# QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS

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## Introduction

1. The guidelines in this document are intended for the monitoring of pesticide residues in the European Union. The guidelines describe the analytical quality control (AQC) requirements to support the validity of data used for checking compliance with maximum residue limits (MRLs), enforcement actions, or assessment of consumer exposure to pesticides.

The key objectives are:

(i) to ensure that false positives or false negatives are not reported
(ii) to ensure that acceptable accuracy is achieved
(iii) to harmonize cost-effective AQC in the EU
(iv) to support compliance with ISO/IEC 17025 (accreditation standard)

2. These guidelines are complementary and integral to the requirements in ISO/IEC 17025.

3. These guidelines supersede Document No. **SANCO/10476/2003**.

4. The glossary (Appendix 1) should be consulted for explanation of terms used in the text.

## Accreditation

5. In accordance with the provisions of Directive 93/99/EEC, laboratory operations should meet the requirements of a recognised accreditation scheme, complying with ISO/IEC 17025. The quality requirements described in this document are intended as guidance for accreditation purposes.

## Sampling, transport, processing and storage of samples

*Sampling*

6. Laboratory samples should be taken in accordance with Directive 2002/63/EC or superseding legislation. Where it is impractical to take primary samples randomly within a lot, the method of sampling must be recorded.

*Laboratory sample transportation*

7. Samples must be transported to the laboratory in clean containers and robust packaging. Polythene bags, ventilated if appropriate, are acceptable for most samples but low-permeability bags (e.g. nylon film) must be used for samples to be analysed for residues of fumigants. Samples of commodities prepacked for retail sale should not be removed from their packaging before transport. Very fragile or perishable products (e.g. ripe raspberries) may have to be frozen to avoid spoilage and then transported in “dry ice” or similar, to avoid thawing in transit. Samples that are frozen at the time of collection must be transported without thawing. Samples that may be damaged by chilling (e.g. bananas) must be protected from both high and low temperatures.

8. Rapid transportation to the laboratory, preferably within one day, is essential for samples of most fresh products. The condition of samples delivered to the laboratory should approximate to that acceptable to a discerning purchaser, otherwise samples should normally be considered unfit for analysis.

9. Samples must be identified clearly and indelibly, in a way that prevents inadvertent loss or confusion of labelling. The use of marker pens containing organic solvents should be avoided for labelling bags containing samples to be analysed for fumigant residues, especially if an electron capture detector is to be used.

*Sample preparation and processing prior to analysis*

10. On receipt, each laboratory sample must be allocated a unique reference code by the laboratory.

11. Sample preparation, sample processing and sub-sampling to obtain analytical portions must take place before visible deterioration occurs. Canned, dried or similarly processed samples should be analysed within the stated shelf life.

12. Sample preparation must be in accordance with the definition of the commodity and the part(s) to be analysed.

13. Sample processing and storage procedures should be demonstrated to have no significant effect on the residues present in the analytical sample. Where labile residues could otherwise be lost, samples may be comminuted frozen (e.g. in the presence of "dry ice"). Where comminution is known to affect residues (e.g. dithiocarbamates or fumigants) and practical alternative procedures are not available, the test portion should consist of whole units of the commodity, or segments removed from large units. All analyses should be undertaken within the shortest time practicable, to minimise sample storage. Analyses for residues of very labile or volatile pesticides should be started, and the procedures involved in potential loss of analyte completed, on the day of sample receipt.

14. If a single analytical portion is unlikely to be representative of the analytical sample, replicate portions must be analysed, to provide a better estimate of the true value.

**Pesticide standards, calibration solutions, etc.**

*Identity, purity, and storage of standards*

15. "Pure" standards of analytes and internal standards should be of known purity and each must be uniquely identified and the date of receipt recorded. They should be stored at low temperature, preferably in a freezer, with light and moisture excluded, i.e. under conditions that minimise the rate of degradation. Under such conditions, the supplier's expiry date, which is often based on less stringent storage conditions, may be replaced, as appropriate for each standard, by a date allowing for storage up to 10 years. The pure standard may be retained if its purity is shown to remain acceptable. The purity should be checked by the allocated time after which a "pure" standard may be retained if its purity is shown to remain acceptable and a new expiry date is allocated. Ideally, the identity of freshly acquired "pure" standards should be checked if the analytes are new to the laboratory.

*Preparation and storage of stock standards*

16. When preparing stock standards (solutions, dispersions or gaseous dilutions) of "pure" standards of analytes and internal standards, the identity and mass (or volume, for highly volatile compounds) of the "pure" standard and the identity and amount of the solvent (or other diluents) must be recorded. The solvent(s) must be appropriate to the analyte (solubility, no reaction) and method of analysis. Moisture must be excluded during equilibration of the "pure" standard to room temperature before use and concentrations must be corrected for the purity of the "pure" standard.

17. Not less than 10 mg of the "pure" standard should be weighed using a 5 decimal place balance. The ambient temperature should be that at which the glassware is calibrated, otherwise preparation of the standard should be based on mass measurement. Volatile liquid analytes should be dispensed by weight or volume (if the density is known) directly into solvent. Gaseous (fumigant) analytes may be dispensed by bubbling into solvent and weighing the mass transferred, or by preparing gaseous dilutions (e.g. with a gas-tight syringe, avoiding contact with reactive metals).

18. Stock standards must be labelled indelibly, allocated an expiry date and stored at low temperature in the dark in containers that prevent any loss of solvent and entry of water. Currently available data show that stock standards of the large majority of pesticides in toluene and acetone are stable for at least 5 years in the freezer when stored in tightly closed glass containers.

19. When a stock standard is prepared for the first time, and for suspensions (e.g. dithiocarbamates) and solutions (or gaseous dilutions) of highly volatile fumigants that must be prepared freshly, the accuracy of the solution should be compared with a second solution made independently at the same time.

*Preparation, use and storage of working standards*

20. When preparing working standards, a record must be kept of the identity and amount of all solutions and solvents employed. The solvent(s) must be appropriate to the analyte (solubility, no reaction) and method of analysis. The standards must be labelled indelibly, allocated an expiry date and stored at low temperature in the dark in containers that prevent any loss of solvent and entry of water. Septum closures are particularly prone to evaporation losses (in addition to being a source of contamination) and should be replaced as soon as practicable after piercing, if solutions are to be retained. Following equilibration to room temperature, solutions must be re-mixed and a check made to ensure that no analyte remains undissolved, especially where solubility at low temperatures is limited.

21. At method development or validation, or for analytes new to the laboratory, the response detected should be shown to be due to the analyte, rather than to an impurity or artefact. If the techniques used can lead to degradation of the analyte during extraction, clean-up or separation, and they generate a product that is commonly found in samples but which is excluded from the residue definition, positive results must be confirmed using techniques that avoid this problem.

*Testing and replacement of standards*

22. Whenever any standard reaches its expiry date or is replaced, its purity should be checked. Existing stock and working solutions may be tested against

newly prepared solutions by comparing the detector responses obtained from appropriate dilutions of individual standards or mixtures of standards. The purity of an old "pure" standard may be checked by preparing a new stock standard and comparing the detector responses obtained from freshly prepared dilutions of old and new stock standards. Inexplicable differences in apparent concentration between old and new standards must be investigated.

23. The means from at least five replicate measurements for each of two solutions should not normally differ by more than ±5%[1]. The mean from the old (existing) solution is taken to be 100%. However, if the number of replicate determinations required to distinguish a difference of ±5% is unacceptably large for problematic analytes, the acceptable range may be increased to ±10%. The use of an internal standard may reduce the number of replicate injections required to achieve a ±5% difference. If a response of the old standard differs by more than ±5% (or ±10% in the case of problematic analytes) from the new, storage time or conditions must be adjusted as necessary on the basis of the results.

**Extraction and concentration**

*Extraction conditions and efficiency*

24. Test portions should be disintegrated thoroughly during extraction to maximise extraction efficiency, except where this is known to be unnecessary or inappropriate (e.g. for determination of fumigants or surface residues). Temperature, pH, etc., must be controlled if these parameters affect extraction efficiency, analyte stability or solvent volume.

*Extract concentration and dilution to volume*

25. Great care must be exercised when extracts are evaporated to dryness, as trace quantities of many analytes can be lost in this way. A small volume of high boiling point solvent may be used as a "keeper" and the evaporation temperature should be as low as practicable. Frothing and vigorous boiling of extracts, or dispersion of droplets, must be avoided. A stream of dry nitrogen or vacuum centrifugal evaporation is generally preferable to the use of an air stream for small-scale evaporation, as air is more likely to lead to oxidation or to introduce water and other contaminants.

26. Where extracts are diluted to a fixed volume, accurately calibrated vessels of not less than 1 ml capacity should be used and further evaporation avoided. Alternatively, an internal standard may be used, particularly for small volumes.

27. Analyte stability in extracts should be investigated during method validation. Storage of extracts in a refrigerator or freezer will minimise degradation but potential losses at the higher temperatures of an autosampler rack should not be ignored.

**Contamination and interference**

[1]Alternatively, a t-test of the means should not show a significant difference at the 5% level.

*Contamination*

28. Samples must be separated from each other, and from other sources of potential contamination, during transit to, and storage at, the laboratory. This is particularly important with surface or dusty residues, or with volatile analytes. Samples known, or thought, to bear such residues should be doubly sealed in polythene or nylon bags and transported and processed separately.

29. Pest control in, or near, the laboratory must be restricted to pesticides that will not be sought as residues.

30. Volumetric equipment, such as flasks, pipettes and syringes, must be cleaned scrupulously, especially for re-use. As far as practicable, separate glassware, etc., should be allocated to standards and sample extracts, in order to avoid cross-contamination. Avoid using excessively scratched or etched glassware. Solvents used for fumigant residues analysis should be checked to ensure that they do not contain the analyte.

31. Where an internal standard is used, unintended contamination of extracts or analyte solutions with the internal standard, or *vice versa*, must be avoided.

32. Where the analyte occurs naturally in, or is produced from, samples (e.g. inorganic bromide in all commodities; sulfur in soil; or carbon disulfide produced from the *Cruciferaceae*), low-level residues from pesticide use cannot be distinguished from natural levels. Natural occurrence of these analytes must be considered in the interpretation of results. Dithiocarbamates, ethylenethiourea or diphenylamine can occur in certain types of rubber articles and this source of contamination must be avoided.

*Interference*

33. Equipment, containers, solvents (including water), reagents, filter aids, etc., should be checked as sources of possible interference. Rubber and plastic items (e.g. seals, protective gloves, wash bottles), polishes and lubricants are frequent sources. Vial seals should be PTFE-lined. Extracts should be kept out of contact with seals, especially after piercing, by keeping vials upright. Vial seals must be replaced quickly after piercing, if re-analysis of the extracts is necessary. Analysis of reagent blanks should identify sources of interference in the equipment or materials used.

34. Interference from natural constituents of samples is frequent. The interference may be peculiar to the determination system used, variable in occurrence and intensity, and may be subtle in nature. If the interference takes the form of a response overlapping that of the analyte, a different clean-up or determination system may be required. Interference in the form of suppression or enhancement of detection system response is dealt with in paragraph 45**.** If it is not practicable to eliminate interference, or to compensate for it by matrix-matched calibration, the overall accuracy (bias) and precision of analysis should nonetheless comply with the criteria in paragraphs and 60 and 65–65.

**Analytical calibration, representative analytes, matrix effects and chromatographic integration**

*General requirements*

35. Correct calibration is dependent upon correct identification of the analyte (see paragraphs 69–82). Bracketing calibration should be used unless the determination system has been shown to be free from significant drift in its

absolute (external standardisation) or relative (internal standardisation) response. In a batch of parallel determinations (e.g. ELISA with 96-well plates), the calibration standards should be distributed to detect differences in response due to position. Responses used to quantify residues must be within the dynamic range of the detector.

36. Batch sizes for determination should be adjusted so that detector response to a single injection of bracketing calibration standards does not drift >20% at ≥2 x LCL, or >30% at 1–2 x LCL (if the LCL is close to the LOQ). If the drift exceeds these values, repeat of determinations is not necessary where the samples clearly contain no analyte, providing that the LCL response remains measurable throughout the batch.

37. Extracts containing high-level residues may be diluted to bring them within the calibrated range but, where calibration solutions must be matrix-matched (paragraph 46) the concentration of matrix extract may have to be adjusted.

*Calibration*

38. Residues below the lowest calibrated level (LCL) should be considered uncalibrated, and therefore reported as <LCL, whether or not a response is evident. If it is desirable to report measurable residues below the original LCL, determinations must be repeated with a lower LCL. If the signal to noise ratio produced by the target LCL is inadequate (less than 5:1), a higher level must be adopted as the LCL. An additional calibration point, for example at two times the target LCL, provides a back-up LCL if there is a risk that the target LCL will not be measurable. Validation of analytical methods should include determination of recovery at the proposed LCL.

39. Calibration by interpolation between two levels is acceptable providing the difference between the 2 levels is not greater than a factor of 4, and where the mean response factors, derived from replicate determinations at each level, indicate acceptable linearity of response with the higher being not more than 120% of the lower response factor (110% in cases where the MRL is approached or exceeded).

40. Where three or more levels are utilised, an appropriate calibration function may be calculated and used between the lowest and highest calibrated levels. The calibration curve (which may or may not appear to be linear) should not be forced through the origin. The fit of the calibration function must be plotted and inspected visually, avoiding reliance on correlation coefficients, to ensure that the fit is satisfactory in the region relevant to the residues detected. If individual points deviate by more than ±20% (±10% in cases where the MRL is approached or exceeded) from the calibration curve in the relevant region, an alternative calibration function must be used.

41. Single-level calibration may provide more accurate results than multi-level calibration if the detector response is variable with time. When single-level calibration is employed, the sample response should be within ±10% of the calibration standard response if the MRL is exceeded. If the MRL is not exceeded, the sample response should be within ±50% of the calibration response, unless further extrapolation is supported by evidence of acceptable linearity of response. Where analyte is added for recovery determination at a level corresponding to the LCL, recovery values <100% may be calculated using a single point calibration at the LCL. This particular calculation is intended only to indi-

cate analytical performance achieved at the LCL and does not imply that residues <LCL should be determined in this way.

*Representative analytes*

42. The determination system must be calibrated with the representative analytes for every batch of analyses. The minimum frequency for calibration of representative and all other analytes is given in Table 1.

**Table 1.** Minimum frequencies for calibration and recovery

| | Representative analytes | All other analytes |
|---|---|---|
| Minimum frequency of calibration | Calibration in each batch of analyses.<br><br>At least at the level corresponding to the reporting limit. | A rolling programme to include all other analytes at least every 12 months, but preferably every 6 months*.<br>At least at the level corresponding to the reporting limit. |
| Minimum frequency of recovery<br>(Paragraph 62) | Determination in each batch of analyses. | Each analyte, at the same time as the corresponding calibration series, as above.<br>At least at the level corresponding to the reporting limit. |

*The minimum requirements are (i) at the beginning and end of a survey or programme and (ii) when potentially significant changes are made to the method.

43. Reliance on representative analytes is associated with an increased risk of incorrect results, especially false negatives. Therefore representative analytes must be chosen very carefully, to provide evidence that acceptable screening is achieved for all other analytes. The choice should be made according to the physico-chemical characteristics of the analytes, subject to inclusion of the following:

(i) all analytes likely to be detected in the samples analysed;

(ii) analytes likely to give the poorest and most variable response and/or recovery.

44. Where an analyte that is not a representative analyte is detected in a sample, the result must be considered tentative until calibrated (see paragraphs 36–41). When the screening result indicates that an MRL might be exceeded, or in the case of other violative residues, the sample must be re-analysed and accompanied by acceptable recovery (see paragraphs 60 and 61) of the detected analyte.

45. If the rolling programme (Table 1) of recovery or calibration of a representative analyte produces unacceptable results at the first attempt, all results produced after the previous successful recovery or calibration of that analyte must be treated as potentially erroneous.

*Matrix effects and matrix-matched calibration*

46. The potential for matrix effects to occur should be assessed at method validation. They are notoriously variable in occurrence and intensity but some techniques are particularly prone to them. If the techniques used are not inherently free from such effects, calibration should be matrix-matched routinely, unless an alternative approach can be shown to provide equivalent or superior accuracy. Extracts (or samples, for calibration of headspace and SPME analysis) of blank matrix may be used for calibration purposes. The best way to negate each matrix effect is to calibrate by standard addition (see paragraphs 49 and 50).

47. A potential problem is that different samples, different types of extract, different commodities and different "concentrations" of matrix may exhibit matrix effects of different magnitudes. Where a slight risk of erroneous calibration is acceptable, a representative matrix (see glossary) may be used to calibrate a wide range of sample types.

48. If required in GC analysis, priming should be performed immediately prior to the first series of calibration determinations in a batch of analyses.

*Standard addition*

49. Standard addition may be used as an alternative approach to the use of matrix-matched calibration standards. Standard addition normally involves the addition of a known quantity of an analyte to one of two duplicate analytical samples immediately prior to extraction. The difference in response from the two sample extracts (spiked and unspiked) obtained from the detector notionally calibrates the response to the known quantity of added analyte and compensates for recovery. The quantity of analyte present in the "unspiked" sample extract is calculated by simple proportion. Matrix effects are thus compensated. This technique assumes some knowledge of the likely concentration of the analyte in the sample, so that the amount of added analyte is similar to that already present in the sample. If the concentration of the analyte is completely unknown then it may be necessary to "spike" a number of replicate samples with increasing quantities of analyte, so that a calibration curve can be constructed in a similar way to normal standard calibration. This technique automatically adjusts for both recovery and calibration. Standard addition will not, of course, overcome chromatographic interferences caused by overlapping/unresolved peaks from co-extracted compounds.

50. Addition of a known quantity of analyte to an aliquot of sample extract, etc., immediately prior to the final determination is another form of standard addition, but in this case adjustment is for calibration only. When an instrumentally based method (e.g. GC-MS, LC-MS, etc.) is used, the spiked sample extract is often referred to as a "syringe" or "injection" standard, because it compensates for injection volume variability.

*Effects of pesticide mixtures on calibration*

51. Calibration using mixed analyte solutions made up in pure solvent, etc. should be checked at method validation (paragraphs 56–58) for similarity of detector response to that obtained from the separate analytes. If the responses differ significantly, or in cases of doubt, residues must be quantified using individual calibration standards in matrix, or better still, by standard addition**.**

*Calibration for pesticides that are mixtures of isomers, etc.*

52. Where a calibration standard is a mixture of isomers, etc., of the analyte, detector response generally may be assumed to be similar, on a molar basis, for each component. However, enzyme assays, immuno-assays and other assays with a biological basis may give calibration errors if the component ratio of the standard differs significantly from that of the measured residue. An alternative detection system should be used to quantify such residues. In those cases where the response of a "selective" detector to isomers differs (e.g. the electron-capture efficiency of HCH isomers), separate calibration standards must be used. If separate standards are not available for this purpose, an alternative detection system should be used to quantify residues.

*Calibration using derivatives or degradation products*

53. Where the pesticide is determined as a degradation product or derivative, the calibration solutions should be prepared from a "pure" standard of that degradation product or derivative, if available. Procedural standards should only be used if they are the only practical option.

*Chromatographic integration*

54. Chromatograms must be examined by the analyst and the baseline fitting checked and adjusted, as required. Where interfering or tailing peaks are present, a consistent approach must be adopted for the positioning of the baseline. Peak height or peak area data may be used; whichever yields the more accurate and repeatable results.

55. Unless biosensor detection is employed, calibration by mixed isomer (or similar) standards may utilise summed peak areas, summed peak heights, or measurement of a single component, whichever is the more accurate.

## Analytical methods and analytical performance

### *Method validation*

56. Within laboratory method validation should be performed to provide evidence that a method is fit for the purpose for which it is to be used. Method validation is a requirement of accreditation bodies, and must be supported and extended by performance verification (on-going analytical quality control). All procedures (steps) that are undertaken in a method should be validated, if practicable. If a method is to be accredited, then before any validation data are generated, the appropriate accreditation body should be consulted. Different accreditation bodies may demand different criteria for method validation.

57. For multi-residue methods, representative matrices may be used. However, representative matrices must be chosen carefully on the basis of their biological or "analytical" similarity. This may be with regard to their water, lipid or sugar contents, pH, etc. So, for example, oranges may be chosen as being representative of citrus fruits, and lettuce as representative of green leafy vegetables, etc.

58. The method must be tested to assess for sensitivity, mean recovery (as a measure of trueness or bias) and precision. This effectively means that spiked recovery experiments to check the accuracy of the method should be undertaken. A minimum of 5 replicates is required (to check the precision) at both the reporting limit (to check the sensitivity of the method), and at least

another higher level, perhaps an action level, for example the MRL. Where the residue definition incorporates two or more analytes, the method should be validated for all analytes included in the residue definition. Table 2 sets out the minimum criteria for repeatability and mean recovery that should be attained for a quantitative method for the analysis of pesticide residues in foods.

59. If the analytical method does not permit determination of recovery (for example, direct analysis of liquid samples, SPME, or headspace analysis), the precision is determined from repeat analyses of calibration standards. The bias is usually assumed to be zero, although this is not necessarily so. In SPME and headspace analysis, the trueness and precision of calibration may depend on the extent to which the analyte has equilibrated, particularly with respect to the sample matrix. If these methods depend upon equilibrium, this must be demonstrated during method development.

**Table 2.** Criteria for quantitative methods

| Concentration range (mg/kg) | Repeatability | | Mean recovery range (%) |
|---|---|---|---|
| | $RSD_A$% | $RSD_L$% | |
| 0.001 – 0.01 | 30 | 32 | 70 – 110 |
| >0.01 – 0.1 | 20 | 22 | 70 – 110 |
| >0.1 – 1 | 15 | 18 | 70 – 110 |
| >1 | 10 | 14 | 70 – 110 |

$RSD_A$%: relative standard deviation of analysis, excluding any contribution due to sample heterogeneity.

$RSD_L$%: relative standard deviation of the laboratory result, including 10% sub-sampling heterogeneity.

***Acceptability of analytical methods – method validation***

60. The analytical method should be demonstrated at validation as being capable of providing mean recovery within the range given in Table 2, for all compounds sought by the method and at appropriate levels (see paragraphs 56–58). The mean recovery at each spiking (fortification) level and for each representative commodity should be in the range 70–110% (in certain justified cases recoveries outside this range will be accepted). Where the method does not permit this, and there is no satisfactory alternative, the relatively poor mean recovery must be considered before taking enforcement action. Exceptionally, where recovery is low but consistent (i.e. demonstrating good precision) and the basis for this is well established (e.g. due to pesticide distribution in partition), a mean recovery below 70% may be acceptable. However, a more accurate method should be used, if practicable.

*Methods for determination of fat or dry weight content*

61. Where results are expressed on the basis of dry weight or fat content, the method used to determine the dry weight or fat content must be consistent. Ideally it should be validated against a widely recognised method.

## Routine recovery determination

62. Where practicable, recovery of all analytes determined should be measured with each batch of analyses. If this requires a disproportionately large number of recovery determinations, the minimum acceptable frequency of recovery may be as given in Table 1. Analysis of reference materials is an acceptable, though rarely practical, alternative providing that the materials contain relevant analytes at appropriate levels.

63. Analyte recovery should normally be determined by fortification within a range corresponding to 1–10 times the LCL, or at the MRL, or at a level of special relevance to the samples analysed. The level of addition may be changed intermittently or regularly, to provide information on analytical performance over a range of concentrations. Recovery at levels corresponding to the LCL and MRL is particularly important. In cases where blank material is not available (e.g. where inorganic bromide is to be determined at low levels) or where the only available blank material contains an interfering compound at an acceptably low level, the spiking level for recovery should be ≥3 times the level present in the blank material. The analyte (or apparent analyte) concentration in such a blank matrix should be determined from multiple test portions. If necessary, recoveries should be corrected by blank values. Blank values and uncorrected recoveries must also be reported. They must be determined from the matrix used in spiking experiments and the blank values should not be higher than 30% of the residue level corresponding to the LCL.

64. As far as practicable, the recovery of all components defined by the MRL should be determined routinely. Where a residue is determined as a common moiety, routine recovery may be determined using the component that either normally predominates in residues or is likely to provide the lowest recovery.

### *Acceptability of analytical performance for routine recoveries*

65. Acceptable limits for single recovery should normally be in the range 60–140% and may be adjusted using repeatability (validation) and intra laboratory reproducibility (routine on-going recovery) data. Recoveries outside this range usually require re-analysis of the batch but may be acceptable in certain justified cases. Where the routine recovery is unacceptably high and no residues are detected, it is not necessary to re-analyse the samples to prove the absence of residues. However, consistently high recovery should be investigated. If a significant trend occurs in recovery, or potentially unacceptable (beyond the %RSDs given in Table 2) results are obtained, the cause(s) must be investigated.

66. Data on violative residues must be supported by mean recovery within the ranges given in Table 2, at least for the confirmatory analyses. If recovery within this range cannot be achieved, enforcement action is not necessarily precluded, but relatively poor accuracy must be taken into account.

## Proficiency testing and analysis of reference materials

67. The laboratory must participate regularly in relevant proficiency tests. Where the accuracy achieved in any of the tests is questionable or unacceptable, the problem(s) should be investigated and, particularly for unacceptable performance, rectified before proceeding with further determinations of the analyte/matrices combinations involved.

68. In-house reference materials may be analysed regularly to help provide evidence of analytical performance. Where practicable, exchange of such mate-

rials between laboratories provides an additional, independent check of accuracy.

## Confirmation of results

*Principles of confirmation*

69. Negative results (residues below the reporting limit) can be considered confirmed if the recovery and LCL measurement for the batch are acceptable (paragraphs 38 and 65). Negative results for represented analytes are supported only indirectly by the recovery and LCL data for representative analytes and must be interpreted with caution.

70. Positive results (residues at or above the reporting limit) usually require additional confirmation to that given in paragraph 69. In addition to the general requirements of paragraphs 71–80, confirmation of positive results for represented analytes (i.e. those with no concurrent calibration and recovery) should be supported by the appropriate concurrent calibration and recovery determinations. Confirmation is not mandatory for all positive results, and must be decided by the laboratory on a case-by-case basis.

71. Suspected MRL exceedances or unusual residues must be identified by the least equivocal technique, or combination of techniques, available and must be quantitatively confirmed by analysis of at least one additional test portion. Different combinations of clean-up, derivatisation, separation, and detection techniques may also be used to support confirmation. The use of a highly specific detection system, such as mass spectrometry, is recommended.

72. Selective detectors employed with GC or LC such as ECD, FPD, NPD, DAD and fluorescence, offer only limited specificity. Their use, even in combination with different polarity columns, can only provide limited confirmatory evidence. These limitations may be acceptable for frequently found residues, especially if some results are also confirmed using a more specific detection technique. Such limitations in the degree of confirmation should be acknowledged when reporting the results.

*Chromatographic separation*

73. Mass spectrometric determination of residues is usually carried out in conjunction with a chromatographic separation technique to simultaneously provide

i) retention time;

ii) ion mass/charge ratio; and

iii) abundance data

For GC-MS procedures, the chromatographic separation should be carried out using capillary columns. For LC-MS procedures, the chromatographic separation can be performed using any suitable LC column. In either case, the minimum acceptable retention time for the analyte(s) under examination should be at least twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the sample extract must match that of the calibration standard (may need to be matrix matched) within a specified window after taking into consideration the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of a suitable internal

standard, i.e. the relative retention time of the analyte, should correspond to that of the calibration solution with a tolerance of ±0.5% for GC and ±2.5% for LC.[2]

*Confirmation by mass spectrometry (MS)*

74. The term "confirmation by mass spectrometry" normally refers to overwhelming evidence that a sample actually contains the analyte, i.e. proof of identity. Confirmation of the quantity of analyte present can only be achieved by analysis of a second test portion.

75. Reference spectra for the analyte should be generated using the instruments and techniques employed for analysis of the samples. If major differences are evident between a published spectrum and that generated within the laboratory, the latter must be shown to be valid. To avoid distortion of ion ratios, the quantity of analyte must not overload the detector.

76. Diagnostic ion chromatograms should have peaks (with minimum 3 data points exceeding, S/N 3:1) of similar retention time, peak shape and response ratio to those obtained from a calibration standard analysed in the same batch. Where chromatograms of unrelated ions show peaks with a similar retention time and shape, or where unrelated ion chromatograms are not available (e.g. with SIM), additional confirmation may be required. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon to quantify or identify residues.

77. Careful subtraction of background spectra may be required to ensure that the resultant spectrum of the chromatographic peak is representative. Whenever background correction is applied, this must be applied uniformly throughout the batch and should be clearly indicated. Where ions unrelated to the analyte in a peak-averaged "full-scan" spectrum (i.e. from *m/z* 50 to 50 mass units greater than the "molecular ion") do not exceed a quarter of base peak intensity in EI spectra, or one-tenth for all other ionisation methods, the spectrum may be accepted as sufficient evidence of identity. Where unrelated ions exceed these limits, and they derive from chromatographically overlapping species, additional evidence should be sought. With EI, the absence of unrelated ions can be used to support identification if the analyte spectrum is very simple. Intensity ratios for principal ions should be within the tolerance limits shown in Table 3. Where an ion chromatogram shows significant chromatographic interference, it should not be used to determine an intensity ratio. The ion that shows the best signal-to-noise ratio and no evidence of significant chromatographic interference, should normally be used for quantification.

78. EI-MS or MS/MS, performed with acquisition of spectra, may provide good evidence of identity and quantity in many cases. In other cases, as with mass spectra produced by other processes (e.g. CI, API) that can be too simple for absolute confirmation of identity, further evidence may be required. If the isotope ratio of the ion(s), or the chromatographic profile of isomers of the

[2] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/757/EC).

analyte, is highly characteristic it may provide sufficient evidence. Otherwise, the evidence may be sought using:

(i) a different chromatographic separation system;

(ii) a different ionisation technique;

(iii) MS/MS;

(iv) medium/high resolution MS; or

(v) inducing "in-source" fragmentation in LC-MS.

79. Where the increased sensitivity obtained by scanning a limited mass range or by SIM is essential, the general minimum requirement is for data from two ions of *m/z* >200; or three ions of *m/z* >100, preferably including the molecular ion. For a few analytes, where these minimum requirements may not be achievable, ions with *m/z* <100 may also provide supporting evidence. However, ions arising from common moieties may be of little use, as are cationised molecules or adducts, such as $[M+NH_4]^+$, formed in LC-MS. Intensity ratios obtained from the more characteristic isotopic ions, e.g. those containing Cl or Br, may be of particular utility. The selected diagnostic ions should not exclusively originate from the same part of the parent molecule.

80. For full scan and SIM the relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion or transition, should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. Matrix-matched calibration solutions may need to be employed. Table 3 below indicates the maximum tolerances.

**Table 3.** Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques [2]

| Relative intensity (% of base peak) | EI-GC-MS (relative) | CI-GC-MS, GC-MS$^n$, LC-MS, LC-MS$^n$ (relative) |
|---|---|---|
| > 50 % | ± 10 % | ± 20 % |
| > 20 % to 50 % | ± 15 % | ± 25 % |
| > 10 % to 20 % | ± 20 % | ± 30 % |
| ≤ 10% | ± 50 % | ± 50 % |

Larger tolerances are more likely to lead to a larger percentage of false positive results. Likewise, if the tolerances are decreased, then the likelihood of false negatives increases[3]. The relative intensities of diagnostic ions and/or precursor/product ion pairs have to be identified by comparing spectra or by integrating the signals of the single mass traces.

When full scan spectra are recorded in single mass spectrometry, a minimum of four ions should be present with a relative intensity of ≥10% of the base peak. The molecular ion must be included if it is present in the reference spectrum

[3] Eugenia Soboleva, Karam Ahad and Árpád Ambrus, Applicability of some mass spectrometric criteria for the confirmation of pesticide residues, Analyst, 2004, 129, 1123-1129.

with a relative intensity of ≥10%. At least four ions should lie within the maximum permitted tolerances for the relative ion intensities (Table 3). Computer-aided library searching may be used. In this case, the comparison of mass spectral data in the test samples with that of the calibration solution has to exceed a critical match factor. This factor should be determined during method validation for every analyte. Variability in the spectra caused by the sample matrix and the detector performance must be checked.

*Confirmation by an independent laboratory*

81. Where practicable, confirmation of results in an independent expert laboratory provides strong supporting evidence of quantity. If different determination techniques are used, the evidence will also support identification.

**Reporting of results**

*Expression of results*

82. Results should normally be expressed as the chemical name defined by the MRL and in mg/kg. Residues below the Reporting Limit should be reported as <RL mg/kg.

*Calculation of results*

83. In general, residues data are not to be adjusted for recovery. If they are adjusted for recovery, then this must be stated. In this case they should be adjusted using the mean value from 3 recoveries performed in the same matrix, and analysed in the same batch of samples.

84. Where confirmed data are derived from a single test portion (i.e. the residue is not violative), the reported result should be that derived from the detection technique considered to be the most accurate. Where results are obtained by two or more equally accurate techniques, the mean value may be reported.

85. Where two or more test portions have been analysed, the arithmetic mean of the most accurate results obtained from each portion should be reported. Where good comminution and/or mixing of samples has been undertaken, the RSD of results between test portions should not exceed 30% for residues significantly above the LOQ. Close to the LOQ, the variation may be higher and additional caution is required in deciding whether or not a limit has been exceeded. Alternatively, the limits for repeatability, or reproducibility, given in Annex VI to Directive 91/414/EEC, may be applied, although these do not incorporate sub-sampling error (which is particularly important when undertaking dithiocarbamate or fumigant analyses).

*Rounding of data*

86. It is essential to maintain uniformity in reporting results. In general, results ≥0.01 and <10 mg/kg should be rounded to two significant figures; results ≥10 mg/kg may be rounded to three significant figures or to a whole number. Reporting limits should be rounded to 1 significant figure at <10 mg/kg and two significant figures at ≥10 mg/kg. These requirements do not necessarily reflect the uncertainty associated with the data. Additional significant figures may be recorded for the purpose of statistical analysis. In some cases the rounding may be specified by, or agreed with the customer/stakeholder of the monitoring.

*Qualifying results with uncertainty data*

87. It is a requirement under ISO/IEC 17025 that laboratories determine and make available the uncertainty associated with analytical results. To this end, laboratories should have available sufficient data derived from method validation/verification, inter-laboratory studies (e.g. proficiency tests) and in-house quality control tests, which are applied to estimate the uncertainties.[4]
Measurement uncertainty is a quantitative indicator of the confidence in the analytical data and describes the range around a reported or experimental result within which the true value can be expected to lie within a defined probability (confidence level). Uncertainty ranges must take into consideration all sources of error.

88. Uncertainty data should be applied cautiously to avoid creating a false sense of certainty about the true value. Estimates of typical uncertainty are based on previous data and may not reflect the uncertainty associated with analysis of a current sample. Typical uncertainty may be estimated using an ISO (Anonymous 1995,'Guide to the expression of uncertainty in measurement' ISBN 92-67-10188-9) or Eurachem (EURACHEM/CITAC Guide, Quantifying Uncertainty in Analytical Measurement, 2nd edition, (http://www.measurementuncertainty.org/mu/guide/index.html) approach. The values used may be derived from in-house validation data, the analysis of reference materials, from collaborative method development data, or estimated based on judgment. Reproducibility RSD (or repeatability RSD if reproducibility data are not available) may be used as the basis, but the contribution of additional uncertainty sources (e.g. heterogeneity of the sample, from which the analytical test portion has to be taken [due to differences in the procedures used for sample preparation, sample processing and sub-sampling], extraction efficiency, differences in standard concentrations) should be included. These RSD values may be derived from recovery data or the analysis of reference materials. Uncertainty data relate primarily to the analyte and matrix used to generate them and should be extrapolated to other analytes and matrices with caution. Uncertainty tends to be greater at lower levels, especially as the LOQ is approached. It may therefore be necessary to generate uncertainty data for a range of concentrations if typical uncertainty is to be provided for a wide range of residues data.

Another practical alternative for a laboratory to estimate its measurement uncertainty and to verify its estimation based on own intra-laboratory data is by evaluating its performance during proficiency tests. Proficiency test results can provide an important indication about the contribution of inter laboratory bias to the measurement uncertainty of an individual laboratory and indirect justification of the used measurement uncertainty.

89. Replicate analyses of a specific sample combined with concurrent recovery determinations, can improve the accuracy of the single-laboratory result and justify the use of a refined figure for the measurement uncertainty. In that case, care should still be taken with the influence of inter-laboratory bias. These uncertainty data will embrace the repeatability of sub-sampling and analysis. , This practice will be typically applied when the analytical results are extremely important (e.g. doubt about MRL compliance and economical interests).

[4] Report of the thirty-seventh session of the Codex Committee on Pesticide Residues, The Hague, The Netherlands, 18-23 April 2005, ALINORM 05/28/24, Appendix XII. Proposed draft guidelines on estimation of uncertainty of results

90. The use of reporting limits based on the LCL eliminates the need to consider uncertainty associated with residue levels found <reporting limits.

*Interpretation of results*

91. Assessment of whether or not a sample contains a violative residue is generally only a problem in cases where the level is relatively close to the MRL. The decision should take account of concurrent AQC data and the results obtained from replicate test portions, together with any assessment of typical uncertainty. The possibility of residue loss or cross-contamination having occurred before, during or after sampling must also be considered.

92. Considering the results so far from EU proficiency tests (for fruits and vegetables, using multiresidue methods), a default expanded uncertainty figure of 50% (corresponding to 95% confidence level), in general covers the inter-laboratory variability between the European laboratories and is recommended to be used by regulatory authorities in cases of enforcement decisions (MRL-exceedances). This is in agreement with the recommendation of the Codex Committee on Pesticide Residues (CCPR 2005, ALINORM 05/28/24). A prerequisite to be allowed to use a 50% default expanded uncertainty is that the laboratory proves its own calculated expanded uncertainty to be less than 50%. In cases where exceedances of an MRL at the same time cause an exceedance of the acute reference dose, an expanded uncertainty with a lower confidence level can be applied as a precautionary measure.

93. If laboratories experience, in individual cases, unacceptably high intra-laboratory repeatability- or reproducibility-RSD's (e.g. at very low concentration levels), or unsatisfactory z-scores during proficiency tests, the use of a correspondingly higher uncertainty figure must be considered, on a case-by-case basis.[4]

For results obtained with single-residue methods (in particular, if stable isotopically labelled internal standards are used), lower expanded uncertainties can be justified, if supported by correspondingly better inter laboratory-reproducibility RSD's (<25%).
It is common practice, that pesticide analysis results are not normally corrected for recovery, but should be corrected if the average is significantly different from 100% (typically if <70%, with good precision). In those cases, the uncertainty associated with recovery correction should also be taken into account.

94. If required, the result should be reported together with the expanded uncertainty (U), as follows: Result = x ± U (units).

**Additional recommended guidance**

Report of the thirty-seventh session of the Codex Committee on Pesticide Residues, The Hague, The Netherlands, 18-23 April 2005, ALINORM 05/28/24, Appendix X,
Proposed draft guidelines on the use of mass spectrometry (MS) for identification, confirmation and quantitative determination of residues.

**Appendix 1. Glossary**

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| accuracy | Closeness of agreement between a test result and the true, or the accepted reference value. When applied to a set of test results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias) (ISO 5725-1). |
| analyte | The chemical species of which the concentration (or mass) is to be determined. For the purposes of these guidelines: a pesticide or a metabolite, breakdown product or derivative of a pesticide. |
| analytical sample | Sometimes referred to as a "test portion", or "test sample".<br>A sample prepared from the laboratory sample and from which "test portions" or "analytical portions" are taken (ISO 78/2, 1982). See also Directive 2002/63/EC. |
| analytical portion | Sometimes referred to as "test portion".<br>The quantity of material (usually homogenised) taken from the analytical sample, and on which the analysis/test is performed (ISO 78/2, 1982). |
| API | Atmospheric pressure ionisation (for LC-MS). A generic term including electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). |
| AQC | Analytical quality control. Measurement and recording requirements intended to demonstrate the performance of the analytical method in routine practice. The data supplement those generated at method validation. AQC data may be used to validate the extension of methods to new analytes, new matrices and new levels. Synonymous with the terms internal quality control (IQC) and performance verification. Concurrent AQC data are those generated during analysis of the batch in which the particular sample is included. |
| batch<br>(analysis) | For extraction, clean-up and similar processes, a batch is a series of samples dealt with by an analyst (or team of analysts) in parallel, usually in one day, and should incorporate at least one recovery determination. For the determination system, a batch is a series undertaken without a significant time break and which incorporates all relevant calibration determinations (also referred to as an "analysis sequence", a "chromatography sequence", etc.). With formats such as 96-well plates, a plate or group of plates may form a batch. A determination batch may incorporate more than one extraction batch.<br>This document does not refer to "batch" in the IUPAC or Codex sense, which relates to manufacturing or agricultural production batches. |

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| bias | Also referred to as “accuracy” .The difference between the mean measured value and the true value, i.e. the total systematic error. |
| blank | (i) Material (a sample, or a portion or extract of a sample) known not to contain detectable levels of the analyte(s) sought. Also known as a matrix blank.<br>(ii) A complete analysis conducted using the solvents and reagents only, in the absence of any sample (water may be substituted for the sample, to make the analysis realistic). Also known as a reagent blank or procedural blank. |
| bracketing calibration | Organisation of a batch of determinations such that the detection system is calibrated immediately before and after the analysis of the samples. For example, calibrant 1, calibrant 2, sample 1.....sample *n*, calibrant 1, calibrant 2. |
| calibration | Determination of the relationship between the observed signal (response produced by the detection system) and known quantities of the analyte. In the present document, calibration does not refer to calibration of weighing and volumetric equipment, mass calibration of mass spectrometers, and so on. |
| calibration standard | A solution (or other dilution) of the analyte (and internal standard, if used) used for calibration of the determination system. May be prepared from a working standard and may be matrix-matched. |
| certified reference material (CRM) | See reference material. |
| CI | Chemical ionisation (for GC-MS). |
| comminution | The process of reducing a solid sample to small fragments. |

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| confirmation | The process of generating sufficient evidence to ensure that a result for a specific sample is valid. Analytes must be identified correctly in order to be quantified. The identity and quantity of residues should be confirmed. It is impossible to confirm the complete absence of residues. Adoption of a "reporting limit" at the LCL avoids the unjustifiably high cost of confirming the presence, or absence, of residues at unnecessarily low levels.<br><br>The nature and extent of confirmation required for a positive result depends upon importance of the result and the frequency with which similar residues are found.<br><br>Assays based on colorimetry, ELISA, TLC or ECD tend to demand confirmation, because of their lack of specificity.<br><br>Mass spectrometric techniques are often the most practical and least equivocal approach to confirmation.<br><br>AQC procedures for confirmation should be rigorous. |
| contamination | Unintended introduction of the analyte into a sample, extract, internal standard solution etc., by any route and at any stage during sampling or analysis. |
| determination system | Any system used to detect and determine the concentration or mass of the analyte. For example, GC-FPD, LC-MS/MS, LC with post-column derivatisation, ELISA, TLC with bioassay. |
| ECD | Electron-capture detector. |
| EI | Electron ionisation. |
| ELISA | Enzyme-linked immuno-sorbent assay. |
| EU | European Union. |
| false negative | A result wrongly indicating that the analyte concentration does not exceed a specified value. |
| false positive | A result wrongly indicating that the analyte concentration exceeds a specified value. |
| FPD | Flame-photometric detector (may be specific to sulphur or phosphorus detection). |
| GC | Gas chromatography (gas-liquid chromatography). |

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| interference | A positive or negative response produced by a compound(s) other than the analyte, contributing to the response measured for the analyte, or making integration of the analyte response less certain or accurate. Interference is also loosely referred to as "chemical noise" (as distinct from electronic noise, "flame noise", etc.). Matrix effects are a subtle form of interference. Some forms of interference may be minimised by greater selectivity of the detector. If interference cannot be eliminated or compensated, its effects may be acceptable if there is no significant impact on accuracy (bias) or precision. |
| internal quality control (IQC) | see AQC |
| internal reproducibility | see reproducibility |
| internal standard | A chemical added, in known quantity, at a specified stage in analysis to facilitate determination of the identity and/or quantity of the analyte. The analyte concentration is deduced from its response relative to that produced by the internal standard. The internal standard should have similar physico-chemical characteristics to those of the analyte. Isotopically labelled analytes form ideal internal standards, where available. For all other types of internal standard, the relative responses must be calibrated for each batch of analyses. Standard addition could be regarded as a special form of ideal internal standardisation. |
| laboratory sample | The sample sent to and received by the laboratory. |
| LC | Liquid chromatography (primarily high performance liquid chromatography, HPLC). |
| LCL | Lowest calibrated level. The lowest concentration (or mass) of analyte with which the determination system is successfully calibrated, throughout the analysis batch. See also "reporting limit". |
| LC-MS | Liquid chromatographic separation coupled with mass spectrometric detection. |
| level | In this document, refers to concentration (e.g. mg/kg, µg/ml) or quantity (e.g. ng, pg). |

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| limit of detection | The minimum concentration or mass of the analyte that can be detected with acceptable certainty, though not quantifiable with acceptable precision. Various definitions are used but, for convenience, it is often the quantity of analyte that generates a response 3 times greater than the noise level of the detection system. Definitions based on standard deviation of blank values can be difficult to apply in chromatographic analysis. With most methods and determination systems, the limit of detection has no fixed value. The term is usually restricted to the response of the detection system but, in principle, it should be applied to the complete analytical method. |
| LOD | Limit of determination (see LOQ below). |
| LOQ | Limit of quantitation (quantification) (also known as limit of determination, LOD). The minimum concentration or mass of the analyte that can be quantified with acceptable accuracy and precision. Should apply to the complete analytical method. Variously defined but must be a value greater than the limit of detection. With most methods and determination systems, the LOQ has no fixed value.<br><br>LOQ is preferable to LOD because it avoids possible confusion with "limit of detection". However, in legislation MRLs that are set at the limit of quantification/determination are referred to as "LOD MRLs", not "LOQ MRLs". |
| matrix blank | See blank. |

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| matrix effect | An influence of one or more undetected components from the sample on the measurement of the analyte concentration or mass. The response of some determination systems (e.g. GC, LC-MS, ELISA) to certain analytes may be affected by the presence of co-extractives from the sample (matrix). Partition in headspace analyses and SPME is also frequently affected by components present in the samples. These matrix effects derive from various physical and chemical processes and may be difficult or impossible to eliminate. They may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte. The presence, or absence, of such effects may be demonstrated by comparing the response produced from the analyte in a simple solvent solution with that obtained from the same quantity of analyte in the presence of the sample or sample extract. Matrix effects tend to be variable and unpredictable in occurrence, although certain techniques and systems (e.g. HPLC-UV, isotope dilution) are inherently less likely to be influenced. More reliable calibration may be obtained with matrix-matched calibration when it is necessary to use techniques or equipment that are potentially prone to the effects. Matrix-matched calibration may compensate for matrix effects but does not eliminate the underlying cause. Because the underlying cause remains, the intensity of effect may differ from one matrix or sample to another, and also according to the "concentration" of matrix. Isotope dilution or standard addition may be used where matrix effects are sample dependent. |
| matrix-matched calibration | Calibration intended to compensate for matrix effects and acceptable interference, if present. The matrix blank (see "blank") should be prepared as for analysis of samples. In practice, the pesticide is added to a blank extract (or a blank sample for headspace analysis) of a matrix similar to that analysed. The blank matrix used may differ from that of the samples if it is shown to compensate for the effects. However, for determination of residues approaching or exceeding the MRL, the same matrix (or standard addition) should be used. |
| method | A sequence of analytical procedures, from receipt of a sample through to the calculation of results. |
| method development | The process of design and preliminary assessment of the characteristics of a method, including ruggedness. |

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| method validation | The process of characterising the performance to be expected of a method in terms of its scope, specificity, accuracy (bias), sensitivity, repeatability and reproducibility. Some information on all characteristics, except reproducibility, should be established prior to the analysis of samples, whereas data on reproducibility and extensions of scope may be produced from AQC, during the analysis of samples. Wherever possible, the assessment of accuracy (bias) should involve analysis of certified reference materials, participation in proficiency tests, or other inter-laboratory comparisons. |
| MRL | Maximum residue limit. MRL* is set at or about the LOQ. |
| MS | Mass spectrometry. |
| MS/MS | Tandem mass spectrometry, here taken to include $MS^n$. An MS procedure in which ions of a selected mass to charge ratio (*m/z*) from the primary ionisation process are isolated, fragmented usually by collision, and the product ions separated (MS/MS or $MS^2$). In ion-trap mass spectrometers, the procedure may be carried out repetitively on a sequence of product ions ($MS^n$), although this is not usually practical with low-level residues. |
| NPD | Nitrogen-phosphorus detector. |
| performance verification | see analytical quality control (AQC) |
| priming (of GC injectors and columns) | Priming effects resemble long-lasting matrix effects and are typically observed in gas chromatography. Typically, an aliquot of sample extract that has not been subjected to clean-up may be injected after a new column or injector liner is fitted, or at the beginning of a batch of determinations. The objective is to "deactivate" the GC system and maximise transmission of the analyte to the detector. In some cases, large quantities of analyte may be injected with the same objective. In such cases it is critically important that injections of solvent or blank extracts are made before samples are analysed, to ensure the absence of carryover of the analyte. Priming effects are rarely permanent and may not eliminate matrix effects. |
| procedural blank | See blank. |
| procedural standard | A calibration standard of a derivative, degradation product, etc., of the analyte which is generated from a precursor, as part of the analytical method. Procedural standards are often employed in cases where the derivative, degradation product, etc., is not available as a "pure" standard. The term is not applied to transient species generated in the detector, e.g. fragments in mass spectrometry. However, it is applicable to the products of post-column reactions generated prior to detection in HPLC. |

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| reagent blank | See blank. |
| recovery (of analyte through an analytical method) | The proportion of analyte remaining at the point of the final determination, following its addition (usually to a blank sample) immediately prior to extraction. Usually expressed as a percentage.<br><br>Routine recovery refers to the determination(s) performed with the analysis of each batch of samples. |
| reference material | Material characterised with respect to its notionally homogeneous content of analyte. Certified reference materials (CRMs) are normally characterised in a number of laboratories, for concentration and homogeneity of distribution of analyte. In-house reference materials are characterised in the owner's laboratory and the measurement accuracy (bias) may be unknown. |
| reference spectrum | A spectrum of absorption (e.g. UV, IR), fluorescence, ionisation products (MS), etc., derived from the analyte and which may be characteristic of it. The reference mass spectrum preferably should be produced from the "pure" standard (or a solution of the "pure" standard) by the instrument used for analysis of the samples, and similar ionisation conditions must be used. |
| "pure" standard | A relatively pure sample of the solid/liquid analyte (or internal standard), of known purity. Usually >90% purity, except for certain technical pesticides. |
| repeatability | The precision (standard deviation) of measurement of an analyte (usually obtained from recovery or analysis of reference materials), obtained using the same method on the same sample(s) in a single laboratory over a short period of time, during which differences in the materials and equipment used and/or the analysts involved will not occur.<br><br>May also be defined as the value below which the absolute difference between two single test results on identical material, obtained under the above conditions, may be expected to lie with a specified probability (e.g. 95%). |
| reporting limit or reporting level | The lowest level at which residues will be reported as absolute numbers. It may represent the practical LOQ, or it may be above that level to limit costs. It must not be lower than the corresponding LCL. For EU monitoring purposes where samples for surveys are analysed over a 12-month period, the same reporting limit should be achievable throughout the whole year. |
| representative analyte | An analyte used to assess probable analytical performance in respect of other analytes notionally sought in the analysis. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes must include those for which the worst performance is expected. |

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| representative matrix | Sample material or an extract of a commodity used as an indicator of method performance, or for matrix-matched calibration, in the analysis of broadly similar commodities. Similarity is usually determined according to the content of water, acids, sugars, lipids, secondary plant metabolites, etc., physical characteristics, or matrix effects. |
| represented analyte | Analytes notionally sought but for which no concurrent quality control data are generated. Quality control data obtained from representative analytes are assumed to show whether or not analytical performance is acceptable for these analytes. Relative responses must be reasonably consistent to ensure that calibration is meaningful. Accuracy (recovery bias) is assumed to be no worse than that of the worst-case representative analyte(s). |
| represented matrix | Sample material or an extract of a commodity sufficiently similar to the representative matrix that analytical quality control data (or matrix-matched calibration) generated from the latter can be considered valid for the former. Where potentially unacceptable residues are detected, method performance data should be generated from the represented matrix. |
| reproducibility | The precision (standard deviation) of measurement of an analyte (usually by means of recovery or analysis of reference materials), obtained using the same method in a number of laboratories, by different analysts, or over a period in which differences in the materials and equipment will occur.<br>Internal reproducibility is that produced in a single laboratory under these conditions.<br>May also be defined as the value below which the absolute difference between two single test results on identical material, obtained under the above conditions, may be expected to lie with a specified probability (e.g. 95%). |
| response | The absolute or relative signal output from the detector when presented with the analyte. |
| RSD | Relative standard deviation (coefficient of variation). |
| sample | A general term with many meanings but, in these guidelines, refers to laboratory sample, test sample, test portion, or an aliquot of extract. |
| sample preparation | The first of two processes which may be required to convert the laboratory sample into the test sample. The removal of parts that are not to be analysed, if required. |
| sample processing | The second of two processes which may be required to convert the laboratory sample into the test sample. The process of homogenization, comminution, mixing, etc., if required. |

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| SD | Standard deviation. |
| selectivity | The ability of the extraction, the clean-up, the derivatisation, the separation system and (especially) the detector to discriminate between the analyte and other compounds. GC-ECD is a selective determination system providing no specificity. |
| SFE | Supercritical fluid extraction. |
| SIM | Selected ion monitoring (MS). |
| solid phase dilution | Dilution of a pesticide by distribution within a finely divided solid, such as starch powder. Normally used only for insoluble analytes such as the complex dithiocarbamates. |
| S/N | Signal-to-noise ratio. |
| specificity | The ability of the detector (supported by the selectivity of the extraction, clean-up, derivatisation or separation, if necessary) to provide signals which effectively identify the analyte. GC-MS with EI is a fairly non-selective determination system capable of high specificity. High resolution mass MS and $MS^n$ can be both highly selective and highly specific. |
| spike or spiking | Addition of analyte for the purposes of recovery determination or standard addition. |
| SPME | Solid phase micro-extraction. |
| standard | A general term which may refer to a "pure" standard, stock standard, working standard, or calibration standard. |
| stock standard | The most concentrated solution (or solid dilution, etc.) of the "pure" standard or internal standard, from which aliquots are used to prepare working standards or calibration standards. |
| test portion | Also referred to as the "analytical portion".<br>A representative sub-sample of the test sample, i.e. the portion which is to be analysed. |
| test sample | Also referred to as the "analytical sample".<br>The laboratory sample after removal of any parts that are not to be analysed, e.g. bones, adhering soil. It may or may not be comminuted and mixed before withdrawing test portions. See also Directive 2002/63/EC. |
| TLC | Thin layer chromatography. |
| trueness | The measure of trueness is normally expressed as 'bias'.<br>The closeness of agreement between the average value obtained from a series of test results (i.e. the mean recovery) an an accepted reference or true value (ISO 5725-1). |

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| uncertainty<br>(of measurement) | A range around the reported result within which the true value can be expected to lie with a specified probability (confidence level, usually 95%). Uncertainty data should encompass trueness (bias) and reproducibility |
| unit (sample) | A single fruit, vegetable, animal, cereal grain, can, etc. For example, an apple, a T-bone steak, a grain of wheat, a can of tomato soup. |
| validation | see method validation |
| violative residue | A residue which exceeds the MRL or is unlawful for any other reason. |
| working standard | A general term used to describe dilutions produced from the stock standard, which are used, for example, to spike for recovery determination or to prepare calibration standards. |