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APPENDIX I

CALCULATION OF MAXIMUM RESIDUE LEVELS AND SAFETY INTERVALS
e.g. Pre-harvest Intervals

1 Introduction

The application for authorization of a plant protection product contains a request to the applicant for proposed safety intervals e. g. pre-harvest intervals and maximum residue levels (MRLs). Pre-harvest intervals, which are the time between the last application of a plant protection product and harvest or the earliest possible use of the treated product, are the result primarily of the needs of agricultural practice and are no shorter than Good Agricultural Practice (GAP) requires. They are set by an experienced expert after he has consulted all the available documentation and information on the residue situation of the envisaged application of the product.

Maximum residue levels are set on the basis of supervised trials in which GAP is observed and must not pose an unacceptable risk to human health. In practice, these levels are also subject to the requirements of international trade with agricultural products.

The following text describes two methods for calculating proposed pre-harvest intervals and maximum residue levels. In 1981 Weinmann and Nolting published a proposal for an evaluation of residue tests. The proposed method has proved its worth in many cases and is described here, with a few minor amendments, as method I. In addition, the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig/Germany has developed a useful method of calculation which is included here as method II.

The relatively small amount of data used in the authorization process is normally not enough for statistical evaluation. However, experience has shown that statistical methods have proved to be useful tools in the derivation of MRLs but these should not replace scientific judgement bases on all the available data.

2 Data

The residue levels of a plant protection product's active substance, in and on plants of a given species and at the same stage of development, primarily depend on the dosage rate per unit surface area and the weather conditions. Factors such as differences in formulation, application methods, etc., are of secondary significance. So all the residue data recorded for the active substance in the region of the maximum dosage rate and number of applications envisaged are used to determine the maximum residue level in a crop. For comparability of data see elsewhere.

2.1 Sorting the residue results on a time basis

To evaluate the data, it is necessary to look at the results of several residue tests together. This means sorting the residue values (R) according to the time of sampling (t). Missing values within residue decline studies can be determined by means of linear and other interpolation methods (Timme, G. et al.). As an alternative, the residue values from various sampling times can be combined and classified together on one day according to the pre-harvest interval categories explained below (Table 2). In this case it is necessary to take into account the crop development stage. Combining the different sampling times is only worthwhile if the same stages of development are present at least at the time of application.

Example: The residue values for samples taken at 6, 7 and 8 days were included unchanged in the calculation as if all the samples were taken on the seventh day after the last application.

For longer pre-harvest intervals, long time intervals still give good results; for example, sampling times of 25 to 30 days or 53 to 60 days can be combined to 28 and 56 days respectively, etc. For comparability of data see elsewhere.

If the residue decline studies show that there is no notable reduction in residue levels in the range in question, the residue levels from several time intervals can be combined.

Grouping residue values can affect the result (particularly the k-factor in method I) and should therefore be indicated in the report. If there are various possibilities for grouping, all the variants should be calculated.

Before grouping data it may be worthwhile using a statistical test to show that there is no significant degradation within the time interval in question. The Wilcoxon test is suitable for small samples (Sachs, L.).

3 Correction of residue results

The mean figures of repeated analyses given in the residue reports are used for the calculations. Results from replicated trials should not be averaged (mean). For the purpose of statistical calculation of the MRL, residue results expressed as < LOD should be assumed to be at the LOD.

4 Method I

Method I is a calculation method which assumes a normal distribution for the measured residues. The calculation requires an arithmetic mean, standard deviation and maximum residues per sampling time. This permits calculation of a maximum residue for a given pre-harvest interval or the pre-harvest interval for a given maximum residue.

4.1 Elimination of outliers

Before calculating maximum residue levels it may be necessary to check for outliers, particularly with large data sets (i. e. 8 and more data points). The Dixon Q-test can be used for this. It checks whether an extreme figure which may be an outlier belongs to a different parent population from the other sample figures.

First of all the measurement X_1 to X_n are assigned to a parent population in accordance with their magnitude, where X_1 is the possible outlier. Then the range $X_1 - X_{n-1}$ between the second smallest and largest values and the difference $X_1 - X_2$ between the extreme value and next lower value are determined and the quotient is calculated:

$$Q = \frac{X_1 - X_2}{X_1 - X_{n-1}} \quad \text{for n = 8 - 10}$$

(See Annex 1 for a detailed calculation including values for n > 10)

This value for Q is now compared with a value from a statistical table (see Annex 1). If Q is greater than or equal to the table value, then there is a 90 % certainty that it is an outlier and should be deleted, in other words: the zero hypothesis - that there are no outliers - is set at the 10 % level (always assuming a normal distribution).

Use of the outlier test for samples which are not normally distributed can easily lead to the elimination of real residue values, so this method should be used with extreme caution, particularly with small data sets. Residue levels must be rejected from the set when there is a clear problem with the trial, for example if the analytical methodology is suspect or the crops may have been overdosed. Where outliers are assumed the recommendation is that calculations be done both with and without them.

4.2 Calculation of the maximum residue level for a given pre-harvest interval

4.2.1 Residue data on the pre-harvest interval available

After correction of the values and consideration of the outliers the mean values R for the residue figures at each test time and the related standard deviations can be determined. s can be calculated according to the formula:

$$s = \sqrt{\frac{\Sigma(R_i - R)^2}{n - 1}}$$

R_i = residue figure at the individual test times
R = mean value of all residue figures at the individual test times
n = number of residue figures at the individual test times

Using the mean value R and standard deviation s it is now possible to calculate a tolerance level for the individual sampling times within which a specific percentage γ of the parent population can be expected to occur with a set probability S. For the assumed distribution of the parent population these levels are given by

$$R \pm k \cdot s$$

Where k is a suitable statistical value to be taken from table 1.

Here only the upper tolerance limit $R + k \cdot s$ will be considered, which is designated below R_{max} = maximum residue value. To calculate it, therefore, the factors for one-sided tolerance limits are used. It is deemed sufficient to use γ and S = 0.95, i.e. a 95 % confidence range.

Table 1: k-factors for the one-sided tolerance range for γ and S = 0.95 of sample means of normally distributed populations (Owen, D. B.)

n	k	n	k
2	26.260	19	2.423
3	7.656	20	2.396
4	5.144	21	2.371
5	4.210	22	2.350
6	3.711	23	2.329
7	3.401	24	2.309
8	3.188	25	2.292
9	3.032	30	2.220
10	2.911	35	2.166
11	2.815	40	2.126
12	2.736	45	2.092
13	2.670	50	2.065
14	2.614	60	2.022
15	2.566	70	1.990
16	2.523	80	1.965
17	2.486	90	1.944
18	2.453	100	1.927

4.2.2 Residue data on pre-harvest interval unavailable (interpolation method)

If no residue data are available for the desired pre-harvest interval t_{WZ} (WZ = PHI) and if values missing within a residue decline study are not calculated via a suitable interpolation method R_{max}-values are calculated for the adjacent sample times t_1 and t_2 (see 4.1). This method assumes that the degradation behaviour within the time interval in question can be sufficiently approximately reproduced by a first order process, i.e. the degradation rate is at all times proportional to the relevant concentration (R) of the reacting substance R:

$$\frac{-d(R)}{dt} = \delta(R)$$

After transformation and integration the following is obtained:

$$\ln(R) = \ln(R_1) - \delta_{1.2}(t_2 - t_1)$$

Where (R_1) is the concentration of R at time $t = t_1$.

These hypotheses are auxiliary constructions which can be regarded as permissible within the method given here.

In the time interval t_1 , t_2 the local constant 1.2 of the rate of the degradation reaction can be determined from the calculated maximum residue values R_{1max} and R_{2max}:

$$\delta_{1.2} = \ln \frac{R_{1max}}{R_{2max}} \Big/ (t_2 - t_1)$$

The maximum residue level R_{WZ} at the pre-harvest interval t_{WZ} (for $t_1 < t_{WZ} < t_2$) can be calculated:

$$\ln R_{WZ} = \ln R_{1max} + \delta_{1.2} (t_2 - t_1)$$

or

$$R_{WZ} = R_{1max} \cdot e^{\delta 1.2 (t2 - t1)}$$

4.3 Calculation of pre-harvest interval for a given maximum residue level

If there is already a maximum residue level or if a provisional level is known which lies within the range R_{1max} and R_{2max}, this case also requires interpolation. The pre-harvest interval t_{WZ} is calculated as follows:

$$t_{WZ} = \left[\ln \frac{R_{max}}{MRL} \Big/ \delta_{1.2} \right] + t_1$$

The pre-harvest intervals and/or maximum residue levels can also be derived directly from a semilogarithmic graph (Fig. 1).

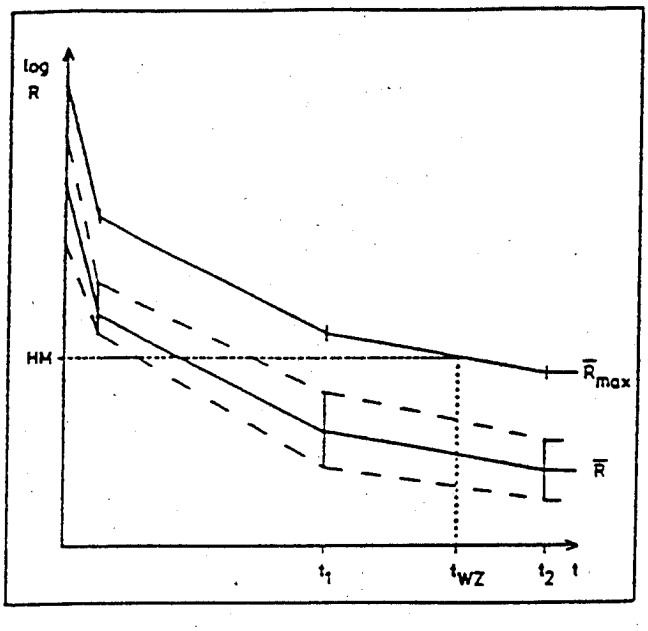

Fig. 1: Graph of the residual behaviour

R: Curve of the means (the residues for the test series lie within the area between the broken lines)
R_{max}: Maximum residue
HM: Maximum residue level (MRL)
t_{WZ}: pre-harvest interval

4.4 Method I Special Case

The k-factor from Table 1 approximates to the value 2 for a large number of values (n > 30). If the variation coefficient is about 100 %, i.e. if the mean and standard deviation are approximately the same, the equation

$$R_{max} = R + k \cdot s$$

can be simplified to

$$R_{max} \cong 3\ R$$

So for a large number of test results with arithmetic means and standard deviations of approximately the same Rmax can be assumed to be about three times the mean.

5 **Method II**

Method II takes into account that a normal distribution cannot always be assumed with sufficient certainty for results from residue tests. The method uses a distribution-free parameter (quantile) and is designed to produce maximum residue levels in the normal order of magnitude given the amount of data material which is usually available (approximately 8 trials per crop).

5.1 Calculation method

The calculation method is relatively tolerant to outliers so no figures need to be excluded. As a rule, all figures within a degradation series are assumed to be true. Complete degradation series will therefore only be eliminated as outliers if there are obvious defects in the test set-up, method, sampling or analysis.

Median and quantiles are the obvious distribution-free parameters for the metric data available. With the few test series available, a quantile greater than the 75 % one is not practicable. The 75 % quantile is to be preferred to the median because it gives a more accurate representation of the frequently negative skew of the distribution function. Lastly, the maximum residue levels should primarily take into account the maximum values possible and not reflect the central distribution trend.

There are various methods available for calculating the 75 % quantile (Lorenz, R. J.; Faulbaum, F. et al.). The following method is proposed:

$$R(0.75) = (1 - G) \cdot R(J) + G \cdot R(J+1)$$
where $(n+1) \cdot P = J + G$

n = number of values
P = T/100, in this case 0.75
T = percentile, in this case 75 %
J = whole-integer proportion of $(n+1) \cdot P$
G = fraction of $(n+1) \cdot P$
R (J) = residue value at point J
R (J+1) = residue value at point J + 1
R (0.75) = desired quantile
R (n) = the residue values ordered according to size at the pre-harvest interval

This calculation has the advantage over other quantile calculations in that a weighted average is determined at point $R (P \cdot (n+1))$ taking into account the figures recorded empirically. The method is relatively insensitive to a variation in n.

Example:

Measured figures (mg/kg) at pre-harvest interval:
0.3 1.1 1.2 1.4 1.5 1.8 4.9 8.1

Measured figure at point
(order): 1 2 3 4 5 6 7 8

n = 8
P = 0.75
$(n+1) \cdot P = J + G$
$9 \cdot 0.75 = 6 + 0.75 = 6.75$
$R(0.75) = (1 - G) \cdot R(J) + G \cdot R(J+1)$
$R(0.75) = 0.25 \cdot 1.8 + 0.75 \cdot 4.9 = 0.45 + 3.675 = 4.125$

5.2 Formula for calculating the proposed maximum residue level:

$R(ber) = 2 \cdot R(0.75) = 2 \cdot [(1 - G) \cdot R(J) + G \cdot R(J+1)]$

R(ber) = calculated figure for the proposed maximum level
R(0.75) = 75 % quantile

The 75 % quantile obviously charcterizes the figure which is not exceeded in 75 % of the cases. Given the required test set-up (worst case) higher figures than this would scarcely be expected in practice. The factor 2 represents a safety margin.

If there are no residue data available for the envisaged pre-harvest interval, interpolation must be carried out between the adjacent R(ber) figures (see point 4.2.2).

5.3 Proposed pre-harvest interval

If there is already a maximum residue level (MRL) or if a provisional level is known, which lies within the range between R1(ber) and R2(ber), interpolation can be carried out as in point 4.3:

$$t_{WZ} = \left[\ln \frac{R_{1\,(ber)}}{MRL} \Big/ \delta_{1.2} \right] + t_1$$

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6 Final assessment

The figures R(max) and R(ber) determined according to the two methods originate in samples from a parent population which is difficult to define. The calculation results should not therefore be seen as precise figures. It has proved better to round off the figures, order them in a system of pre-harvest intervals and maximum residue levels and regard the relevant class as the final result.

In deciding on the appropriate maximum residue level, due consideration should be given to the extent to which the data points reflect Good Agricultural Practice. For example if most of the data points are from trials in which the dose rates are lower than the registered use, then figures arrived at by Method I and II should be rounded up rather than down in order to ensure that there is no conflict between the Maximum Residue Levels and registered uses.

6.1 Pre-harvest interval classes

Practice over the last two decades has shown that there is no**t normally a** need for a continuous range of pre-harvest intervals. On the contrary, a series of values has been found which does not correspond to any mathematical rules but satisfies the requirements of practical crop protection measures.

The following class distribution has been obtained after a number of corrections - by omitting rarely used values and adapting to a continuous system.

Table 2: Pre-harvest interval classes (in days)

1	2	3	4	7	10	14	21	28	35	42	49	56	90	120	F*

*F: The pre-harvest interval(s) for the envisaged area(s) of application is/are covered by the application conditions and/or the growing period remaining between the envisaged application and use (e.g. harvest); it is not necessary to lay down/indicate a pre-harvest interval in days (F).

6.2 Maximum residue level of classes

Where there is not yet a maximum residue level for the active substance and crop in question, the result of the residue test is evaluated to provide a proposed maximum residue level. Here, too, it has been found that a continuous range of levels is neither necessary nor useful. A sequence of levels has developed over the years.

The following classes, arrived at from the observed distribution of the test results, seems worthwhile:

Table 3: Maximum residue level classes (in mg/kg)

0.01	0.02	0.05	0.1	0.2	**0.3**	0.5	1	2	**3**	5	10	20	50	100	>100

At an international level, further maximum level classes are being proposed. For example, a World Health Organization table lists additional values of 15 and 30 mg/kg.

Individual cases may deviate from Table 3, for example, where it is necessary to harmonize with international maximum residue levels for toxicological reasons. If at the end of the pre-harvest interval the analytical results from all residue tests are below the limit of determination, the maximum residue level is proposed at or about the practical limit of determination unless there are specific reasons to the contrary.

7 **Selection of the suitable method**

Method I has proved itself in an adequate number of residue tests and for early application timings (applications at early growth stages, e.g. where there is still no harvestable material which can be affected by the treatment).

Method II provides practicable results where there are few data (8 - 12 residue tests for evaluation) and for applications close to harvesting with a proportionately higher level of residue and uncertain statistical distribution. The final results from both methods often coincide.

It is recommended initially to use both methods together. Time will show in which cases method I or II is to be preferred. It is doubtful whether it will be possible in the future to develop a uniform method meeting all requirements, given the small database, variety of active substances and different application conditions.

8 **Special cases**

8.1 Limited data base

In special cases (e.g. minor crop situation) it might be necessary to calculate a MRL or PHI on the basis of 3 - 4 results. This limited data base is not sufficient for a statistical calculation using the methods recommended here as a tool. Nevertheless there is a need to access and/or propose PHIs or MRLs on that basis.

8.2 Extensive data base

Often in cases where extensive data are available two questions arise:

1. Which Good Agricultural Practice (GAP) can be assumed to represent the worst case?
2. Which data have to be taken into account to calculate a MRL?

1st Example: Use of aldicarb on potatoes. GAP in England (UK), The Netherlands (NL) and Spain (ES), 190 results available.

UK data:
n = 26 mean = 0.088 s = 0.116 k = 2.292
Rmax = 0.354 Rber = 0.21
MRL-proposal **0.3** mg/kg (unacceptable as 3 of 26 results = 11.5 % exceed the proposed MRL)
MRL-proposal **0.5** mg/kg

NL data:
n = 76 mean = 0.067 s = 0.065 k = 1.99
Rmax = 0.197 Rber = 0.175
MRL-proposal **0.2** mg/kg

ES data (all results):
n = 74 mean = 0.0601 s = 0.100 k = 1.99
Rmax = 0.260 Rber = 0.125
MRL-proposal **0.2** mg/kg

ES data (early potatoes, PHI 80 - 125 days):
n = 18 mean = 0.131 s = 0.154 k = 2.453
Rmax = 0.507 Rber = 0.415
MRL-proposal **0.5** g/kg

Summary (decision):
On the basis of results on data from Spain on early potatoes a MRL of **0.5** mg/kg is recommended.

2nd Example: Use of aldicarb on citrus fruits.

Oranges
n = 19 man = 0.06 s = 0.072 k = 2.4.53
Rmax = 0.235 Rber = 0.20
MRL-proposal **0.2** mg/kg

Lemons (mature)
n = 7 mean = 0.053 s = 0.026 k = 3.401
Rmax = 0.142 Rber = 0.12
MRL-proposal **0.1** mg/kg (with one residue out of seven being at the proposed level)
MRL-proposal **0.2** mg/kg

Summary (decision):
On the basis of results on data from oranges and lemons a MRL of **0.2** mg/kg is recommended for citrus fruit.

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Annex I:

Outlier test according to DIXON
Significance limits for testing outliers according to Dixon, W. J.

No. of values	Q for = 0.10	Test parameter
8	0.479	$\frac{X_1 - X_2}{X_1 - X_{n-1}}$
9	0.441	
10	0.409	
11	0.517	$\frac{X_1 - X_3}{X_1 - X_{n-1}}$
12	0.490	
13	0.467	
14	0.492	
15	0.472	
16	0.454	
17	0.438	
18	0.424	$\frac{X_1 - X_3}{X_1 - X_{n-2}}$
19	0.412	
20	0.401	
21	0.391	
22	0.382	
23	0.374	
24	0.367	
25	0.360	

Annex 2:

Example for the evaluation of residue results

Crop: pome fruit Active substance: .....

$\frac{t \text{ (day)}}{R \text{ (mg/kg)}}$

Report-Nr.	0	6	7	8	13	14	15	17	20	21	26	28	30
	Days after last application												
0815/01	1.7		0.6					0.04					
0815/02	1.64		1.4			0.52				0.47			0.46
0815/03	0.90			0.38			0.33			0.18			
0815/04	0.58	0.47			0.40				0.40				
0815/05			0.71			0.53				0.51		0.45	
0815/06	0.58		0.74			0.81				0.54		0.47	
0815/07	0.77			1.26			1.13			0.39	0.19		
0815/08	0.41		0.44			0.39				0.23		0.43	
0815/09	1.29			1.46			1.13			0.57	0.75		
0815/10	0.55		0.80			0.67				0.19		0.20	

Sorting (days after final application).
0 7 (6-8) 14 (13-17) 21 (20-21) 28 (26-30)

Method I:

day	n	R	s	k-factor	R(max)
0	9	0.936	0.489	3.032	2.42
7	10	0.825	0.414	2.911	2.03
14	10	0.595	0.348	2.911	1.61
21	9	0.387	0.152	3.032	0.85
28	7	0.421	0.189	3.401	1.06

Method II:

day	n	R(0.75)	R(ber)
0	9	1.465	2.93
7	10	1.305	2.61
14	10	0.89	1.78
21	9	0.525	1.05
28	7	0.47	0.94

Result/conclusion:
In practice a pre-harvest interval of 21 days is necessary. A maximum residue level of 1 mg/kg is proposed.

Note: In the calculation according to method I no values were eliminated as outliers.