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Guideline on determination of withdrawal periods for milk

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This guideline replaces the Note for guidance for the determination of withdrawal periods for milk (EMEA/CVMP/473/98-FINAL).

Keywords	***Withdrawal periods, milk, statistical approach***

*The current revision consists of administrative changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated.

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Guideline on determination of withdrawal periods for milk

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1. Introduction (background)

For the establishment of withdrawal periods for milk different methods were available and used by Member States. A harmonised method has been developed for decentralised applications for marketing authorisations, as well as for centralised procedures, in order to facilitate that harmonised milk withdrawal periods can be fixed in EU Member States.

The Note for Guidance Approach towards Harmonisation of Withdrawal Periods (EMEA/CVMP/036/95-FINAL) [1] later replaced by the Guideline on determination of withdrawal periods for edible tissues (EMA/CVMP/SWP/735325/2012) [21] provides detailed guidance on how to establish withdrawal periods for edible tissues and was developed by the CVMP in order to provide a standardised approach within the European Union. However, because the character of milk depletion data and the statistical aspects of calculations with these data differ from those of meat residue data, a separate methodological approach was necessary.

A harmonised method should fulfil the following criteria:

- It should provide safe withdrawal periods, derived from the depletion data in a scientifically justified way.
- It should be applicable for most, if not all, realistic data sets, which meet certain reasonable and feasible minimal criteria.
- It should enable Member States to establish equal withdrawal periods if depletion data are identical.
- Its use and application should be transparent
- The information necessary to apply the method should be made generally available.

The harmonised method for the determination of withdrawal periods for milk is the Time To Safe Concentration (TTSC) method. With the TTSC method (fully described in 2.2) tolerance limits on the number of milkings per animal - necessary for the residue concentration in the milk of most animals to reach the safe concentration (most often the Maximum Residue Limit or MRL) - are calculated. The described method is a modified version of a previously published method [2]. The harmonised method assumes a log-normal distribution (of individual times to safe concentration), it corrects by monotonic regression for increasing concentrations found during the depletion phase, and in a second monotonic regression step it smoothes the relation between MRL (or other reference value)[1] and resulting withdrawal period. In accordance with the position already taken with respect to the calculation of withdrawal periods for meat [21], it is recommended to calculate the withdrawal period as the 95/95 tolerance limit, i.e. the upper 95% confidence limit of the 95th percentile of the population.

In a preliminary comparative study the method was found to be applicable in the largest number of realistic cases and resulted in withdrawal periods comparable to (or slightly longer than) those resulting from other tested methods (i.e. in cases where those methods were applicable). As the TTSC method is also applicable if many concentrations are below the limit of quantification, if depletion is non-linear and if variability is heterogeneous, it may be expected that it will be applicable for most data sets. Available results showed that the assumption of log-normality and inclusion of monotonic regression in case of increasing concentrations during depletion gave satisfactory results with most of the tested data sets.

The harmonised method laid down in this guideline is applicable to new products. It is recognised that some data sets may not lend themselves to statistical analysis and, in accordance with the position already taken with respect to the calculation of withdrawal periods for meat [21], in those cases a

[1] In the absence of a numerical MRL, another reference value may be used as the relevant limit for residue concentrations. This is applicable whenever "MRL" is mentioned further below.

statistical approach other than the harmonised method may be acceptable, but only on the condition that the applicant provides data which adequately show that the proposed alternative is more appropriate than the harmonised method. On these occasions it can be considered appropriate to extend the calculated withdrawal period with an additional safety factor.

2. Harmonised method for the determination of withdrawal periods for milk in the European Union

2.1. Definitions and basic principles

2.1.1. Definition of "withdrawal period for milk"

The definition given in Regulation (EU) 2019/6 [3] shall apply: " 'withdrawal period' means the minimum period between the last administration of a veterinary medicinal product to an animal and the production of foodstuffs from that animal which under normal conditions of use is necessary to ensure that such foodstuffs do not contain residues in quantities harmful to public health ". It should be noted, that the relevant value for the determination of withdrawal periods will be most often the MRL. However, for substances without a MRL the relevant safe concentration might be based on an ADI or other scientifically justified health based guidance value.

For example, a milk withdrawal period of 108 hours means that all the milk up to and including the last milking before 108 hours after treatment must be discarded. Depending on the time of treatment in a 12-hours milking cycle the last milk to be discarded may be from the milking at any time point at or after 96 hours after treatment but earlier than 108 hours after treatment. In this example milk from the first milking at or after 108 hours is considered safe. Similarly, a milk withdrawal period of 12 hours means that all milkings within a 12 hour period from the last treatment must be discarded and only milk taken at or after 12 hours is considered safe.

2.1.2. Milking interval in the experiment, and unit in which the withdrawal period for milk should be expressed

The withdrawal period for milk is initially calculated in milkings and rounded up to the first higher full number of milkings. Since the predominant milking scheme is twice a day, experiments for the determination of withdrawal periods for milk should be carried out with animals milked twice a day. For reasons of consistency, between milking intervals of 12 hours are to be preferred. However, because a different milking frequency can be used in practice, the final unit of the milk withdrawal period should be real time. For this reason, the final withdrawal period is rounded up to multiples of 12 hours or whole days and expressed in hours or days, respectively. If there are indications that this procedure does not provide appropriate withdrawal periods for animals milked according to other milking schemes, data from residue experiments with animals milked according to such other schemes might be of interest.

2.1.3. Sampling protocol

Withdrawal periods in the European Union, for all milk producing species, are established for individual animals and not for tank milk because milk from individual or few animals is used for consumption and for small-scale production of dairy products on farm level. Furthermore, too many assumptions are needed to calculate withdrawal periods for tank milk: the number of animals and the fraction of treated animals is variable and therefore assumptions of fixed numbers of animals introduce risks of underestimating withdrawal periods.

In accordance with VICH GL 48 [5], four-quarter composite samples should be collected from individual cows at each time point. For multiple doses products, samples should be taken after the last treatment, for products that may qualify for a 0-day milk discard time, samples should also be collected during treatment.

2.1.4. Tolerance vs. prediction limits

A tolerance limit gives 100q % confidence that at least 100p % of the individuals in a population is below that limit. At the prediction limit we expect that 100p % of the individuals in a population is below that limit. Consequently 95 % tolerance limits give more protection against incorrect results than 95 % prediction limits. It has been claimed that 99 % prediction limits would give similar results as 95 % tolerance limits [6], but this result has no general validity, and Chester et al. [2] noted that with the TTSC approach the 99 % prediction limit consistently did not maintain its designed characteristics. It has also been shown that prediction limits behave very strangely with severe extrapolation due to the skewness of the underlying coverage distribution [7]. Based on these findings it is concluded that tolerance limits are preferable to prediction limits.

2.1.5. 95 % vs 99 % tolerance limits

The EMA/CVMP approach [21] for tissue has adopted 95 % tolerance limits (with 95 % confidence level). The choice between 95 % and 99 % tolerance limits should first of all be made by the responsible authorities based on risk management decisions. Nevertheless, the following statistical truth should be borne in mind: the higher the chosen percentile, the more statistical units (animals) will be needed in experiments to establish a withdrawal period with sufficient accuracy. In general, samples from a population allow better inference for statistics concerning the central part of the distribution (e.g. median, quartiles, 95 % percentiles) than for statistics in the tails of the distribution (e.g. 99 % percentiles).

2.1.6. Parametric vs. non-parametric tolerance limits

Ideally an approach for establishing withdrawal periods should make as few as possible statistical assumptions. In the approaches described in Annex I of this guideline the form of the statistical distribution is assumed to be known: log-normal for concentrations in the SCLR (Safe Concentration-Linear Regression) and SCPM (Safe Concentration Per Milking) approaches, and log-normal, normal or Weibull in the respective variations of the TTSC approach. Especially in the TTSC case there is no underlying theory which predicts the type of distribution: the choice made is empirical (over many data sets; data sets are too small to allow a separate choice for each data set). This prompts the question whether it would not be preferable to apply non-parametric (also called distribution-free) methods. Of course, not to use a distributional assumption when actually it is true, will lead to loss of power, and therefore longer withdrawal periods.

Non-parametric tolerance limits are based on order statistics, which means that the tolerance limit is just one of the original observations in the sample (e.g. the highest, or the next-highest observation). Non-parametric tolerance limits are not exact, because a choice has to be made from a finite set of values. Conservative non-parametric 100p % tolerance limits provide a confidence level of at least 100q %. This can only be achieved for a certain minimal sample size, which can be calculated as $n \geq \ln(1-q) / \ln(p)$ (see e.g. [9], p. 93). Therefore, with confidence level q=0.95, we will need at least 59 observations (animals) for a 95 % non-parametric tolerance limit, and no less than 299 observations (animals) for a 99 % non-parametric tolerance limit. It is clear that the number of animals that can be included in depletion experiments in practice will be too small to use non-parametric methods, and therefore one has to rely on the distributional assumptions of parametric methods.

2.1.7. Selection of animals in experiments

From a statistical perspective, a sample size n= 20 is the very minimum to allow empirical estimates of a 95 % percentile. A smaller sample size (i.e. number of animals) amounts to the use of extrapolation in the coverage. A sample size larger than 20 is advisable to give the desired confidence level (95 %) at least some empirical basis (n=59 would be necessary to allow a completely non-parametric approach, see 2.1.6).

With respect to stratification, it has been suggested to include both high yielding cattle at an early stage of lactation, and low yielding cattle at a late stage of lactation in a residue depletion study. This should guarantee that at least some of the between animal variability is included in the study. However, this is only a partial solution to the problem of how to take inter animal variability into account since, for instance, differences in races or food regimes may be important as stratifying factors, too. Therefore, it seems best not to prescribe stratification, but only to require a representative sample from the relevant population of animals. Selection of animals should be made with attention for at least those factors, which are known to be important, such as milk yield. In a representative sample the inter animal variability will be an honest estimate of the inter animal variability in the population. The inter animal variability in the study now is artificial; therefore the 95 % percentile, which is central in the statistical approach, now refers to an artificial population of animals. In principle, this can be corrected, if the true proportion of high/low yielding animals in the complete population of cows would be known.

Therefore, a sufficient number of animals (minimum number: n = 20) should be sampled. It is important that also a representative sample of animals should be taken from the relevant target population (i.e. the distribution of milk yields should be realistic, the animals should be kept under normal zootechnical conditions).

The principles mentioned in VICH GL 48 should be followed.

2.1.8. Time of last administration

A withdrawal period is actually a discrete variable. The milk from two subsequent milkings may be unsafe and safe, respectively, and it is not sensible to define safety at intermediate time points. It is recommended that, in depletion studies, the last administration of the compound shall be 12 hours (one milking period) before milking 1. If this condition is not met, the data from milking 1 should be discarded, because there is no suitable model or data for within milking cycle kinetics. In general, milk collected in a 12 hours milking scheme, but less than 12 hours after treatment will be a mixture from the periods before and after treatment, and may therefore have a lower concentration than milk collected 12 hours after treatment. Consequently, the lowest possible withdrawal period for general use is the time of the first milking at or after one full milking period.

2.1.9. Shortest possible withdrawal period

In some cases all milk residue concentrations may be below or at the MRL[1] from the first milking after treatment on. In this case the harmonised TTSC method cannot be used (because there is no variability in times to safe concentration). If a sufficiently large proportion of the concentrations is between the LOQ and MRL[1]the data might still be processed statistically, e.g. by calculation of the tolerance limit for the first milking (see annex 1: SCPM method). If a withdrawal period of 1 milking results from an experiment with an interval of 12 hours between the treatment and the first milking, the withdrawal period should thus be 12 hours. If most or all residue concentrations are below the LOQ, no meaningful calculation of a 95/95 tolerance limit is possible (unless n≥59, see 2.1.6), and, provided that the LOQ is sufficiently below or equal to the MRL[1], it is acceptable to establish a withdrawal period of 12 hours.

Note that a withdrawal period of 12 hours means that no milk at all has to be discarded if a single treatment is given just after a milking and 12 hours before the next milking. However, in a setting where usual milking is more frequent (*e.g.* 3 times per day, or at the animal's choice by a milk robot), milking during the first 12 hours should be avoided or the milk discarded.

The only way to obtain withdrawal periods shorter than the standard milking interval (12 hours) is to conduct residue experiments with a shorter interval between 0th milking/last treatment and first milking.

2.1.10. Withdrawal periods for intramammary products

In experiments for the establishment of withdrawal periods for products intended for intramammary treatment at drying off, all quarters should be treated, i.e. normally four quarters in bovine. Although, in practice, it is unlikely that all quarters will be treated with a product for intramammary treatment during lactation at the same time, this should however be done in a residue study to represent a worst case situation.

For products intended for intramammary treatment at drying off, the principles laid down in this note for guidance are applicable. Statistical methods should be applied on the residue concentrations found in the milk after birth. However, the variation in the length of the dry period may cause a large variability between animals. Therefore, the experiment should be designed in such a way that a sufficient number of animals give birth in a limited time interval. For instance, if an applicant wishes to establish a withdrawal period for cows calving 30 days after treatment, data are needed from at least 20 cows calving between e.g. 20 and 30 days after treatment. However, if the applicant wishes to establish a withdrawal period for animals calving after the more common dry period of 60 days, data are needed from at least 20 cows calving before 60 days after administration, e.g. between 40 and 60 days after treatment.

The applicant should try to keep the differences between dry periods between animals within the experiment as small as possible, in order to keep the variability as small as possible. This can, for instance, be done by drying-off and infusing animals with the formulation when they are at the targeted number of days from the expected date of giving birth, provided that appropriate consideration is given to animal welfare issues.

Since the differences in residue concentrations between animals which differ little in length of dry period are often in the same order of magnitude as the relatively large differences found already between animals with equal dry periods, differences between animals with slightly differing length of dry period may not add too much to the large inter-individual differences which are unavoidable anyway. A number of data sets are currently being evaluated in order to reconsider a new statistical approach when dealing with animals that have different lengths of dry period.

2.2. Full description of the Time-To-Safe-Concentration (TTSC) method

The Time-To-Safe-Concentration (TTSC) method calculates a tolerance limit on the number of milkings per animal. This tolerance limit is the time necessary for the residue concentration in the milk of most animals to reach the safe concentration (most often the MRL[1]). The method assumes a log-normal distribution (of individual times to safe concentration), it corrects by monotonic regression for increasing concentrations found during the depletion phase, and in a second monotonic regression step it smoothes the relation between MRL[1] and resulting withdrawal period. The withdrawal period is calculated as the 95/95 tolerance limit, i.e. the upper 95% confidence limit of the 95th percentile of the population of individual times to safe concentration. This section gives a full stepwise description of the calculations of the method.

For the purpose of statistical analysis, true milk withdrawal period is interpreted here as the time period from the time of last administration to the time point at and where after 95 % of the population of

interest has residue levels not higher than the MRL[1] The population of interest is taken to be all individual animals, which could potentially be treated with the medicinal product.

In general the true milk withdrawal period is unknown. It has therefore to be estimated from empirical data from representative treated animals. The estimated milk withdrawal period (*WP*) is chosen such that there is at least 95 % confidence (under the model assumptions) that the true withdrawal period is not higher than the estimated withdrawal period. Such estimators are known as tolerance limits. A theoretical comparison with other type of estimators (prediction limits, expected percentiles) has been made for the tissue case, and is available on request [7].

The described approach is a modification and extension of the method described in [2]. In its simplest form one takes, for each animal, the first time point where the measured concentration is at or below the MRL[1], and stays below the MRL[1] at later times. This time point is labelled the time-to-safe-concentration (*TTSC*). With replicate measurements on each milk sample geometric mean concentrations are used in this step. The resulting set of *TTSC* points is then used to calculate a tolerance limit. A critical issue with this method is the choice of distributional assumption for the *TTSC* points. Chester et al. [2] assume a normal distribution. In the harmonised method described here *TTSC* points are assumed to arise from a log-normal distribution, which has been shown to be a better fit for a number of real data sets from industrial practice (see Annex I.5.3.1).

The method contains a pre-processing step in which monotonic regression is applied to the log concentration data versus time. This least squares procedure replaces the data values of each animal by fitted values under the only condition that these fitted values should be non-increasing with time. This step incorporates the prior knowledge that during the depletion period residue concentrations are decreasing. This pre-processing step removes the influence of variability due to incidental increases in measured values during the depletion phase.

The method also contains a post-processing step in which monotonic regression is applied to preliminary estimates of withdrawal period as a function of the MRL[1] considered as a variable. Using one and the same data set, it is reasonable to expect a longer (or equal) withdrawal period when the MRL[1] would be lowered (and vice versa). However, due to statistical fluctuations, this is not guaranteed with the TTSC method as described so far. Therefore, an additional monotonic regression is applied to obtain such a relation.

The TTSC method consists of the steps described below. An example, using the calculations described in this chapter, can be found in annex II. A computer program, assisting in the calculations laid down in this guideline, is available at EMA homepage[2].

Step 1. Notation and censoring indicator

Data should be available for a representative sample of n animals, with $n \geq 20$. Suppose there are data from J milkings per animal, and K replicated measurements on each milk sample (in practice, K will often equal 1). Let c_{ijk} denote the k'th measured concentration in a milk sample from animal i and milking j.

Some of the concentrations may be given as '<*LIM*', where *LIM* is just the lower limit for reporting a numerical value. In practice, the limit of quantification (*LOQ*) is often used. Also in this paper 'the *LOQ*' is used to denote the reporting limit, but we assume no statistical properties of the *LOQ*. *MRL*[1] denotes the maximum residue limit for the marker residue.

In order to distinguish measured real values from results reported as 'below LOQ' a censoring indicator variable z is constructed as follows:

$z_{ijk} = 0$ if $c_{ijk} \geq LOQ$

[2] https://www.ema.europa.eu/en/determination-withdrawal-periods-milk

$z_{ijk} = 1$ if $c_{ijk} < LOQ$

Measured concentrations $c_{ijk} < LOQ$ are temporarily replaced by LOQ (this is for the data preprocessing in steps 2 and 3 only). Note that values equal to LOQ may have $z_{ijk}=0$ or $z_{ijk}=1$ at this point.

Step 2. Logarithmic transformation of concentrations and means of replicate measurements

Natural logarithms of the concentrations are taken:

$y_{ijk} = ln(c_{ijk})$

If there are $K>1$ replicate measurements on any milk sample, y is averaged over the last index:

$y_{ij} = \Sigma^{K}_{k=1} y_{ijk} / K$

The geometric mean concentration of milk sample ij is

$c_{ij} = e^{y_{ij}}$

The censoring indicator for sample ij is

$z_{ij} = min_k (z_{ijk})$

i.e. the measurement on milk sample ij is considered to be below the LOQ only if *all* measurements on that sample are below the LOQ. If any measurement on this sample is uncensored, the conservative (high) concentration estimate c_{ij} is used in the following as a real measurement.

Step 3. Monotonic regression concentration vs. time

For each animal i separately, a set of non-increasing log-concentration values is obtained by monotonic regression. Monotonic regression does not change the data unless they are in the wrong order: during the depletion phase we expect concentrations decreasing with time, therefore only concentrations at time periods showing an increase with time will be changed. Basically, log-concentration values in the wrong order (a high value following a low value) are replaced with their average. When more than two values are out of order, new values can be found from an easy iterative algorithm. Start with weights $w_j=1$ for all time points j. Then, for any adjacent pair $(j,j+1)$ with increasing concentration values ($y_{ij}<y_{i,j+1}$), replace the pair by one value, the weighted average

$(w_j y_{ij}+w_k y_{i,j+1})/(w_j+w_{j+1})$

and set the new weight equal to w_j+w_{j+1}. Repeat this procedure until the resulting averages per animal do not show increases in concentration over time. The new averages then replace the values y_{ij} in the following steps. Average values are valid for each of the underlying time points. For more information see e.g. [10].

The censoring indicator z_{ij} is set to 0 if a value $y_{ij}=LOQ$ is replaced by a higher value in the monotonic regression. This will happen when censored observations are followed by values above the LOQ.

Step 4. Set values for graphical display of data below the limit of quantification

For samples with $z_{ij}=1$, the concentrations c_{ij} are set to $^{1}/_{2}$ of the LOQ, and the log-concentrations y_{ij} to $ln(^{1}/_{2} LOQ)$. This is done for graphical purposes only, so that censored observations are shown in plots as points below the LOQ line. This step has no influence on the estimation of the withdrawal period in steps 5-10.

Step 5. Calculate times to safe concentration

For each animal i identify the first time point t_j (in milkings) with $c_{ij} \leq MRL^1$ and $c_{ik} \leq MRL^1$ for all $k>j$. This time point is labelled as the time-to-safe-concentration for animal i, and is denoted $TTSC_i$.

Note: if the last concentration in the data set is still above MRL[1], $TTSC_i$ cannot be calculated. In that case the TTSC method is not applicable to the data set.

Step 6. Change to logarithmic scale

Calculate for each animal i the natural logarithm of the time to safe concentration:

$x_i = ln(TTSC_i)$

Step 7. Tolerance limit calculation

The calculation is based on the assumption of a normal distribution for x. First calculate the mean and the standard deviation of the n values x_i:

$m = (1/n) \Sigma^{n}_{i=1} x_i$

$s_x = \{1/(n-1)\} \Sigma^{n}_{i=1} (x_i - m)^2$

Values x_i are from a discrete set (times of milking), whereas the tolerance calculations assume a continuous variable. To avoid zero standard deviations (when all x_i are equal) s_x is not allowed to become lower than a minimal value which represents the rounding error. The minimal standard deviation of x is approximately equal to the minimal coefficient of variation of $TTSC$ values: it is set to $(1/\sqrt{12}) / e^m$.

The tolerance limit is calculated as

$x_{tol} = m + k\, s_x$

where the tolerance limit factor k for a 95/95 tolerance limit and a specific value of n can be found in Table 1[3].

Table 1. One-sided tolerance limit factors k for standard 95/95 tolerance limit calculations (p=0.95; 1-α=0.95). Data from [13], and calculated.

n	***k***	***n***	***k***
2	26.260	21	2.371
3	7.656	22	2.350
4	5.144	23	2.329
5	4.210	24	2.309
6	3.711	25	2.292
7	3.401	26	2.275
8	3.188	27	2.260
9	3.032	28	2.246
10	2.911	29	2.232
11	2.815	30	2.220
12	2.736		
13	2.670	40	2.126
14	2.614	50	2.065
15	2.566	60	2.022
16	2.523	70	1.990
17	2.486	80	1.965
18	2.453	90	1.944
19	2.423	100	1.927
20	**2.396**	∞	1.645

[3] More generally k can be calculated as $k = t'_{n-1}(1-\alpha;\delta) / \sqrt{n}$, where $t'_{n-1}(1-\alpha;\delta)$ is the $100(1-\alpha)$ percentile of the non-central t distribution with $n-1$ degrees of freedom and non-centrality parameter $\delta = z_p \sqrt{n}$. In the latter expression z_p is the $100p^{th}$ percentile in the standard normal distribution (e.g. $z_{0.95}$=1.645). A standard reference for the non-central t distribution is [11]. Standard algorithms are available for calculating cumulative probabilities in the non-central t distribution, for example algorithm AS5 in the Applied Statistics collection available in STATLIB on the Internet, and described in [12]. Percentiles of the non-central t distribution can be calculated by applying a simple search algorithm to the algorithm of AS5, e.g. consisting of a fixed-step search plus a bisection search. Approximative formulas to calculate tolerance limits exist, see e.g. Guideline on determination of withdrawal periods for edible tissues (EMA/CVMP/SWP/735325/2012) [21]

Step 8. Un-rounded withdrawal period

The tolerance limit is converted to an un-rounded withdrawal period (*UWP*) at the ordinary time scale (in milkings):

$UWP = e^{Xtol}$

Step 9. Monotonic regression on UWP vs. MRL^1 relation

Calculations of step 5-8 are repeated for a range of MRL^1 values. In principle, all MRL^1 values in the range of the data are investigated. In practice, it is sufficient to calculate *UWP* for the real MRL^1, and for MRL^1 values equal to all concentration values in the data set (with the exception of MRL^1 values that are too low to allow all animals to arrive at a safe concentration at the last time point).

A monotonic regression is applied to the resulting set of (MRL^1,*UWP*) pairs. If the *UWP* values are arranged according to increasing MRL^1 values using an index *j*, then the fitted values from monotonic regression ($MUWP_j$) are calculated by iterated weighted averaging of pairs where *UWP* increases with MRL^1. Start with $MUWP_j=UWP_j$ and weights $w_j=1$, for all *j*. Then, for any adjacent pair (*j,j+1*) with $MUWP_j<MUWP_{j+1}$, replace the pair by one value, the weighted average

$(w_jMUWP_j+w_{j+1}MUWP_{j+1})/(w_j+w_{j+1})$

and set the new weight equal to w_j+w_{j+1}. Repeat this procedure until the resulting averages satisfy the imposed negative relation between MRL^1 and *MUWP*. The resulting weighted averages are valid for all underlying MRL^1 values.

Step 10. Calculate withdrawal period

Finally, the *MUWP* value corresponding to the real MRL^1 is rounded upward to an integer number of milkings. This should be converted to the withdrawal period in real time.

$WP = (\Delta t)\ int\ (MUWP+1)$

where Δt is the interval in hours between milkings in the experiments (e.g. 12 hours).

See Annex II for an example of the application of this procedure.

Annex I: Comparison of several approaches for establishing milk withdrawal periods

I.1 Introduction

The point of departure was to examine the linear regression method developed by the US Food and Drug Administration (FDA) for its suitability to be used in the EU. However, as this method had certain characteristics which made it difficult to be applied in the European situation (in the European situation e.g. withdrawal periods are established for individual animals, the data sets are often not suited for linear regression, and usually no data are available on intra-individual variability), and because developments in statistical science should be taken into account, a modification of the FDA method and alternatives for this method were investigated. The FEDESA ad hoc Working Party on Harmonisation of Withdrawal Periods provided useful additional information with regard to suitable alternatives and data sets to test the different methods.

The methods were applied to eleven real data sets (not shown here due to confidentiality), and the results were evaluated to select the most appropriate method. The results were compared with each other and with results achieved by applying two so-called "simple" methods as used previously in different Member States (i.e., first milking with all concentrations below MRL[1], and the first milking with all concentrations below MRL[1] plus a "safety span". A detailed description of these methods lies beyond the scope of this Note for Guidance).

I.2 SCLR method: Safe concentrations, based on linear regression, and allowing for measurements below the limit of quantification (LOQ)

This is a modification of the FDA method [8,14]. In principle the method fits a regression line to the log concentration data of each cow. The fitted lines are used to estimate the distribution of log concentrations at each time point. Estimates are made of between-animal variance and of measurement error variability, and these are then used to calculate a log concentration tolerance limit at each time point. The estimated withdrawal period is the first time point where the tolerance limit is at or below the MRL[1]. Referral is made to the FDA guideline for computational details.

The method used here deviates from the FDA method on the following points:

- Calculation of 95% tolerance limits (FDA chooses 99% tolerance limits)
- All animals used in the withdrawal time calculation are assumed to have been treated (FDA assumes that if the product is used to treat mastitis, no more than one-third of the milk comes from treated animals).
- No requirement for the number of animals (FDA requires at least 20 animals).
- No requirement for the number of replicate analyses per milk samples (FDA requires triplicate assays). When no replicate measurements were available an external estimate of assay variance was used.
- Regression lines are calculated by maximising the combined normal likelihood of values at or above the LOQ and measurements reported as 'below the LOQ' (FDA excludes the latter measurements as well as other data from time points with less than three remaining values). The likelihood to be optimised has the form

$$\Pi i \in A\ (2\pi\sigma^2)^{-½}\ \exp\{(y_i-\beta_0-\beta_1 t)^2/\sigma^2\}\ \Pi i \in B\ \Phi\{(y_{LOQ}-\beta_0-\beta_1 t)/\sigma\}$$

where y_i is the log concentration of animal/time combination i, y_{LOQ} is the natural logarithm of the LOQ, A is the set of values {i; $y_i \geq y_{LOQ}$} and B is the set of censored values {i; $y_i < y_{LOQ}$}. β_0 and β_1 are the regression line slope and intercept, and σ is the residual standard error. Φ denotes the cumulative standard normal distribution.

- No checks on log-linearity (FDA prescribes lack-of-fit F tests per animal to select points to be used for subsequent calculations).

I.3 SCPM method: Safe concentrations, based on data per milking, allowing for data below the limit of quantification

This approach is similar to the approach described in [15]. Essentially, a tolerance limit is calculated from the measured values at each time point separately. A normal distribution is assumed for the log concentrations. Allowance for data below the LOQ is made by using a maximum likelihood method.

In some cases a shorter withdrawal period can be obtained by a pre-processing step termed monotonic regression. This least squares procedure replaces the data values of each cow by fitted values under the only condition that these fitted values should be non-increasing with time.

The tolerance limit is calculated as

$$y_{tol} = \hat{\mu} + k\,\hat{\sigma}$$

$$k = t_{\nu,\delta,\alpha}/\sqrt{n}$$

$$\nu = n-1$$

$$\delta = z_p\,\sqrt{(n/m)}$$

where n is the number of cows observed and m is the number of cows contributing milk to a bulk tank, z_p is the $100p^{th}$ percentile point of the standard normal distribution (e.g. $z_{0.95} = 1.645$), and $t_{\nu,\delta,\alpha}$ is the $100(1-\alpha)^{th}$ percentile of the non-central t distribution with ν degrees of freedom and non-centrality parameter δ. $\hat{\mu}$ and $\hat{\sigma}$ are estimates of the mean and standard deviation of the normal distribution at this time point. For uncensored data these are just the usual mean and sample standard deviation calculated from the data. With some of the data below the LOQ the estimates $\hat{\mu}$ and $\hat{\sigma}$ are made by maximising the likelihood

$$\Pi i \in A\ (2\pi\sigma^2)^{-1/2}\ \exp\{(y_i-\mu)^2/\sigma^2\}\ \Pi i \in B\ \Phi\{(y_{LOQ}-\mu)/\sigma\}$$

where y_i is the log concentration of animal i, y_{LOQ} is the natural logarithm of the LOQ, A is the set of values {i; $y_i \geq y_{LOQ}$} and B is the set of censored values {i; $y_i < y_{LOQ}$}. μ and σ are the mean and the standard error. Φ denotes the cumulative standard normal distribution.

The withdrawal period is the first time point where $y_{tol} \leq y_{MRL}$, and where this condition is not violated at later time points in the data set.

Some complications involving the censored values arise a) with replicate measurements; b) when monotonic regression is used as a pre-processing step.

When replicate measurements (per milk sample) are available, a first step is to take the mean of the log concentration values. Also the monotonic regression procedure requires the calculation of (weighted) means of log concentration values across two or more time points whenever the data are increasing with time. How should censored values be treated when calculating these means? As a conservative approach, censored data are set equal to y_{LOQ}. Any value remaining equal to y_{LOQ} after these two pre-processing steps is reset to a censored observation (set B) in the ML method.

When at any time point all observations are censored, the theoretical maximum likelihood estimate of log concentration is -∞. However, in the program used for this study, the optimisation program left the estimate at its initial value. The standard deviation of the log concentration distribution was set, quite arbitrarily, to the smallest non-zero standard deviation in the original data set (which was the data set with censored values entered arbitrarily as LOQ).

I.4 TTSC method: Based on times-to-safe-concentration

This is the approach described as the harmonised approach in the main document of this Note for Guidance (chapter 2.2).

I.5 Discussion of results

I.5.1 Safe concentration from linear regression (SCLR) approach

I.5.1.1 Linearity

The main characteristic of the SCLR approach is the assumption of a linear relation between log concentration and time. If this assumption is sufficiently close to the truth then linear regression gives the most accurate estimates of log concentration at any time point or of the time to reach a safe concentration for each cow.

However, the assumption of linearity may fail for the following reasons:

1. There may be need for two (or more) pharmacokinetic compartments in an appropriate model, implying that concentration should be modelled as a sum of exponentials. Although such a model could be fitted in principle, in practice the data are often too scarce to allow a proper choice between one- or more-component models, or even to fit a more-component model.

   The practical alternative advocated by the FDA [8] is to use only points in the final linear phase of the depletion curves. Lack-of-fit F tests may be used to decide which points to exclude. For these tests an estimate of 'pure error' variance is needed, either from replicated assays [8] or as external information to be supplied by the applicant.

2. Binding of the substance to e.g. plasma proteins may be relatively higher at low concentrations. This may cause upward deviations from the final log concentration depletion line.

3. Circadian (or other) biorhythms may cause cyclic deviations from the values predicted by the linear depletion model.

Deleting points to achieve linearity may be sensible in case 1, but does not seem to provide a solution in cases 2 and 3.

Of course, in practice deviations from linearity may be small enough to ignore them.

I.5.1.2 Data below the limit of quantification

There are two problems with the SCLR approach when there are data below the LOQ (known in statistics as censored data):

1. Fitting the regression lines.

   Simple approaches are replacement of censored data with 0, $^{1}/_{2}$ LOQ, or LOQ, or deletion of time points with data <LOQ. The latter approach is advocated in the FDA method. Such methods have no

theoretical basis, and have been found to perform poorly in many studies (see e.g. [16] and references therein).

Maximum likelihood estimation (MLE) is a general statistical technique, which can also be used for estimating regression functions in the presence of censored data (see e.g. [17,18]). The application of MLE requires the optimisation of a non-linear function, which is easily performed by standard statistical programs. According to [16] MLE methods are commonly used in environmental disciplines such as air quality studies and geochemistry.

Users of MLE methods should be warned that the theory behind MLE is based on large samples. Therefore, data sets should not be too small: for small sample sizes (n=5, 10, 15) estimates may have large bias and poor precision [16]. The ML estimate of the residual variance (needed in the tolerance limit calculations) will be badly biased when the number of uncensored observations is small [17].

2. Estimating the inter animal variability

At each time point the data show animal variability and assay variability, where the former is often much larger than the latter. In the SCLR approach between animal variability will be estimated from the fitted values at that time point. When all animals have measurements above the LOQ this is a reasonable procedure. However, with many data below the LOQ, one may obtain a widely diverging bundle of regression lines on the log concentration scale. It then occurs that at a certain time point all fitted values are below the LOQ, but they are widely different, ranging over e.g. a factor 1000 for the ratio of concentrations. In the subsequent tolerance calculations the large inter animal variance will make it impossible to guarantee that 95 % of the population is below MRL[1]. Essentially, the SCLR approach requires that the linearity assumption is accepted for all unobserved values below the LOQ, and that no upper limit is put on the (partly or wholly unobserved) inter animal variability of the log concentrations.

I.5.2 Safe concentration per milking (SCPM) approach

The advantage of the SCPM approach over the SCLR approach is that no linearity needs to be assumed in cases where this seems questionable. Therefore, problems regarding the linearity lack-of-fit tests and any artificially enlarged inter animal variability, if many data are below the LOQ, are avoided. Another advantage is that the variance at each time point is allowed to be different.

The disadvantage is that information is not optimally used whenever linearity and homogeneity of variance are valid assumptions, especially if the number of animals is small. Moreover, extrapolation beyond the range of observed time points in the experiment is impossible.

An intermediate approach between linear regression and pure per-milking is the use of monotonic regression (also termed isotonic regression, see [10]). In this note the use of monotonic regression is considered as a variation of the PM approach. In monotonic regression, data points that contradict the prior assumption of concentration decreasing with time are replaced by weighted averages of data points. Technically, the data points y_{ij} of a cow i (averaged over replicated analyses if appropriate) are replaced by fitted values equal to $\min_{s \le j} \max_{t \ge j} Av(s,t)$, where Av(s,t) is the average value of $y_{is}, y_{i(s+1)}, ..., y_{it}$. In practice most values are left unaltered, with only those few that show an increasing instead of decreasing trend being replaced by averages. Nevertheless, the influence on the estimated withdrawal period may be large.

Handling data below the LOQ may be more difficult with the SCPM approach as compared to the SCLR approach. Technically the MLE procedure is almost the same, but the number of uncensored observations

is in most cases much smaller than with the SCLR approach. Consequently, the ML estimates of standard errors and tolerance limits may be very biased.

I.5.3 Time-to-safe-concentration (TTSC) approach

The TTSC approach has the same advantage as the SCPM approach: no assumption of linear depletion is needed. Moreover, there are in principle no problems with data below the LOQ: a data point below the LOQ just counts as a point below MRL[1]. Therefore, there is no need for the MLE approach with its uncertain behaviour in small samples.

A disadvantage of the TTSC approach is that we need a distributional assumption for the TTSC values. Whereas standard theory supports the assumption of log-normality for concentrations, no such theory exists for the times necessary to reach a safe concentration. In practice, therefore, we need an empirical choice for a distribution, which fits the data well enough. A further problem is that the TTSC values per animal are discrete (milkings), whereas proposed distributions (normal, log-normal, Weibull) are defined for continuous variables. Treating a discrete variable as if it were a continuous variable may lead to withdrawal period estimates, which are too short.

A comparison has been made between several distributional assumptions. 95 % tolerance limits are statements about the 95 % percentile of the population. Therefore, large differences are expected when distributions with the same mean and variance, but different skewness are fitted to some data. Based on the linear regression model it is expected that TTSC values will show a right-skewed distribution, and this is indeed almost always true. Normal-theory tolerance limits may therefore be expected to be too low.

Chester et al. (2) propose to use tolerance limit calculations based on a normal distribution anyway. They investigated the performance in the case of normal and non-normal distributions using Monte Carlo simulations from continuous g-and-h distributions [19]. They simulated TTSC values with 95 % percentiles of 5.50, 5.75, 6.00 and 6.25 (milkings), and concluded that the 95 % tolerance limit provided at least 95 % confidence for all but the most skewed distributions (g=0.6, h=0.3), when the target 95 % percentile was 6.25. It may be concluded that in these simulations, where withdrawal period was considered as a continuous variable, the downward bias from using normal-theory calculations was compensated in most cases by the upward bias from using TTSC values rounded upward to whole milkings. It is unclear whether this would also be the case with simulations from a discrete distribution, or in cases where the withdrawal period is a larger multiple of 12 hours milking periods (so that rounding effects are relatively small).

Figure:

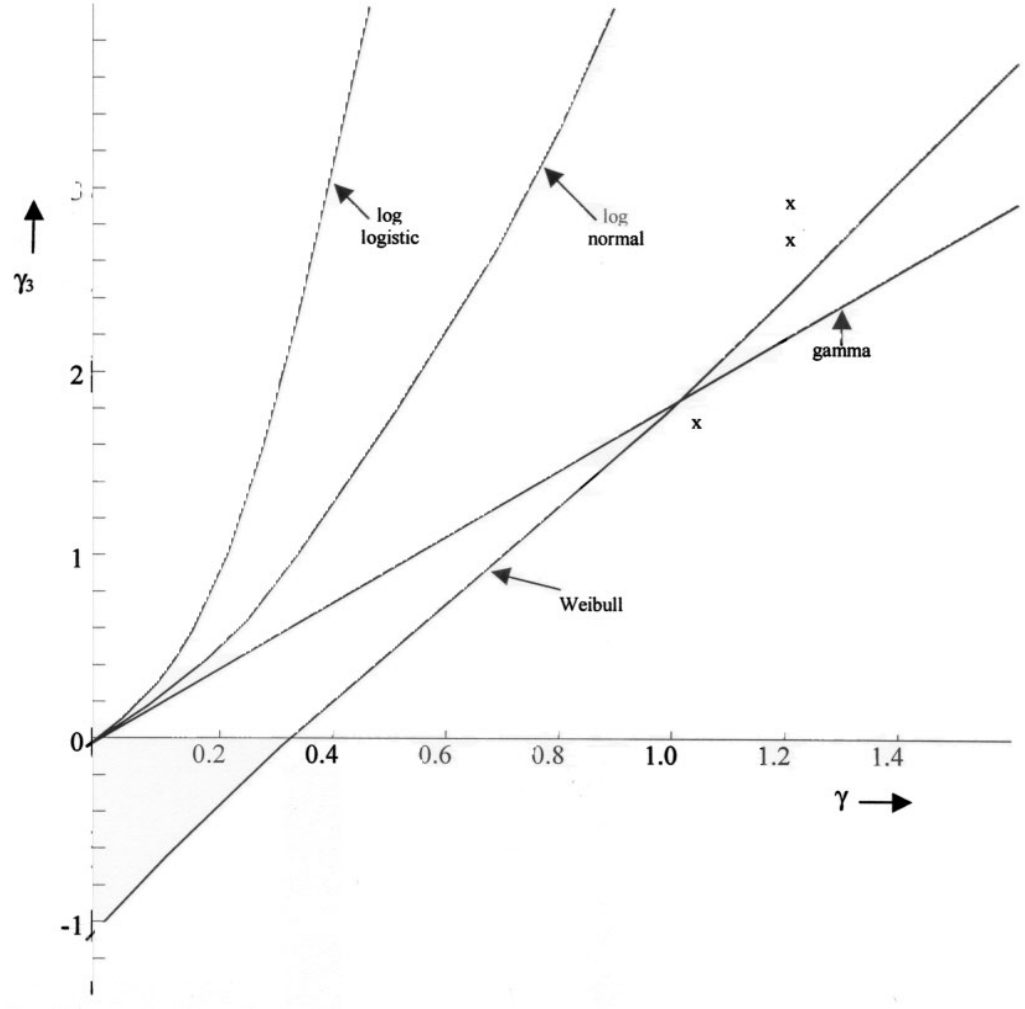


Figure 1. Standardised third moment, γ_3, vs. coefficient of variation, γ for log logistic, log-normal, gamma and Weibull distributions. Exponential distribution is at point (1,2). Reproduced from [20].

For several standard distributions (log-normal, log-logistic, gamma, Weibull) the skewness is a function of the coefficient of variation only (see Figure 1 reproduced from [20]). In general the skewness, expressed as the standardised third moment $\gamma_3=\mu_3/s^3$, increases with the coefficient of variation $\gamma=\sigma/\mu$. A high value of γ_3 implies a relatively long right tail. At a certain coefficient of variation the tail of the log-logistic distribution is longer than that of a log-normal distribution, which in turn is longer than the tail of a gamma distribution. It is noteworthy that the Weibull distribution is actually skewed to the left instead of to the right for coefficients of variation less than approximately 30 %. A practical advantage of the log-normal distribution is that calculations remain just as simple as in the case of a normal distribution: one simply works with ln(TTSC) instead of TTSC values.

In an empirical evaluation on real data sets the distributional assumption made a difference for the established withdrawal period in most of the data sets. The pattern always was: increasing withdrawal periods in the order Weibull, normal, log-normal. This is in accordance with theory. Figure 2 shows the position of 11 real data sets in the plot of standardised third moment against coefficient of variation, together with points for data sets of size n=25 simulated from Weibull or log-normal distributions. The normal distribution (simulations not shown) gives points around a horizontal line with standardised third moment 0 (symmetric distribution).

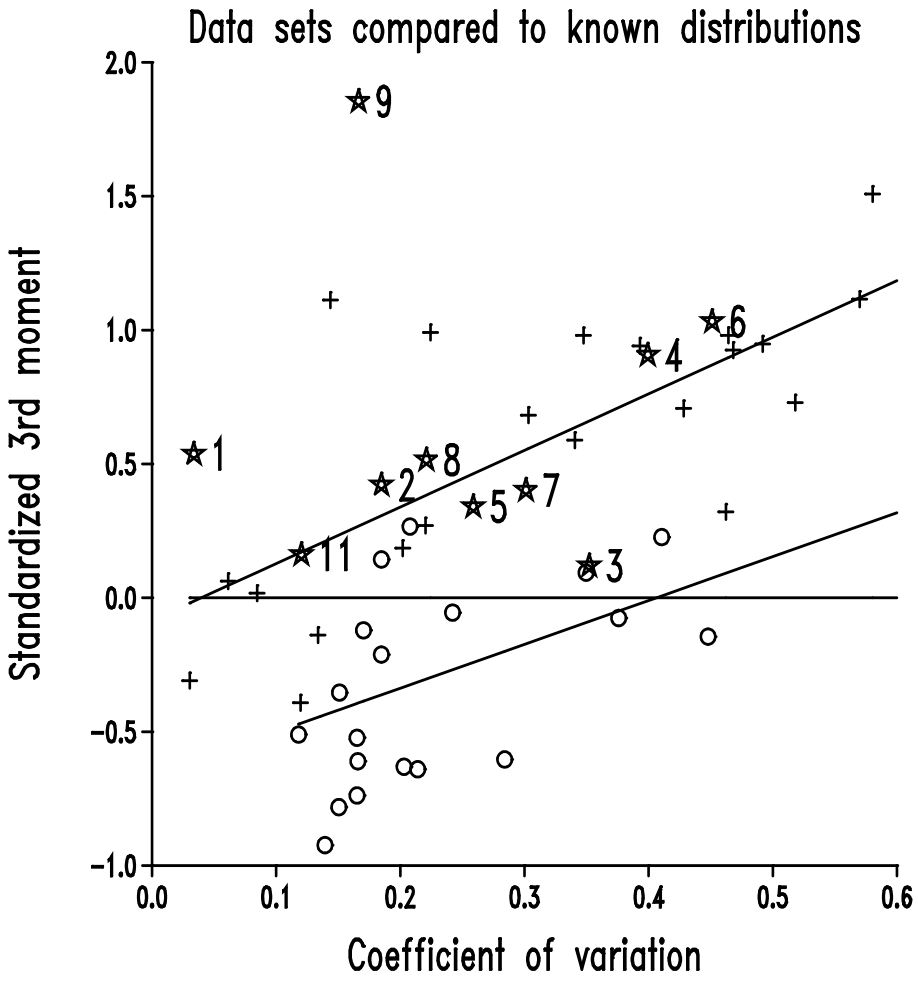


Figure 2. Skewness against variability for data sets (stars) and simulations from log-normal (crosses and upper regression line) and Weibull (circles and lower regression line) distributions.

It can be seen that most data sets are more like simulations from a log-normal distribution than from a Weibull distribution. The most deviating data sets are 1, 3 and 9. Data set 1 is a set with just n=6 cows. Data sets 9 and 3 are cases with a short withdrawal period and therefore a large distortion from rounding to integer values.

Annex II: Example of the TTSC approach

Figure 3 and Table 2 show data for n = 25 animals and 8 milkings (in a 12-hours milking scheme, with the last treatment 12 hours before the first milking). These artificial data were generated by simulation based on a real data set. In the simulation normal error was added to linear regression estimates of the log concentration values in a real data set.

Monotonic regression pre-processing was applied to the example data (see Table 3). Consider for example the data and graph for cow 1, where the log concentrations of milkings 2 and 3 were averaged (ln(0.402) is the average of ln(0.341) and ln(0.473)), as well as the log concentrations of milkings 4, 5 and 6.

The MRL in this example is 0.1. At the last milking, all cows have a (pre-processed) value not higher than MRL, therefore the TTSC method can be applied. TTSC values per cow are now calculated, and summarised in Table 4.

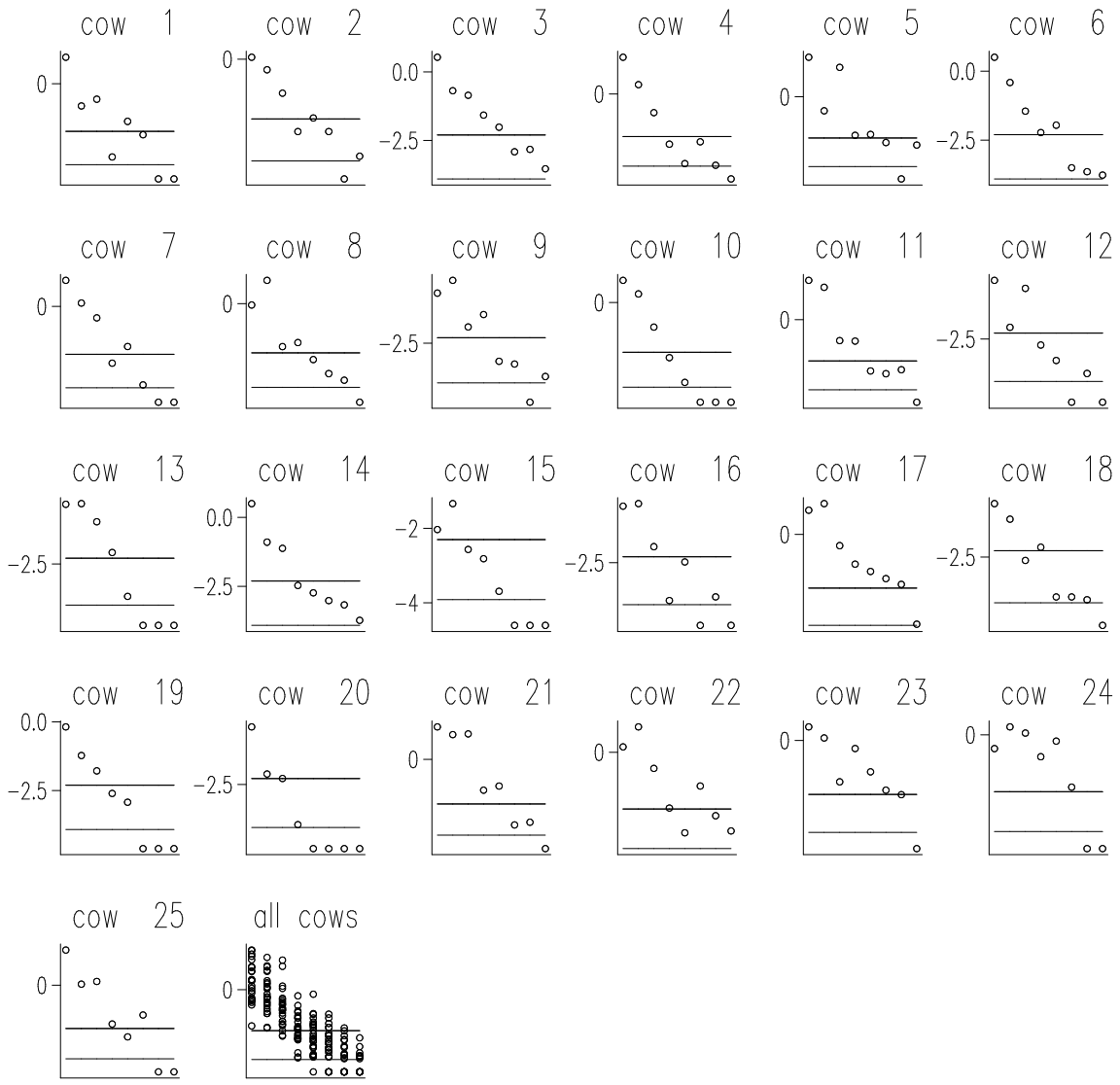
The mean m and standard deviation s of the ln(TTSC) values are 1.556 and 0.2779, respectively. With a tolerance limit factor k = 2.292 (see Table 1) this leads to a 95/95 tolerance limit of $1.556+2.292*0.2779 = 2.193$ on the logarithmic scale, corresponding to $e^{2.193} = 8.962$ on the milking interval scale.

If the TTSC method would be applied without the last monotonic regression step, the tolerance limit 8.962 would be rounded upward to obtain a withdrawal period of 9 milking intervals, or $9\times12=108$ hours.

In the last monotonic regression step of the TTSC method the calculations above are repeated with MRL values chosen over the range of the data. In practice MRL values are chosen equal to all concentration values where at least one of the TTSC values changes. The resulting set of (MRL,UWP)-pairs is shown in Table 5 and Figure 4, together with fitted UWP values after applying monotonic regression. At the true MRL of 0.1 the fitted UWP is slightly lower, 8.886 instead of 8.962. In this example the resulting withdrawal period of 9 milking intervals is unaltered.

The stabilising effect of the last monotonic regression step can be illustrated by noting the effect on withdrawal period of changes in MRL. For a real MRL of 0.15 the withdrawal period would remain 9 (instead of 8) milking intervals, for a real MRL of 0.20 it would still be 9 (instead of 10) milking intervals.

Thus, the withdrawal period is fixed at $9\times12=108$ hours. Milk from milkings at or after 108 hours after treatment is considered safe. With a regular 12-hours milking scheme the first safe milk is milk from the 9^{th} milking if treatment in practice is given 12 hours before the first milking, and milk from the 10^{th} milking if treatment is given less than 12 hours before the first milking.



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635 Figure 3. Example data (simulated). Log concentration vs. time (milking). Horizontal lines mark
636 MRL=0.1 and LOQ=0.02. Data <LOQ are shown at LOQ.

Table 2. Example data set. 25 animal (rows) and 8 time points (columns). MRL=0.1 and LOQ=0.02. Data below LOQ were entered as 0.01.

3.609	0.341	0.473	0.029	0.162	0.085	0.010	0.010
1.077	0.665	0.270	0.062	0.104	0.062	0.010	0.024
1.714	0.503	0.426	0.206	0.133	0.054	0.059	0.029
7.342	1.656	0.362	0.066	0.023	0.075	0.021	0.010
9.201	0.454	5.220	0.116	0.122	0.077	0.010	0.067
1.662	0.663	0.234	0.108	0.141	0.030	0.026	0.023
3.482	1.176	0.576	0.065	0.145	0.023	0.010	0.010
0.942	2.961	0.134	0.162	0.073	0.038	0.028	0.010
0.492	0.774	0.147	0.229	0.043	0.039	0.010	0.025
2.766	1.483	0.320	0.078	0.025	0.010	0.010	0.010
8.963	6.073	0.311	0.303	0.057	0.049	0.061	0.010
0.577	0.121	0.442	0.067	0.040	0.010	0.026	0.010
0.635	0.649	0.348	0.122	0.027	0.010	0.010	0.010
1.646	0.408	0.327	0.085	0.065	0.049	0.042	0.024

Table 2. Example data set. 25 animal (rows) and 8 time points (columns). MRL=0.1 and LOQ=0.02. Data below LOQ were entered as 0.01.

0.131	0.263	0.077	0.060	0.025	0.010	0.010	0.010
0.545	0.593	0.140	0.023	0.084	0.010	0.026	0.010
2.848	3.779	0.619	0.280	0.204	0.150	0.117	0.021
0.425	0.263	0.074	0.111	0.024	0.024	0.022	0.010
0.832	0.294	0.168	0.074	0.054	0.010	0.010	0.010
0.547	0.116	0.100	0.022	0.010	0.010	0.010	0.010
5.333	3.578	3.717	0.203	0.251	0.034	0.039	0.010
1.242	2.800	0.518	0.104	0.038	0.253	0.076	0.041
1.780	1.110	0.171	0.708	0.262	0.120	0.099	0.010
0.573	1.380	1.075	0.412	0.776	0.120	0.010	0.010
6.483	1.060	1.225	0.127	0.064	0.205	0.010	0.010

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Table 3. Example data from Table 2 pre-processed by monotonic regression against time.

3.609	0.402	0.402	0.074	0.074	0.074	0.020	0.020
1.077	0.665	0.270	0.080	0.080	0.062	0.022	0.022
1.714	0.503	0.426	0.206	0.133	0.056	0.056	0.029
7.342	1.656	0.362	0.066	0.042	0.042	0.021	0.020
9.201	1.539	1.539	0.119	0.119	0.077	0.037	0.037
1.662	0.663	0.234	0.123	0.123	0.030	0.026	0.023
3.482	1.176	0.576	0.097	0.097	0.023	0.020	0.020
1.670	1.670	0.147	0.147	0.073	0.038	0.028	0.020
0.617	0.617	0.183	0.183	0.043	0.039	0.022	0.022
2.766	1.483	0.320	0.078	0.025	0.020	0.020	0.020
8.963	6.073	0.311	0.303	0.057	0.055	0.055	0.020
0.577	0.231	0.231	0.067	0.040	0.023	0.023	0.020
0.642	0.642	0.348	0.122	0.027	0.020	0.020	0.020
1.646	0.408	0.327	0.085	0.065	0.049	0.042	0.024
0.186	0.186	0.077	0.060	0.025	0.020	0.020	0.020
0.568	0.568	0.140	0.044	0.044	0.023	0.023	0.020
3.281	3.281	0.619	0.280	0.204	0.150	0.117	0.021
0.425	0.263	0.091	0.091	0.024	0.024	0.022	0.020
0.832	0.294	0.168	0.074	0.054	0.020	0.020	0.020
0.547	0.116	0.100	0.022	0.020	0.020	0.020	0.020
5.333	3.647	3.647	0.226	0.226	0.036	0.036	0.020
1.865	1.865	0.518	0.104	0.098	0.098	0.076	0.041
1.780	1.110	0.348	0.348	0.262	0.120	0.099	0.020
0.947	0.947	0.947	0.565	0.565	0.120	0.020	0.020
6.483	1.140	1.140	0.127	0.115	0.115	0.020	0.020

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Table 4. Time to safe concentration (TTSC) values per cow.

TTSC	ln(TTSC)	frequency	cow numbers
3	1.099	3 ***	15, 18, 20
4	1.386	9 *********	1, 2, 4, 7, 10, 12, 14, 16, 19
5	1.609	5 *****	8, 9, 11, 13, 22
6	1.792	4 ****	3, 5, 6, 21
7	1.946	3 ***	23, 24, 25
8	2.079	1 *	17

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Table 5. Un-rounded withdrawal period (UWP) and monotonic fit (MUWP) at varying MRL values. UWP and MUWP values are valid from the tabulated MRL up to the next tabulated MRL value, *e.g.* MRL=0.20 gives UWP=9.044 and MUWP=8.035.

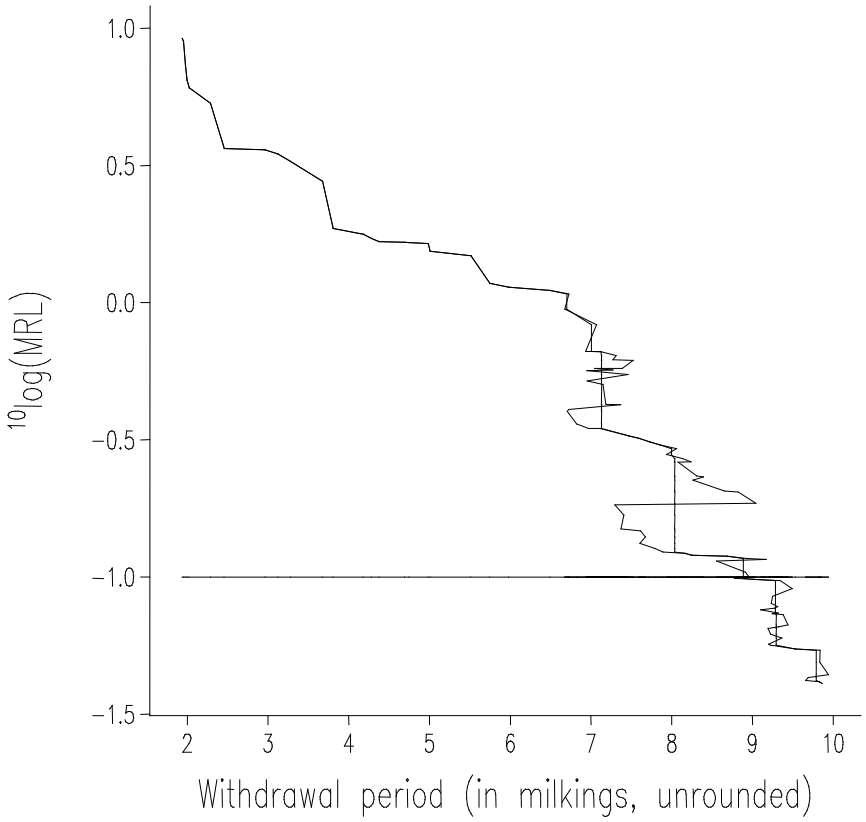
MRL	UWP	MUWP
0.0410	9.861	9.861
0.0415	9.826	9.826
0.0420	9.657	9.792
0.0430	9.692	9.792
0.0440	9.942	9.792
0.0490	9.834	9.792
0.0540	9.836	9.792
0.0547	9.534	9.534
0.0564	9.219	9.293
0.0570	9.201	9.293
0.0600	9.364	9.293
0.0620	9.228	9.293
0.0650	9.191	9.293
0.0660	9.323	9.293
0.0670	9.440	9.293
0.0730	9.381	9.293
0.0736	9.246	9.285
0.0740	9.319	9.285
0.0760	9.104	9.285
0.0770	9.272	9.285
0.0780	9.311	9.285
0.0803	9.232	9.285
0.0850	9.248	9.285
0.0906	9.490	9.285
0.0971	9.345	9.285
0.0981	9.011	9.011
0.0990	8.777	8.886
0.1000	8.962	8.886
0.1040	8.919	8.886
0.1145	8.558	8.886
0.1160	9.170	8.886
0.1170	8.930	8.886
0.1190	8.688	8.688
0.1200	8.249	8.249
0.1220	8.164	8.164
0.1234	7.892	8.035
0.1270	7.794	8.035
0.1330	7.607	8.035
0.1400	7.675	8.035
0.1473	7.612	8.035
0.1500	7.373	8.035
0.1680	7.409	8.035
0.1835	7.297	8.035
0.1856	9.044	8.035
0.2040	8.824	8.035
0.2060	8.655	8.035
0.2257	8.261	8.035
0.2313	8.395	8.035

MRL	UWP	MUWP
0.2800	7.937	7.997
0.2940	8.057	7.997
0.3030	7.852	7.852
0.3110	7.728	7.728
0.3200	7.599	7.599
0.3270	7.467	7.467
0.3479	7.114	7.130
0.3480	6.970	7.130
0.3620	6.823	7.130
0.4016	6.706	7.130
0.4080	6.725	7.130
0.4250	7.367	7.130
0.4260	7.182	7.130
0.5030	7.148	7.130
0.5180	6.955	7.130
0.5470	7.460	7.130
0.5654	6.948	7.130
0.5685	7.272	7.130
0.5760	7.047	7.130
0.5770	7.388	7.130
0.6171	7.524	7.130
0.6190	7.273	7.130
0.6420	7.312	7.130
0.6630	7.126	7.126
0.6650	6.935	7.003
0.8320	7.071	7.003
0.9473	6.675	6.702
1.0770	6.728	6.702
1.1100	6.494	6.494
1.1395	5.982	5.982
1.1760	5.748	5.748
1.4830	5.513	5.513
1.5394	5.010	5.010
1.6460	4.985	4.985
1.6560	4.746	4.746
1.6620	4.694	4.694
1.6701	4.376	4.376
1.7140	4.285	4.285
1.7800	4.181	4.181
1.8648	3.807	3.807
2.7660	3.675	3.675
3.2806	3.277	3.277
3.4820	3.125	3.125
3.6090	2.965	2.965
3.6468	2.457	2.457
5.3330	2.289	2.289
6.0730	2.024	2.024
6.4830	1.998	1.998

MRL	UWP	MUWP
0.2340	8.311	8.035
0.2620	8.079	8.035
0.2630	8.238	8.035
0.2700	8.135	8.035

MRL	UWP	MUWP
7.3420	1.976	1.976
8.9630	1.957	1.957
9.2010	1.938	1.938

644



645

646 Figure 4. Effect of final monotonic regression to obtain monotonically increasing withdrawal periods for
647 decreasing MRLs. Vertical line segments are monotonic fits *MUWP* replacing calculated *UWP*
648 *values*. Horizontal line indicates the true MRL=0.1 for this example, and intersects the *MUWP*
649 curve at 8.886, which upon rounding gives a withdrawal period (*WP*) of 9 milkings.

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