



# **QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS**

**Guidelines for Residues Monitoring in the European Union**

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by Alan Hill (UK) and the organising committee, following discussions at the  
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# QUALITY CONTROL PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS

## Introduction

**1** This document describes analytical quality control (AQC) requirements to support the validity of data used for checking compliance with maximum residue limits (MRLs), to support enforcement actions, or to assess consumer exposure to pesticides. The objectives are (i) to ensure that false positives or false negatives are not reported, (ii) to ensure that acceptable accuracy (bias) and precision are achieved and (iii) to achieve harmonisation of cost-effective AQC in the EU. Where options are provided, achievement of higher accuracy and precision will generally require application of the more stringent requirements.

**2** These guidelines supersede document 7826/VI/97, also published as Annex II of Commission Recommendation 1999/333/EC (Official Journal L128, p.25, 21 May 1999) and as Annex II of EFTA Recommendation No. 153/99/KOL (Official Journal L74, p.21, 2 July 1999).

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

## Accreditation

**4** In accordance with the provisions of Directive 93/99/EEC, laboratory operations should meet the requirements of a recognised accreditation scheme, complying with ISO 17025 or Good Laboratory Practice (GLP). The quality requirements described in this document are intended as guidance for accreditation purposes. The accuracy and precision of weight and volume measurements must be consistent with achievement of the overall accuracy and precision requirements of paragraphs 58-63.

## Sampling, transport, processing and storage of samples

### *Sampling*

**5** Samples should be taken in accordance with Directive 79/700/EEC 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*

**6** 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 pre-packed 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 which are frozen at the time of collection must be transported without thawing. Samples which may be damaged by chilling (e.g. bananas) must be protected from both high and low temperatures.

**7** 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.

**8** Samples must be identified clearly and indelibly, in a way which 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.

*Sample preparation and processing prior to analysis*

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

**10** Sample preparation, sample processing and sub-sampling to obtain test portions must take place before visible deterioration occurs. Canned, dried or similarly processed samples must be analysed within the stated shelf-life, unless stored in deep freeze.

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

**12** Sample processing and storage procedures should be demonstrated to have no significant effect on the residues present in the test 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. Determination of very labile or volatile residues should be started, and the procedures involving potential loss of analyte completed, on the day of sample receipt.

**13** If a single test portion is unlikely to be representative of the test sample, replicate portions must be analysed, to provide a reliable indication of the mean value.

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

*Identity and purity of standards*

**14** "Pure" standards of analytes and internal standards should be of known purity. Each "pure" standard must be uniquely identified, the date of receipt recorded, and an expiry date allocated if the supplier has not provided this. After the expiry date, a "pure" standard may be retained if its purity is shown to remain acceptable and a new expiry date is allocated. Otherwise it must be replaced. The relative purity of the new and old "pure" standards may be determined by comparing the detector responses obtained from freshly-prepared dilutions. Inexplicable differences in apparent concentration or identity between old and new "pure" standards must be investigated. Ideally, the identity of freshly acquired "pure" standards should be checked if the analytes are new to the laboratory.

**15** At method development or validation, the response detected must 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 which is commonly found in residues but which is excluded from the residue definition, positive results must be confirmed using techniques which avoid this problem<sup>1</sup>.

#### *Storage of standards*

**16** “Pure” standards should be stored according to the suppliers’ instructions (where given), to minimise degradation. Generally, storage at low temperature (refrigerator or freezer) in the dark is satisfactory. The containers must be sealed to avoid entry of water, which is especially likely during equilibration to room temperature. If a “pure” standard changes visibly during storage it must not be used without checking the purity, unless the change is due to freezing and melting.

#### *Preparation, use and storage of stock and working standards*

**17** Preparation of stock and working standards (solutions, dispersions or gaseous dilutions) of “pure” standards of analytes and internal standards requires careful attention to detail. Any inaccuracy in their preparation may not be apparent from checks of calibration or recovery but will directly affect the accuracy of the residues data. The identity and mass (or volume, for highly volatile compounds) of the “pure” standard, the identity of the solvent (or other diluent), and the volumes and dilution steps employed, must be recorded. Stock and working standards must be labelled indelibly. Concentrations must be corrected for the purity of the “pure” standard.

**18** The analyte (and internal standard) must not react with, and should have adequate solubility in, the solvent(s) used to prepare solutions. The solvent(s) must be appropriate to the method of analysis and be compatible with the determination system used.

**19** Unless suitably accurate facilities are available, not less than 5-10 mg of the “pure” standard should be weighed. 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).

**20** Analyte solutions (or other dilutions) must be allocated an expiry date, after which they should normally be discarded. Newly prepared stock standards should be diluted (if necessary) and compared with those to be discarded. If the mean measurement for the new solution differs by more than  $\pm 5\%$  from the old one<sup>2</sup>, the new solution should be checked for accuracy against a further newly prepared one. If the number of replicate determinations required to distinguish a difference of

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<sup>1</sup> This requirement applies where the product of analytical degradation must be distinguished from the chemically identical metabolite in the sample, in order to determine the residue level according to the definition. For example, 4,4'-dichlorobenzophenone from dicofol, tetrahydrophthalimide from captan and captafol, phthalimide from folpet, 2-chlorobenzonitrile from clofentazine.

<sup>2</sup> Alternatively, a t-test of the means should not show a significant difference at the 5% level.

$\pm 5\%$  is unacceptably large for problematic analytes, the acceptable range may be increased to  $\pm 10\%$ . If the old standard produces  $\leq 95\%$  (or 90% in the case of problematic analytes) of the response obtained from the new standard, the storage period for solutions must be shortened or the storage conditions improved. If the responses from old and the new standards do not differ significantly, a longer storage period may be considered. Aqueous suspensions of dithiocarbamates and solutions (or gaseous dilutions) of highly volatile fumigants must be prepared freshly. The concentration of such a standard may be checked by comparison with one prepared independently.

**21** Solutions should not be exposed to direct sunlight and should be stored at low temperature in the dark, in a refrigerator or freezer, sealed to avoid loss of solvent and entry of water. Solutions removed from low temperature storage must be equilibrated to room temperature and re-mixed before use. If solubility at low temperatures is limited, great care must be taken to ensure that the analyte is completely re-dissolved after storage of solutions.

**22** Unless standard solutions are internally standardised, solvent losses by evaporation are unacceptable. Solvent losses from small volumes are difficult to monitor and, in the absence of an internal standard, great care is required to avoid evaporation. 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.

### **Extraction and concentration**

#### *Extraction conditions and efficiency*

**23** Test portions should be disintegrated thoroughly during extraction to maximise extraction efficiency, except where this is known to be unnecessary (e.g. some SFE extractions) 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 losses.

#### *Extract concentration and dilution to volume*

**24** 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.

**25** 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. The requirements of paragraph 22 also apply to extracts.

**26** 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**

### *Contamination*

**27** 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.

**28** Pest control in, or near, the laboratory must be restricted to pesticides which will not be sought as residues.

**29** 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. Badly scratched or etched glassware should be avoided. Solvents used for fumigant residues analysis should be checked to ensure that they do not contain the analyte.

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

**31** 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 *Cruciferaeae*), 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*

**32** 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.

**33** 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 paragraphs 45-46. If it is not practicable to eliminate interference, or to compensate for it by matrix-matching

calibration, the overall accuracy (bias) and precision of analysis should nonetheless comply with the criteria in paragraphs 53 and 58-63.

## **Analytical calibration and chromatographic integration**

### *General requirements*

**34** Correct calibration is dependent upon correct identification of the analyte (see paragraphs 64-73). 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.

**35** The determination system must be calibrated for every batch of analyses. If calibration for all analytes sought implies an unacceptably large number of calibration determinations, the system may be calibrated with representative analytes<sup>3</sup> during each batch of analyses. The minimum frequency for calibration of representative and represented (i.e. all other) analytes is given in Table 1.

Table 1. Minimum frequencies for calibration and recovery determination

	Representative analytes <sup>3</sup>	Represented (i.e. all other) analytes
Minimum frequency of calibration	Calibration in each batch of determinations	A rolling programme to include all represented analytes at least every 6 months*  ≥2 levels, including the LCL
Minimum frequency of recovery	Determination in each batch of analyses	One for each analyte, synchronised with the corresponding calibration series, as above  ≥2 levels, including the LCL

\* 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 separation/determination system.

**36** 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 represented 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;

<sup>3</sup> Known as “reference pesticides” in the first edition of these guidelines.

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

**37** Where a represented analyte is detected in a sample, the result must be considered tentative. The sample should be re-analysed and accompanied by acceptable recovery and calibration of the detected analyte. This is essential where the screening result indicates that an MRL might be exceeded.

**38.** If the rolling programme (Table 1) of recovery or calibration of a represented 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 false negatives.

**39** 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 (see glossary, S/N), 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 incorporate determination of recovery and response linearity at the proposed LCL.

**40** Calibration by interpolation between two levels is acceptable where the mean response factors, derived from replicate determinations at each level, indicate acceptable linearity of response. The higher response factor should not be more than 120% of the lower response factor (110% in cases where the MRL is approached or exceeded).

**41** 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  $\pm 20\%$  ( $\pm 10\%$  in cases where the MRL is approached or exceeded) from the calibration curve in the relevant region, a more satisfactory calibration function must be used or the determinations must be repeated.

**42** More frequent single-level calibration may provide more accurate results than less frequent multi-level calibration, if the detector response is variable with time. When single-level calibration is employed, the sample response should be within  $\pm 10\%$  of the calibration standard response if the MRL is exceeded. If the MRL is not exceeded, the sample response should be within  $\pm 50\%$  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 indicate analytical performance achieved at the LCL and does not imply that residues <LCL should be calculated in this way.

**43** Extracts containing high-level residues may be diluted to bring them within the calibrated range but, where calibration solutions must be matrix-matched (paragraphs 45-46), the concentration of matrix extract may have to be adjusted accordingly.

**44** Batch sizes for determination should be adjusted so that detector response to bracketing calibration standards does not drift >20% at  $\geq 2 \times$  LCL, or >30% at  $< 2 \times$  LCL (if the LCL is close to the LOD, otherwise the 20% value applies). In cases where the MRL is approached or exceeded, these maximum drift values should be 10% and 15%, respectively. If the drift exceeds these values, repeating the determinations is not necessary where the samples clearly contain no analyte, providing that the LCL response remains measurable throughout the batch. However, positive results and recovery values should be quantified by repeating the determinations in a batch in which the drift is acceptable.

#### *Matrix effects and matrix-matched calibration*

**45** 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 analysis) of blank matrix may be used for calibration purposes. The best way to eliminate each matrix effect is to calibrate by standard addition.

**46** A potential problem is that different samples of the same commodity, 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 may be used to calibrate a wide range of sample types.

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

#### *Effects of pesticide mixtures on calibration*

**48** Calibration using mixed analyte solutions, etc., should be checked at method validation 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 which are mixtures of isomers etc.*

**49** Where a calibration standard is a mixture of isomers, etc., of the analyte, detector response is usually 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 residues. In those cases where the response of a

more conventional detector to isomers differs<sup>4</sup>, 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*

**50** 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 may be used if they are the only practical option.

#### *Chromatographic integration*

**51** 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.

**52** With the exception of cases where the detector response to the components of mixed isomer (or similar) standards differs on a molar basis, calibration may utilise summed peak areas, summed peak heights, or measurement of a single component, whichever is the more accurate. If none of these is sufficiently accurate, a more satisfactory detection system must be used.

### **Analytical methods and analytical performance**

#### *Acceptability of analytical methods*

**53** The analytical method should be demonstrated at validation as being capable of providing mean recovery within the range 70-110%, for all compounds sought by the method and at appropriate levels (paragraph 56). Where the method does not permit this, and there is no satisfactory alternative, the relatively poor accuracy must be considered before taking enforcement action. Where the residue definition incorporates two or more analytes, the method should be validated for all analytes.

#### *Methods for determination of fat or dry weight content*

**54** 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*

**55** 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 determination 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.

**56** Analyte recovery should normally be determined by addition within a range corresponding to 1-10 times the LCL, or at the MRL, or at a level of special

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<sup>4</sup> For example, the differing electron-capture efficiency of HCH isomers in ECD, or the differing proton affinity of abamectin isomers in electrospray ionisation.

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  $\geq 5$  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.

**57** 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 which either normally predominates in residues or is likely to provide the lowest recovery.

#### *Acceptability of analytical performance*

**58** Routine recovery within the range 60-140% is acceptable. Where a routine recovery result is unacceptably low, the batch of samples should normally be re-analysed. Exceptionally, where recovery is low but precision is good and the basis for this is well established (e.g. due to pesticide distribution in partition), a mean recovery below 60% may be acceptable. However, a more accurate method should be used, if practicable. 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 recovery is slightly beyond the 60-140% range, the results for samples in the batch(es) affected may be considered semi-quantitative. If recovery is outside this range, the residues data may have little value, especially if low recovery is involved.

**59** Data on violative residues should be supported by recovery within the range 70-110%, at least for the confirmatory analyses. If recovery within this range cannot be achieved, enforcement action is not necessarily precluded but the relatively poor accuracy must be taken into account.

**60** If a significant trend occurs in recovery, or potentially unacceptable (beyond 3 SD) results are obtained, the cause(s) must be investigated. Acceptable limits for recovery may be adjusted when repeatability (validation) data are supplemented by internal reproducibility (routine recovery) data but a substantial decline in precision must be investigated.

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

#### **Proficiency testing and analysis of reference materials**

**62** Determination of recovery does not demonstrate the overall accuracy or overall bias of results, so the laboratory must participate regularly in relevant proficiency tests. Where the accuracy achieved in a test is questionable or unacceptable, the problem(s) should be investigated and, particularly for unacceptable performance, rectified before proceeding with further determinations of the analytes involved.

**63** In-house reference materials may be analysed regularly to help provide evidence of analytical performance. Where practicable, exchange of such materials between laboratories provides an additional independent check of accuracy.

### **Confirmation of results**

#### *Principles of confirmation*

**64** Negative results can be considered confirmed if the recovery and LCL measurement for the batch are acceptable (paragraphs 58 and 39). Negative results for represented analytes are supported only indirectly by the recovery and LCL data for representative analytes and must be interpreted with caution.

**65** Positive results require additional confirmation to that given in paragraph 64. In addition to the general requirements of paragraphs 66-72, confirmation of positive results for represented analytes (i.e. those with no concurrent calibration and recovery) must be supported by the appropriate concurrent calibration and recovery determinations. Additional confirmation requirements for all positive results, especially for those close to LODs, must be decided on a case-by-case basis. However, where reasonable doubt remains, further confirmation must be sought.

**66** Potentially violative 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 extraction, clean-up, derivatisation, separation, and detection techniques may also be used to support confirmation.

**67** If detectors of limited specificity are employed, GC or LC with a second chromatographic column of different polarity provides only limited confirmatory evidence. Limitations in the quality of the confirmation provided should be acknowledged in the reporting of results. Such limitations may be acceptable for frequent residues, especially if some results are also confirmed by a more specific technique, but the general use of a more specific technique is advisable.

#### *Confirmation by mass spectrometry (MS)*

**68** 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.

**69** Diagnostic ion chromatograms should have peaks (minimum 3 data points, minimum S/N 3:1, see glossary, S/N) 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.

**70** Careful subtraction of background spectra is required to ensure that the resultant spectrum of the chromatographic peak is representative. 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 70-130% of those obtained from the standard. Where an ion-chromatogram shows significant chromatographic interference, it should not be used to determine an intensity ratio. The most abundant ion that shows no evidence of chromatographic interference, and the best signal-to-noise ratio, should normally be used for quantification.

**71** EI-MS or MS/MS, performed with acquisition of spectra, may provide good evidence of identity and quantity in many cases. Mass spectra produced by other processes (e.g. CI, API) can be too simple for confirmation of identity and further supporting evidence may be required. If the isotope ratio of the ion(s), or the chromatographic profile of isomers of the 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) altering fragmentation by changing the "cone voltage" in LC-MS. The ions selected for medium/high resolution MS or MS/MS should be characteristic of the analyte, not common to many organic compounds.

**72** Where the increased sensitivity obtained by scanning a limited mass range or by SIM is essential, the minimum requirement is for data from two ions of  $m/z > 200$ ; or three ions of  $m/z > 100$ . Intensity ratios obtained from the more characteristic isotopic ions may be of particular utility. Additional supporting evidence (see paragraph 71) should be provided where these requirements cannot be met.

#### *Confirmation by an independent laboratory*

**73** Where practicable, confirmation of results in an independent 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*

**74** Results should normally be expressed as defined by the MRL, with the concentration in mg/kg. Residues below the LCL should be reported as <(LCL) mg/kg.

### *Calculation of results*

**75** In general, residues data should not be adjusted for recovery.

**76** 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 for a single test portion are obtained by two or more equally accurate techniques, the mean value may be reported.

**77** 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 is undertaken, the RSD of results between test portions should not exceed 30% for residues significantly above the LOD. Close to the LOD, 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 Directive 93/94/EC (Annex VI to Directive 91/414/EEC), may be applied, although these do not incorporate sub-sampling error. Sub-sampling error can contribute significantly to the uncertainty of results, especially in the case of dithiocarbamates or fumigant determination (paragraph 12).

**78** For a pesticide having a residue definition that includes two or more analytes determined separately, adoption of a single reporting limit is problematic. Three options are illustrated in Table 2, although there is only one option if no analyte is detected. Options (i) and (ii) are scientifically correct, whereas option (iii) is not strictly correct. The choice of option should be made according to the purpose of the results. Where the “pure” standard contains two or more components producing similar molar responses but which differ in concentration, for example chlorfenvinphos isomers, results may be calculated on the basis of the component producing the largest response. If this approach is adopted, the lack of a characteristic component profile supporting the identification of residues at or about the reporting limit may require the use of a more rigorous confirmation technique.

Table 2. Reporting results for multi-component residues, using endosulfan as an example

Results, mg/kg*	Options for reporting endosulfan, mg/kg
alpha-endosulfan <0.05 beta-endosulfan <0.05 endosulfan sulfate <0.1	(i) <0.2
alpha-endosulfan 0.05 beta-endosulfan 0.05 endosulfan sulfate <0.1	(i) <0.2 (ii) ≥0.1 but <0.2 (iii) 0.1
alpha-endosulfan 0.05 beta-endosulfan <0.05 endosulfan sulfate <0.1	(i) <0.2 (ii) ≥0.05 but <0.2 (iii) 0.05
alpha-endosulfan <0.05 beta-endosulfan <0.05 endosulfan sulfate 0.1	(i) <0.2 (ii) ≥0.1 but <0.2 (iii) 0.1

\* LCLs assumed to correspond to 0.05 mg/kg for alpha- and beta-endosulfan and 0.1 mg/kg for endosulfan sulfate.

#### *Rounding of data*

**79** Results <0.1 mg/kg should be rounded to one significant figure; results ≥0.1 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 purposes of statistical analysis.

#### *Qualifying results with uncertainty data*

**80** Measurement uncertainty is a useful quantitative indicator of the confidence, when analytical data are to be compared with legal limits. Uncertainty ranges must take into consideration all sources of error, including inter-laboratory bias if several laboratories analyse the same sample.

**81** 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<sup>5</sup> or EURACHEM<sup>6</sup> approach. The values used may be derived from in-house

<sup>5</sup> Anonymous (1995), "Guide to the expression of uncertainty in measurement" (ISBN 92-67-10188-9). ISO, Geneva, Switzerland.

<sup>6</sup> The EURACHEM/CITAC Guide, Quantifying Uncertainty in Analytical Measurement, 2<sup>nd</sup> edition, is accessible at <http://www.vtt.fi/ket/eurachem/quam2000-p1.pdf>.

validation data, the analysis of reference materials, from collaborative method development data, or estimated based on judgement. 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. laboratory sample heterogeneity, extraction efficiency, bias in standard concentrations) should be included if possible. Where proficiency test performance indicates that the assigned true value lies outside the typical uncertainty for the result obtained, typical uncertainty data must be reviewed. Uncertainty data relate primarily to the analyte and matrix used to generate them. They should not be extrapolated to represented analytes and should be extrapolated with caution to represented matrices. Uncertainty tends to be greater at lower levels, especially as the LOD 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.

**82** For a specific sample, uncertainty data may be derived from replicate analysis of 5-10 test portions and the mean from corresponding concurrent recovery data. This is practical only in cases where the results are extremely important and where there is doubt about compliance with an MRL. These uncertainty data will embrace the uncertainty of sub-sampling and analysis for the particular sample within the laboratory. These uncertainty data will not indicate the bias, or the uncertainty of bias, of the laboratory.

**83** The use of reporting limits based on the LCL eliminates the need to consider uncertainty associated with results  $<LCL$ .

#### *Interpretation of results*

**84** 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.

#### *Retention of information*

**85** Sample data records, laboratory notebooks, chromatograms, tables of results, disks bearing chromatographic or spectral data, etc., must be retained for scrutiny. The period of retention should be in accordance with national or accreditation requirements.

## Appendix 1. Glossary

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.
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 (analysis)	<p>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, or part of one. In the latter case, determination of recovery is incorporated into one of the determination batches.</p> <p>This document does not refer to “batch” in the IUPAC or Codex sense, which relates to manufacturing or agricultural production batches.</p>
bias	The difference between the mean measured value and the true value.

blank	<p>(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.</p> <p>(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.</p>
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 <i>n</i> , 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 disintegrating a solid sample.

confirmation	<p>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.</p> <p>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.</p> <p>Assays based on colorimetry, ELISA, TLC or ECD tend to demand confirmation, because of their lack of specificity.</p> <p>Mass spectrometric techniques are often the most practical and least equivocal approach to confirmation.</p> <p>AQC procedures for confirmation should be rigorous.</p>
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 densitometry, or bioassay.
ECD	Electron-capture detector.
EI	Electron ionisation (formerly also known as electron-impact 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).

interference	A positive or negative response produced by a compound(s) other than the analyte, which contributes to or detracts from the response measured for the analyte, or which makes measurement of the analyte response less certain or less 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 use of a detector with greater selectivity. 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. It is the level below which there is no experimental evidence to demonstrate that residues will have been detected and calibrated satisfactorily. It will normally correspond to the 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).

LOD	<p>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 which 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 LOD has no fixed value. The term LOD is usually restricted to the response of the detection system but, in principle, it should be applied to the complete analytical method.</p>
LOQ	<p>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 precision. Should apply to the complete analytical method. Variously defined but must be a value greater than the LOD. With most methods and determination systems, the LOQ has no fixed value.</p>
matrix blank	See blank.

matrix effect	<p>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.</p>
matrix-matched calibration	<p>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 analyte 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.</p>
method	<p>A sequence of analytical procedures, from receipt of a sample through to the calculation of results.</p>

method development	The process of design and preliminary assessment of the characteristics of a method, including ruggedness.
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 <sup>n</sup> . 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 <sup>2</sup> ). In ion-trap mass spectrometers, the procedure may be carried out repetitively on a sequence of product ions (MS <sup>n</sup> ), although this is not usually practical with low-level residues.
negative result	A result demonstrating that the analyte was not found in a sample, at or above the reporting limit. It does not demonstrate the complete absence of the analyte and does not imply that the result is arithmetically negative. “Null result” is a more appropriate term, though rarely used. A negative result is reported as less than the reporting limit.
NPD	Nitrogen-phosphorus detector.
performance verification	see analytical quality control (AQC)
positive result	A result demonstrating that the analyte was found in a sample, at or above its reporting limit.

priming (of GC injectors and columns)	<p>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 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.</p>
procedural blank	See blank.
procedural standard	<p>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.</p>
reagent blank	See blank.
recovery (of analyte through an analytical method)	<p>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. The mean recovery provides a measure of part of the bias in results. It may or may not be the most significant source of bias in any particular case.</p> <p>Routine recovery refers to the determination(s) performed with the analysis of each batch of samples.</p>
reference material	<p>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.</p>

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	<p>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.</p> <p>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%).</p>
reporting limit	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 LCL. For EU monitoring purposes, it should refer to an LCL that is applicable to all data obtained in the year.
representative analyte (known as “reference pesticide” in the first edition of the guidelines)	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.
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	<p>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.</p> <p>Internal reproducibility is that produced in a single laboratory under these conditions.</p> <p>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%).</p>
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.
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. A ratio of 3:1 is often considered acceptable. However, the values for S/N produced by integration software can be questionable. In addition, the acceptable S/N may be influenced by the relative frequency of the noise. Chemical noise (interference) must also be considered. The overriding aim should be to meet the criteria of paragraphs 39 and 58-60, as far as practicable.
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 <sup>n</sup> 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.

standard addition (for calibration only)	Addition of a known quantity of analyte to an aliquot of a sample extract, etc., immediately prior to the final determination. Additions of different quantities may be made to separate aliquots. The difference in response obtained with and without the addition calibrates the response to the known quantity. The quantity present without the addition is calculated by simple proportion, or graphically as the intercept. This form of calibration compensates fully for matrix effects but it requires some knowledge of the likely concentration of analyte in the sample, and that the “concentration” of matrix is similar with and without addition of the analyte.
standard addition (for automatic adjustment of recovery and calibration)	Addition of a known quantity of analyte to one of two duplicate test portions of the sample to be analysed, immediately prior to extraction. The difference in response obtained from the determination system notionally calibrates the response to the known quantity and compensates for recovery. The quantity present without the addition is calculated by simple proportion. Matrix effects are compensated. As with other approaches to adjustment for recovery, the calculated result for each sample is affected by the precision of the method, because of the addition and relative measurement error of the internal standard. The technique assumes some knowledge of the likely concentration of analyte in the sample, and that the “concentration” of matrix is similar with and without addition of the analyte.
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	A representative sub-sample of the test sample, i.e. the portion which is to be analysed.
test sample	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.
TLC	Thin layer chromatography

<p>uncertainty (of measurement)</p>	<p>A range around the reported result within which the true value can be expected to lie with a specified probability (usually 95%). An important concept but approaches to, and the estimation, expression and interpretation of, uncertainty are still evolving. ISO rules for evaluating and expressing uncertainty in measurement require identification of the potential sources of uncertainty that influence the result. Uncertainty data should encompass accuracy (bias) and reproducibility. Published procedures enable values to be calculated for typical uncertainty, rather than the uncertainty associated with an individual result.</p> <p>The use of LCL as the reporting limit effectively removes the need for assessment of the uncertainty associated with negative results.</p>
<p>unit (as part of sample)</p>	<p>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.</p>
<p>validation</p>	<p>see method validation</p>
<p>violative residue</p>	<p>A residue which exceeds the MRL or is unlawful for any other reason.</p>
<p>working standard</p>	<p>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.</p>