

MANUAL FOR CALCULATING WITHDRAWAL PERIOD (MILKINGS) using the PROAST web application

PROASTweb is an online tool with the purpose of simplifying the use of the Benchmark dose approach. It is used for dose-response analysis of toxicological data and derivation of a BMD confidence interval; the lower bound of which (the BMDL) is used as a Point of Departure for risk assessment.

PROASTweb includes the option to estimate the time that is needed to achieve a residue concentration in milk lower than the MRL in 95% of the population of the species considered. This time will be called the TT95, where TT stands for True Time, i.e. the unknown value in the infinite population. Since the data only reflect a limited sample of test animals, the TT can only be estimated approximately, and therefore the 90%-confidence interval (5% for each of both tails) for the TT95 is calculated. The upper bound of that confidence interval (the tolerance limit, i.e. the time point where the concentration in milk is lower than the MRL in 95% of the population, with 95% confidence) can be used as a basis for setting the withdrawal period for milk.

Principle of the method

A regression function is fit to the decaying milk residue concentrations in each individual animal, taking into account that the animals may vary in the parameters of the regression function, such as the intercept (concentration at $t=0$) or the rate of decay. The model fitting is done by PROAST, a package within R (which is a general software tool). Next, the R-package MILKINGS evaluates the uncertainties (estimation errors) of the parameters in the model (e.g. intercept, slope, within-animal variation). This is translated into the uncertainty (confidence interval) of the TT95. By using the upper bound of the confidence interval (= tolerance limit) as the withdrawal period, the confidence that 95% of the population achieves a residue concentration in milk lower than the MRL at this time point, will be the same for weak or good data. However, with relatively weak data (e.g. relatively few animals, relatively high LOQ) there will be more uncertainty, which will be reflected by relatively large confidence intervals and consequently the upper bound (the withdrawal period) may be higher than needed.

In other words, a better study with good data will result in a smaller confidence interval, possibly with a lower upper bound, resulting in a lower withdrawal period.

The confidence interval is calculated by the bootstrap method. Briefly, it may be imagined that replicate studies are simulated by the computer, each of which result in a somewhat different estimate of the TT95. The variation in these TT95 values represent the impact of sampling error, as the data only reflect a limited sample of test animals, and together provide the information for setting the confidence interval.

How to work with the tool

1. Go to the PROAST website: proastweb.rivm.nl
2. After accepting the Terms and Conditions, you arrive at the page "Overview". This page shows the current status of your work (so, it is empty when first using the tool).

3. Click on “Create new analysis”, and you arrive at the page “Analysis; Upload a file”. In this window you can upload a dataset. The file should be saved as a tab-delimited text in .txt format. For further information on how to present the data, see the help text in the webtool.
4. As a first exercise, you might use the example dataset available in the web application, by first clicking on “Use example data sets”, and then from the scroll bar “Data set” select “Example dataset 1 for withdrawal period (milkings)”, or “Example dataset 2 for withdrawal period (milkings)”. The selected example dataset now appears at the bottom of the page.

If you want to use another dataset available on your own computer, you can upload that file by clicking on “Browse” in the input field “Data set file”, find the dataset, and click on “open” in your browser. Then, click on “Upload”. You will now see the dataset at the bottom of the web page. The help button provides information on how to format the dataset.

5. In the input field “Analysis name”, type a name. This name will be used in the Overview window, so that you can find the results of each specific analysis.
6. At “Purpose” select “Withdrawal period (milkings)”.
7. Click on “Next: Specify”, and you will arrive at the “Specify” page. Here, you can select some settings.
8. In general, you should keep “Effect of dry period” active. If you have a dataset of milkings without dry periods, this option will automatically be ignored. If you do have data with dry periods, the program will check if the factor dry period has a (statistically) significant impact on the residue concentrations in milk. If the dry period does not have a significant impact, the program only calculates one confidence interval of the TT95 (the upper bound of which may be used as a withdrawal period). Otherwise, the program presents a table with the upper bounds of the confidence interval for the various dry periods, i.e. the withdrawal period will then depend on the dry period of the animal.
9. In the fields “Limit of quantification” and “MRL” you can fill in the values (in $\mu\text{g}/\text{kg}$) for the specific data you have. For both example dataset, the values are 2 and 4, respectively.
10. The program takes into account the uncertainties of the parameters in the model (e.g. intercept parameter a , shape parameter d , within-animal variation) and uses that for calculating the confidence interval of the TT95. For calculating the withdrawal period to be used in a regulatory context, the uncertainty in parameter d (which reflects the deviation from a linearity in the decay function) needs to be included in the calculations.
11. The default number of bootstrap runs (number of simulated replicate studies) is 200. However, with 200 runs the calculation time may be quite long. Therefore, it may be convenient to first do a “pilot” calculation with fewer runs (e.g. 10 or 20), to make sure that the calculations are running without difficulties. You only need to change the number of bootstrap runs and re-run the calculations. However, for the final calculation of the withdrawal period, the number of bootstrap runs should be 200.
12. Click on ‘Run’. You now return to the Overview page. This page now shows that your “job” is queued, and next if it is in progress or is finished. When the state is “finished”, click on the third symbol . This gives you a brief report of the result.

13. Examine the content of the report (which is an HTML page, the content of which can be copied into Word). The section “Input values” gives an overview of the settings used. In the section “Graphical output” you will see two plots for the example dataset 1. The first plot shows the confidence interval of the TT95 as a function of dry period: the lower curve is the lower bound, the upper curve the upper bound. The second plot (i.e. “fitted decay curves”) shows the observed residue concentrations against time of milking, with the fitted curves for each individual animal. In this plot, the horizontal dotted line indicates the LOQ, and the marks below this line represent the observations below LOQ.

NOTE: Data below the LOQ are plotted at $\frac{1}{2}$ LOQ in this plot. However, in the statistical calculations, data below LOQ are *not* replaced by $\frac{1}{2}$ LOQ (or any other single number), as this procedure is statistically invalid. Instead, a measurement below LOQ, presented as 0 in the dataset, is taken into account as an (unknown) value between zero and the LOQ. PROAST uses statistical methods which can take such information into account, by assuming that the data are lognormally distributed (and including the associated cumulative distribution function in the log-likelihood function). See also the help button for ‘limit of quantification’.

14. The section “Withdrawal Period” provides the final results. In the case of the example dataset 1, a table of the dry period and the associated withdrawal period (i.e. the tolerance limit) is presented, because a significant impact of the dry period on the withdrawal period was observed for this example product. To verify your results, see Table 1 below showing the results when using 200 bootstrap runs.
15. In case there is no significant impact of the dry period, only the plot with the fitted decay curves will be shown and a single confidence interval will be presented, the upper bound of which can be used to establish the withdrawal period. To illustrate the type of output from the web application that you will get in case no significant impact of the dry period is found, you may analyze example dataset 2 (again with LOQ=2 and MRL = 4). In the section “Graphical output” only one plot (i.e. “fitted decay curves”) is displayed which shows the observed residue concentrations against time of milking, with the fitted curves for each individual animal. You will now see that no significant impact of dry period was found, therefore only one single confidence interval for the TT95 is calculated, i.e. (5.756, 11.69). In this example, the upper bound limit of the confidence interval, rounded up to 12 days, would be the withdrawal period, and be considered valid for all dry periods.

Table 1. Tolerance limit (withdrawal period) as a function of the dry period, resulting from the example dataset 1, with LOQ=2 and MRL=4, using 200 bootstrap runs.

Withdrawal Period

dry period	withdrawal period (day)
8.00	57.46
9.00	55.18
10.00	52.89
11.00	50.60
12.00	48.31
13.00	46.07
14.00	43.83
15.00	41.59
16.00	39.36
17.00	37.24
18.00	35.13
19.00	33.01
20.00	30.90
21.00	28.99
22.00	27.09
23.00	25.18
24.00	23.27
25.00	21.64
26.00	20.00
27.00	18.37
28.00	16.73
29.00	15.39
30.00	14.05
31.00	12.71
32.00	11.37
33.00	10.31
34.00	9.25
35.00	8.19
36.00	7.13
37.00	6.29
38.00	5.45
39.00	4.61
40.00	3.78
41.00	3.08
42.00	2.38
43.00	1.68
44.00	0.98
45.00	0.35
46.00	0.00
47.00	0.00
48.00	0.00