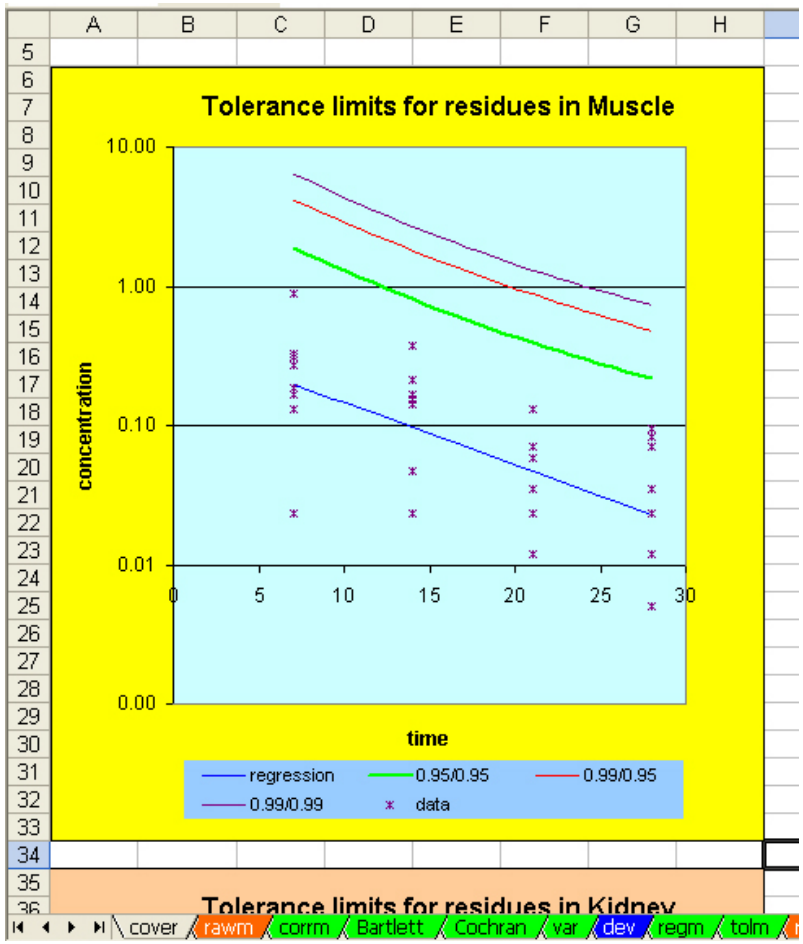
“graph” (continued)	
Description of the function of the worksheet	Comments found on the worksheet
Procedure for the completion of the graphs: Tissue by tissue select the first cell under the headline “data” and sort the data by clicking on the symbol for ascending sorting (arrow from A to Z) in the tool bar. Tissue by tissue click on the plot. When the menu “chart” appears select “add data”. When the dialog box appears select at the same time all data containing cells (***no more*****!**) under and including the headlines “time” and “data”. Complete the information in the checkbox before clicking on “ok”	



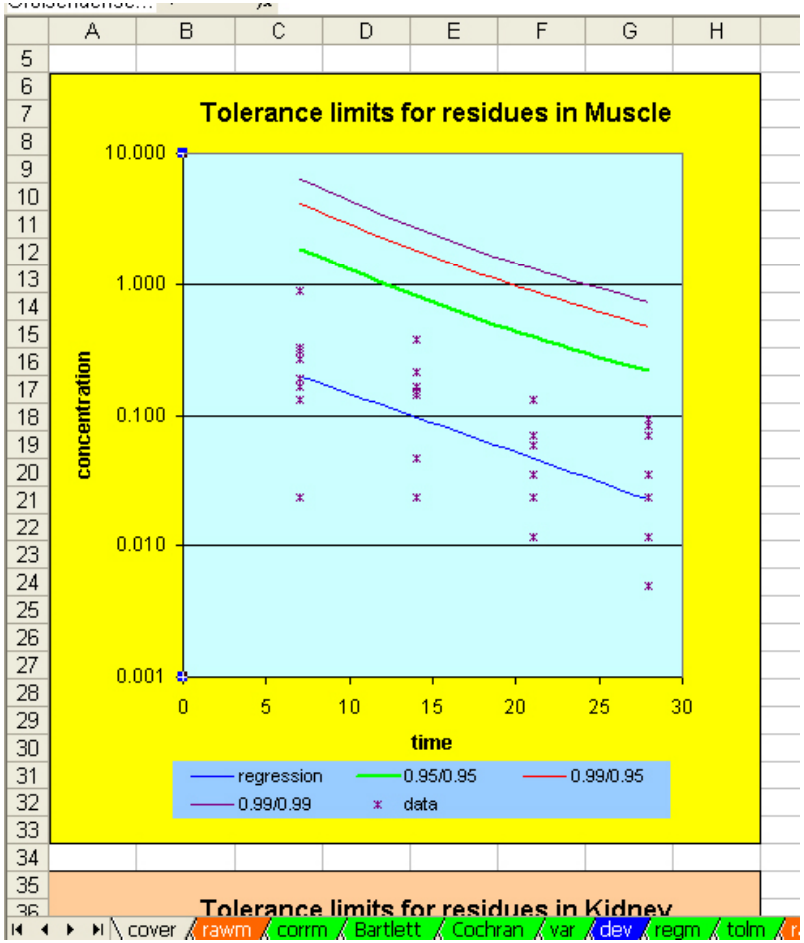
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
66	Muscle				Liver					Kidney				Fat				
67																		
68	time	data			time	data				time	data			time	data			
69	28	0.005			28	0.005				21	0.005			28	0.034			
70	28	0.005			28	0.005				21	0.005			28	0.034			
71	21	0.012			21	0.013				28	0.005			28	0.034			
72	28	0.012			28	0.013				28	0.005			28	0.052			
73	7	0.024			28	0.013				28	0.005			21	0.053			
74	14	0.024			14	0.025				21	0.013			28	0.069			
75	21	0.024			21	0.025				28	0.013			21	0.084			
76	28	0.024			21	0.025				21	0.027			28	0.086			
77	21	0.035			7	0.038				28	0.027			21	0.095			
78	21	0.035			21	0.038				28	0.027			28	0.103			
79	21	0.035			28	0.038				7	0.040			7	0.105			
80	28	0.035			28	0.038				14	0.040			14	0.105			
81	14	0.047			14	0.050				14	0.040			21	0.105			
82	14	0.047			21	0.063				21	0.040			21	0.126			
83	21	0.059			14	0.075				21	0.053			28	0.138			
84	21	0.071			21	0.075				14	0.067			21	0.147			
85	28	0.071			21	0.088				21	0.080			21	0.158			
86	28	0.082			28	0.100				28	0.080			21	0.168			
87	28	0.094			28	0.113				14	0.093			28	0.172			
88	7	0.129			14	0.138				28	0.093			28	0.172			
89	21	0.129			14	0.138				14	0.107			7	0.200			
90	14	0.141			7	0.188				7	0.120			7	0.211			
91	14	0.153			7	0.213				14	0.173			14	0.211			
92	7	0.165			7	0.225				21	0.173			14	0.211			
93	14	0.165			21	0.225				7	0.187			7	0.232			
94	7	0.188			14	0.238				7	0.187			14	0.232			
95	14	0.212			7	0.263				14	0.187			14	0.232			
96	7	0.271			14	0.263				7	0.267			14	0.284			

cover | rawm | corrm | Bartlett | Cochran | var | dev | regm | tolm | ratio | intake | graph | rawt | regt

Without rescaling



After rescaling



“rawt”	
Description of the function of the worksheet	Comments found on the worksheet
This worksheets serves for entering concentration data of total residue if such a study is available. Its design is similar to the design of worksheet “rawm”. The “empty” template shows only the basic structure of the worksheet. In order to use and view the tables the user has to unfold them by clicking on the “+” buttons	Cells N14, N 28, N42, N56: For this table the worksheet sheet will accept: - either a numerical value, - or an empty cell.

“regt”	
Description of the function of the worksheet	Comments found on the worksheet
This worksheets performs a linear regression analysis of a total residue study if such a study is available. Its design is similar to the design of worksheet “regm”. The “empty” template shows only the basic structure of the worksheet. In order to view the tables the user has to unfold them by clicking on the “+” buttons	