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RESEARCH ARTICLE

SETTING MRLS FOR PESTICIDE RESIDUES IN FOOD BASED ON EU UNIFORM CRITERIA

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ABSTRACT

Objective: Pesticides are used worldwide. They are applied during the growing season and part of the amount may remain in or on treated crop. Pesticides can drift to nearby areas and contaminate crops that were not treated. The MRLs are the maximum amount of a pesticide permitted in or on food (& feed), expressed in mg/kg or ppm and always defined for the active substance – crop combination. MRL should be set according to residue trials under Good Agricultural Practice (GAP) and under the condition that: Daily Consumer Intake < Acceptable Daily Intake. The residue definition of active substance is derived from plant and animal metabolism. Supervised trials are the primary source of information for estimating maximum residue levels and calculating International Estimated Daily Intake. The agricultural practice the worst-case situation should be used to generate data from supervised trials to define the MRL. As the number of controlled field tests, and hence the data for MRL proposal is not large, the usual statistical models are inappropriate.

Methods: Two methods for calculating proposed pre-harvest intervals (PHI) and maximum residue levels are used. The first one (Rmax) has proved its worth in many cases since 1981, and the second (Rber) has been developed by the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig/Germany. Both methods are described briefly. The first one is suitable for a larger number of data. It assumes a normal distribution of random variables, which are not always assumed with sufficient certainty. The second one uses a non-parametric distribution- and it is appropriate for more limited number of trials. Several case studies are discussed. The MRL Regulation on pesticide residues in food is a great progress towards better protection of children and consumer in general.

Conclusions: Uniform criteria have to be used for evaluation of residue trials and MRLs setting process. Knowledge of the European approach in establishing the pesticide residue levels is useful for all countries in the world that have a policy of restriction of persistent organic pollutants in the environment aiming towards food safety for people of all ages.

Key words: Food, MRL, Pesticide residues, Plant Protection Products, Risk assessment

INTRODUCTION

Pesticides are widely used worldwide. They are applied during the growing season and part of the amount may remain in or on treated crops. Pesticides can drift to nearby areas and contaminate crops that were not treated. Pesticides are found as contaminants in soils and sediments, and animals such as fish accumulate pesticides obtained through the food chain over time in their bodies. Usually these are pesticides that have been approved and used in the past and which have a very long life. These are known as persistent organic pollutants or POPs (DDT, DDE).

Commercial plant protection product (PPP) consists of one or more active substances and other additives to improve the quality of use - adhesives, additives and others. The label of PPP specifies the conditions and manner of use. The rules and requirements for good agricultural practice in the use of PPP are "at the harvest, the pesticide residue levels to have values, lower than the maximum residue limits for residues of the active substance in the crop"

Why it is so important to establish a maximum residue limit (MRL)?

Firstly, MRLs allow the free trade. As of September 1st, 2011 MRLs are fully harmonized under Regulation (EC) 396/2005 in Annex I, which means that the MRL requirements are the same in all the EU Member States. This ensures free trade within the EU and European Free Trade Association (EFTA).

Second, MRLs provide safeguards for consumers. Regulation (EC) 396/2005 sets MRLs for pesticides permitted in products of animal and plant origin intended for human or animal consumption and replaces the variety of national MRLs with unique MRLs at EU level.

Third, MRLs are set under the Good Agricultural Practice (GAP). They are a guarantee of compliance with it. MRLs are derived after a thorough assessment of the properties of the active substance and residue levels resulting from good agricultural practices as defined for the treated crops. A necessary condition for the establishment of MRLs is to demonstrate through a risk assessment the safety for consumers (consumer intake should not exceed the toxicological reference values). Harmonisation gives a chance for a great progress towards better protection of consumers, especially children.

How were EU MRLs set?

It is necessary to perform the following assessments:

- Estimation of the residue level in or on an agricultural crop treated with the pesticide under conditions of the Good Agricultural Practice (GAP) in supervised trials.
- Estimation of the total daily intake of the specific pesticide using appropriate consumer intake models and the established residue levels [14,15, 17].
- Estimation of an 'acceptable daily intake' (ADI) using data from toxicological tests. This involves finding the highest dose that would produce no adverse effects over a lifetime (chronic) exposure period and then applying appropriate safety factors [14,15,17].

Maximum Residue Level (MRL) should be set from residue trials under Good Agricultural Practice (GAP) under the condition that:

Daily Consumer Intake < Acceptable Daily Intake

In fact MRL is the maximum amount of a pesticide, permitted in or on food (& feed), expressed in mg/kg or ppm, always defined for the combination active substance – crop.

The residue definition of active substance derives from the plant and animal metabolism.

The supervised trials are the primary source of information for estimating the maximum residue levels and calculating International Estimated Daily Intake. The term "supervised trials"

includes the application of a pesticide according to the authorized methods with the use of reliable experimental design and sampling. Residue trials are performed under the terms of the FAO Guidelines on Producing Residues Data from Supervised Trials. New supervised trials should be planned, implemented, documented and reported according to the OECD [8, 9] (or comparable) GLP principles (OECD, 1992; 1993) or in compliance with national regulations which ensure the quality of residue data. Maximum Residue Limits are largely derived from residue data obtained from supervised trials designed to determine the nature and level of residues in conditions of the registered or approved pesticide use. The trials should be based on the usage intended for registration. For estimating maximum residue levels of pesticide residues in commodities, results of supervised trials representing the typical agriculture practices as well as the growth and the climatic conditions during at least two growing seasons are needed. These controlled field trials are very expensive and their results are limited in number.

In the case of the outdoor applications it is assumed that for the carrying out of residue trials, the climatic conditions play a decisive role. EU countries are divided into two regions - North & Central, and South & the Mediterranean. Controlled field trials conducted in the same region are comparable with each other. However, trial data should be representative of the areas where Community authorization is granted or envisaged. Northern and Central Europe: include Sweden, Norway, Iceland Finland, Denmark, United Kingdom, Ireland, northern France, Belgium, The Netherlands, Luxembourg, Germany, Poland, Czech Republic, Slovakia, Austria, Hungary, Switzerland, Estonia, Latvia, Lithuania, Romania, Slovenia. Southern Europe and the Mediterranean: Spain, Portugal, Southern France, Italy, Greece, Malta, Croatia, Serbia, Bosnia and Herzegovina, FYROM (Former Yugoslav Republic of Macedonia), Turkey, Bulgaria, Cyprus [14, 15, 17]. In the additional annex the distribution of France between the two regions and the corresponding crop distribution is illustrated. Data from different countries within the same region may reflect different cultural practices and it might therefore be rejected. The agricultural practice defining the worst-case situation should be used to generate data to define the MRL. Results from regions, that are not climatically comparable, cannot in general serve as a total substitute for trials, carried out in comparable regions [6]. However, they add knowledge about the residue behaviour of the active substance. The evaluation of intended uses within the EU should be based on the residue data mainly generated within the EU. Data from other climatic zones (e.g., in the USA) may, however, in individual cases provide supporting evidence for the evaluation of the residue situation in the Member States of the EU. An estimate of comparable climates can be looked up in a relevant compendium on geography (e.g. Müller-Hohenstein, 1981, [7]). The results from green houses and/or seed treatments are independent from climatic conditions [1, 14-17].

The scope of the trials data have to be in the range of ± 25% GAP, in the appropriate climatic region and with the same or compatible formulation. The number of trials have to be as is shown it table 1.

In the case when the value is not equal to LOQ (Limit of Quantification of the analytical method for residue determination), all other values are required to be supported by evidence from metabolism studies.

As the number of controlled field tests, and hence the data for MRL proposal is not large, the usual statistical models are inappropriate. Two methods for calculating proposed pre-harvest intervals (PHI) and maximum residue levels are used.

Table 1: Number of trials need for setting MRL [4]

Type of crop	Number of trials	Number of seasons
Major	8 trials	2 seasons /1 season for protected use
Minor	4 trials	2 seasons /1 season for protected use
Very minor	4 trials	2 seasons /1 season for protected use

The first one is proven to be effective in many cases since 1981 and is described here, with a few minor amendments, as method I.

The second has developed by the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig/Germany.

Data needed

Only data for the applied dose and climatic conditions are important to evaluate the pesticide residues. All comparable data on these factors can be used in calculating the MRL. Details regarding the type of formulation or method of application are considered minor and will not participate in the selection of relevant values. If the PHI is longer than 7 days, the results, received by formulations type EC (Emulsifiable concentrates), WP (Wettable powders), WG (wettable granulas) and SC (Solutions and water soluble concentrates) are comparable [10].

Missing values in time of sampling may be supplemented by linear or another extrapolation. Values near the time of sampling can be grouped. If there are different possibilities for clustering - all options should be calculated. The mean value for repeat analyses per trial should be used. Results with values below LOQ are accepted as equal to the LOQ.

Standard criteria have to be used for evaluation of residues trials.

EU Method 1 (Rmax)

Method 1 (calculation method) assumed a normal distribution of residuals. It requires the average, standard deviation and maximum residue for a given PHI. It allows calculating the maximum residue for a given PHI or PHI to propose a maximum residue. [1, 14]

This method is inconvenient for large differences in the data. The outliers should be excluded from the sample through a statistical test.

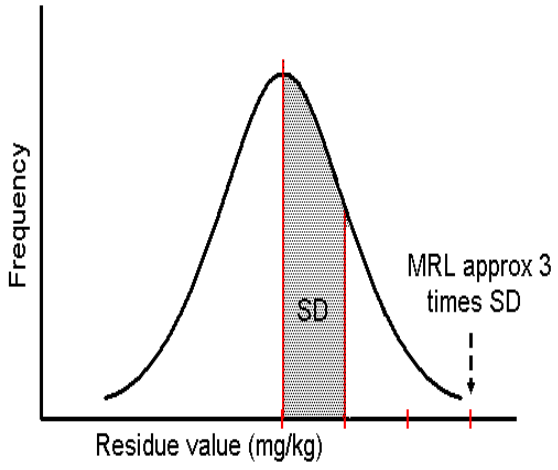

Fig.1: Rmax method. Normal distribution of data is assumed. The MRL is approximately equal to 3 times of SD (Standard Deviation) [1, 15, 17]

Residue data on the pre-harvest interval should be available. By using the mean value R and standard deviation S, it is now possible to calculate a tolerance level for the individual sampling time, within which a specific percentage γ (usually 75%) 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 :

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

Where k is a statistical value (can to be taken from table). Dependence of coefficient k of the number of trials n is graphically shown on Figure 2.

Only the upper tolerance limit

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

will be considered, which is designated as MRL. It is deemed sufficient to use γ and S = 0.95 (95 % confidence interval). The values of k-factors are for the one-sided tolerance range for γ and S = 0.95 of sample means of normally distributed populations.

It is seen (Figure 2) that the coefficient k assumes values close to 2 for a large number of trials - (20≤n≤100). If S is approximately 100%, the Rmax≈3R (about three times the mean value).

Method 1 is appropriate for a large number of experiments. It supposes a normal distribution of random variables, which are not always to be assumed with sufficient certainty.

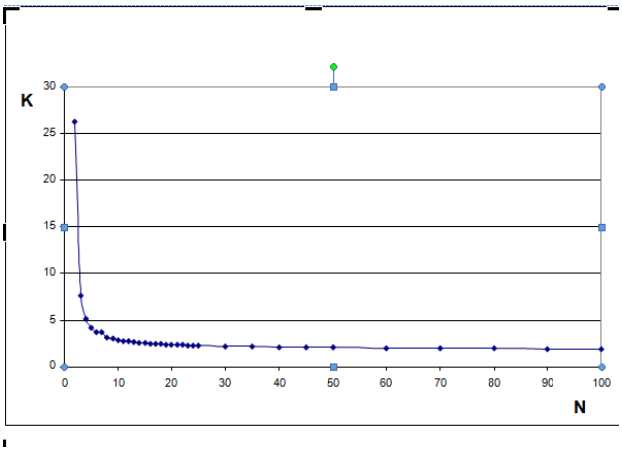

Fig. 2: Relation between the coefficient k and the number of trials N

EU Method 2 (Rber)

The method 2 uses a nonparametric distribution. MRL proposal is justified on the limited number of analyzes (often 8 or 4 are available). The outlier values should not be excluded. All available data is used. Nonparametric distribution is characterized by the values of median and quartile. The "75% quintile" is preferred to be the median, because it gives a more accurate representation of the frequently negative skew of the distribution function. Furthermore, the purpose is to consider the maximum values and not to determine the underlying trend in the distribution (Figure 3) [1, 15, 17].

EU Approach

Rmax and Rber should not be as exact figures. They are taken into account when deciding on the MRL. MRL is chosen to be the closest value from the table of possible values [1]. As of June 2010 the new table of classes for the setting MRLs is available by SANCO 10634/2010 Rev 0 [12]. Additional intermediate classes may be also used, if and when justified. For rounding calculated results to new classes, international accepted approach should be used, i.e. for example given calculation between 0.401 to 0.449 mg/kg would be rounded down to 0.4 mg/kg and calculations between 0.450 and 0.499 mg/kg would be rounded up to 0.5 mg/kg.

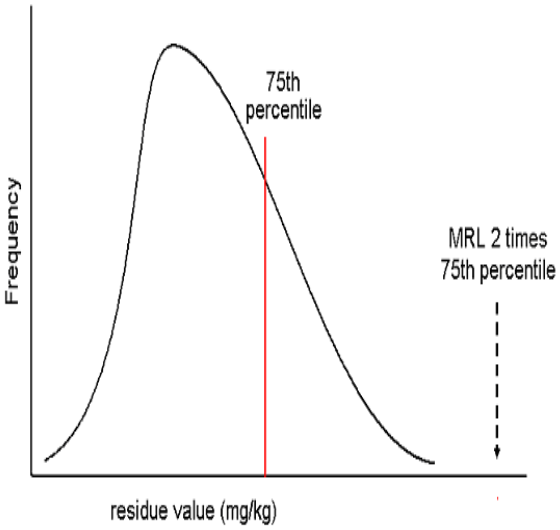

Fig 3: Rber method. Non-parameter (quantille) distribution of data is assumed. The MRL approximately equal to 2 times of 75% percentile [1, 15, 17]

It is known that the Rmax works well for large and sufficient data sets and early application timings, whereas the Rber practically gives good results where data sets are limited (n=8-12), the residues are higher and the distribution is uncertain. It is recommended initially to use the both methods together. Time will show in which cases the method I or II is preferable. 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.

Examples from practice

Example 1: The data from residue trials under critical GAP are (8 trials): 0.26; 0.32; 0.35; 0.45; 0.48; 0.52; 0.46; 0.64 mg/kg

Table 1: Data for Example 1 and MRL proposal.

Number trials, n	ofHR	STMR	EU Method1 (Rmax)	EU Method 2 (Rber)	MRL, mg/kg
8 trials	0.640	0.455	0.822	1.020	*It has to make a choice between* 0.8 and 1.0

What value should be chosen for MRL – 0.8 or 1.0? Because Method 2 is suitable for a small number of trials (in this case n=8) and it should be applied in the worst case, the value 1 mg/kg for MRL is an appropriate proposal.

Example 2: Available data from supervised trials under critical GAP are: 0.01; 0.01; 0.01; 0.01; 0.02; 0.02; 0.02; 0.02 mg/kg (8 trials).

Table 2: Data for Example 2 and MRL proposalsss

Number of trials, n	HR	STMR	EU Method 1 (Rmax)	EU Method 2 (Rber)	Proposal MRL, mg/kg
8 trials	0.02	0.015	0.032	0.040	0.04

Example 3: Citrus [2]: Available data from supervised trials: Oranges, 8 trials: 0.02; 0.04; 0.07; 0.14; 0.07; 0.03; 0.05; 0.08 and Mandarins, 8 trials: 0.04; 0.03; 0.02; 0.07; 0.12; 0.10; 0.25; 0.07

Table 3: Data for Example 3 and MRL proposal

Crop	HR	STMR	EU Method 1 (Rmax)	EU Method 2 (Rber)	Proposal MRL, mg/kg
Oranges – 8 trials	0.140	0.060	0.183	0.155	**0.2**
Mandarins – 8 trials	0.250	0.070	0.323	0.230	**0.5**
All 16 trials for Citrus	0.250	0.070	0.222	0.190	**0.3**

MRL for oranges will be set at 0.2 mg/kg, for mandarins – 0.5 mg/kg.
But there are enough data for setting MRL for whole group "citrus" – 0.3 mg/kg.

Example 4: Tomatoes: Available data from supervised trials: 8 trials on tomato in North region (indoor, glass): 0.1; 0.1; 0.3; 0.4; 0.5; 0.5; 0.7; 0.8 and 8 trials on tomato in South region (outdoor, field): 0.01; 0.01; 0.03; 0.04; 0.05; 0.05; 0.07; 0.08

Table 4: Data for Example 4 and MRL proposal

Crop	HR	STMR	EU Method 1 (Rmax)	EU Method 2 (Rber)	Proposal MRL, mg/kg
Tomatoes – NORT region 8 trials	0.8	0.45	1.238	1.300	2.0
Tomatoes – SOUT region 8 trials	0.08	0.045	0.124	0.130	0.2
Tomatoes					2,0

The proposed MRL of 2 mg/kg should be chosen in condition of the critical GAP (North region EU).

MRLs are safe limits

Regulation (EC) No 396/2005 [11] envisaged a full harmonisation for all pesticide Maximum Residue Levels (MRLs) and replaced the previous legislation concerning MRLs for about 250 active substances. The European Commission has taken forward a food standard programme in order to harmonise the MRLs of the remaining 900 pesticides, which could potentially be present as residues in or on food. According to the Regulation these harmonised MRLs should be based on existing national provisions in EU Member States.

The major priorities the MRL Regulation are

- No pesticide authorisation without an established MRL
- all pesticide/commodity combinations without an established MRL = 0.01 mg/kg („zero limit" a priori)
- Improved transparency by publishing annual reports about the situation in the European countries
- The children and the unborn have to be protected. As Recital 5 of MRL Regulation [11] says*: „...MRLs should be set at the lowest achievable level consistent with good agricultural practice for each pesticide with a view to protecting vulnerable groups such as children and the unborn".*
- The safety limit (ADI) must take into account the sensitive groups such as children.
- The MRL setting must take into account a second safety limit – The Acute Reference Dose (ARfD)
- The pesticide dose that can be ingested over a short period of time, usually during one day, without appreciable health risks (taking into account sensitive groups within the population) has to be established.

The novelty in this field is that with the goal of harmonizing the calculation of MRLs across the OECD, it was proposed a new MRL calculation procedure [8]. The guiding principles of this procedure are that the procedure is a practical implementation of sound statistical methods, simple to use without requiring extensive statistical knowledge from a user. It produces a clear and unambiguous MRL proposal for most residue datasets produced by field trials; and, the procedure should be harmonized

by the EU and NAFTA procedures as much as possible. The OECD MRL Calculator is available on the OECD public website http://www.oecd.org/env/pesticides under Pesticide Publications/Publications on Pesticide Residues Sheet under "Residues (mg/kg)". The data for residues under LOQ is included in calculation as "Censored data" (residue values that are less than the limit of quantification or LOQ) and is entered by listing the LOQ values (example, 0.01 mg/kg) along with an asterisk in the adjacent column. The order in which the data is entered does not impact the results [3, 5]. If several analytical measurements have been carried out for the same sample, the mean value should be evaluated and used for input data in the calculator. For residue trials with replicate field samples, the mean of the replicate values should be used for input in the calculator.

CONCLUSION

Harmonization of MRLs for pesticide residues in food is a great progress towards better protection of children and consumer in general but there are a number of open questions how the EU and the Member States will implement this Regulation. Uniform criteria have to be used for evaluation of residue trials and MRLs setting process.

Knowledge of the European approach in establishing the pesticide residue levels is useful for all countries in the world that have a policy of restriction of persistent organic pollutants in the environment aiming towards food safety for people of all ages.

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