RIVM report 613340007/2004

Guidelines for selection and presentation of residue values of pesticides

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This investigation has been performed by order and for the account of VWS/GZB, in connection within the framework of project V/613340/02/SW: advice on pesticides VWS. In 2003 the project number was changed into V/320203/01/SW.

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Abstract

Pesticide residue assessments are executed to establish legal limits, called Maximum Residue Limits (MRLs). MRLs are derived from the results of these pesticide residue trials, which are performed according to critical Good Agricultural Practice. Only one residue value per residue trial may be selected for the MRL derivation. Here, a proposal is described for the selection and presentation of residue values in advisory reports, drafted in The Netherlands either by order of the Dutch Board for the Authorisation of Pesticides or the Food and Agricultural Organisation of the United Nations. In these advisory reports, residue values from each submitted residue trial are presented in a table. Independent and replicate residue trials are distinguished. Residue trials carried out at the same location and same point in time with the same equipment are considered as one residue trial with several replicates (when the area of application, formulation, dose rate, number of applications and crop variety are the same). For a residue trial consisting of replicate trials, all individual residue values are presented, but only the maximum residue value is selected. Furthermore, one or more field samples can be taken per residue trial and each field sample can be subdivided into one or more laboratory samples, which in turn can be subdivided into one or more analytical portions. For a residue trial consisting of replicate field samples, all individual residue values are presented, but only the mean residue value is selected. Finally, for a residue trial consisting of replicate laboratory samples or replicate analytical portions, only the mean residue values are presented and selected.

Preface

The guidelines presented in this report have been discussed by:
a. Dr. F.X.R. van Leeuwen (chairman), Ir. G.J. Schefferlie (vice-chairman), Dr. A.J. Baars, and Dr. P van Zoonen, members of the residue peer review group of the Centre of Substances and Integrated Risk Assessment (SIR) from the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands).
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The present report is an English translation from the Dutch RIVM report 613340004/2002 "Richtlijnen voor selectie en weergave van residugehaltes van bestrijdingsmiddelen" by the same authors and published in 2002. The translation was carried out by Mrs. P. Hoogerhuis, RIVM, The Netherlands. Because some of the issues raised in the original Dutch report were incorporated into the updated FAO manual, the text in the present report is adapted to match with the updated version of the FAO manual (2nd edition, 2002) and the updated FAO guidelines (1990). Further some extra items have been added: §3.3.2.4 (seed coating), §5.5 (2 GAPs, same trial), §6.1 (reporting figures), §6.5 (collection at different treatment times) and §6.6 (collection at different growth stages). The report can be downloaded from the internet at http://www.rivm.nl

The guidelines in the present report have been presented by poster at the 10th IUPAC International Congress on the Chemistry of Crop Protection in Basel, Switzerland, 2002.

Contents

Samenvatting

Residubeoordelingen van bestrijdingsmiddelen worden uitgevoerd om wettelijke residulimieten (MRLs = maximum residue limits) vast te leggen. MRLs worden afgeleid uit de resultaten van die residuproeven met bestrijdingsmiddelen die volgens kritisch “Good Agricultural Practice” zijn uitgevoerd. Er mag slechts één residugehalte per residuproef geselecteerd worden voor de afleiding van de MRL. Het huidige rapport beschrijft een voorstel voor de selectie en weergave van residugehaltes in adviesrapporten die in Nederland worden opgesteld hetzij in opdracht van het College voor de Toelating van Bestrijdingsmiddelen hetzij in opdracht van de “Food and Agricultural Organisation of the United Nations”. In deze adviesrapporten worden de residugehaltes van elke aangeleverde residuproef weergegeven in een tabel. Bij residuproeven wordt onderscheid gemaakt tussen onafhankelijke en herhaalde residuproeven. Residuproeven die op dezelfde locatie op hetzelfde tijdstip met dezelfde apparatuur zijn uitgevoerd worden beschouwd als één residuproef met meerdere herhalingen (mits ook het toepassingsgebied, formulering, dosering, aantal toepassingen en gewasvariëteit dezelfde zijn). Als een residuproef bestaat uit herhaalde residuproeven, worden alle individuele residugehaltes weergegeven, maar alleen het maximum residugehalte wordt geselecteerd. Daarnaast kunnen per residuproef één of meer veldmonsters zijn genomen en elk veldmonster kan verder worden verdeeld in één of meer laboratoriummonsters, die op hun beurt kunnen worden verdeeld in één of meer analytische porties. Als een residuproef bestaat uit herhaalde veldmonsters, worden alle individuele residugehaltes weergegeven, maar alleen het gemiddelde residugehalte wordt geselecteerd. Als een residuproef bestaat uit herhaalde laboratoriummonsters of herhaalde analytische porties, wordt alleen het gemiddelde residugehalte weergegeven en geselecteerd.

Summary

Pesticide residue assessments are executed to establish legal limits, called Maximum Residue Limits (MRLs). MRLs are derived from the results of these pesticide residue trials, which are performed according to critical Good Agricultural Practice. Only one residue value per residue trial may be selected for the MRL derivation. Here, a proposal is described for the selection and presentation of residue values in advisory reports, drafted in The Netherlands either by order of the Dutch Board for the Authorisation of Pesticides or the Food and Agricultural Organisation of the United Nations. In these advisory reports, residue values from each submitted residue trial are presented in a table. Independent and replicate residue trials are distinguished. Residue trials carried out at the same location and same point in time with the same equipment are considered as one residue trial with several replicates (when the area of application, formulation, dose rate, number of applications and crop variety are the same). For a residue trial consisting of replicate trials, all individual residue values are presented, but only the maximum residue value is selected. Furthermore, one or more field samples can be taken per residue trial and each field sample can be subdivided into one or more laboratory samples, which in turn can be subdivided into one or more analytical portions. For a residue trial consisting of replicate field samples, all individual residue values are presented, but only the mean residue value is selected. Finally, for a residue trial consisting of replicate laboratory samples or replicate analytical portions, only the mean residue values are presented and selected.

1. Introduction

Residue assessments of pesticides are executed to establish legal residue limits (MRLs = maximum residue limits). MRLs have the purpose to uniform trade and to protect public health. By determining the MRLs the starting point is a high protection level for the consumer. Within the agricultural use attempts are being made to use MRLs as low as possible. The starting point for the determination of the MRLs is Good Agricultural Practice (GAP); this means that crops are cultivated according to common agricultural practice and that a pesticide is used according to the legal instructions for use.

The residue assessment of pesticides is executed on three levels: national, European and worldwide. In The Netherlands the assessments on national (NL) and European level in connection with the EC directives are, by order of the CTB (Dutch Board for the Authority of Pesticides), executed by several “evaluating authorities” among which is the RIVM (National Institute for Public Health and the Environment).
The assessments on worldwide level are executed by order of the FAO (Food and Agricultural Organisation of the United Nations) for the benefit of the JMPR (FAO/WHO Joint Meeting of Pesticide Residues, the scientific advisory committee of the CCPR (Codex Committee on Pesticide Residues)). In The Netherlands the last mentioned assessments are financed by VWS (Dutch Ministry of Health, Welfare and Sport) and are executed by the RIVM.

The residue assessment on national (NL) and European level is executed according to the guidelines that are originally recorded in the so-called “Lundehn document”. This Lundehn document is continuously adjusted to the newest insights [1].
The residue assessment on worldwide level is executed according to guidelines that are included in the updated FAO manual [2] and the updated FAO guidelines [3]. The FAO documents are also continuously adjusted to the newest insights. Supplements to the FAO documents are published in the so-called JMPR reports [4].

Specific guidelines with respect to the selection and presentation of residue values based on submitted residue trials are missing from the Lundehn document as well as from the FAO documents. Therefore a start was given within the residue assessment group of the RIVM to formulate guidelines with respect to the selection and presentation of residue values for the benefit of the own assessment group. Because the RIVM residue assessment group deals with all three assessment levels, the current guidelines intend to be applicable for all three levels. VWS perceived the necessity of making this document, which led to an assignment and financing. VWS shall make this document available for the CTB with the purpose to increase the consistency of the executed risk assessments, which were made under their auspices. In a later stadium this document could also be introduced in the European Union and in the JMPR.

In connection with the residue assessment of a pesticide, from each residue trial carried out according to the critical GAP, only one residue value may be selected.
Chapter 2 indicates what is meant with residue trials and which residue value from a residue trial is selected during replicate residue trials and/or samples.
If from a specific field only one field sample is taken and the field sample is worked out to one laboratory sample and after that to one analytical portion, the selection is simple; there is only one residue value. But if replicate residue trials are carried out and/or replicate field samples, replicate laboratory samples and/or replicate analytical portions are taken, the agreement must be made which residue value to select: the maximum or the mean. In chapter 3 and 4 the conceptions replicate residue trials, replicate field samples, replicate laboratory samples and replicate analytical portions are described in more detail. In chapter 5 a few specific cases are discussed. Chapter 6 indicates how residue values from (replicate) residue trials and (replicate) samples are presented in an advisory report.

2. Selection of residue values from a residue trial

2.1 Residue trials

Residue trials in agricultural products are carried out for the benefit of the registration of a pesticide product, especially for determining an MRL for the treated vegetable product. The definition of a residue trial is represented in the FAO manual ([2] Appendix II): *"Supervised trials for estimating maximum residue levels are scientific studies in which pesticides are applied to crops or animals according to specified conditions intended to reflect commercial practice after which harvested crops or tissues of slaughtered animals are analysed for pesticide residues. Usually specified conditions are those which approximate existing or proposed GAP"*.

Residue trials can be divided into 2 types of treatments:

a) pre-harvest treatment: pesticide application shortly before or during crop cultivation. This may be cultivation in the open air or cultivation under glass or plastic.
b) post-harvest treatment: pesticide application on the harvested crop. This may be before or during storage.

In a study report submitted by the notifier one or more residue trials are reported. Sometimes several residue values are reported in a study report at the relevant harvest times, whereby it is not always clear immediately if these residue values come from one or from more residue trials. In this case reported residue values can come from independent residue trials, replicate residue trials or from replicate samples (field samples, laboratory samples and/or analytical portions).

All the submitted residue trials are summarised in an advisory report that the evaluating authority must compose. But only the residue trials carried out according to the critical GAP may be used in the derivation of the MRL, STMR (supervised trials median residue) and HR (highest residue). From each residue trial carried out according to the critical GAP, only one residue value may be selected.

2.2 Use of the MRL, STMR and HR

For the benefit of the enforcement the MRL is used as ultimate limit to show that pesticides are not applied according to the legal instructions for use. The STMR and HR are not used in enforcement. The MRL is used as an ultimate limit that cannot be exceeded.

In the FAO manual ([2] chapter 8) is written with respect to the MRL:
"By definition an MRL is a limit not to be exceeded. The burden of proof is on the monitoring authority to establish, with a high degree of assurance, whether the residue in the lot being examined exceeds the MRL in order to make any regulatory actions."

To avoid rejection at a permitted application (according to GAP), it is desired for enforcement, to select the maximum residue value per residue trial for the derivation of the MRL. The choice for maximum residue values comes from the concern for the representativeness of the residue trials. The MRL is derived from a small number of residue trials (sometimes only 4-8 residue trials). Because this random check must be extrapolated to common use, the maximum residue value per residue trial is chosen. The MRL derived like this contains all possible worst case situations and exceeding the MRL indicates illegal use.

NB
For JMPR assessments the proposed MRL is always higher than the measured residue values in a specific crop, because the maximum residue value from a series of residue values is always rounded upwards. For NL and EU assessments a statistic calculation combined with rounding upwards or downwards is used so that the proposed MRL can lie below the actual measured maximum residue value. For NL and EU assessments the proposed MRL does not contain all possible worst case situations. However, usually rounding upwards is chosen if higher measured residue values are present in the series of selected residue values.

For the benefit of the risk assessment the MRL is only used to make a rough assessment of the chronic exposure of the consumer. If the ADI (acceptable daily intake) is exceeded, using the STMR refines the chronic exposure calculation. Next to this the STMR and HR are used to make a rough assessment of the acute exposure of the consumer. If exceeding of the ARfD (acute reference dose) is found, the acute exposure calculation may be refined by using a probabilistic method [5, 6, 7, 8].

For the initial risk assessment it is desired to start from the worst case situation and therefore it is desired to start from the maximum residue values per residue trial for the derivation of the MRL, STMR and HR. Because all residue values come from normal use, all risks are anticipated.

2.3 Selection of residue values from a residue trial

From §2.2 it becomes clear that for the selection of a residue value from a residue trial the selection of the maximum residue value per residue trial is preferred. If the worst case situation is really desired, the maximum residue value of a replicate analytical portion as well as from a replicate laboratory sample as well as from a field sample as well as from a replicate residue trial must be chosen.

The FAO manual [2] and the Lundehn document, appendix I [1] are in two minds about the selection of a residue value. For replicate residue trials the maximum residue value is selected, while for replicate analyses (= replicate laboratory samples and/or replicate analytical portions) the mean residue value is selected. In the FAO manual the mean residue value is selected for replicate field samples. It is not clear what the EU does with replicate field samples.

In the Lundehn document ([1] Appendix I § 3) is written with respect to the selection of residue values:
"*The mean figures of **replicate analyses** given in the residue reports are used for the calculations."*
*"The results from **replicated trials** should not be averaged (mean)."*

In the FAO manual ([2] Chapter 6) is written with respect to the selection of residue values:
"*The mean of the residues from replicate laboratory samples or replicate field samples should be taken as the single value for the trial, while the highest residue value from replicate plots or sub-plots or replicate trials should be taken as the single value for the purpose of identifying the STMR or HR value or recommending the maximum residue level.*"

In the above-mentioned quotation from the Lundehn document is said that replicate residue trials may not be averaged but there is no description what to do with them. Because the refined method of chronic risk assessment and with this the introduction of the concept STMR took place on international level (JMPR/CCPR), for the residue assessment of pesticides the definition of the STMR is used in principal like it was put by the JMPR. Therefore the above-mentioned quotation from the FAO manual also counts for the selection of the residue values for the NL and EU assessments (CTB assignment).

Based on the above the residue values for the evaluation on behalf of the JMPR and CTB (NL, EU) are selected as follows:

replicate residue trials:	maximum residue value;
replicate field samples:	mean residue value (FAO manual) no guidelines are given in the Lundehn document; §4.1 explains how to deal with this;
replicate laboratory samples:	mean residue value;
replicate analytical portions:	mean residue value.

In chapter 3 and 4 the concepts replicate residue trials, replicate field samples, replicate laboratory samples and replicate analytical portions are described in more detail.

3. Independent and replicate residue trials

3.1 Definition and presentation of independent residue trials

Residue trials are always seen as independent if one or more of the eight points mentioned hereafter apply (see Lundehn document [1] appendix D §3):

1. area of application: the area of application is different: open field, greenhouse (under glass or covered with plastic), climate chamber, storage room;
2. formulation: the formulations used are different: SC versus WP, SC 450 versus SC 500, or SC500 with different concentrations of adjuvantia;
3. application rate: the dose rate is different: 1.0 kg ai/ha, 2.0 kg ai/ha;
4. number of applications: the number of applications is different: 1 x 2.0 kg ai/ha, 2 x 1.0 kg ai/ha;
5. crop variety: the variety is different: Golden Delicious apple and Jonagold apple;
6. time of application: the time of application is different (see §3.3.1);
7. location: the geographical location is different (see §3.3.2);
8. application method: the application method is different (see §3.3.3).

Independent residue trials can be mentioned in different study reports, but also in the same study report. In an advisory report for JMPR or CTB (NL, EU) independent residue trials are presented in a table on different lines (see §6.2). For the derivation of the MRL, STMR or HR each separate residue value counts.

3.2 Definition and presentation of replicate residue trials

Residue trials carried out at the same time (see §3.3.1) on the same location (see §3.3.2) with the same equipment are seen as one residue trial with more replicates provided that none of the first five points mentioned under independent residue trials (see §3.1) apply.

For a post-harvest application a replicate residue trial is a trial where the same treatment is repeated once or several times on a different sample lot on the same location at the same application time (example 1).

Example 1 (post-harvest application)
With a drench treatment with thiabendazole (for storage) a tank is filled with a solution in which portions of oranges (basket, crate or truck container) are drenched (each time in a fresh solution). The treatment takes place in the same area on the same place. In this case drenched basket, crate or truck container can be seen as a replicate residue trial.

Samples stored in separate storage rooms that get a treatment as a whole can not be seen as a replicate residue trial, because each storage room has its own climate and therefore they are both seen as an independent residue trial.

Replicate residue trials are usually mentioned in the same study report but incidentally they are mentioned in different study reports

In an advisory report for JMPR or CTB (NL, EU) each separate residue value of a replicate residue trial is presented in the same table on the same line (see §6.2). For the derivation of the MRL, STMR or HR the maximum residue value per line is selected (see §2.3). However there are two other things that need attention while presenting the replicate residue trials: see §3.2.1 and §3.2.2.

3.2.1 Refreshing pesticide solution and calibration of equipment

In the Lundehn document ([1] appendix D § 2.3) is written with respect to replicate residue trials: *"Comparative trials at a single trial site must be organised in such a way that to the greatest possible extent genuinely comparable conditions can be expected."*

- The amount of active substance could decrease as a pesticide solution stands longer (e.g. by break down under the influence of sunlight or adverse pH conditions). This means that with every later spraying less active substance will be found on the treated crops. Refreshment of the solution can prevent this.
- With a post-harvest dip or drench application, the pesticide solution can contain less and less active substance, because it remains on the treated crops. Refreshment of the solution can prevent this.
- With dosing equipment set up once for all trials, each time the same (deviating) concentration can be sprayed, the set up of the equipment can elapse or the equipment can get clogged. This way more or less of the active substance then intended can get on the crop. Recalibration of the equipment prevents this.

It is desirable that with every replicate residue trial the solution is refreshed and the equipment is recalibrated. Refreshment of the solution and (re) calibration of the equipment takes care that comparable conditions (in this case the amount of active substance) per residue trial are guaranteed. If there are reasons to assume that these conditions are not guaranteed (because for example the residue value becomes lower after treatment), only those residue values, for which the same conditions are guaranteed, will be presented in the advisory report (usually only the first obtained residue value is presented, the other residue values are not mentioned). The above illustrates the necessity to select the maximum residue value for replicate residue trials.

3.2.2 Dutch residue trials with four repetitions carried out before 1993

In the past (before1991) a notifier could deliver residue trials with several repetitions (usually 4), for the admittance of a pesticide in The Netherlands. Each repetition of such a residue trial was counted as a separate residue trial. The introduction of the EU regulation on pesticides (directive 91-414/EC) has made an end to this way of trial set up and at the same time the requirements for the number of residue trials per crop became stricter with this directive. With the harmonisation of the residue limits (MRLs) a problem could develop for old substances for which admittance was honoured in The Netherlands based on only a few residue trials with 4 repetitions (not enough residue trials according to EU guidelines). Therefore an (oral) exception rule was included within the EU. This unwritten rule is described as follows:

Residue trials carried out before 1993 in The Netherlands with 4 repetitions count as 2 separate residue trials. The highest and the lowest residue value of these 4 replicate residue trials may be used for the derivation of the MRL.

This exception rule [9]:

- only counts for NL and EU assessments (CTB) and not for JMPR assessments.
- only counts for the Dutch residue trials carried out with 4 repetitions and not for a residue trial with 2 or 3 repetitions.
- only counts for those crops for which a permitted application only exists in The Netherlands.
  With the NL assessments it is not known if admissions also exist in other countries and therefore it is assumed in this case the admission only counts for The Netherlands.
  With the EU assessments it is known if admissions exist in other countries. If an admission does exist in another EU country, this exception rule does not count.
- does only apply when less than 8 (for major crops) or less than 4 (for minor crops and very minor crops) residue trials are available for that crop.

3.3 Problems in acknowledging replicate/independent residue trials

If residue trials are carried out on apparently the same location at the same application time with the same equipment, the description of the residue trials (either in the same study report or in different study reports) must be looked at critically, because sometimes the notifier has indicated some residue values as independent residue trials wrongly (this means: put on different lines in a table).

The following situations could occur if none of the first five points mentioned under independent residue trials (see §3.1) apply:

a) same location and same time and same equipment: replicate residue trials;

b) different locations and same time and same equipment: independent residue trials;
c) same location and different times and same equipment: independent residue trials;
d) same location and same time and different equipment: independent residue trials.

Because a replicate residue trial can never be carried out on the exact same location or at exactly the same time, the granting of a replicate or an independent residue trial strongly depends on the definitions of the same time and the same location. The granting of a replicate or an independent residue trial could also depend on what is understood by the same equipment.

3.3.1 Definition of the same time

In the FAO manual [2] (Chapter 3) a replicate residue trial is defined as:
"…. in close vicinity and treated on the same day with the same equipment using the same formulation at the same nominal rate."
In the Lundehn document no definition is given, different from what is mentioned in §3.2.1.

Based on the FAO definition the same time is defined as "on the same day".

If none of the first five points mentioned under independent residue trials apply, residue trials carried out on the same location on the same day with the same equipment are seen as replicate residue trials. Here it is assumed that the weather conditions in this short period of time do not differ much.

Example 1
If an application is carried out on the same location, but the time of application differs half a day, these residue trials are seen as replicate residue trials.

Example 2 (field trial)
Table 1 Example of a field trial with melons

Field	Landowner	Time of application	Actual rate (kg ai/ha)	Sample size
1	JVV	04-05-97; 17:30 u	0.9	5 melons
2	JVV	06-05-97; 7:30 u	0.9	5 melons

In this field trial the location is the same. Table 1 indicates that field 1 and field 2 are treated on different days.
Presentation: as independent residue trials.

3.3.2 Definition of the same location

In the FAO manual ([2] Chapter 3) a replicate residue trial is defined as:
"in close vicinity and treated on the same day with the same equipment using the same formulation at the same nominal rate."

In the Lundehn document ([1] appendix D § 2.3) only an indication is given with regard to the execution of residue trials in general:
"Owing to the largely unpredictable weather conditions, trials at several different sites, with a sufficient regional spread are necessary as a general principal."

Based on the FAO definition the location does not have to be exactly the same.

3.3.2.1 Definition of the same location for a field trial

With a field trial the same location is defined as "the location with the same place name". Because zip codes and addresses are usually not given in a study report and because it is impossible for a reviewer to check if different place names are or are not located in close vicinity, the same location is equal to a location with the same place name.

If none of the first five points mentioned under independent residue trials apply (see §3.1), the field trials with the same place name carried out on the same day with the same equipment, are seen as replicate residue trials.

However for pesticides absorbed by the plant via the soil (e.g. soil insecticides or herbicides) another exception situation applies. For the above-mentioned definition of the location with the same place name it is assumed that the soil conditions are not so different on such a short distance. The moment there are distinct differences in soil specimens, the residue trials may be seen as independent after all.

A soil specimen is characterised by the texture (according to USDA classification (see Appendix 3) based on %clay (particle size <2 μm), %silt (particle size 2-50 μm), %sand (particle size >50 μm)) and organic matter [1]. But depending on the physical and chemical properties of the pesticide the pH and/or the percentage clay can also be of importance. Regarding the pesticide's physical and chemical properties three cases can be distinguished [10]:

[1] If the organic material is expressed as organic carbon (org. C), the % of org. C must first be calculated to % organic matter (om) with the formula: A % org. C = (A*100/58) % om.

- pesticides without acid/base properties (no pK_a or K_a);
- pesticides with acid/base properties (pK_a = - log K_a given) that do bind to organic matter;
- pesticides with acid/base qualities (pK_a = - log K_a given) that do not bind to organic matter. Pesticides that do not bind to organic matter are not very common and usually contain a positive charge (NH_2 transformed to NH_3^+). In environmental assessments a correction is made for organic matter and a K_{om} is derived. In environmental assessments a so-called pseudo K_{om} or K is derived when pesticides do not bind to organic matter.

For pesticides without acid/base properties, clear differences in soil specimens are assumed if one or two of the points mentioned below apply:

1. the texture according to USDA classification is different (e.g. clay loam, sandy clay);
2. the percentage organic matter differs 0.5% or more. Soil specimens with percentage organic matter <0.5% and >15% are not relevant for The Netherlands.

For pesticides with acid/base properties that do bind to organic matter, clear differences in soil specimens are assumed if one or more of the points 1 till 3 apply:

3. the pH of the soil differs 0.5 units or more in the area of 2 pH units under the pK_a till 2 pH units above the pK_a of the pesticide (pK_a-2 <= pH <= pK_a+2). Some pesticides can have more than one pK_a, because they contain more acid or base groups; the pH stretch for differences in soil specimens becomes larger.

For pesticides with acid/base properties that do not bind to organic matter, clear differences in soil specimens are assumed if one or more of the points 1 till 4 apply:

4. the percentage clay difference is 5% or more. The percentage clay can be specified more precisely by the CEC (=cation exchange capacity). But because the CEC is seldom stated in residue trials, the percentage clay is used as criterion.

For an environmental assessment the information of four different soil specimens are needed with respect to biodegradability and sorption. From these trials one can deduce if the biodegradability and sorption depend on the soil specimen. The worst case soil specimen for bioavailability for plants cannot be derived from environmental information immediately (pesticide must remain in the soil, but must also be bio-available for plants). Therefore it is useful if some field trials are carried out on a number of extreme soil specimens, however specific requirements do not exist.

3.3.2.2 Definition of the same location for a greenhouse trial

With a greenhouse trial the same location is defined as “the same greenhouse”.
For practical reason, treatments in plastic tunnels, cold greenhouses and climate-regulated greenhouses are all seen as a greenhouse trial. Residue trials carried out in different greenhouses or plastic tunnels, even if they are situated on the same location, are seen as independent. Residue trials carried out in the same greenhouse, but where the greenhouse is divided in climate compartments that can be regulated separately, are also seen as independent. It is assumed that there is a different climate in each tunnel, greenhouse or separately regulated greenhouse compartment. If there is no specification if the residue trial was carried out in different tunnels, greenhouses or separately regulated greenhouse compartments, the location is assumed to be the same. Therefore the latter residue trials are seen as replicate residue trials.

3.3.2.3 Definition of the same location for a post-harvest application

With a post-harvest application the field location (=location where the crops are grown) does not count, only the location where the treatment took place (=treatment room). With a post-harvest application the same location is defined as “same treatment or storage room”.
Residue trials carried out in different rooms, even if these rooms are in the same building, are seen as independent. Here is it assumed that there is different equipment in every treatment and/or storage room and/or each storage room has a different (own) climate (if applicable).
If there is no specification if the residue trials were carried out in different treatment areas, the location is assumed to be the same. Therefore the residue trial is seen as replicate residue trial.

3.3.2.4 Definition of the same location for seed coating

For seed coating very often one batch of seed is coated with a pesticide and thereafter several residue trials are carried out with this single batch of treated seed. Because seed coating is carried out with high precision by professional seed coating companies, differences in concentration of the active substance will be negligibly small. Seed coating within the same company with the same equipment is therefore considered to be equal to seed coating with different equipment and/or different companies.

With seed coating the location where the seed was treated does not count, only the location where the seeds are sown. The same location is defined as “the field location with the same place name or the same greenhouse where the seeds are sown”.
When one batch of seed is sown at different field locations or in different greenhouses, this is seen as independent seed coating trials.
When one batch of seed is sown at different plots on a location with the same place name or in the same greenhouse this is seen as replicate seed coating trials.
When one batch of seed is sown in the same greenhouse and thereafter the seedlings are planted on different field locations after one month, this is seen as replicate seed coating trials. Here it is assumed that the conditions during cultivation of the seedlings are critical for

the concentration of the residues in the plants. During the cultivation period of the seedlings, the same location was used and therefore the trials are considered as replicate trials.

3.3.2.5 Examples

Example 1 (field trial)
Given a pesticide is not absorbed via the soil. If two residue trials are carried out on the same day in Bilthoven and in De Bilt, these residue trials are seen as independent residue trials. Bilthoven and De Bilt are situated in the same municipality and the places are situated next to each other, but the place names are different. Because a reviewer cannot know all the regional divisions, the place name is used as criterion.

Example 2 (field trial)
Given a pesticide is not absorbed via the soil. If two residue trials are carried out in Bilthoven on the same day, these residue trials are seen as replicate residue trials, even when the address and/or the owner and/or the soil specimens of each trial field are different.

Example 3 (field trial)
Given a pesticide is absorbed via the soil and this pesticide has no pK_a. Two residue trials are carried out on the same day. Field trial 1 consists of sandy soil with 1.0% organic matter and pH=4.5 and field trial 2 consists of sandy soil with 1.0% organic matter and pH=5.0. In this example the texture (here each sandy soil) and the percentage organic matter (here each 1.0%) are important. These are the same for both field trials and therefore the residue trials are seen as replicate residue trials.

Example 4 (field trial)
Given a pesticide is absorbed via the soil and this pesticide has a pK_a =5 and binds to organic matter. Two residue trials are carried out in Bilthoven on the same day. Field trial 1 consists of sandy soil with 1.0% organic matter and pH=4.5 and field trial 2 consists of sandy soil with 1.0% organic matter and pH=5.0. In this example the texture (here each sandy soil), the percentage organic matter (here each 1.0%) and the pH (difference 0.5 pH units) are important. Because the difference in pH is 0.5 pH units, the residue trials are seen as independent.

Example 5 (field trial)
Given a pesticide is absorbed via the soil and this pesticide has a pK_a=7 and does not bind to organic matter. Two residue trials are carried out in Lelystad on the same day. Field trial 1 consists of sandy clay with 2.0% organic matter and pH=6.5 and a percentage clay of 40% and field trial 2 consists of sandy clay with 2.0% organic matter, pH=6.5 and a percentage clay of 45%. In this example the texture (here all sandy clay), the organic percentage matter (here each 2.0%), the pH (here each 6.5) and the percentage clay (difference 5%) are important. Because the difference in the percentage clay is 5%, the residue trials are seen as independent.

Example 6 (field trial)
Given a pesticide is absorbed via the soil and this pesticide has a pK_a=7 and binds to organic matter. Two residue trials are carried out in Lelystad on the same day. Field trial 1 consists of sandy clay with 2.0% organic substance and pH=6.5 and a percentage clay of 40% and field trial 2 consists of sandy clay with 2.0% organic matter, pH=6.5 and a percentage clay of 45%. In this example the texture (here all sandy clay, the organic percentage matter (here 2.0% each) and the pH (here 6.5 each) are important. There is no difference and therefore the residue trials are seen as replicate residue trials.

Example 7 (greenhouse trial)
Report 1 and report 2: The trial was carried out in the municipal area of Lucena del Puerto, in the province of Huelva, on the property known as “E F” which belongs to Mr. J M D.
On closer examination of the two reports, the macro tunnels of report 1 and report 2 appear to be situated next to each other. There is a difference of 30 min in time between the treatment of the macro tunnel in report 1 and 2.
In this greenhouse trial the location is different, because the treatment is carried out in two different macro tunnels. In each macro tunnel there can be a different climate and therefore the residue trials are considered as independent.

Example 8 (field trial)
“The experimental design of the trial was replicated with two repetitions. The two sprayed plots were separated from the non sprayed controls with a plastic sheet, the distance between the sprayed plots and the controls was 25 m. A sample of 5 melons of every sprayed and non sprayed plot was collected.”
In this field trial the location is the same and therefore the trials are seen as replicate residue trials.

Example 9 (field trial)
Report 1 and report 2: The trial was carried out in the municipal area of Bonares, in the province of Huelva, on the property known as “E P” which belongs to Mr. A M M.
A road separates the plots from report 1 and report 2. There is a difference in time of one hour between the treatment of plots in report 1 and report 2.
The fields are separated widely from each other but are situated on the land of the same landowner and therefore situated on a location with the same place name. Therefore the location is the same. The spraying is carried out on different times but on the same day. Therefore the application time is the same.
Presentation: as replicate residue trials.

Example 10 (post-harvest application)
The trial was carried out at the Arcosegre centre in Sudanell. Samples were taken at random from 2-3 kg of fruit for each of the three treatments. Residues: 1.64, 1.73, 2.16 mg/kg.
Each time a sample lot of 2-3 kg is treated in the same treatment area and from each sample lot one “field sample” is taken. There is no explicit indication if the treatments took place on the same day and therefore it is assumed that the application time is the same.
Presentation: as replicate residue trials

Example 11 (post-harvest application)
Fruit was collected from two sites in the major avocado producing regions of Costa Rica. The treatment location in Dulce Nombre was used. A bulk sample of at least 240 fruits was collected from each site. Each bulk sample was divided into sub-samples of 24 fruits. For the spray mist application, 24 fruits, representing a sample, were placed on a suspended wire mesh tray. Spray was applied to the bottom and over the top of the tray.

Table 2 Example of a post-harvest application on avocados

Trial number	Field location	Treatment location	Residues
3006R	1	Dulce Nombre	8.9 mg/kg
3008R	3	Dulce Nombre	7.0 mg/kg

In this example the fruit (same variety) comes from two different field locations. However the treatment of each sample lot (here 24 avocados) took place in the same treatment area. The samples of different field locations were placed behind each other on a wire mesh tray and were treated with the same spraying equipment. The origin of the fruit does not matter here and the location is seen as the same. Because the exact application time is not mentioned, it is assumed that the application time is the same.

Presentation: as replicate residue trials.

Example 12 (seed coating)
A commercial seed coating company coated 1 kg of lettuce seeds with 1.1 kg 70WS formulation. After 19-26 days coated seeds were sown in nursery pots and placed in an air conditioned chamber for 3 days. Then pots were placed in a greenhouse for 18 days before transplanting in the field on 4 different locations. Mature lettuce head samples were collected 72 days after sowing.
In this example the seeds were sown in the same greenhouse and transplanted thereafter in the field. The 4 different field locations are seen as replicate field trials, because the cultivation of the seedlings (on the same location) is seen as critical step for residue levels. Presentation: as replicate seed coating trials.

3.3.3 Definition of the same equipment

In the FAO manual ([2] Chapter 3) a replicate residue trial is defined as:
"in close vicinity and treated on the same day with the same equipment using the same formulation at the same nominal rate."

In the Lundehn document ([1] appendix D § 2.3) the following is mentioned about a replicate residue trial:
"Comparative trials at a single trial site must be organised in such a way that to the greatest possible extent genuinely comparable conditions can be expected."

The same equipment is defined as "equipment for the same application method".

If none of the first five points mentioned under independent residue trials (see §3.1) apply, residue trials carried out on a location with the same place name on the same day with equipment for the same application method, are seen as replicate residue trials.

Here the assumption is made that the residue values are comparable for this equipment. In this case the making of a (between times) fresh spraying solution is not seen as a reason to expect other residue values (see §3.2.1). If it can be made plausible that more active substance per crop is expected with a specific type of equipment, because the equipment is more efficient than other equipment that is also used for the same application method, the residue trials are seen as independent
In this case for example manual spraying with a backpack sprayer, mechanical spraying with a boom sprayer, a drift limited spraying with a tunnel sprayer and spraying with an aeroplane are seen as different equipment. Also equipment for ultra low volume spraying and high volume spraying is seen as different equipment. But also the soil treatment method for soil insecticides can make a difference: granulate sprinkled on unprocessed ground, granulate tilled 5 cm deep, or granulate tilled 10 cm deep is seen as different application methods.

Example 1 (field trial)
Two residue trials were carried out on the same day in Bilthoven, but field trial 1 is sprayed with the spraying equipment of farmer A and field trial 2 is sprayed with (almost) the same spraying equipment of farmer B. The equipment is seen as the same. Because the location, the application time and the equipment are the same, the residue trials are seen as replicate residue trials.

Example 2 (field trial)
Two residue trials were carried out on the same day in Bilthoven, but field trial 1and field trial 2 are sprayed with spray solutions that were made on different times. Because refreshment of a spraying solution is seen as a condition for replicate residue trials (§3.2.1), the residue trials are seen as replicate residue trials.

Example 3 (field trial)
Two residue trials were carried out on the same day in Bilthoven, but field trial 1 is sprayed with a tunnel sprayer and field trial 2 is sprayed with normal spraying equipment. Even though spraying is used in each trial spraying with a tunnel sprayer is more efficient than the one with normal spraying equipment. The equipment is not the same and therefore the residue trials are seen as independent residue trials.

Example 4 (field trial)
"The experimental design of the trial was replicated with two repetitions. The two sprayed plots were separated from the non sprayed controls with a plastic sheet, the distance between the sprayed plots and the controls was 25 m. A sample of 5 melons of every sprayed and non sprayed plot was collected."
The study report indicated that a fresh solution was made for every plot.
In this field trial the location is the same. There is an explicit indication that the spraying was carried out with a fresh solution. There is no explicit indication that the same equipment was used.
Presentation: as replicate residue trials.

Example 5 (field trial)
"The trial plot was located in the area of La Mojonera, (Almería, Spain) and the landowner was A L M. Application 04-05-97; 19.00 u; 1.804 L product/ha. A sample of five melons was collected"
"The trial plot was located in the area of La Mojonera, (Almería, Spain) and the landowner was J V V. Application 04-05-97; 17:30 u; 1.824 L product/ha. A sample of five melons was collected"
The treatment was carried out in the same region on the land of different owners. The place name is the same and therefore the location is the same. The soil specimens are not indicated. There is a difference in time in the applications but the applications were carried out on the same day. Therefore the application time is the same. There is no explicit indication that a fresh solution or different equipment was used. What does differ a little is the amount of product per hectare: it is assumed that this is the consequence of making a fresh solvent.
Presentation: as replicate residue trials (comparable with example 2 from §3.3.2 and example 1 from §3.3.3).

Example 6 (post-harvest application)
Fruit was collected from a major avocado-producing region of Costa Rica. The treatment location in Dulce Nombre was used. A bulk sample of at least 240 fruits was collected. Each bulk sample was divided into sub-samples of 24 fruits. A fresh dip solution was prepared for each sample.

Table 3 Example of a post-harvest application with avocados

Trial number	Field location	Treatment location	Residues
3007R	3	Dulce Nombre	6.2 mg/kg
3008R	3	Dulce Nombre	4.8 mg/kg

In this example the fruit (same variety) comes from the same field location and the treatment of each sample lot (in this case 24 avocados) took place in the same treatment area. The location is therefore the same.

Each sample was treated separately with a fresh dip solution. Because the making of a fresh solution is a condition for guaranteeing the same circumstances per trial, the application method is the same. The exact application time is not mentioned and therefore it is assumed that the application time is the same.
Presentation: as replicate residue trials.

4. Replicate field samples, laboratory samples and analytical portions

With sampling three types of samples are distinguished: field samples, laboratory samples and analytical portions.

4.1 Definition and presentation of replicate field samples

A field sample is a representative sub-sample from a treated field or a treated sample lot (with post-harvest applications before or during storage).
With replicate residue trials, a representative sample is taken per field or per sample lot (with post-harvest application). Field samples obtained this way may not be mixed but must undergo further handling and analysis as separate samples
With replicate field samples, a representative sample is taken several times from a specific field, a specific sample lot or a specific storage room. Replicate field samples may not be mixed but must undergo further handling and analysis as separate samples.

Representative means that the field sample comes from the entire field, the entire sample lot or the entire storage room, either by a sampling scheme or by random sampling. Guidelines for a sampling scheme are given in the Lundehn document (appendix B [1]) guidelines regarding the sample size and the crop parts that have to be sampled are mentioned in the FAO manual (appendix V and VI, [2]), the FAO guidelines [3] and the Lundehn document (appendix B [1])

A (replicate) field sample may not come from a specific part of the field or from a specific part of the sample lot or the storage room. However this is sometimes the case. To make a distinction two names are being used:

- at random obtained replicate field samples (called replicate field samples by FAO): here the separate sample is representative for the entire field or the entire sample lot.
- non-random obtained replicate field samples (called replicate sub-plots or split-plots by FAO): the sampling is not carried out according to guidelines of the FAO manual or the Lundehn document. With non-random obtained field samples the field is (after treatment) divided into two or more compartments and from each compartment a separate sample is taken. Alternatively the samples come from specific parts of a sample lot or a storage room. Here an individual sample is not representative for the entire field or for the entire sample lot.

Replicate field samples are necessary in situations where much intra-trial variation is expected like with the sampling of fruit trees, sampling of greenhouses and with the sampling of large storage rooms. Especially for sample lots treated in storage rooms, replicate “field”

samples are very important because there can be a difference in residue value from samples coming from the top, middle, bottom, front and back in the storage room.

Field samples from certain crops undergo a pre-treatment. For example with root and tuber vegetables the sand may be removed with a brush or by washing with tap water. Pre-treatment procedures are described in the FAO manual, appendix VI [2] and the Lundehn document, appendix B [1].

In the FAO manual [2] the mean residue value is selected for randomly obtained replicate field samples and the maximum residue value is selected for non-randomly obtained replicate field samples (replicate sub-plots). It is not clear what the EU does with replicate field samples.

An argument to choose for the maximum residue value is that the replicate field samples can tell just as much about the variability from a treatment as replicate residue trials. Often replicate field trials are taken if a lot of variability is expected, for instance if the treatment is inhomogeneous (e.g. spraying of large fruit trees with many leaves) or if the field samples are small in proportion to the field.

In the FAO guidelines [3] (part 3; §3.1) is written:
"In selecting sampling points and/or the sampling method, all factors that control the residue distribution over the entire experimental plot must be considered. [….] The samples must be representative to enable the analytical result to be applied to the entire experimental unit. The greater the number of plants sampled in a field plot, the more representative the sample will the sample be. However economics and the practical problems involved in handling large samples affect the magnitude of the sampling programme. The size of sample suggested is the minimum that experience has shown is needed to give a representative, valid sample"

The quotation mentioned above indicates that the size and the number of field samples are connected with the size of the field. However a relation was not included: only the minimum size of the sample was included (see Lundehn document, appendix B [1] and the FAO manual, appendix V [2]). This means that with replicate field samples of for instance 2 kg each, more variation is expected if the samples come from a 10,000 m^2 field than samples coming from a 100 m^2 field. To get a representative residue value for the larger field more (random) field samples are necessary and the mean must be taken from these (random) field samples.

In the FAO guidelines [3] (part 3, §2.1.3) is written with regard to the variability of replicate residue trials:
"Since the variations in residue levels between replicates at individual sites are small compared with those found in data from different sites, it is usually not necessary to replicate treatments at individual sites. However it is useful to have three or four replicates at one site to study experimental uniformity and determine the within-site variations."

Something like this is also written in the Lundehn document ([1] appendix B §5.3)
"Duplicate trials carried out at the same site are useful but experience shows that intra-site variations in residue levels are smaller than inter-site variations in levels."

In the FAO manual [2] (Chapter 3) is written:
"… the variability of residues within a store (i.e. intra-store variability) can be particularly high, for instance in situations such as fogged potatoes in box stores. For this reason sampling procedures must be designed to obtain a sample representative of the lot."

In the FAO guidelines [3] (part 3, §3.1.2) is written:
"In certain cases where there is likely to be considerable within-plot variation, such as orchard and glasshouse trials, there should be at least three sample replicates per plot at or near harvest. Sample integrity should be maintained throughout to leave it to the discretion of the analyst to analyse individual or composite samples"

The most important consideration is that the field samples must represent the entire plot. Representativeness is only obtained if an average is taken of the results from the field samples. Non-randomly obtained field samples (from replicate sub-plots) can still be representative for the entire field if all field samples together cover the entire field so that after taking the average of the results a representative residue value for the entire field can be obtained. Conclusion: select the mean residue value per residue trial for the randomly as well as for the non-randomly obtained field samples.

This conclusion is different from the FAO manual [2, Chapter 6] where the mean residue value is selected for randomly obtained replicate field samples and the maximum residue value is selected for non-randomly obtained replicate field samples (replicate sub-plots). However, we feel that the mean residue gives the most representative value for the entire field or sample lot.

An exception to this rule is a fogging treatment in box store. Potatoes can be treated with a fogging treatment (such as chlorpropham or carvone) in bulk stores as well as in box stores. From such treatments it is known that the residue values between the top and bottom stored potatoes are not equal. With the bulk store treatment the potatoes are mixed before they go to the consumer. Potatoes coming from bulk store are first assembled in a shoot and are then transported to a distribution machine by an assembly line. As an alternative the potatoes are

dumped in a truck and are divided into little bags at a distribution centre. The chance that a consumer gets potatoes from a specific part of this storage is negligibly small [11]. With bulk store treatment the mean residue value per residue trial is selected for randomly as well as non-randomly obtained field samples. For box store treatment the choice for the maximum or the mean or the residue value depends on the way the potatoes are distributed after treatment. In The Netherlands potatoes coming from box store are treated the same way as potatoes coming from bulk store [11]. In other countries (for instance the USA) this is not the case and potatoes are divided from the treated boxes into the bags. Therefore the chance that a consumer gets potatoes from a specific part of storage in a bag is not negligible.

Because of the uniformity within the EU and because the distribution of potatoes after box store treatment is not known for other countries outside The Netherlands, the maximum residue value per residue trial is selected for box store treatment for all assessment levels (CTB (NL, EU) and JMPR (worldwide)), for randomly as well as non-randomly obtained field samples. A non-random sampling is preferred with the box store treatment.

If there is a lot of variation between the field samples a remark must be made about this in the text of the advisory report. Variation is usually expressed as relative standard deviation (=s/mean), also called coefficient of variation. A relative standard deviation (RSD) of >40% indicates that the variation is quite large.

In the FAO manual ([2] Chapter 8) is written with regard to the relative standard deviation: *"The experiments show that, on average, the expected minimum coefficient of variation of residue results of supervised trials is around 0.3-0.4 (=30%-40%). In this estimate the variation of replicate analyses accounted for only 10%."*

Replicate field samples are always mentioned in the same study report. In an advisory report for the JMPR or CTB (NL, EU) the separate residue values as well as the mean residue value of a replicate field sample are presented on the same line. For the derivation of the MRL, STMR and HR the mean residue value is selected (see §5.1 en §6.2).

Example 1 (post-harvest application)

Application:	At the packing house A M C in Carcer (Valencia). A commercial drencher was used which is designed for drenching a stack of 36 boxes per cycle (700 kg; 04 December 1998; batch number 547/1).
Specimen collection:	Two specimens (3.0 and 2.8 kg), picked randomly by hand (24 fruits per specimen).
Residues:	sub-specimen 1: 3.27 mg/kg; sub-specimen 2: 4.62 mg/kg.

This concerns two replicate field samples from one large sample lot that has had the same treatment in one time.
Presentation: as randomly obtained replicate field samples

Example 2 (bulk store treatment of chlorpropham on potatoes)
In this example potatoes are treated in bulk store (on a large heap) with a fogging product. Potatoes were sampled from the bottom (b), in the middle (m) and from the top (t). Each sample was analysed separately. The results are stated in table 4.

Table 4 Example of a bulk store treatment from potatoes

Results per residue trial	Mean	Selected residue value	RSD
6.2 (b); 5.0 (m); 3.5 (t)	4.9	4.9	28%
5.3 (b); 4.2 (m); 1.4 (t)	3.6	3.6	56%
8.3 (b); 4.6 (m); 3.3 (t)	5.4	5.4	48%

In this example a non-random sampling was carried out. From this sampling it becomes clear that the treatment was not homogeneous. But with fogging this can be expected (also see the remark mentioned above from the FAO manual [2]). Because there is little chance that the consumer will get a bag of potatoes only from the bottom of the heap, the mean residue value is selected for the derivation of the MRL.

Example 3 (box store treatment of chlorpropham on potatoes)
In this example potatoes in box store (in separate boxes) are treated with a fogging product. Potatoes were sampled from boxes that were on the bottom (b), in the middle (m) and on top (t) of the pile. Each sample was analysed separately. The results are stated in table 5.

Table 5 Example of a box store treatment of potatoes

Results per residue trial	Mean	Selected residue value	RSD
6.2 (b); 5.0 (m); 3.5 (t)	4.9	6.2	28%
5.3 (b); 4.2 (m); 1.4 (t)	3.6	5.3	56%
8.3 (b); 4.6 (m); 3.3 (t)	5.4	8.3	48%

In this example a non-random sampling was carried out. From this sampling it becomes clear that the treatment was not homogeneous. But with fogging this can be expected (also see the remark mentioned above from the FAO manual [2]). Because there is a chance that the consumer will get a bag of potatoes only from the bottom of the pile, the maximum residue value is selected for the derivation of the MRL.

4.2 Definition and presentation of replicate laboratory samples

A laboratory sample is a representative sub-sample of the field sample; in most cases the laboratory sample is just as large as the field sample. While making a laboratory sample the product items from which the field sample is build, must remain intact. A laboratory sample may not have had a treatment, such as cutting or grinding at the moment of receipt at the laboratory.

In the FAO guidelines [3] (part 3 §6) is written with regard to laboratory samples:
"Ideally, the field sample should be submitted intact for analysis. The requirements of the analyst should not influence the sampler to take a smaller sample than is necessary for a valid field sample. In practice, a valid field sample is often much larger than the sample needed by the analyst and cannot be handled economically […]. In such cases, a reduction in the size of the field sample is desirable. […] For samples consisting of small units, such as cereal grains or small fruit, there is little difficulty in valid sample reduction. […] With samples of medium sized products such as beans and peas in the pod, there is an increased risk of losing sample validity by sample reduction […]. The problem of maintaining sample validity during sample size reduction is greater with large fruit and vegetables such as cabbage or melons."

In connection with contamination the preparation of a laboratory sample must take place in an area arranged especially for this purpose (neither in the field, nor in the analytical area). The minimum demands for the sample size for the laboratory sample are mentioned in the Lundehn document, appendix B [1].

With a replicate laboratory sample a representative sub-sample is taken several times from a field sample. It is not common to take a replicate laboratory sample for registration purposes (residue trials). The laboratory sample will be send to the laboratory in labelled bags or containers immediately after the sample is taken (cooled or in frozen condition) or the laboratory sample will be stored first (in the deep-freezer) and will then be send (in frozen condition) to the laboratory.

Replicate laboratory samples are always presented in the same study report. In an advisory report for JMPR or CTB (NL, EU) the mean residue value of replicate laboratory samples is presented in a table and is used for further calculations (see §5.1 and §6.2).

4.3 Definition and presentation of replicate analytical portions

At the laboratory each laboratory sample is handled further as a whole. The further handling of the laboratory sample successively results in an analytical sample, a sample homogenate and in (replicate) analytical portions.
Analytical sample: First sample preparation takes place: for instance removing stones from stone fruits, removing sand from potatoes, removing dead or rotten leaves from lettuce or removing the top of carrots. The sample that is left behind after sample preparation is called the analytical sample [12, 13]. From one laboratory sample only one analytical sample can be obtained.
Sample homogenate: The analytical sample is then homogenised as a whole (so a sub-sample may not be taken from it before the sample is homogenised) by mixing, chopping and

grinding. The obtained sample is called a sample homogenate [12, 13]. Always only one sample homogenate is obtained from a laboratory sample.
Analytical portion: Then a representative sub-sample is taken from a homogenised analytical sample; this is the analytical portion. The size of the analytical portion depends on the homogeneity of the homogenised analytical sample (this must be determined experimentally), but for an acceptable sampling reproducibility an analytical portion of at least 30 g is required [14]. With replicate analytical portions (analytical replicates) a representative sub-sample is taken several times from the sample homogenate.
Each analytical portion is handled separately (i.e. extraction, clean-up, concentration). This concentrated extract is injected once or several time (replicate injections) on a GC column (gas chromatography) or a HPLC column (high performance liquid chromatography). With replicate injections the mean result counts as the result of one analytical portion.

Replicate analytical portions are always mentioned in the same study report. In an advisory report for JMPR or CTB (NL, EU) the mean residue value of the analytical portion is presented in a table and is used for further calculations (see §5.1 and §6.2).

Example 1
A laboratory sample is divided into different portions (=analytical portions); each portion is handled and analysed separately. Residue values: 1.89-2.00-1.39-1.51 mg/kg
In this example a laboratory sample is analysed in quadruplet, therefore these are replicate analytical portions.
Presentation: as replicate analytical portions (the mean residue value).

5. Special cases

5.1 Combined repetitions

A special case exists if on a specific residue trial more repetitions are carried out, thus replicate residue trials, replicate field samples, replicate laboratory samples and/or replicate analytical portions. For selection of the final residue value, the calculation is performed in reverse order. First an average of the results of the replicate analytical portions is taken per laboratory sample. Then the average of the results of the replicate laboratory samples is taken per field sample. After that the average of the results of the replicate field samples is taken per replicate residue trial. Then the maximum residue value of the replicate residue trials is selected for the MRL dataset. This calculation is presented schematically in table 6.

Table 6 Schematic presentation of calculation and selection of residue values at combined repetitions

Replicate analytical portions	Replicate laboratory samples	Replicate field samples	Replicate residue trials	Selected residue value
A11a; A11b; A11c	$A11_{mean}$	$A1_{mean}$	A_{mean}	maximum (A,B)
A12a; A12b; A12c	$A12_{mean}$			
A13a; A13b; A13c	$A13_{mean}$			
A14a; A14b; A14c	$A14_{mean}$			
A21a; A21b; A21c	$A21_{mean}$	$A2_{mean}$		
A22a; A22b; A22c	$A22_{mean}$			
A23a; A23b; A23c	$A23_{mean}$			
A24a; A24b; A24c	$A24_{mean}$			
B11a; B11b	$B11_{mean}$	$B1_{mean}$	B_{mean}	
B12a; B12b	$B12_{mean}$			
B13a; B13b	$B13_{mean}$			
B14a; B14b	$B14_{mean}$			
B21a; B21b; B21c	$B21_{mean}$	$B2_{mean}$		
B22a; B22b; B22c	$B22_{mean}$			
B23a; B23b; B23c	$B23_{mean}$			
B24a; B24b; B24c	$B24_{mean}$			

Example 1 (post-harvest application)

Application:	At the packing house A in Picassent a commercial drencher was used which is designed for drenching whole truck loads (600 kg; batch number 547/1).	
Date of application:	report 1: 07 December 1998.	
Date of application:	report 2: 02 December 1998.	
Specimen collection:	Two specimens picked randomly by hand (12 fruits per specimen).	
Residues:	report 1; sub-specimen 1 (2.0 kg; 09 December 1998):	1.96 mg/kg;
	report 1; sub-specimen 2 (1.8 kg; 09 December 1998):	2.16 mg/kg;
	report 2; sub-specimen 1 (2.1 kg; 04 December 1998):	3.66 mg/kg;
	report 2; sub-specimen 2 (2.2 kg; 04 December 1998):	2.68 mg/kg.

The treatment of report 1 and report 2 is carried out on the same location. The treatment of report 1 and report 2 took place on different days. Because the application time differs report 1 and report 2 may be considered as independent residue trials. Per report sub-specimen 1 and 2 are considered as replicate field samples.

Calculation: mean of 1.96 and 2.16 = 2.06 = 2.1 (rounded) selected residue value report 1;
mean of 3.66 and 2.68 = 3.17 = 3.2 (rounded) selected residue value report 2.
Presentation: per report as replicate field samples (i.e. separate residue values plus mean)

Example 2 (post-harvest application)

Application:	At the packing house A M C in Carcer (Valencia). A commercial drencher was used which is designed for drenching a stack of 36 boxes per cycle (700 kg; 04 December 1998; batch number 547/1).	
Specimen collection:	Two specimens picked randomly by hand (24 fruits per specimen).	
Residues:	report 1; sub-specimen 1 (3.0 kg; 04 December 1998):	3.27 mg/kg;
	report 1; sub-specimen 2 (2.8 kg; 04 December 1998):	4.62 mg/kg;
	report 2; sub-specimen 1 (1.7 kg; 04 December 1998):	2.83 mg/kg;
	report 2; sub-specimen 2 (2.4 kg; 04 December 1998):	4.39 mg/kg;

The results from report 1 (sub-specimen 1 and 2) probably come from a different drench round than the results from report 2 (sub-specimen 1 and 2). The results of report 1 and report 2 are obtained on the same day at the same location, therefore the results of report 1 and report 2 must be seen as two replicate residue trials. Sub-specimen 1 and 2 are seen as replicate field samples per report. There is no explicit indication that different solutions are used (the batch number of the pesticide is the same, per sub-specimen as well as per report), but there is also no clear decreasing trend between the four results, therefore all the results may be used for the calculation.
Calculation: mean of 3.27 and 4.62 = result report 1 = 3.945;
mean of 2.83 and 4.39 = result report 2 = 3.61;
maximum of 3.945 and 3.61 = 3.945 = 3.9 (rounded) selected residue value.
Presentation: as replicate field samples (per report) and as replicate residue trials (two reports). This means four separate residue values, of which the maximum is underlined.

5.2 Significantly different repetitions

Which residue values must be selected if one or more results of the replicate analytical portions, replicate laboratory samples, replicate field samples or replicate residue trials appear to be significantly different from the remaining results of the same residue trial?

With replicates it usually concerns a limited number of figures (usually 2-4 residue values), so that a statistic outlier test (e.g. Dixon's Q-test, Grubb's test, Sign test, Wilcoxon signed rank test) or a statistic test on differences in population (e.g. Student's t-test, ANOVA, Mann-Whitney U test, Cochran's maximum variance test) is not very useful because of the wide confidence interval with a low number of observations. Therefore the starting point in residue assessments is that the possible differences in results represent the natural variation.

- If the author of the study report does not make a remark about outlying results, all residue values are used. This means the selection of the mean of all the results with replicate analytical portions, replicate laboratory samples and replicate field samples and selection of the maximum residue value with replicate residue trials.
- If the author of the study report indicates that something went wrong with the analyses or sampling of a specific analytical portion, a specific laboratory sample or a specific field

sample, the relating residue value is not used for further calculation of the mean residue value, presented in the table.

- If the author of a study report indicates that something went wrong with the analysis, sampling or treatment (e.g. dose rate, number of applications, equipment adjustment) the relating residue value is not presented in the table.

Example 1 (post-harvest application)
From a treated sample lot two field samples are taken; one sample goes to laboratory X, the other one goes to laboratory Y. In each laboratory the sample is analysed several times.
Residue value laboratory X: 1.5-1.6-1.7-1.7-1.7-1.7-1.9-2.0 mg/kg: mean 1.7 mg/kg.
Residue value laboratory Y: 2.3-2.5-2.6-2.7 mg/kg: mean 2.5 mg/kg.
In this example 8 analytical portions per field sample were taken in laboratory X and in laboratory Y 4 analytical portions per field sample were taken and analysed. All residue values from laboratory Y are higher than the ones from laboratory X. The author of the study report did not indicate if something went wrong with the analysis of sampling in laboratory X or laboratory Y. This means that there is no reason to reject residue values from one or both laboratories. Presentation: as replicate field samples (mean of 1.7 and 2.5).

Example 2 (field trial; JMPR 1994; captan on mandarins)

Table 7 Significantly different residue values between samples coming from laboratory A and B

Trial	DAT	Captan (mg/kg), laboratory A	Captan (mg/kg), laboratory B
Spain, 1989	0	0.80; 0.63; 0.72; mean 0.72	0.37; 0.34; 0.40; mean 0.37
	7	0.68; 0.59; 0.62; mean 0.63	0.25; 0.28; 0.25; mean 0.26
	14	0.55; 0.56; 0.52; mean 0.54	0.20; 0.18; 0.24; mean 0.21
	21	0.57; 0.57; 0.55; mean 0.56	0.15; 0.17; 0.22; mean 0.18
	28	0.48; 0.40; 0.36; mean 0.41	0.19; 0.12; 0.15; mean 0.15
	49	0.15; 0.24; 0.28; mean 0.22	0.13; 0.20; 0.11; mean 0.15

In this example two field samples per DAT are taken: one sample goes to laboratory A, the other sample goes to laboratory B. On each laboratory the samples were analysed in triplet. In this example 3 analytical portions per field samples were taken and analysed in each laboratory. For every DAT the residue values of laboratory A are higher than the residue values of laboratory B. The author of the study report did not indicate if something went wrong with the analysis of sampling at laboratory A or laboratory B. This means that there is no reason to reject residue values from one of the laboratories.
Presentation: as replicate field samples per DAT (mean of two mean residue values per DAT).

5.3 Selection of residue values in a decline study

A decline trial has the purpose to study the fate of the substance in the living plant on several time points. There are two ways to conduct a decline trial:

- Normal residue decline study. A large field is treated once and after that divided in parts. Each part is harvested at a different time after treatment (see example 1). This decline trial has the advantage that the treatment has been equal for all plots, but has the disadvantage that the harvest time has not been optimal for all samples (e.g. unripe or too small crops on DAT=14 and overripe crops on DAT=28).
- Reverse residue decline study. Different plots are pointed out and are treated on different days before harvest so that the day of harvest is equal for all plots (see example 2). This

decline trial has the advantage that the crops are harvested on the same time, but has the disadvantage that the treatment per trial can be different (e.g. due to weather conditions).

A decline study is seen as one residue trial; the residue values are presented per PHI (pre-harvest interval) or DAT (days after treatment). Although in the first example it is clear that it only concerns one independent residue trial (treatment on the same day) in the second example you could say that it concerns more independent residue trials (treatment on different days). In the table, mentioning the date(s) of treatment indicates the differences between both decline studies.

Example 1

Dates of application:	August 19, 1982		
Date of sampling	September 2, 1982 (A); September 9, 1982 (B); September 16, 1982 (C)		
Analytical results:	A time interval 14 days: 0.098 mg/kg		
	B time interval 21 days: 0.066 mg/kg		
	C time interval 28 days: 0.062 mg/kg		
Presentation:	as a decline trial within the same line of the table		
	Treatment date 19-08-1982	DAT = 14	0.098 mg/kg
	Treatment date 19-08-1982	DAT = 21	0.066 mg/kg
	Treatment date 19-08-1982	DAT = 28	0.062 mg/kg

Example 2

Dates of application:	August 5, 1982 (A); August 12, 1982 (B); August 19, 1982 (C)		
Date of sampling	September 2, 1982		
Analytical results:	A time interval 28 days: 0.062 mg/kg		
	B time interval 21 days: 0.066 mg/kg		
	C time interval 14 days: 0.098 mg/kg		
Presentation:	as a decline trial within the same line of the table		
	Treatment date 19-08-1982	DAT = 14	0.098 mg/kg
	Treatment date 12-08-1982	DAT = 21	0.066 mg/kg
	Treatment date 05-08-1982	DAT = 28	0.062 mg/kg

With each type of decline study only one residue value is selected: the highest residue value within the defined PHI interval. If there is no definition of the PHI, the highest residue value from a decline study is selected.

If the PHI is established as for example 7 days, only the residue values belonging to a PHI interval of 7±25% = 5-9 days may be selected. In table 8 this would be two residue values per residue trial (i.e. PHI = 5 and PHI = 7 days). But also in this case only one residue value is selected, the maximum residues value.

Table 8 Examples of residue trials, whereby two residue values lie in the selected PHI interval

Residue trial	PHI	parent (mg/kg)	
Trial of glyphosate on wheat straw (EU-monograph); PHI = 7 ± 25%[a] (= 5-9) days	0	10.9	
	5	15.8	selection of highest residue value within PHI interval
	7	13.1	
Trial of diflubenzuron on orange (FAO 2002); PHI = 30 ± 30%[a] (= 21-39) days	0	0.38	
	7	0.28	
	14	0.36	
	21	0.32	selection of highest residue value within PHI interval
	28	0.27	

a. Trials that fall within the 25% limit (FAO 30%) of the shortest PHI as defined in the instructions for use may be seen as carried out according to the critical GAP (see §6.2)

With a decline study the thought is that the residue values from a longer PHI are lower than the ones from shorter PHIs. However it sometimes can happen that within one residue trial a residue value from a longer PHI is higher than the residue values from the selected interval (see table 9). In that case the highest residue value is chosen, also if the PHI lies outside the selected interval.

Table 9 Example of a residue trial, where residue values from a lower PHI are higher than the one in the selected PHI interval ((figures adopted from EU monograph glyphosate on wheat)

Residue trial	PHI	glyphosate (mg/kg)	
Trial 2; PHI = 7 (5-9); grain	0	2.81	
	3	3.42	
	5	3.54	
	7	3.86	
	10	4.25	
	14	5.44	selection at a higher PHI
	21	4.01	

5.4 Selection of residue values for mushrooms that are harvested several times

With a mushroom cultivation the compost is first inoculated with the desired mushroom culture. After about 2 weeks the compost is covered with a casing. Mushrooms grown out of this are harvested. After the harvest new mushrooms will grow out of this. This way mushrooms can be harvested 3-5 times (flushes) from the same compost/casing mixture. An insecticide like for instance diflubenzuron is used on the compost as well as on the casing. Are the mushrooms from different flushes seen as independent residue trials?
Actually this situation can be compared with a decline study (see §5.3) where a PHI is not indicated. In this example the same treatment is used and samples are taken on different time points. The residue values are presented per DAT (days after treatment) only one residue value is selected: the highest residue value from the whole residue trial.

Example 1 (from JMPR assessment diflubenzuron)
The casing layer was treated with diflubenzuron during the covering. The residue is defined as DFB, the PHI is not defined and 4 flushes of mushrooms are harvested on respective DAT=16-23-33-45 (days after treatment). Per harvest two samples are taken (=replicate field samples). The results are mentioned in table 10. Because a PHI is not defined the highest mean residue value is selected from the residue trial.

Table 10 Example of a residue trial where mushrooms were harvested in 4 flushes after a casing treatment with diflubenzuron (at DAT = 16-23-33-45) and where the parent (DFB) as well as metabolites (CPU, PCA and DFBA) were measured

DAT	DFB, mg/kg	CPU, mg/kg	PCA, mg/kg	DFBA, mg/kg
16	2.6, 3.8; mean 3.2	<0.01, 0.02; mean 0.02	0.20, 0.30; mean 0.25	0.03 (2); mean 0.03
23	2.7, 6.2; mean 4.5	0.03, 0.05; mean 0.04	0.31, 0.42; mean 0.36	0.05 (2); mean 0.05
33	1.4, 1.9; mean 1.6	0.02 (2); mean 0.02	0.14, 0.29; mean 0.22	0.03, 0.04; mean 0.04
45	0.32, 2.0; mean 1.1	0.02 (2); mean 0.02	0.04, 0.12; mean 0.08	0.02, 0.04; mean 0.03

DFB = diflubenzuron (parent); CPU = 4-chlorophenylurea; PCA = 4-chloroaniline; DFBA = 2,6-difluorobenzoic acid

5.5 Selection of residue values in case different GAPs apply to the same residue trial

For FAO and EU assessments residue trials are selected based on the GAPs available. It can happen that one and the same decline study can be used for different GAPs. In that case, the GAP that results in the highest residue is selected. The same trial may not be used twice in setting the MRL, STMR and HR.

Example 1 (FAO-evaluation 2002, diflubenzuron in pome fruit)
Different GAPs were submitted for different European countries:
France: critical GAP is PHI = 15 ± 30% (=10-20) days at 0.01 ± 30% (=0.007-0.013) kg ai/hL.
Spain: critical GAP is PHI = 30 ± 30% (=21-39) days at 0.015 ± 30% (=0.010-0.020)kg ai/hL.
Results for residue trials are presented in the table

Location	kg ai/hL	DAT (days)	Residue values parent (mg/kg)
Italy, location X	1x 0.012	0 13 19 26 33 42	0.50 0.22 ← French cGAP 0.23 ← French cGAP 0.15 ← Spanish cGAP 0.21 ← Spanish cGAP 0.19
Italy, location Y	1x 0.012	0 7 11 25 42	0.83 0.44 0.28 ← French cGAP 0.34 ← Spanish cGAP 0.29

A critical GAP for a particular Southern European country (in this case Southern France or Spain) may be compared with residue trials carried out in any of the other Southern European countries. Because only one residue per trial may be selected, the highest residue is selected. In case of location X value 0.23 is selected, corresponding to the French GAP. In case of location Y value 0.34 is selected, corresponding to the Spanish GAP.

6. Presentation of residue values in advisory reports

6.1 Presentation of residue values in figures

Residue values till 100 mg/kg are rounded to 2 significant figures (0.023-0.23-2.3-23) and above that in 3 significant figures (233). Also lower residue values are presented in 2 significant figures and not in two decimals, because a very low MRL is needed for some toxic substances (e.g. fipronil)

- If only 1 significant figure is indicated on a certain level in a study report, an extra 0 is not placed behind this (thus 0.01 stays 0.01).
- If in consequence of averaging more figures arise than the originally indicated, rounding on 2 significant figures takes place (<100 mg/kg) or 3 significant figures (>=100 mg/kg), unless only 1 figure was indicated in the study report (than rounding on 1 figure takes place).
- Residue values just above the LOQ of the analytical method in question are also presented in 2 significant figures. Although this is in contradiction to the quotations mentioned below from the FAO manual [2], 2 significant figures are still chosen because also on LOQ level the same analytical accuracy is required as on higher levels [18, 19].

In the FAO manual ([2] appendix X) is written:
"Round numbers in tables to a practical level, usually 2 significant figures. […] Residues should be reported as 0.36 and 4.5 mg/kg, not 0.363 and 4.47 mg/kg. Near the LOQ (limit of quantification) rounding to 1 significant figure is recommended. For example, if the LOQ is 0.05 mg/kg, report residue data from 0.05 to 0.09 mg/kg to 1 significant figure."

For the rounding rules the NEN 1047 [15] is used. This rule states that the figures 1, 2, 3, 4 are rounded downwards and that the figures 6, 7, 8, 9 are rounded upwards. If a figure is exactly 5, it will be rounded to the nearest even number. Rounding is done in one step and only after the mean is calculated.

Example 1
0.0234 becomes 0.023 (4 is rounded downwards)
0.02349 becomes 0.023 (rounding in one step, 49 is less than 50 and is therefore rounded downwards)
0.0235 becomes 0.024 (rounding to the nearest even number)
0.0245 becomes 0.024 (rounding to the nearest even number)
0.02451 becomes 0.025 (rounding in one step, 51 is more than 50 and is therefore rounded upwards)
0.0246 becomes 0.025 (6 is rounded upwards)
0.245-0.556-0.678-0.893 becomes (0.245+0.556+0.678+0.893)/4=0.593 becomes 0.59 (rounding after taking the average).

Residue values below the limit of quantification of the analytical method in question are presented as <0.01 mg/kg (if LOQ =0.01 mg/kg) and not as actual measured value (e.g. 0.003 mg/kg). If residue values must be averaged, first take the average and then round.

Example 2
If the LOQ=0.01 mg/kg and the residue values 0.005-0.007-0.012-0.013 mg/kg are given, the following applies: (0.005+0.007+0.012+0.013)/4=0.00925 mg/kg becomes <0.01 mg/kg in the table.

If average residue values must be taken and a few residue values are indicated with for example <0.01 mg/kg, the calculation is done with 0.01 mg/kg.

Example 3
If LOQ=0.01 mg/kg and the residue values <0.01-<0.01-0.01-0.02 mg/kg are given apply: (0.01+0.01+0.01+0.02)/4=0.0125 mg/kg becomes 0.01 mg/kg in the table (no presentation in 2 figures, because the original study only indicates 1 figure).

Very often residue values are presented as values measured as such and/or values corrected for control samples and/or recovery. It is preferred to tabulate the values measured as such and add a footnote with the results for control samples and concurrent method recovery (mean and range). In this way residue values can be excluded from selection for derivation of the MRL, STMR and HR when the validity of that particular residue value is questioned.

6.2 Presentation in table form

In an advisory report for a CTB assignment (NL, EU) or a JMPR assignment the residue trials are presented in table form.
The results of residue trials (=residue value in a specific crop on a specific time) are indicated as follows:

- The residue values are presented in mg/kg.
- In the table heading is indicated how the residue is expressed or which residue it concerns (parent of metabolite).
- With each residue trial the residue values are presented for all harvest times (DAT of PHI).

For the derivation of an MRL, STMR of HR of the residue trials carried out according to the critical GAP only one residue value per residue trial is used for the relevant PHI (or DAT). Residue values used to establish the dataset for the MRL, STMR and HR are underlined in the table.

Guidelines that indicate when a residue trial may be included in the dataset for the MRL, STMR and HR are described in detail in appendix D of the Lundehn document [1] and chapter 5 and 6 of the FAO manual [2]. Here the 25% rule is indicated: trials that fall within the 25% limit (FAO 30%) of the shortest PHI as defined in the instructions for use, or the highest dose rate or the maximum number of applications, may be seen as carried out according to the critical GAP.

The general starting point is that each independent residue trial is presented in the table on a separate line. Residue values of independent residue trials carried out according to the critical GAP are underlined. Only the underlined residue values count for the derivation of the STMR, MRL or HR

In the FAO manual ([2] appendix X) is written:
"…. report individual residues as far as possible."

All residue values of replicate residue trials are presented on the same line in the table. For replicate residue trials carried out according to the critical GAP only the maximum residue value is underlined. Only the underlined residue value counts for the derivation of the STMR, MRL or HR. An exception to this is the residue trial with four replicates carried out before 1993 in The Netherlands for CTB assignments (NL and EU, but not JMPR). With these residue trials the minimum as well as the maximum residue value is underlined if not enough residue trials were carried out (see §3.2.2). In this case these replicate residue trials are seen as two separate residue trials.

In the Lundehn document [1] or in the FAO manual [2] no indications are given about the presentation of residue values for replicate field samples.

In the FAO manual ([2] Chapter 3) is written:
"Samples taken from replicate plots (…) and replicate samples taken from a single plot should be clearly distinguished."

All residue values of replicate field samples are presented and the mean is placed behind this [16]. If the corresponding residue trial is carried out according to the critical GAP, the mean is underlined.

In the FAO manual ([2] Chapter 3) is written:
"The analytical replicates (obtained by analysing replicate portions of the same laboratory sample) should be distinguished from results of replicate samples. The average value of the analytical replicates should be included in the summary table."

In the above mentioned the analytical replicates are interpreted as replicate analytical portions and the replicate samples are interpreted as replicate laboratory samples.

From replicate laboratory samples only the mean residue value is presented in the table (and not the separate results). If the residue trial is carried out according to the critical GAP, this residue value is underlined.

From replicate analytical portions only mean residue value is presented in the table (and not the separate results). If the corresponding residue trial is carried out according to the critical GAP, this residue value is underlined.

Table 11 gives an overview of the way the residue values are presented when it is clear which type of residue trial or which type of sample it concerns (see chapter 3 and 4).

Table 11 Overview of the way residue values are presented

Type of residue trial or type of sample	Authority	Presentation in the table
Independent residue trials	JMPR; CTB	residue values on different lines; residue value according to critical GAP underlined
replicate residue trials	JMPR	residue values next to each other on the same line; maximum residue value according to critical GAP underlined
	CTB	residue values next to each other on the same line; maximum residue value according to critical GAP underlined **exception** if not enough residue trials were taken the following counts: in quadruple replicate residue trials in The Netherlands before 1993: minimum and maximum residue value according to critical GAP underlined (this counts as two independent residue trials)
at random obtained replicate field samples	JMPR; CTB	residue values next to each other on the same line, plus mean; mean residue value according to critical GAP underlined
non-random obtained replicate field samples (replicate sub-plots)	JMPR; CTB	residue values next to each other on the same line, plus mean; mean residue value according to critical GAP underlined
replicate laboratory samples	JMPR; CTB	mean of the residue values mean according to critical GAP underlined or used for further calculations
replicate analytical portions	JMPR; CTB	mean of the residue values mean according to critical GAP underlined or used for further calculations

In table 12 an example is given for a table in an advisory report for JMPR or CTB (NL, EU). If residue values in the same residue trial appear several times, the residue value will only be mentioned once and between brackets. If a field sample is the mean of several laboratory samples and/or analytical portions, a footnote indicates how many repetitions that particular field sample consists of.

In the FAO manual ([2] appendix X) is written:
"If there are a number of values at the same level they can be recorded as <0.05 (7), where there are 7 values of <0.05 mg/kg."

Table 12 Example presentation for residue values (only third column) in an advisory report for JMPR or CTB NL, EU); LOQ = 0.01 mg/kg

Description residue trial	DAT	parent compound (mg/kg)
BE 1990; 1 residue trial (not according to critical GAP; in the report residue values were indicated in three decimals)	0 14	0.015 0.032
BE 1990; 1 residue trial (not according to critical GAP; in the report residue values were indicated in two decimals)	0 14	<0.01 0.02
DE 1990; 4 replicate residue trials (PHI = 14; according to critical GAP; in the report residue values were indicated in three decimals)	0 7 14 21	1.8, 1.9, 2.0, 2.1 1.2, 1.3 (2), 1.4 1.1, 1.2, 1.3, 1.4 0.43, 0.45, 0.47, 0.52
NL 1990; 4 replicate residue trials (only for NL and EU, not JMPR); (PHI = 14; according to critical GAP; in the report residue values were indicated in three decimals); this only counts if not enough residue trials were carried out	0 7 14 21	1.8, 1.9, 2.0, 2.1 1.2, 1.3 (2), 1.4 1.1, 1.2, 1.3, 1.4 0.43, 0.45, 0.47, 0.52
UK 1996; 4 replicate field samples (sampled at random); (PHI = 14; according to critical GAP; in the report residue values were indicated in two decimals)	0 14	1.8, 1.9 (2), 2.0, mean 1.9 1.1, 1.2, 1.3, 1.4, mean 1.2
UK 1996; 4 replicate field samples (not sampled at random); (PHI = 14; according to critical GAP; in the report residue values were indicated in two decimals)	0 14	1.8, 1.9 (2), 2.0, mean 1.9 1.1, 1.2, 1.3, 1.4, mean 1.2
North FR 1994; 4 replicate laboratory samples (PHI = 14; according to critical GAP; in the report residue values were indicated in two decimals)	0 16	2.0 1.2
North FR 1994; 4 replicate analytical portions; (PHI = 14; according to critical GAP; in the report residue values were indicated in two decimals)	0 16	2.1 1.0
North FR, 1979, 2 replicate residue trials with each 2 replicate at randomly sampled field samples, (PHI=14; according to critical GAP; in the report residue values were indicated in two decimals)	14	0.10, 0.17, mean 0.14 0.14, 0.18, mean 0.16
UK, 1979, 2 replicate residue trials with each 2 replicate at randomly sampled field samples, with each 2 replicate laboratory samples and each 2 replicate analytical portions (PHI=14; according to critical GAP; in the report residue values were indicated in two decimals)	14	0.10[1], 0.17[1], mean 0.14 0.14[1], 0.18[1], mean 0.16

[1]Residue value is the mean of 2 replicate laboratory samples each measured with 2 analytical portions.

6.3 Presentation for residue definitions consisting of two or more compounds

It is possible that a residue definition consists of the sum of two or more compounds.
To make the selection of the residue value more clear it is very useful to put the values of the independent compounds as well as the total residue in the table. Especially because it is possible to use the original (not rounded) values for the study assessment so that rounding errors can be prevented as much as possible. When the total residue is calculated it becomes clear if a higher residue value is found at higher PHIs. However it is also useful to mention the values of the independent compounds in case another residue definition is chosen later.

It is possible that two different residue definitions are used: one for the enforcement and one for risk assessment.
Example tolylfluanid. For enforcement the residue definition tolylfluanid without metabolites counts. For risk assessment the residue definition tolylfluanid plus DMST counts, whereby the residue is expressed as tolylfluanid (T + 1.621 * D). In table 13 the separate values of T (expressed as T) and D (expressed as D) are presented, but the total residue value T+1.621*D (expressed as T) is also presented.

Table 13 Example presentation of residue values (only in 3rd-5th column), where two different residue definitions exist and where the residue definition for the risk assessment consists of two compounds

Trial	DAT	T (mg/kg) enforcement	D (mg/kg)	T+1.621*D (mg/kg T) risk assessment
1; PHI=35	0	3.3	0.33	3.9
	14	0.34	0.10	0.50
	21	0.16	0.25	0.57
	28	0.19	<0.05	0.27
	35	0.23	0.07	0.34
	43	0.25	0.05	0.33

For residue values below the limit of quantification a few separate requirements apply if the residue consists of two or more compounds [17].
If the concentration of each compound from the residue definition lies below the (own separate) LOQ of the analytical method(s) in question, the LOQ of the residue will be equal to the highest LOQ of compounds presented. Because a conversion factor is used to convert the compounds into parent, the conversion factor is taken into consideration in the decision which LOQ is the highest.

Example 1 (tolylfluanid), both compounds lie below its own LOQ
Given LOQ=0.02 mg/kg for compound T and LOQ =0.02 mg/kg for compound D. The residue is defined as T + 1.621 D (expressed as mg/kg T).
In a residue trial a residue of <0.02 mg/kg T and <0.02 mg/kg D was found for crop X. The LOQs expressed as T are: 0.02 mg/kg T for compound T and 0.02*1.621=0.03242 mg/kg T = 0.03 mg/kg T (rounded) for compound D. In this case <0.03 mg/kg is placed in the table in the column for the total residue.

If one of the compounds from the residue definition lies on or above the (own separate) LOQ of the analytical method in question, a calculation is made using the measured residue values of each compound. If one of the compounds lies below the (own separate) LOQ of the analytical method in question, a calculation is made using the value of the LOQ.

Example 2 (tolylfluanid), one of the compounds lies above its own LOQ.
Given LOQ=0.02 mg/kg for compound T and LOQ =0.02 mg/kg for compound D. The residue is defined as T + 1.621 D (expressed as mg/kg T).
In a residue trial a residue of 0.17 mg/kg T and <0.02 mg/kg D was found for crop X.
In this case 0.17+1.621*0.02= 0.2024 = 0.20 mg/kg placed in the table in the column for the total residue.

Example 3 (tolylfluanid), one of the compounds lies on its own LOQ.
Given LOQ=0.02 mg/kg for compound T and LOQ=0.02 mg/kg for compound D. The residue is defined as T + 1.621 D (expressed as mg/kg T).
In a residue trial a residue of 0.02 mg/kg T and <0.02 mg/kg D is found for crop X.
In this case 0.02+1.621*0.02= 0.05242 = 0.05 mg/kg is placed in the table in the column for the total residue. The result is presented in 1 significant figure because each of the separate residue values was presented in one figure.

6.4 Untreated control samples

Residue values of untreated control samples are not placed in the table, but are preferably inserted in the summary of analytical methods or else in the footnote of the table. In the analytical method summary an indication must be given in the footnote if an untreated control sample is measured and if so what is the range of the residue value and how many samples are measured (e.g. <0.002-0.022 mg/kg; n=5). Residue values of non-treated control samples below the limit of quantification are presented as actual measured value: 0.002 mg/kg
(if LOQ =0.01 mg/kg). This in relation to the demand that the residue values of untreated control samples must be smaller than 0.3*LOQ and that at least 2 samples must be measured [18, 19].

6.5 Samples collected at different treatment times

In applications with multiple treatments, sometimes the (immature) crops are sampled and analysed just before the next treatment. This kind of information is very valuable in deciding whether a residue accumulates upon multiple treatments and whether applications with more or less treatments than specified in the GAP might be used for derivation of the MRL, STMR or HR.

Example diflubenzuron on apples (FAO 2002)
Samples were treated three times with a spray application: on 23-06-76; on 15-07-76 and on 12-08-76. At several timepoints samples were taken. The information was tabulated as in table 14.

Table 14 Example of sampling at different treatment times

Location	Application	Interval	DAT	parent, mg/kg
Germany	3x 0.020 kg ai/hL	22, 28	0[1]	0.58, 0.82, mean 0.70
			22[1]	0.24, 0.30, mean 0.27
			0[2]	1.2 (2), mean 1.2
			28[2]	0.50, 0.51, mean 0.51
			0[3]	1.2
			12[3]	0.84
			26[3]	0.49
			39[3]	0.62
			54[3]	0.59

1, 2, 3 samples taken after the 1st , 2nd or 3rd treatment

6.6 Samples collected at different growth stages or samples collected from different plant parts

For crops with long growing periods like cereals, pulses and oilseeds, crops can be fed to livestock in an early growth stage (e.g. forage) and at maturity. At maturity both the seeds and the remains (straw/fodder) can be fed to livestock. For such crops, samples have to be taken at different growth stages and for different plant parts.

For a CTB assignment (NL, EU) the results for samples taken at different growth stages and/or from different plant parts are presented within the same line, to indicate that the results are originating from the same trial.
For a JMPR assignment, the results for samples taken at different growth stages and/or from different plant parts are presented in different tables.

In the FAO manual ([2] appendix X) is written:
"Where a crop produces more than one commodity, e.g. cereal crops produce grains and forage and fodder, prepare separate residue data tables for the grain and the forage and the fodder"

Example for CTB assignment (diflubenzuron on soybean, rice and pecans)
Soybean was treated twice. Forage and soybean hay were harvested 32 and 73 days, respectively, after the first application. Mature soybean seeds were harvested 22 days after the second application. The information was tabulated as in table 15.
Rice was treated twice and mature samples were separated into grain (kernel plus hull) and straw using a thresher. The information was tabulated as in table 15.
Pecan trees were treated four times. Mature pecans were mechanically separated into nutmeat, shells, and hulls using separators. The information was tabulated as in table 15.

Table 15 Examples of sampling at different growth stages and sampling of different crop parts

Type	Application	Interval	DAT	Plant part	parent, mg/kg
Trial on soybean	1x 0.074 kg ai/ha; 1x 0.077 kg ai/ha	113	32[1] 73[1] 22[2]	forage hay seed	<0.05, 0.14, mean 0.10 0.40, 0.45, mean 0.42 <0.05 (2), mean <0.05
Trial on rice	2x 0.10 kg ai/ha	30	93 93	grain straw	<0.02 (2); mean <0.02 <0.01 (2); mean <0.01
Trial on pecans	1x 0.060 kg ai/hL; 1x 0.058 kg ai/hL; 1x 0.062 kg ai/hL; 1x 0.12 kg ai/hL;	21, 20, 154	28 28	nutmeat hulls	<0.05 (2); mean <0.05 0.52, 0.64; mean 0.58

1, 2 samples taken after the 1st or 2nd treatment

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7028/VI/95 rev. 3	22/07/1997	Appendix A, Metabolism and distribution in plants
7029/VI/95 rev. 5	22/07/1997	Appendix B, General recommendations for the design, . preparation and realization of residue trials
7524/VI/95 rev. 2	22/07/1997	Appendix C, Testing of plant protection products in . rotational crops
7525/VI/95 rev. 7	12/06/2001	Appendix D, Guidelines on comparability, extrapolation, . group tolerances and data requirements for setting MRLs
7035/VI/95 rev. 5	22/07/1997	Appendix E, Processing studies
7030/VI/95 rev. 3	22/07/1997	Appendix F, Metabolism and distribution in domestic . animals
7031/VI/95 rev. 4	22/07/1997	Appendix G, Livestock feeding studies
7032/VI/95 rev. 5	22/07/1997	Appendix H, Storage stability of residue samples
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56-60 Bureau Rapportenbeheer.
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Appendix 2 List of abbreviations

Code	Meaning
ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
CCPR	Codex Committee on Pesticide Residues
CEC	Cation Exchange Capacity
CTB	(Dutch) Board for the Authority of Pesticides
DAT	Days After (last) Treatment
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
GAP	Good Agricultural Practise
HR	Highest residue
JMPR	FAO/WHO Joint Meeting of Pesticide Residues
K_a	Acid constant of a substance
LOQ	Limit of quantification or Limit of quantitation
MRL	Maximum Residue Limit
NL	Dutch, from The Netherlands
pH	(degree of) acidity of a substance in water ($pH = -\log[H_3O^+]$)
PHI	pre harvest interval
pK_a	$-\log K_a$
RIVM	National Institute for Public Health and the Environment
RSD	Relative Standard Deviation = standard deviation/mean = Coefficient of Variation (CV)
SC	Suspension Concentrate
STMR	Supervised trials median residue
USA	United States of America
VWS	Dutch Ministry of Health, Welfare and Sport
WHO	World Health Organisation of the United Nations
WP	Wettable Powder

Appendix 3 USDA classification for soil specimens

The texture of a soil specimen is classified according to the USDA-classification-triangle (USDA, 1951) based on %clay (particle size <2 μm), %silt (particle size 2-50 μm) and %sand (particle size >50 μm).
Within this classification triangle the following mineral soil specimens are distinguished:
clay; sand; silt; loam;
sandy clay; silty clay;
clay loam; sandy loam; silt loam;
loamy sand; sandy clay loam; silty clay loam